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BIOLOGICAL ACTIVITY AND PERSISTENCE OF ATRAZINE

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BIOLOGICAL ACTIVITY AND PERSISTENCE OF ATRAZINE

by

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ABSTRACT

Cases of atrazine damage to maize, and occasional reports of excessive residue persistence that caused injury to susceptible following crops, prompted research on factors which influence the bioactivity and persistence of the herbicide. Bioassays with several indicator species were conducted in the field and in glasshouses. Measurement of atrazine by chemical means was done in only three of the experiments. Maize seedlings were not more sensitive to atrazine when stressed by insufficient supplies of N, P, K, Ca or Mg. Neither high P supply nor phytotoxic P concentrations in maize seedlings lowered their tolerance to atrazine. These findings on the roles of certain nutrients in the tolerance of maize to atrazine concluded a series of investigations into damage reported in the field. No satisfactory evidence for the factors which rendered maize susceptible to atrazine in the field was ever found. Another investigation showed that atrazine threshold concentrations for certain susceptible species varied from soil to soil, indicating that differential availability of the herbicide for uptake by plants in different soils precludes the allocation of fixed atrazine threshold values to different

crop species. Atrazine bioactivity and persistence varied considerably in a series of field experiments which were conducted to determine the order of importance of certain soil properties in the prediction of short- and long-term bioactivity. Soil organic matter content (% C) was the best predictor in both instances. Soil pH was a poor predictor of short-term bioactivity, but became more prominent with time. Organic matter, pH and P-reversion accounted for 35%, 19% and 14% of the variation in bioactivity measured six months after atrazine application in the field. In view of the variation in atrazine persistence, and therefore, the potential for variable carry-over from soil to soil, the applicability of the recropping interval recommended for dry beans and sunflower were investigated. It was concluded that the specification could be justified for sunflower only, but then only on certain soils. It is suggested that recropping intervals, which are recommended for certain crops after atrazine use in maize, be refined on the basis of differences in follow-up crop sensitivity to atrazine, and relationships between atrazine persistence and certain soil properties. Chemical analysis showed that soil type and soil water content had greater influences on atrazine persistence than temperature. Irrespective of soil type, the rate of atrazine breakdown was faster in soil at a water content of field capacity, and in water-logged soil (2x FC), than in air-dry soil. A bioassay technique was used to estimate the concentration of atrazine and/or its phytotoxic residues in a soil profile. Basically the same procedure was followed in an incubation study with 25 soils to develop the following regression model for prediction of atrazine half-life in soil: $y = -2.29 + 1.77x_1 + 20.81x_2$, where y is half-life in days; x_1 = [soil pH(H_2O)]^2 and x_2 = % C. The bioassay technique proved useful for estimating total amounts of atrazine and its phytotoxic residues in various soil types.

BIOLOGIESE AKTIWITEIT EN NAWERKING VAN ATRASIEN

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UITTREKSEL

Beskadiging van mielies deur atrasien, en sporadiese berigte van oormatige nawerking met gepaardgaande beskadiging van gevoelige opvolggewasse, het aanleiding gegee tot navorsing oor faktore wat die bio-aktiwiteit en nawerking van die onkruiddoder beïnvloed. Biotoetse is met verskillende toetsplante in glashuis- en veldproewe uitgevoer. Bepaling van atrasien deur chemiese analise is in slegs drie proewe gedoen. Mieliesaailinge se verdraagsaamheid teenoor atrasien is nie deur tekorte aan N, P, K, Ca en Mg in die plante beïnvloed nie. Nog hoë P-voorsiening, nog fitotoksiese P-konsentrasies in saailinge, het hul weerstand teen atrasien verlaag. Hierdie bevindings oor die rol van sekere voedingselemente by die verdraagsaamheid van mielies teenoor atrasien het 'n reeks ondersoeke na destydse skade in die veld afgesluit, sonder bevredigende verklarings vir die probleem. In 'n ander ondersoek is gevind dat drumpelwaarde-konsentrasies van atrasien vir bepaalde gevoelige gewasplante van grond tot grond varieer. Differensiële beskikbaarheid van atrasien in grond sal dus die toekenning van 'n vaste drumpelwaarde aan 'n bepaalde gewasplant verhoed. Die

organiese koolstofinhoud van grond was die belangrikste voorspeller van die kort- en langtermyn bio-aktiwiteit van atrasien in 'n reeks veldproewe. Die variasie in bio-aktiwiteit wat ses maande na toediening van die doder deur organiese materiaalinhoud, pH en P-reversie verklaar is, was onderskeidelik 35%, 19% en 14%. Derhalwe het grond-pH, wat 'n swak voorspeller (1% van variasie) van die korttermyn-aktiwiteit van atrasien was, belangriker geraak met tyd. Die bio-aktiwiteit en nawerking van atrasien het aansienlik tussen lokaliteite verskil. Weens die variërende nawerking van atrasien in grond, en gevolglike verskille in die potensiële oordraging van atrasien na 'n volgende seisoen, is die toepaslikheid van die wagperiode wat vir droëbone en sonneblom gestel word vervolgens ondersoek. Die voorgeskrewe wagperiode kon slegs vir sonneblom as toepaslik bevestig word, en dan slegs op sekere grondsoorte. Dit word voorgestel dat wagperiodes verfyn behoort te word op basis van verskille tussen grondsoorte en die verdraagsaamheid van gewasplante teenoor atrasien. Met chemiese analise is bepaal dat grondsoort en -waterinhoud 'n belangriker effek op nawerking van atrasien as temperatuur gehad het. Vergeleke met die afbraaktempo in lugdroë grond het atrasien vinniger afgebreek in grond waar die waterinhoud by veldkapasiteit was, asook wanneer dit twee keer hierdie hoeveelheid water bevat het . 'n Biotoetstegniek is gebruik vir skatting van atrasien- en/of fitotoksiese residu-konsentrasies in 'n grondprofiel. Basies dieselfde prosedure is in 'n inkubasieproef met 25 gronde toegepas vir ontwikkeling van die volgende model vir voorspelling van die halfleeftyd van atrasien in grond: $y = -2.29 + 1.77x_1 + 20.81x_2$, waar y halfleeftyd in dae is; $x_1 =$ [grond-pH(H_2O)]^2 en $x_2 =$ % C. Die biotoetstegniek was effektief vir skattings van die totale hoeveelheid atrasien en fitotoksiese atrasienresidue in grond.

INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-*s*-triazine) is a selective soil-applied herbicide used principally in the maize-producing regions of South Africa. It is the most widely used herbicide in maize (*Zea mays* L.) but is also applied in sugarcane (*Saccharum officinarum* L.), grain sorghum (*Sorghum bicolor* (L.) Moench), pineapples (*Ananas comosus* L.), and for industrial weed control. While the crop safety margin for atrazine in maize is regarded as one of the largest safety margins in herbicide-crop relationships, cases of crop injury have been reported (Thompson, Slife & Butler, 1970). During the 1981/82 and 1982/83 growing seasons reports of atrazine damage to maize, resulting in up to 25% stand loss, were received from certain parts of the main maize growing region in South Africa (Malan, Visser & Van de Venter, 1985).

Of greater concern than infrequent and isolated lapses in selectivity is the frequently recurring problem of excessive persistence of soil residues of atrazine affecting the growth of sensitive follow-up crops. In spite of exhaustive research on atrazine since its discovery in the 1950's, occasional problems of carry-over to sensitive crops (Pestemer, Stalder & Potter, 1983; Shea, 1985; Haigh & Ferris, 1991) and contamination of water resources (Wood, Harold, Johnson & Hance, 1991) are encountered worldwide. The discovery that trace amounts of certain agrochemicals such as atrazine can occur in ground and surface waters has heightened public awareness and interest in the environmental behaviour of pesticides (Leonard, Shirmohammadi, Johnson & Marti, 1988; Schiavon, 1988; Riley, 1991). In Italy the use of atrazine was barred

after residues were detected in ground water (Del Re, Capri, Bergamaschi & Trevisan, 1991), while elsewhere in Europe a limit of 0.1 μg L^{-1} for individual pesticides in ground and surface waters has been imposed through the European Community Drinking Water Directive (Tooby & Marsden, 1991).

Knowledge of factors that govern herbicide uptake by plants, crop sensitivity, and the active lifetime of compounds in soil is required to properly manage the use of potentially persistent herbicides. Persistent herbicides should be administered in ways that acceptably limits residual biological activity, thereby lowering the risk of damage to susceptible follow-up crops. In addition, residues should be prevented from reaching surface-water and groundwater below the rooting zone. From an agronomical viewpoint, the terms "bioactivity" and "persistence" are interrelated. Persistence has been defined as "the continuation of herbicidal bioactivity beyond the time of planting a sensitive following crop". Herein lies the ambiguous nature of the term "persistence" - it is positive in the sense that the ideal herbicide should persist long enough to provide acceptable weed control during the season in which it was applied, but negative in the sense that persistence must not be so long that phytotoxic residues carry over to sensitive following crops. Herbicidal bioactivity and persistence are both determined by the following four factors: characteristics of the compound, application rate, plant sensitivity, and rate of dissipation in the environment (Beyer, Duffy, Hay & Schlueter, 1988; Hance, 1988; Nash, 1988; Leistra & Green, 1990).

Forecasts of the presence and bioactivity of atrazine residues are often perplexing. Behavioural inconsistencies locally and abroad have occasionally manifested in either

poor weed control by the herbicide, loss of selectivity which leads to crop injury, or excessive persistence that causes damage to sensitive following crops. Research presented here formed part of a comprehensive study to identify environmental and plant factors which influence the behaviour of atrazine in South African soils. Although past research on atrazine in this country focused on factors affecting the bioactivity of the herbicide, excessive persistence and the concomitant injury to follow-up crops is the real problem.

Some additional facets of the bioactivity of atrazine were investigated in the present study, but the main aim was to accrue information on factors which govern atrazine persistence in soils. Specific aims were: a) to establish whether growth-retarding levels of N, P, K, Ca and Mg and excessive amounts of P in the growth medium could influence the resistance of maize to atrazine; b) to assess the variability in the resistance shown by several susceptible crops to atrazine in various soils; c) to substantiate under field conditions the order of importance of some soil variables in the prediction of atrazine bioactivity, as found in a previous study in the glasshouse; d) to assess to what extent atrazine persistence varied between soils in the field, and to establish which soil variables predicted the bioactivity of residual atrazine best; e) to demonstrate that recropping periods, which are specified for crops that are susceptible to atrazine, require refinement; f) to show that soil type, soil water content and temperature influence atrazine persistence; g) to assess whether the bioassay technique could be useful in monitoring the movement of atrazine in soil, and h) to establish the importance of selected soil properties on atrazine half-life. It was hoped that the work would contribute towards the improved prediction of atrazine persistence in soils.

CHAPTER 1

LITERATURE REVIEW

Introduction

The fate of a herbicide applied to soil is governed to a large extent by its adsorption on soil colloids, desorption of the adsorbed fraction, its degradation and persistence (Briggs, 1984; Rahman, Krishna & Rao, 1988). Herbicides dissipate from soil by a variety of means. Some of the more important avenues of dissipation are chemical degradation, leaching, microbial degradation, uptake by plants, photodecomposition and volatilization (Bailey & White, 1970; Weber, 1970a,b; Goring & Hamaker, 1971; Swann & Eschenroeder, 1983; Weber, 1991a). Weber (1991a) presented a review on the many chemical, physical and biological processes involved in the dissipation of herbicides in the environment. By definition, dissipation infers that "the herbicide is transformed into by-products, which are further transformed, and that the parent herbicide and its by-products are also transferred to other places and transformed until none exists, or in most cases none is detectable". Hence the many processes in the dissipation of herbicides are categorized as transfer processes or transformation processes (Dubach, 1970; Weber, 1991a).

Transfer processes are characterized by the chemical molecule remaining unchanged. Transfer mechanisms include adsorption to and desorption from soil colloids, absorption, exudation and retention by plants, movement on the soil surface, volatilization, and leaching or capillary movement. The transformation processes cause

loss of integrity of the chemical molecule. These processes include degradation by chemical, photochemical or biological means. Transformation of a chemical can occur during any of the transfer modes.

There is considerable interest in atrazine and other *s*-triazine herbicides because of their extensive use in agriculture, their persistence in soil, effects on succeeding crops, and movement into surface and groundwater. The herbicidal activity, mode of action, and fate of atrazine in biological and soil systems have been extensively reviewed by Esser, Dupuis, Ebert, Marco & Vogel (1975). Erickson & Lee (1989) reviewed factors involved in the degradation of atrazine and related *s*-triazines. Nel & Reinhardt (1984) reviewed factors which affect the activity of atrazine in plants and soil, and also reported on research conducted locally. The present treatise on the documented fate of atrazine in the environment will be concerned primarily with the dissipation of the herbicide in soil. Comprehensive reviews on the effects of soil factors on herbicide activity have been presented by Riley & Morrod (1976), Eagle (1976), Walker (1980), Hance (1983) and Weber (1991a). Similar effects of soil would be expected for herbicides with comparable chemo-physical characteristics, because the movement and uptake of herbicides in soil depend on the soil/chemical interaction; and not on herbicide bioactivity which is only expressed when the chemical reaches the active site in the plant. Hence the literature citations in the present review include those studies in which atrazine featured, and situations in which other compounds behaved in a manner similar to atrazine.

TRANSFER PROCESSES

Adsorption on soil colloids

Herbicide adsorption is dependent on soil chemical and physical properties, structure of the herbicide and environmental factors such as temperature and soil water content. Schiavon (1988) and Hubbs & Lavy (1990) found that adsorption determines movement of the herbicide in soil, including leaching to groundwater. Adsorption also reduces the availability of atrazine for absorption by plants, thereby reducing herbicide bioactivity (Dao & Lavy, 1978).

The availability of atrazine for absorption by plant roots is dependent on the adsorption-desorption equilibrium being shifted towards the desorption side. This equilibrium must be expected to be quite variable due to the involvement of the different, often interrelated, constituents of the solid soil phase. These soil constituents include clays, hydrous oxides of iron and aluminium, organic material and various other organic substances (Dubach, 1970; Hayes, 1970; Weber, 1970a,b; Weber, 1991a). Green & Obien (1969) and Dubach (1970) contend that the dynamic and open nature of soil, absorption by plants and microorganisms, and degradation processes ensure that equilibrium with respect to herbicide concentration between the adsorbed and solution phases is never reached.

It has been shown that herbicides generally, and the triazines particularly, are reversibly adsorbed on the organic matter and clay mineral fractions in soils (Frissel, 1961; Talbert & Fletchall, 1965; Weber, 1970a,b; Hayes, 1970; Schiavon, 1988). Several

studies revealed that soil organic matter is the most important soil factor as far as atrazine adsorption or bioactivity is concerned (Talbert & Fletchall, 1965; Weber, Weed & Ward, 1969; Harrison, Weber & Baird, 1976; Rahman & Matthews, 1979; Anderson, Stephenson & Corke, 1980). In spite of relatively low organic matter contents in South African soils, Smit, Nel & Fölscher (1980), Nel & Reinhardt (1984) and Ehlers, Reinhardt & Nel (1987, 1988) found that the organic carbon content (% C) of soil was strongly and negatively correlated with atrazine bioactivity. In all the above-mentioned studies, organic matter content consistently predicted atrazine availability better than either cation exchange capacity (CEC), total clay content or individual clay minerals. According to Eagle (1983b), a positive relationship usually exists between the clay and organic matter content of soils, but problems tend to occur when this is not the case, and the recommendations for herbicide rates to be applied are based solely on the clay content of soils. In these situations, either excessive bioactivity, which may lead to crop damage, or low bioactivity resulting in poor weed control may be expected.

Weber (1991a) states that organic matter, clay minerals and sesquioxides represent the active fraction of soil, and silt and sand the inactive fraction. The active fraction, which is involved in chemical reactions in the soil, is colloidal in nature, i.e. the constituents representing this fraction are organic and inorganic substances with a small particle size (< 0.001 mm) and a resulting large surface area per unit mass. According to Weber (1991a), it can be expected that the tilled surface soil is a relatively homogeneous mixture of the active and inactive fractions. Weber (1991a) reviews the important characteristics of soil colloids which pertain to their ability to adsorb

herbicides. Properties of soil colloids involved in adsorption of herbicides are briefly discussed here.

Weber (1991a) contends that organic matter is more flexible than clays with respect to the ability to adsorb a wide assortment of herbicides. Some degree of solubility is required of herbicides to become retained at the highly hydrated hydrophilic surfaces of clay particles, whereas organic matter has both hydrophilic and lipophilic characteristics which enables it to react with herbicides of wide ranging solubility and ionizability. According to Kononova (1966), and Weber, Swain, Strek & Sartori (1986), humified substances (humus) make up 17 to 97% of the total organic carbon in a soil. Humus consists predominantly of humic acids which are high molecular mass compounds with functional groups and aromatic rings which are lipophylic in nature and which possess numerous ionizable carboxyl and hydroxyl groups that give the polymer pH dependent exchange properties (Kononova, 1966).

Bohn, McNeal & O'Connor (1985) list some important characteristics of certain clay minerals which adsorb herbicides. The two major types of clay minerals present in soils are the 1:1 and 2:1 (silica:alumina) types (Brady, 1974). Kaolinite (1:1 clay mineral) does not exhibit high-intensity colloidal properties because of its limited adsorptive capacity for cations and a relatively low surface area. The intensive study of Frissel (1961) focused on the important role of montmorillonite and to a lesser extent illite and kaolinite in the adsorption of triazines in soil. Talbert & Fletchall (1965) reported that kaolinite at pH 5 and pH 7 did not adsorb either atrazine or simazine. In the 2:1 expanding type of clay, montmorillonite, there is a very large internal surface

which far exceeds its external surface area. The combined internal and external surfaces (specific surface area) of montmorillonite greatly exceeds the surface area of kaolinite.

The oxides and hydrous oxides of iron and aluminium are intermixed with silicate clays in temperate regions. They are prominent in tropical and sub-tropical soils in several mineralogical forms, including boehmite, gibbsite, goethite and hematite (Brady, 1974; Brown, Newman, Rayner & Weir, 1978). These minerals have a pH dependent charge, and therefore may exist as positive, neutral, or negatively charged particles. The hydrous oxides have a much smaller CEC than kaolinite. In South Africa the amorphous Fe-Al-OH component is commonly found in acid soils in which kaolinite predominates. The Fe subfraction tends to be bonded strongly to crystalline minerals such as kaolinite, whilst the more weakly bonded Al fraction carry active positive charge (Fouché & Brandt, 1973). Smit, Nel & Fölscher (1980) suggested that atrazine can be bonded to the positive charge of Al of the (Fe.Al.OH) component through free electrons on the N atoms of amine groups and/or N atoms in the ring structure of atrazine. In the light of increased atrazine bioactivity that was caused by increases in the pH and the phosphorus level in soil, they construed that increases in OH^- and $H_2PO_4^-$ neutralised the adsorptive capacity of the amorphous component for atrazine.

There are two general sources of CEC for inorganic colloids: (a) permanent negative charge that results from isomorphic substitution, and (b) pH dependent charge which results from the ionization of hydrogens from hydroxyl (OH) groups located at the edges of micelles and from iron and aluminium hydroxides (Anderson, 1983). The

greater surface area of the expanding type clays, combined with a much greater CEC, endows them with a much greater propensity for binding herbicides than the non-expanding types (Weber, 1972; Weber, Shea & Weed, 1986).

Adsorption theories and equations

Atrazine has a low vapour pressure (0.08 mPa at 25°C), which makes adsorption at the solid-water interface, and not adsorption at the solid-gas interface, the dominant phenomenon in soil treated with this herbicide (Calvet, 1980). The herbicide exists in the soil in two phases: solution and sorbed. The total amount of herbicide, solution plus sorbed fractions, decreases with time due to degradation, but at any time follows the mass conservation equation: $m = Vc + Mx$. In this equation, m is the total mass (μg) of the herbicide in the system, V is the volume of water (ml) in the system, c is the herbicide concentration ($\mu g\ ml^{-1}$) in water phase, M is the mass (g) of soil in the system, and x is the herbicide concentration (μg^{-1}) in the sorbed phase. According to Calvet (1980), knowledge of solute concentrations only is deemed sufficient for the adsorption process to be described, usually as an adsorption isotherm, i.e. the relationship between amount adsorbed and solution concentration. Isotherms that describe herbicide adsorption in aqueous solutions (soil-water systems) have been derived empirically. Weber (1991a) states that the relatively low solubility of most herbicides, and the lack of knowledge about adsorption mechanisms also limit the value of equations to describe adsorption.

The effect of adsorption on herbicide availability in the soil solution can be estimated from adsorption coefficients which are derived from adsorption theories. The empirical

Freundlich equation (1) can be rearranged to obtain a distribution coefficient (K_d) for a given herbicide in a given soil as shown in Equation 2 (Weber, 1991a).

$$X = KC^{n^{-1}}$$

X = quantity of herbicide (nmol) adsorbed to a mass (1 g) of soil

C = concentration of herbicide in solution (nmol ml^{-1})

K = constant which reflects adsorption capacity

n^{-1} = constant which reflect intensity of binding

$$K_d = \frac{X}{C^{n^{-1}}}$$

K_d = distribution coefficient

Riley (1978) found that the K_d value for atrazine varies between about 1 and 3, the latter value being typical in loam soil containing 3% organic matter. Herbicides with a $K_d < 10$ are classified as mobile in soil (Riley, 1991).

K_d values are normally highly correlated with the organic matter contents of soils, increasing as organic matter content increases (Weber, 1991a). Knowing the percentage of organic carbon of a soil, and assuming that herbicides are adsorbed only to organic surfaces (most applicable to nonionic, lipophilic herbicides), a K_{oc} value (partition coefficient) for a given herbicide adsorbed by a particular soil may be calculated using Equation 3 (Weber, 1991a).

$$K_{oc} = \frac{K_d}{\%OC} x100 \qquad \text{------3}$$

K_{oc} = partition coefficient

oc = organic carbon content of soil (%)

K_{oc} values calculated for selected herbicides, as well as certain other key properties appear in Table 1.

An assumption associated with the Freundlich equation is that the herbicide adsorption-desorption process is a reversible equilibrium that is attained during the time period of the experiment (Clay, Allmaras, Koskinen & Wyse, 1988). However, it has been reported *inter alia* by Calvet (1980) and Clay & Koskinen (1990) that desorption of organic chemicals from the soil is not satisfactorily predicted by the adsorption Freundlich equation. Difference in the adsorption and desorption isotherms is known as hysteresis. Non-extractable herbicide fractions are termed "fixed". Best & Weber (1974) found that this plant-unavailable fraction range from 4.6 to 23% for atrazine and prometryn, depending on the pH of the system. A literature review by Rahman *et al.* (1988) revealed that much less attention has been paid to desorption as compared to adsorption.

Table 1 Classification and key properties of atrazine and selected ionic herbicides (from Weber, 1991a; originally from Weber, 1972, 1987)

Category	Common name	Species	pKa	Water solubility (mg L^{-1})	Vapour pressure (mPa)	Soil K_{OC}	Half-life (days)
Strong base	paraquat	$R\text{-}N^{+2}$	>9	1×10^6	<0.01	10^5-10^6	100-400
Phosphonic acid	glyphosate	PO_3^{-1}; PO_3^{-2}	3.7; 10	1.6×10^4	<0.001	10^4-10^5	20-40
Moderate base	prometryn	molecule; cation	4.1	52	0.27	300-600	30-60
Weak base	**atrazine**	molecule; cation	1.7	30	0.08	150-300	25-50
Very weak base	metribuzin	molecule; cation	1.0	1300	<0.13	20-60	15-30
Very weak acid	bromacil	molecule; anion	9.1	815	0.033	30-60	60-120
Strong acid	dalapon	molecule; anion	1.5	1×10^6	<0.13	1-2	3-10

Adsorption mechanisms

Details of the types of herbicide adsorption isotherms and mechanisms have been discussed by Weber & Miller (1989). Evidence that Van der Waals forces, ion exchange, hydrogen bonding and complexation are involved in the adsorption of triazines on organic matter is thoroughly reviewed by Hayes (1970). Bailey & White (1970) categorized mechanisms of adsorption of organic chemicals on clay minerals. Postulated mechanisms of adsorption between organic molecules and organic and mineral adsorbants are the following:

(i) Ionic bonds. This type of bonding involves chemical adsorption due to attraction of opposite unit charges between adsorbate and adsorbent (Anderson, 1983). Adsorption by ionic bonds is due to ion exchange, a process in which the N atoms in the atrazine molecule would be protonated, resulting in the molecule existing as a cation in the soil system. In general, chemical adsorption is of high bonding strength.

The findings of Kononova (1966) and Sullivan & Felbeck (1968) suggest that protonated atrazine molecules may be bonded to carboxyl anions ($-COO^-$) on the colloidal surface, and that this adsorption mechanism is pH dependent. In moderately to strong acid soils the hydrogen is apparently tightly held by covalent bonding on the colloidal surface, and is therefore not subject to ready displacement by other cations. A negative colloidal surface charge is therefore not apparent under acid soil conditions (Brady, 1974), thus precluding extensive ionic binding of organic bases. Hayes (1970) is of the opinion that ion exchange can account only for a small fraction of adsorption on organic colloids, since humic acids ionize at pH values lower than those normally encountered in soil. The magnitude of the pH dependent colloidal charge varies with the type of colloid.

It is the dominant type of charge for organic colloids, and accounts for most of the charge of the 1:1 type clay minerals and up to one fourth of that of some 2:1 types (Brady, 1974).

(ii) Coordinate bonding. Coordination compounds (metal atom complexes) form when an atom or ion of a metal is surrounded and bonded by a cluster of ions or molecules. This cluster is referred to as a ligand. Such interactions are possible with the water molecule usually being the exchange ligand. Weber *et al.* (1969) contend that atrazine may be adsorbed by complex formation. However, according to Hance (1971), it is unlikely that atrazine is adsorbed in this way.

(iii) Hydrogen bonds. Hydrogen bonding occurs when a hydrogen atom is attracted to two highly electronegative atoms (Anderson, 1983). Hydrogen bonds of appreciable strength only occur in molecules containing highly electronegative atoms, such as oxygen, nitrogen, bromine, sulphur, carbon and chlorine; so that all herbicides are involved. In spite of the limitation of not very convincing experimental proof (Calvet, 1980), two kinds of hydrogen bonds have been described: (a) between adsorbed water molecules and adsorbed organic molecules; (b) between surface groups (e.g. oxygen atoms) and organic molecules. The first type has been suggested by Calvet & Terce (1975a) to be a mechanism for the adsorption on montmorillonite of atrazine. Hayes (1970) is of the opinion that adsorption by hydrogen bonds between the secondary amino groups of the atrazine molecule and the carbonyl group (-C=O) of organic material (carbonyls and ketones) is more important than is generally accepted.

(iv) London-Van der Waals bonds. These are due to physical forces (dispersion forces) which result from the interaction of the positive and negative charges between neighbouring atoms (Anderson, 1983). In general, physical adsorption is of low binding strength and probably exists with all herbicides (Calvet, 1980). Physical adsorption by Van der Waals forces is temperature-dependent. At high temperatures, the kinetic energy of the atrazine molecule is higher than the energy involved in Van der Waal bonds, thereby rendering this adsorption mechanism irrelevant according to Hayes (1970).

Weber (1991a) explains that the complexity of the soil system due to its many phases, including solids, colloids, soil solution, solutes, gases such as CO_2 and O_2 and vapours of herbicides and other organics, precludes adequate descriptions of adsorption-desorption reactions of herbicides in soils. The many different adsorption mechanisms that are possible, depending on the chemical and physical properties of the herbicides involved, further confound the issue. This aspect is illustrated by the grouping of atrazine and selected herbicides into seven categories (Weber, 1972; Weber, 1987) in Table 1 on the basis of key properties.

Influence of soil pH, water content, electrolyte concentration and temperature on adsorption

The primary roles of the physico-chemical properties of both the herbicide and the soil colloids in the adsorption-desorption phenomenon have been discussed above, but the availability of atrazine for absorption by plant roots is known to be directly or indirectly

influenced by several other factors. Although identification of the individual effects of soil factors involved in herbicide bio-availability is often complicated by interrelationships between them, several factors which affect atrazine adsorption have been documented.

Soil pH

According to Appleby (1985), soil pH has an important influence on the adsorption, and therefore, the availability of atrazine for uptake by plants. Soil pH influences triazine adsorption through its effect on both the chemical structure of the herbicides and soil adsorption sites. Weber & Whitacre (1982) found that atrazine is a weak base in aqueous solution and exists as a molecular species at high pH, and cations at low pH. Only atrazine in the cation form would be subject to adsorption on soil colloids with negative charge, thus explaining its increased adsorption and lower phytotoxicity at low pH in most soils. It was shown by Colbert, Volk & Appleby (1975) that adsorption of atrazine and terbutryn decreased on natural and limed soils as the soil pH increased to pH 8. 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 soil pH level (7.7) compared to the lower pH (5.5). Consistent with the reported decrease in *s*-triazine adsorption with increased pH, Smit *et al.* (1979, 1980) showed that the phytotoxicity of atrazine in some soils was increased by increases in the soil pH from below pH 5 to about pH 6. They attributed this effect to increased availability of atrazine for uptake by plants, and also to increased stability of atrazine molecules at the higher pH levels.

Several studies revealed that adsorption of *s*-triazines increased with a concomitant decrease in soil pH (McGlamery & Slife, 1965; Talbert & Fletchall, 1965; Weber, 1970b; Yamane & Green, 1972; Marshall, Nel & Smit, 1982). Protonation of atrazine molecules, and subsequent adsorption at negatively charged sites on soil colloids, progressively increases as soil pH decreases. Weber (1970b) suggested that atrazine may be bonded by complexation with protons on clay surfaces under acid conditions. Maximum adsorption of atrazine may be expected to occur near the pKa value (1.68) for this herbicide (McGlamery & Slife, 1965; Weber, 1970b). Reduced sorption is possible at pH levels lower than the pKa value, because of increased competition between hydronium ions (H_3O^+) and atrazine (cations$^{(+)}$) for negatively charged sites on soil colloids.

Harris & Hurle (1979) showed that atrazine and simazine adsorption onto clay colloids are highly sensitive to minute changes of soil solution pH and that plants can play an important role here by bringing about such changes in pH through ion exchange. They theorized that since triazine adsorption is so highly sensitive to pH changes, any change in rhizosphere pH through ion exchange or microbial activity must affect the adsorbed/solution herbicide equilibrium and, therefore, the amount of herbicide immediately available to the plant.

Soil water

The availability of atrazine for uptake by underground plant parts is influenced directly by soil water content, since water is the medium in which atrazine is transported and from which it is adsorbed. Green & Obien (1969), Bailey & White (1970) and Appleby

(1985) found an inverse relationship between atrazine adsorption and soil water content. Dao & Lavy (1978) suggested that soil water depletion would increase the atrazine concentration in soil solution, allowing polar atrazine molecules to compete favourably with a reduced number of water molecules for binding sites on soil colloids. Green & Obien (1969) were of the opinion that atrazine mobility in soil, and not fluctuations in atrazine concentration in soil solution *per se,* governed herbicide availability at the root surface. Several studies indicated that atrazine phytotoxicity was linked to soil water content, with increases in bioactivity as soil water content increased (Lavy, 1968; Dao & Lavy, 1978; Nel & Reinhardt, 1984).

According to Ammon (1985), leaching depth is a function of herbicide mobility, persistence, weather factors and percolating water. These parameters need to be considered in estimations of the potential of a herbicide to contaminate underground water. Leaching of a herbicide beyond the root zone is dependent on the amount, frequency and intensity of water received. Apart from temperature, which has a significant effect on microbial activity and hence the persistence of compounds, the most important climatic characteristics relating to pesticide leaching are the duration of the "field capacity period" and the amount of "excess rain" during this period (Hollis, 1991). According to Talbert & Fletchall (1965) and Nel (1975), most of the atrazine applied at normal rates can be leached out of the root zone, but those fractions adsorbed on the internal surfaces of 2:1 swelling type clays, such as montmorillonite, would be least subject to leaching. Based on K_{oc} values, atrazine (K_{oc} = 102 to 163) is classified as moderately to slightly mobile (Leonard *et al.*, 1988). Because of the pH dependent adsorption of atrazine on soil colloids, the herbicide is more susceptible to leaching

under neutral and alkaline than under acid conditions (McGlamery & Slife, 1965; Smit & Nel, 1977).

Coarse-textured soils, which are low in organic matter and clay, would be particularly susceptible to atrazine leaching because these soils have limited sorption capacity. Fleming, Wax & Simmons (1992) contend that continued registration of atrazine for use in these soils may depend on development of formulation or application methods that reduce leaching potential. Controlled release formulations may have promise for reducing the mobility of atrazine. Fleming *et al.* (1992) showed that a starch-encapsulated atrazine formulation (granules 20-40 mesh) should result in relatively fast atrazine release (compared to release from large granules of 14-20 mesh) and improved weed control (compared to weed control provided by the large granules and a commercial dry flowable formulation), while still reducing the leaching potential of atrazine.

According to Spencer & Cliath (1973), herbicides in the unsaturated soil solution may undergo some net upward movement with water as it moves from high to low potential via evaporation from the soil surface. Hubbs & Lavy (1990) states that the magnitude of this effect is proportional to the adsorbed and soil solution amounts of a compound. These researchers found that evaporation of water from the soil surface, and consequent upward movement of atrazine with capillary water, may be important in the dissipation of the herbicide. They also reported significant atrazine losses from plain glass slides and ascribed them to volatilization. However, a soil coating on the glass slides reduced ^{14}C volatility losses for atrazine from 81 to 7%. Although these laboratory studies

suggest that capillary movement is important to the volatilization loss of atrazine, it remains doubtful that volatilization is an important avenue of dissipation of atrazine under field conditions.

Water solubility is often regarded as an indicator of adsorption and leaching. Retention of urea and triazine herbicides in the upper layers of the soil profile was originally attributed to their low water solubility. As discussed by Hartley (1976), it was subsequently recognized that adsorption by soil, not insolubility, was the most important factor retarding the movement of chemicals through soil. According to Briggs (1984), there is an inverse relationship between solubility and K_{ow} and hence K_d for liquids, whilst for solids an additional factor is the energy required to disrupt the crystal structure. This is related to the melting point (T_m) of the chemical (Briggs, 1981a) by the equation:

$$\textbf{log water solubility (mole ml}^{-1}\textbf{)} = -\log K_{ow} - 0.01(T_m - 25) \quad \text{-----4}$$

It can be calculated that there can be a 1000-fold variation in water solubility between a liquid and a high melting point solid of the same K_{ow} and a corresponding 36-fold difference in K_d for compounds of the same water solubility (Briggs, 1984). Nicholls, Briggs & Evans (1984) showed that there are few chemicals for which water solubility is an important feature of their behaviour in soil. After an initial equilibration period all soil-applied herbicides are either adsorbed or dissolved at normal field rates, water solubility being a factor only for simazine and lenacil, each with log $K_{ow} < 2$ and a high melting point (Briggs, 1984). The log K_{ow} values/melting points for atrazine and simazine are 2.0-2.5/173°C and 1.5-2.0/225°C, respectively. From equation (4), the

water solubility of atrazine (33 mg L^{-1} at 25°C) would be expected to be higher than that for simazine (3.5 mg L^{-1} at 20°C).

Weber (1991a) states that organic matter is more universal in its ability to react with a wide assortment of herbicides with varying degrees of solubility and ionizability. Grover (1965) contends that under high soil water conditions, the availability of atrazine will predominantly be determined by the extent of its adsorption on the hydrophobic adsorptive sites on organic colloids. Under high soil water conditions adsorption on hydrophilic sites could be insignificant because atrazine would be readily desorbed from these sites by the overwhelming number of highly polar water molecules. Under low soil water conditions the availability of hydrophilic adsorptive sites will affect the bioactivity of atrazine. As the water content in the soil is decreased, more atrazine will be adsorbed on the now accessible hydrophilic surfaces (Grover, 1965).

Electrolyte concentration in soil solution

The differential availability of nutrients and soil-applied herbicides for uptake by plants depends on differences in their solubility in the soil solution and the extent and strength of adsorption. Hurle & Freed (1972) found that atrazine and simazine adsorption increased in the presence of rather high electrolyte (NH_4^+, K^+, Ca^{++}) concentrations and ascribed this effect to depressed herbicide solubility. Since the replacing power of cations on clay and humus in general follows the order $C^+ < C^{++} < C^{+++}$ (Brady, 1974), the divalent Ca^{++} ions would cover more of the negative adsorption sites and were more strongly adsorbed than the monovalent K^+ and NH_4^+ ions (Hurle & Freed, 1972). Herbicide adsorption could, therefore, be limited by competition between

atrazine and cations for negatively charged adsorption sites, thereby increasing the concentration of atrazine in the soil solution. These findings were corroborated when Dao & Lavy (1978) showed that atrazine adsorption increased with an increase in the concentration of KCl and NH_4Cl in the soil solution. However, the electrolytic effect of $CaCl_2$ was comparatively low, probably because of competition between Ca^{++} and atrazine for adsorption sites (Dao & Lavy, 1978). Hurle & Freed (1972) are of the opinion that the effect of electrolyte concentration is negated under field conditions by constantly changing uptake by plants, dilution or precipitation.

Temperature

Temperature affects herbicide activity in various ways, often interrelated with other environmental factors. According to Appleby (1985), temperature affects the bioactivity of atrazine by influencing its adsorption in soil, absorption by plants, and fate in plants.

Several researchers have shown that increasing temperatures result in decreased adsorption of atrazine on clay colloids (McGlamery & Slife, 1965; Talbert & Fletchall, 1965). In contrast, McGlamery & Slife (1965) also found that adsorption of atrazine on a humic acid increased as temperature increased being nearly twice as great at 40°C than at 0.5°C. This is opposite of what usually occurs with mineral systems (Bailey & White, 1970). McGlamery & Slife (1965) concluded that the adsorption phenomena that occur on isolated humic acids may not be the same as those occurring with natural soil organic matter. There was very little desorption from the humic acid, but desorption of atrazine in a soil increased as temperature increased, desorption being

nearly complete at 30°C. According to McGlamery & Slife (1965), increases in temperature should increase desorption as desorption is endothermic.

Temperature is believed to exert an indirect influence on the adsorption process through its effect on herbicide solubility. Bailey & White (1970) reported the existence of an inverse relationship between the degree of adsorption and solubility within a number of herbicide groups, in particular *s*-triazines. McGlamery & Slife (1965) suggested that solubility play a role in the temperature effect on desorption as the solubility of atrazine is only 22 mg L^{-1} at 0°C, while it is 70 and 320 mg L^{-1} at 27 and 85°C, respectively.

Absorption and retention by plants

Plants respond to herbicides only after absorption and translocation of the compound to the site of action in plants. Lavy (1970) showed that uptake of *s*-triazines occurs primarily from free herbicide in soil water. Herbicides that enter the roots and are readily translocated to the site of action in the plant include amitrole, dalapon, *s*-triazines, TCA and ureas (Anderson, 1983). Soil-applied atrazine is absorbed mainly by the roots and translocated in the apoplast system along a water gradient established by water loss through transpiration (Ashton & Crafts, 1981). Minshall, Sample & Robinson (1977) linked increased uptake of atrazine by tomato (*Lycopersicon esculentum* Mill.) plants to increased flow of the xylem stream. They also found that the uptake mechanism tended to maintain a constant concentration of atrazine in the xylem stream of plants.

Robinson & Dunham (1982) were able to predict the uptake of atrazine and terbuthylazine by oats seedlings on the basis of the mass flow theory. It is conceivable that metabolism of atrazine to hydroxyatrazine in root tissue causes absorption of more atrazine because this conversion reduces the concentration of atrazine in the cells and more atrazine diffuses into the tissue down the concentration gradient (Price & Balke, 1983).

Norris & Fong (1983) suggested that differences in atrazine metabolism, in conjunction with the altered partitioning between polar and nonpolar plant components, could lead to differential herbicide uptake. Phillips, Egli & Thompson (1972) found that soybean (*Glycine max* Merr.) seeds absorbed atrazine in relatively large quantities, and explained it in terms of the compatibility of this nonpolar (lipophilic) herbicide with the oil in the seed.

The site of absorption for a single herbicide can differ among plant species. Atrazine is most effective when placed in the shoot zone of green foxtail (*Setaria viridis* L.), giant foxtail (*Setaria faberi* Herrm.) or radish (*Raphanus sativus* L.) (Knake, Appleby & Furtick, 1967; Knake & Wax, 1968; Shone & Wood, 1976), whereas it appears to enter wild oat (*Avena fatua* L.) plants primarily by root uptake (Nishimoto, Appleby & Furtick, 1969). Since herbicides are carried to the roots by mass flow (fast process) and to the underground shoots by diffusion (slow process), the effect of soil water on the phytotoxicity of atrazine may differ among plant species (Moyer, 1987).

Absorption and retention of herbicides by weeds and crop plants is also dependent on the characteristics of the chemical (Ashton & Crafts, 1981; Fedtke, 1982). Mobility in and out of plants is often correlated with herbicide solubility - mobility increasing as solubility increases (Weber, 1991a). According to Weber (1991a), the amount of a mobile herbicide normally absorbed and retained by plants can reach about 5% of the total amount applied. Best & Weber (1974) found that the total amount of atrazine absorbed and retained by plants over a five-month study ranged from 1.6 to 4.3% of the total amount applied; and was very dependent on the pH of the soil, higher herbicide concentrations occurring in the plants at pH 7.7 than at pH 5.5.

Hoffman & Lavy (1978) showed that plants grown in atrazine-treated soils compete for the plant-available atrazine fraction. In their bioassay studies, high plant populations were not as effective as low plant populations in detecting low levels of atrazine in soil. Conversely, by increasing plant populations or decreasing soil volumes, quantitative measurement of higher atrazine concentrations could be determined. According to Weber (1991a), high weed populations may result in much higher amounts of plant-mobile herbicide being removed from the soil, but amounts rarely exceed 10% of that applied, and that which is taken up by crop plants is normally inactivated. The amount of herbicide and metabolites removed by crop plants is normally less than 5 mg kg^{-1} and the amount deposited in crop seeds is generally non-detectable at levels lower than 1 pg kg^{-1} (Weber, 1991a).

Nelson & Khan (1992) demonstrated that hyphae of vesicular-arbuscular fungi (naturally occurring root symbionts found in many plant species) remove atrazine from soil and transfer the herbicide to maize plants.

Mechanism of action

Vostral, Buchholtz & Kust (1970) suggested that differences in the resistance of plant species to atrazine are due partly to environmental factors that alter atrazine absorption and translocation to stems and leaves. They assumed that if absorption and translocation rates are low, metabolic degradation of the herbicide may prevent accumulation of toxic amounts within the plants. Penner (1971) found increased atrazine phytotoxicity with increasing temperatures from 20 to 30°C. He suggested that a possible relationship exists between increased herbicide transport to the shoot at high temperature and increased phytotoxicity. At the high temperature, increased herbicide absorption, greater translocation from root to shoot, reduced capacity of the enzymes to inactivate the compound, or rate changes in the aforementioned factors might have resulted in accumulation of atrazine to phytotoxic amounts within the plants (Penner, 1971).

In a resistant species like maize (*Zea mays* L.) or grain sorghum (*Sorghum bicolor* L. Moench), atrazine is primarily metabolized to hydroxyatrazine before it reaches the chloroplasts (Norris & Fong, 1983). In susceptible species such as peas (*Pisum sativum* L.), beans (*Phaseolus vulgaris* L.) or oats (*Avena sativa* L.), it is primarily atrazine that reaches and accumulates in the chloroplasts (Shimabukuro & Swanson, 1969; Ezra & Stephenson, 1985).

Plant uptake of phytotoxic amounts of atrazine is shown by symptoms (chlorosis followed by necrosis) which follows the translocation pattern in sensitive plants (Ashton, De Villiers, Glen & Duke, 1977). Good (1961) and Couch & Davis (1966) found that triazines, substituted ureas, uracils and dipyridyls characteristically inhibit photosynthesis through inhibition of photosynthetic electron transport. Initially, Good (1961) and Tischer & Strotmann (1977) postulated that these herbicides exert their phytotoxicity primarily through inhibition of the excitation of electrons in the Hill-reaction of photosynthesis. The site at which herbicides inhibit the Hill-reaction was not identified, but was believed to be associated with the short wavelength absorbing pigment system located in chloroplasts (Moreland, 1965).

Recent findings by Fuerst & Norman (1991) suggest that atrazine binds to the D1 protein of the photosystem II reaction centre situated in thylakoid membranes in chloroplasts, thus blocking photosynthetic electron transport. The oxygen evolving process (Hill-reaction) of photosynthesis is closely associated with the photosystem II reaction centre where atrazine apparently exerts its influence. Treatment of sensitive plants with atrazine blocks the flow of electrons through photosystem II. According to Fuerst & Norman (1991), some weeds developed resistance to atrazine through mutations of serine 264 (an amino acid in the D1 protein) to glycine - a process that prevents atrazine from binding to the D1 protein, thus allowing unrestricted electron flow.

TRANSFORMATION PROCESSES

The above discussion of transfer processes involved in the determination of atrazine activity has dealt with some important components of the complex of factors which governs herbicide bioactivity and persistence in soil. Although degradation of herbicides in soil is only one component of this complex, it is generally considered to be the principal route for loss of most compounds from soil (Walker, 1987, 1989). Soils provide an ideal environment for many types of degradative processes. Soil is a dynamic and complex biological and chemical medium in which numerous variables interact to determine the degradation rate of a compound and, hence, its persistence. In this context, factors such as soil water content, temperature, soil texture, nutrient status, organic matter, pH and microbial activity are regarded as important variables. Degradation processes usually involve direct chemical transformation of the herbicide and the activities of soil microorganisms.

Aspects of soil properties

Organic matter content of soil could be of particular importance in determining the degradation rate and thus the persistence of atrazine. The dominant role of organic matter in the adsorption of atrazine was cited in the above discussion. Organic matter also regulates microbial activity of soil - with generally higher levels of biomass and respiration in more organic soils (Walker, 1989). The clay content of soil may also be important in determining atrazine persistence, because as pointed out earlier, it is an important predictor of atrazine availability in the soil solution. When working with natural soils, it can be difficult to separate the effects of clay and organic matter since

they are often correlated (Eagle, 1983a; Reinhardt & Nel, 1989). Clay content is an important component of soil texture, and soil texture can have a marked effect on other soil properties. Coarse-textured soils with low clay content tend to have less organic matter and lower microbial biomass than fine-textured soils (Walker, 1989). Soil texture will also influence pore size distribution in soil and consequently water relations and aeration characteristics.

Soil pH can have a pronounced effect on atrazine degradation rates. As pointed out earlier, pH exerts a direct effect when the stability of the chemical is pH dependent, or it has an indirect effect through changes in microbial populations, or through colloidal adsorption of the compound.

Aspects of temperature and water

In addition to the variability in herbicide degradation rates between soils, rates of loss may vary in a particular soil according to the water and temperature regimes encountered. In general, rates of herbicide loss increase with increases in temperature and soil water (Walker, 1989). Increased atrazine degradation rates with increasing temperature and soil water have been demonstrated in field experiments (Harris, Woolson & Hummer, 1969) and in laboratory incubation studies (Walker & Zimdahl, 1981). With many pesticides, Briggs (1983) and Walker & Allen (1984) demonstrated a 2 to 2.5 fold increase in half-life with a 10°C decrease in temperature, and a 1.5 to 2.5 fold increase in half-life if soil water is reduced by a factor of two. Weather conditions after herbicide application in the field should have a similar large effect on persistence. The weather, therefore, can have a marked influence on rates of atrazine

loss, and it is essential to be aware of this when evaluating field persistence data from different sites.

Chemical degradation

Chemical hydrolysis of atrazine to hydroxyatrazine is regarded as the main avenue of inactivation of the herbicide in soil (Armstrong, Chesters & Harris, 1967; Gamble & Khan, 1985). The atrazine molecule is stable under neutral pH conditions, but rapid chemical hydrolysis occurred under highly acid or alkaline conditions (Armstrong *et al.*, 1967). According to Weber (1970a), the Cl^- ion of the atrazine molecule is replaced with an OH^- ion through direct nucleophilic substitution under alkaline conditions. Under acid conditions, protonation of a ring or side chain N atom is followed by cleavage of the C-Cl bond by H_2O. Protonation of N would increase the electron deficiency of the C bonded to Cl, thus increasing the tendency for nucleophilic displacement of Cl by H_2O.

In studies with simazine (Walker *et al.*, 1983) and metribuzin (Allen & Walker, 1983), degradation rates decreased as adsorption on soil colloids increased, presumably due to decreased availability for degradation. Adsorption, however, does not always lead to protection from degradation, and examples of adsorption-catalyzed hydrolysis of some chlorotriazines, including atrazine, have been reported (Armstrong & Chesters, 1968; Hance, 1979). Armstrong *et al.* (1967) and Swain (1981) ascribed the major role of organic matter in the inactivation of atrazine in soil to its influence on the rate of atrazine hydrolysis. Organic matter evidently catalyzed hydrolysis by adsorption of

atrazine, accounting for the more rapid rate of hydrolysis in soils with a relatively high organic matter content compared to soil with low organic matter contents.

Microbiological degradation

As atrazine is chemically stable at neutral pH values (Armstrong *et al.*, 1967), it is likely to persist in many soils and chalky groundwater (Wood *et al.*, 1991) unless degraded by microorganisms. Kaufman & Kearney (1970) listed a large number of microorganisms that have the ability to degrade atrazine in pure culture, most of those reported being fungi. There are, however, reports of bacteria including *Arthrobacter* sp., *Bacillus* sp. and *Pseudomonas* sp. (Kaufman & Kearney, 1970; Cain & Head, 1991). Oxidative dealkylation appears to be the major mechanism by which microorganisms degrade atrazine, but degradation has also been observed under anaerobic conditions (Kaufman & Kearney, 1970).

SIGNIFICANCE OF PERSISTENCE

The above discussion has given an indication of how variations in some soil properties can influence rate of degradation through effects on adsorption, chemical transformation, or microbial breakdown. The end result is variability in degradation rate, and therefore, herbicide persistence that varies from soil to soil and from site to site. Residual soil-applied herbicides such as atrazine are an essential component of many crop production systems. The time that herbicides persist in the soil is of particular importance as this has already been shown to have serious implications for the safety

of sensitive following crops (Eagle, 1978; Caverley, 1983; Gottesbüren, Pestemer, Wang, Wischnewsky & Zhao, 1991).

From an agronomic viewpoint, the ideal herbicide should persist long enough to provide season-long weed control but not so long that its residues harm sensitive follow-up crops. Carry-over problems are likely to occur when soil and weather factors which favour reduced herbicide degradation prevail periodically or for a significant length of time subsequent to herbicide application; and when the particular follow-up crop is markedly sensitive to the specific herbicide residue. Excessive persistence tends to occur more frequently in some seasons than in others (Eagle, 1983b), reflecting the influence of climatic factors on rates of loss. Some soils are also more prone to carry-over problems than others. These problems are exacerbated by errors in initial application which lead to overdosing.

The absolute value for residual concentration will be determined by sampling depth; and phytotoxicity of residues will depend on soil type (Eagle, 1978; Williams, 1983), residue distribution in soil as modified by cultivation (Dragun & Helling, 1985) and leaching, and on weather conditions (Eagle, 1983b). High enough residual amounts to damage sensitive plants may be present in soil, but unavailability of the total amount due to adsorption may prevent plants being affected. However, should a particular set of environmental factors render the residues available for uptake by plants in high enough amounts, a phytotoxic effect will occur.

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.

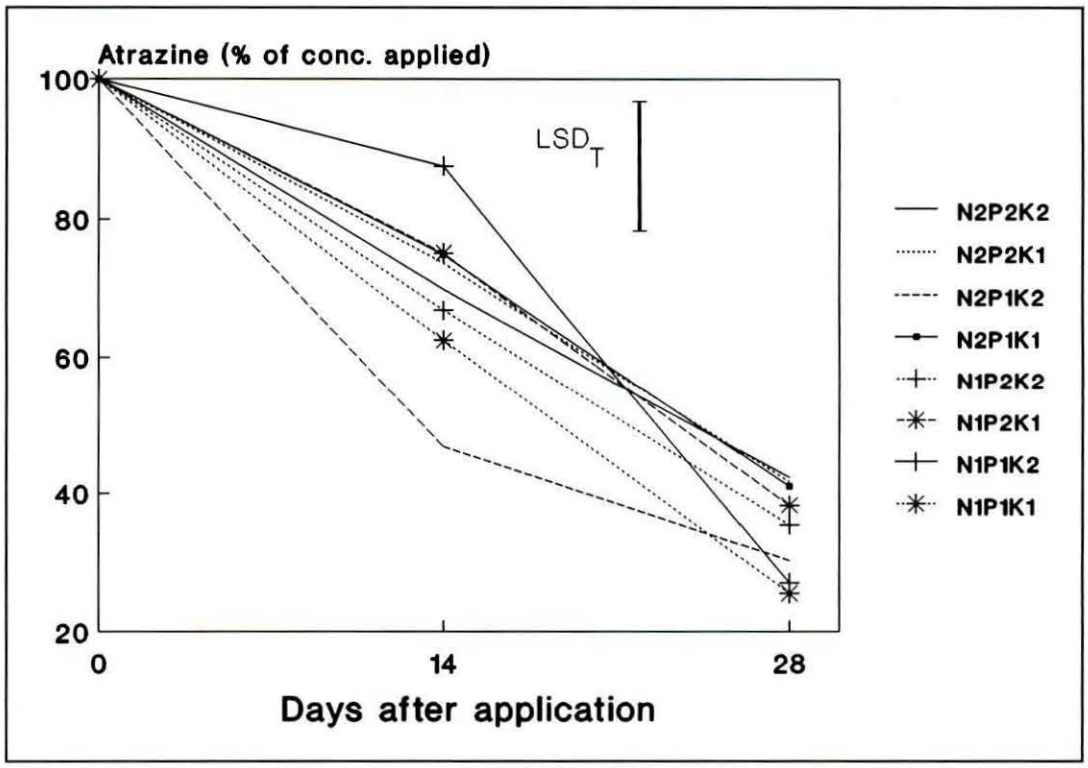

Figure 1 Percent atrazine remaining in NPK-solutions at 14 and 28 days after application (ANOVA in Table 4A)

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.

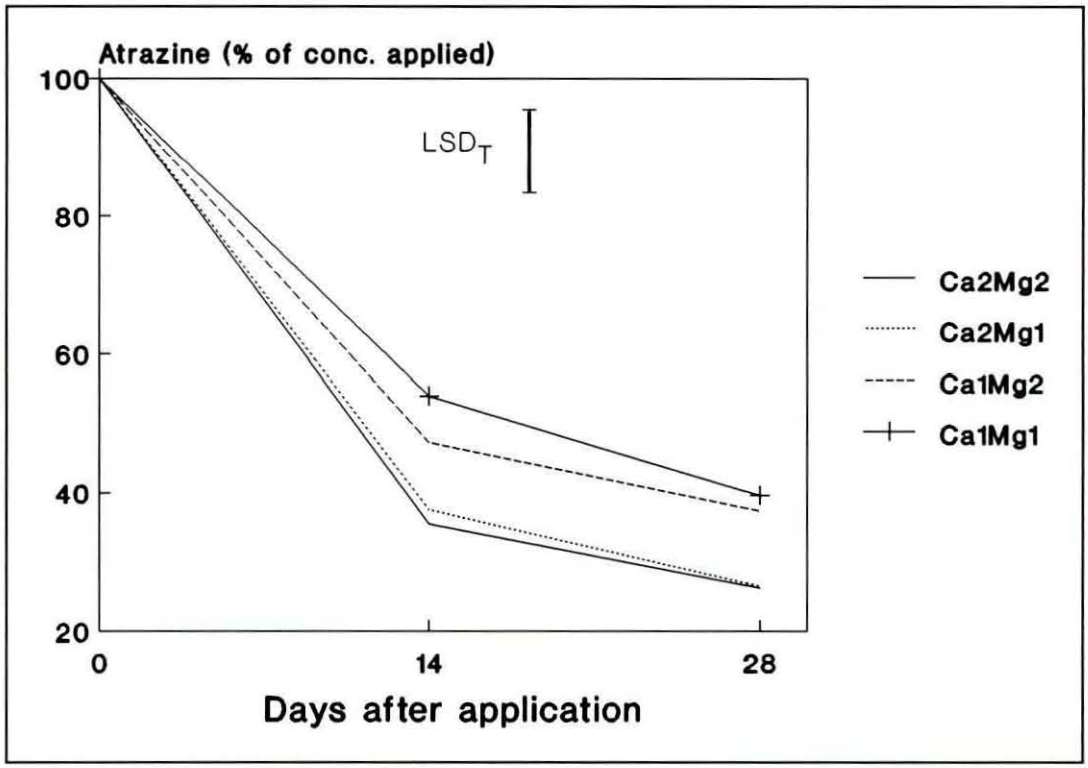

Figure 2 Percent atrazine remaining in CaMg-solutions at 14 and 28 days after application (ANOVA in Table 8A)

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*).

CHAPTER 3

SUSCEPTIBILITY OF FIVE CROP SPECIES TO ATRAZINE IN VARIOUS SOILS

Introduction

Differences in the resistance of plant species to atrazine have been ascribed to variation in the rates of absorption and translocation of the herbicide (Vostral *et al.*, 1970), dissimilarity in metabolic degradation rates (Penner, 1971), and discrepancies in the ability of atrazine molecules to inhibit photosynthetic electron transport (Fuerst & Norman, 1991).

Seed size has been found to influence the resistance of dry bean cultivars and lines (Mennega *et al.* (1990b), as well as that of soybean cultivars (Andersen, 1970) to atrazine. Differential cultivar resistance to atrazine has been demonstrated in crops such as cucumber (*Cucumis sativa* L.)(Werner & Putnam, 1980), dry beans (Mennega *et al.*, 1990b), grain sorghum (Stahlman & Hackerott, 1979), maize (Le Court de Billot & Nel, 1985), soybeans (Andersen, 1970) and sunflower (Mennega *et al.*, 1990a).

Several soil and weather factors have been shown to influence the availability of atrazine for uptake by certain crop species (Nel & Reinhardt, 1984; Ehlers, Reinhardt & Nel, 1987, 1988). Thus a particular crop species may appear to show variable resistance to atrazine across localities because soil and weather differences between them resulted in differential availability of atrazine for uptake by plants.

tolerance to atrazine across localities because soil and weather differences between them resulted in differential availability of atrazine for uptake by plants.

The present study was conducted to evaluate, with various soils, the tolerance to atrazine of some crop species which are commonly grown in rotation with maize. The main aim was to ascertain whether atrazine threshold values, which may be determined in particular soils for sensitive crops, could be useful for making crop choices in cases where the amounts of atrazine that had carried over in diverse soils are known.

Materials and Methods

In bioassays in a glasshouse, dry beans (cv Teebus), grain sorghum (cv NK 222), oats (cv SWK 001), soybeans (cv Forrest) and sunflower (cv SO 222) were used as test species. Lack of space in the glasshouse necessitated separate groupings of bioassays; Experiment I involved dry beans and sunflower, Experiment II - oats and soybeans, and Experiment III - grain sorghum. Test species were grown separately on a total of ten soils. Certain key characteristics of the soils appear in Table 14. Because of unsatisfactory emergence of dry bean and soybean seedlings in the Kroonstad and Pietermaritzburg soils respectively, data from the remaining nine soils were used for statistical analysis in these cases.

Ten atrazine rates were used in all experiments. A distinct difference between the susceptibility of dry beans, oats, soybeans and sunflower, as a group, and grain sorghum demanded the use of different ranges of atrazine rates. Rates used for the group of relatively sensitive crops (dry beans, oats, soybeans and sunflower) were: 0.025, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 and 0.5 mg ai kg^{-1}. In the bioassay

Herbicide concentrations were established by mixing pre-determined volumes of a 50 mg atrazine L^{-1} solution with 500 g soil in each pot. The same volume of water was used to prepare the untreated controls.

Experimental conditions for the different test species were standardized. Water content of the soils was adjusted to 75% of the total amount per pot at field capacity level, through weighing of pots on alternate days. Nutrient supply was in the form of the nutrient solution of Nitsch (1972) - 50 cm^3 was applied on days not designated for weighing of pots. The composition of the Nitsch solution is given in Table 1B (Appendix B). A constant day/night temperature regime of 27/17°C (± 1°C) was maintained for a 12/12 h thermoperiod in a glasshouse. Supplemental lighting was used to extend the daylight period to a minimum of 12 hours. The growth period was 21 days, from seeding until harvesting of top growth. Data were expressed as percent damage, i.e. percentage reduction in shoot dry mass compared to the untreated controls. Pots were arranged according to a completely randomized design in the glasshouse because treatments took two days to complete, thus compelling handling of replicates on different days. Analyses of variance were performed on combined data for dry beans/sunflower (Exp. I) and oats/soybeans (Exp. II), as well as for individual test species.

Table 14 Selected characteristics of soils[1]

Locality	Clay %	Organic C %	pH H_2O	CEC cmol(+) kg^{-1}	P-reversion mg P kg^{-1}	Clay mineral content (%)		
						Kaolinite	Montmoril.	Illite
Bapsfontein A	37	0.84	5.8	7.7	35	70	15	10
Ermelo	16	0.80	5.3	8.5	82	65	-	10
Nelspruit	4	0.29	6.8	2.5	170	40	-	5
Kroonstad	19	0.36	5.6	4.8	75	70	15	10
Pietermrzb.	31	2.74	6.0	14.1	6	29[a]	-	-
Pretoria	25	0.42	6.4	4.6	151	70	-	15
Redhill	50	0.98	5.0	3.3	5	50	-	15
Viljoenskroon	8	0.19	6.5	1.9	118	87	-	8
Vryheid	53	2.04	5.5	15.8	25	80	5	5
Warmbad	35	0.50	7.8	26.6	69	10	80	-

[1]Standard soil analyses were performed by NWK Laboratory, P O Box 107, Lichtenburg 2740. Clay mineral measurements are semi-quantitative and were determined by the National Building Research Institute of the CSIR, P O Box 395, Pretoria 0002.
[a]In addition to 29% kaolinite, the Pietermaritzburg soil contained 55% chlorite.

Results and Discussion

Data for dry beans and sunflower (Exp. I) are given in Table 15, and data for oats and soybeans (Exp. II) appear in Table 16. Data from the experiment with grain sorghum (Exp. III) are given in Table 21. The grouping of test species in separate experiments, because of limited space and for reasons of practical handling, dictates that statistically valid comparisons of the tolerance of species to atrazine can only be made for the combinations dry beans/sunflower and oats/soybeans. The second order interaction of Atrazine rate x Test species x Soil type was significant in Experiments I & II (Tables 15 & 16).

Sunflower was generally more susceptible than dry beans. Significant differences in tolerance between the dry bean cultivar and the sunflower cultivar first occurred at the Nelspruit, Pretoria, Viljoenskroon and Warmbad soils treated with 0.1 mg atrazine kg^{-1} (Table 15). At the next herbicide rate these differences were significant for only the Nelspruit, Pretoria and Warmbad soils. Except at the Pretoria and Warmbad soils, subsequent increases in the atrazine rate tended to reduce dry matter yield to the same extent for both crops. The Vryheid soil was the exception, however, with a significant difference in susceptibility being recorded at the maximum atrazine rate only.

In general, the oats cultivar was more susceptible than the soybean cultivar. The first significant difference in the tolerance of these species was recorded at the Vijoenskroon soil treated with the lowest atrazine rate (Table 16). Already at the next atrazine rate (0.05 mg kg^{-1}), oats was significantly more susceptible than soybeans at an additional

two soils, Kroonstad and Nelspruit. This trend was observed in other soils (Bapsfontein, Pretoria, Warmbad and Redhill) as the atrazine rate increased to around 0.2 mg kg^{-1}. As was the case for dry beans and sunflower, further increases in herbicide rate tended to eliminate differences in tolerance between oats and soybeans. However, in the Vryheid soil the first significant difference in susceptibility was recorded at the 0.3 mg atrazine kg^{-1} rate.

The response of individual test species to ten atrazine rates in different soils are given in Table 17 (dry beans), Table 18 (oats), Table 19 (soybeans), Table 20 (sunflower) and Table 21 (grain sorghum). The reduction in growth caused by atrazine generally increased with increasing herbicide rates, and the damage caused by a particular atrazine rate varied from soil to soil. The dose-response of a particular test species can be expected to vary between different soils because the amount of herbicide absorbed by plants would be determined by, for example, the organic matter and clay contents of soils. Consequently, the threshold value at which atrazine caused damage to test species generally increased with an increase in the adsorptive capacity of soils. Strong negative correlations have been found between the organic matter content and, to a lesser degree, the clay content of soils and atrazine bioactivity (Anderson *et al.*, 1980; Ehlers *et al.*, 1987, 1988; Reinhardt, Ehlers & Nel, 1990). It therefore appears pointless to assign atrazine threshold concentrations to specific crop species, irrespective of the soil and climatic conditions they are grown under.

Although determination of herbicides by chemical means is usually more accurate and less time-consuming than bioassay techniques, analytical measurements of residues in soils would be of limited value in predicting crop reaction. For crop growers, knowledge of total residues (adsorbed part + part available for plant uptake) obtained by extraction with organic solvents and determined instrumentally is of secondary interest (Stalder & Pestemer, 1980). The best approach would be to link residue concentration in a particular soil to test plant response in bioassays conducted with that soil. Bioassay techniques have been shown to be useful and valid tools in atrazine residue studies. Stalder & Pestemer (1980) state that for the assessment of the risk involved in recropping with susceptible crops a quick determination of the part of the total residues in soils available to the plant is needed. They described a simple extraction method with water which allowed the quantitative determination of the residues of certain herbicides (including atrazine) which are potentially available to plants. Nyffeler, Gerber, Hurle, Pestemer & Schmidt (1982) evaluated different bioassay methods in order to improve the reproducibility of the bioactivity of certain soil-applied herbicides, including atrazine.

Except for grain sorghum (Table 21), which was treated with relatively high doses, atrazine elicited virtually no response from the two more susceptible test species (dry beans and sunflower) grown on the Pietermaritzburg soil (Table 15), probably due to inactivation of the relatively small amounts of herbicide in this soil (31% total clay; 2.74% C) through sorption on relatively many atrazine adsorptive sites.

Table 15 Percent reduction in dry mass of the top growth of dry bean and sunflower seedlings exposed to ten atrazine rates in nine soils (ANOVA in Table 15A)

Atrazine rate		Locality								
(mg kg^{-1})	Test crop	Baps.A	Nelsp.	Vryh.	Ermelo	Pieter.	Redhill	Viljoen.	Pta.	Warmb.
0.025	Dry beans	-25	4	-2	9	-17	6	1	-2	-7
	Sunflower	-12	16	-10	12	-7	-2	37	-4	16
0.05	Dry beans	-11	2	10	10	-15	7	52	2	-2
	Sunflower	5	20	-11	13	-13	-6	84	4	17
0.1	Dry beans	-9	25	12	13	-14	6	64	0	2
	Sunflower	41	75	-6	22	-10	0	86	41	27
0.15	Dry beans	7	60	12	10	-1	7	70	8	-2
	Sunflower	44	86	-2	16	-4	1	85	75	60
0.2	Dry beans	20	71	17	18	-11	11	66	16	20
	Sunflower	59	85	15	39	-3	7	86	77	80
0.25	Dry beans	35	75	14	21	-12	10	67	42	27
	Sunflower	59	89	16	47	-16	17	86	83	84
0.3	Dry beans	43	71	16	26	-11	14	67	58	56
	Sunflower	78	89	19	62	-4	35	86	87	84
0.35	Dry beans	59	72	19	28	-18	16	68	58	60
	Sunflower	80	87	26	70	-5	37	88	88	84

Continued overleaf

Table 15 continued

0.4	Dry beans	61	67	19	39	-20	18	69	62	56
	Sunflower	84	89	27	82	-17	43	89	84	86
0.5	Dry beans	56	71	18	46	-19	31	65	73	57
	Sunflower	86	89	59	87	-1	72	88	86	85
LSD_T (P=0.05)					Atrazine rate x Test crop x Soil = 24					

Table 16 Percent reduction in dry mass of the top growth of oats and soybean seedlings exposed to ten atrazine rates in nine soils (ANOVA in Table 16A)

Atrazine rate		Locality								
(mg kg^{-1})	Test crop	Baps.A	Krnst.	Nelsp.	Vryheid	Ermelo	Redhill	Viljoen.	Pta.	Warmb.
0.025	Oats	0	11	19	7	4	1	76	4	3
	Soybeans	2	10	5	13	12	1	11	6	5
0.05	Oats	0	66	62	7	-5	12	83	26	20
	Soybeans	7	13	6	23	10	2	25	21	13
0.1	Oats	40	73	77	21	2	22	85	67	59
	Soybeans	14	22	14	21	8	10	63	27	15
0.15	Oats	60	78	75	18	22	43	84	71	66
	Soybeans	12	46	61	19	6	20	69	41	30

Continued overleaf

Table 16 continued

0.2	Oats	63	80	76	29	55	56	84	76	75
	Soybeans	26	52	59	21	6	14	66	43	40
0.25	Oats	58	80	78	43	64	66	86	78	77
	Soybeans	41	61	64	31	14	14	66	49	51
0.3	Oats	64	78	79	56	65	65	83	78	78
	Soybeans	64	60	63	20	16	13	67	54	66
0.35	Oats	65	80	79	73	70	66	84	74	78
	Soybeans	58	65	65	29	16	14	68	56	64
0.4	Oats	67	79	80	73	70	66	84	74	78
	Soybeans	61	63	66	18	25	17	69	61	65
0.5	Oats	71	80	81	66	68	66	84	76	79
	Soybeans	63	62	66	21	67	25	71	62	67

LSD_T (P=0.05) Atrazine rate x Test crop x Soil = 19

Table 17 Percent reduction in dry mass of dry bean (cv Teebus) seedlings caused by 10 atrazine rates in nine soils (ANOVA in Table 17A)

Atrazine rate	Locality								
(mg kg^{-1})	Baps.A	Nelsp.	Vryh.	Ermelo	Pieter.	Redhill	Vilj.	Pta.	Warmb.
0.025	-25	4	-2	9	-17	6	1	-2	-7
0.05	-11	2	10	10	-16	7	52	2	-3
0.10	-9	25	12	13	-14	6	64	0	2
0.15	7	60	12	10	-1	7	70	8	-2
0.20	20	71	17	18	-11	11	66	16	20
0.25	35	75	14	21	-12	10	67	42	27
0.30	43	71	16	26	-11	14	67	58	56
0.35	59	72	19	28	-18	16	68	58	60
0.40	61	67	19	39	-20	18	69	62	56
0.50	56	71	18	46	-19	31	65	73	57
Mean	24	52	14	22	-12	13	59	32	27
$LSD_T(P=0.05)$	Atrazine rate x Soil = 25								

Table 18 Percent reduction in dry mass of oats (cv SWK 001) seedlings caused by ten atrazine rates in ten soils (ANOVA in Table 18A)

Atrazine rate	Locality									
(mg kg^{-1})	Bap.A	Krnst.	Nels.	Vryh.	Erm.	Piet.	Redh.	Vilj.	Pta.	Warmb.
0.025	0	11	19	7	4	-8	1	76	4	3
0.05	0	66	61	7	-5	-8	12	83	26	20
0.10	40	73	77	21	2	-5	22	85	67	59
0.15	60	78	75	18	22	2	43	84	71	66
0.20	63	80	76	29	55	4	56	84	76	75
0.25	58	80	78	43	54	-4	66	86	78	77
0.30	64	78	79	56	65	2	65	83	78	78
0.35	65	80	79	70	72	-3	64	83	76	78
0.40	67	79	80	73	70	0	66	84	74	78
0.50	71	80	81	73	68	13	66	84	76	79
Mean	49	71	71	39	42	0	46	83	63	61
$LSD_T(P=0.05)$	Atrazine rate x Soil = 19									

Table 19 Percent reduction in dry mass of soybean (cv Forrest) seedlings caused by ten atrazine rates in nine soils (ANOVA in Table 19A)

Atrazine rate	Locality								
(mg kg^{-1})	Bap.A	Krnst.	Nels.	Vryh.	Erm.	Redh.	Vilj.	Pta.	Warmb.
0.025	2	10	5	13	9	1	11	6	5
0.05	7	13	6	23	10	2	25	21	13
0.10	14	22	14	21	8	10	63	27	15
0.15	12	46	61	19	6	20	69	41	29
0.20	26	52	59	21	6	14	66	43	40
0.25	41	61	64	31	14	14	66	49	51
0.30	64	60	63	20	16	13	67	54	66
0.35	58	65	65	29	16	14	68	56	64
0.40	61	63	66	18	25	17	69	61	65
0.50	63	62	66	21	67	25	71	62	67
Mean	35	45	47	22	17	13	58	42	42
$LSD_T(P=0.05)$	Atrazine rate x Soil = 18								

Table 20 Percent reduction in dry mass of sunflower (cv SO 222) caused by ten atrazine rates in ten soils (ANOVA in Table 20A)

Atrazine rate	Locality									
(mg kg^{-1})	Bap.A	Krnst.	Nels.	Vryh.	Erm.	Piet.	Redh.	Vilj.	Pta.	Warmb.
0.025	-12	-7	16	-10	12	-7	-2	37	-4	16
0.05	5	17	20	-11	13	-13	-6	84	4	17
0.10	41	69	75	-6	22	-10	0	86	41	27
0.15	44	78	86	-2	16	-4	1	85	75	60
0.20	59	84	85	15	39	-3	7	86	77	80
0.25	59	87	89	16	47	-16	17	86	83	84
0.30	78	86	89	19	62	-4	35	86	87	84
0.35	80	87	87	26	70	-5	37	88	88	84
0.40	84	87	89	27	82	-17	43	89	84	86
0.50	86	86	89	59	87	-1	72	88	86	85
Mean	52	67	73	13	45	-8	21	81	62	62
$LSD_T(P=0.05)$	Atrazine rate x Soil = 22									

Table 21 Percent reduction in dry mass of grain sorghum (cv NK 222) caused by 10 atrazine rates in nine soils (ANOVA in Table 21A)

Atrazine rate	Locality								
(mg kg^{-1})	Bap.A	Krnst.	Nels.	Vryh.	Erm.	Piet.	Vilj.	Pta.	Warmb.
0.1	2	8	2	3	6	1	1	3	6
0.2	4	18	4	3	10	10	10	12	17
0.4	6	33	23	6	15	8	37	21	26
0.8	15	43	46	6	19	19	68	42	42
1.0	14	44	54	12	26	23	71	51	46
1.4	40	58	70	16	32	20	84	64	62
1.8	48	73	79	28	34	23	87	76	63
2.4	53	74	81	32	32	26	88	77	70
2.8	55	78	89	37	45	27	91	83	72
Mean	26	48	50	16	24	18	60	48	45
$LSD_T(P=0.05)$	Atrazine x Soil = 18								

Results presented above show that prediction of potential damage to susceptible crops should reflect the differential availability of atrazine residues in different soils. Also, amounts of residues available for uptake by plants in a particular soil, and not merely total residue concentration in that soil should regulate crop choice. Thus differential availability of residues for uptake by plants in different soils precludes the allocation of fixed herbicide threshold values to specific crops. Ideally, knowledge of a particular cultivar's response to known amounts of atrazine residues in a specific soil should determine crop choice. Recropping intervals that are specified on labels of atrazine products neither reflect the variability in atrazine threshold values for crop species nor differential threshold concentrations for different soils.

Own research reported in subsequent chapters was focused on determining the relative importance of certain soil characteristics on the bioactivity and persistence of atrazine. Basically the same procedures as those described under *Materials and Methods* were employed in subsequent bioassays that were conducted in glasshouses. Certain crop cultivars which had been employed in the present investigation were used as indicators of the availability of atrazine and/or its phytotoxic residues for uptake by plants.

CHAPTER 4

BIOACTIVITY OF ATRAZINE AS AFFECTED BY SELECTED SOIL PROPERTIES: FIELD STUDY

Introduction

The greatest drawback of field studies is that results often apply to one particular location and season. There are valid reasons why attempts to extrapolate results obtained under controlled conditions to the field situation are subject to criticism. Firstly, the level of any one environmental parameter seldom stays the same for an extended period of time in the field. Secondly, levels of many factors are changing in this way, resulting in the exposure of plants to an almost infinite number of permutations of environmental conditions. Thirdly, plants grown and treated indoors almost certainly differ in a number of ways from plants cultivated in the field (e.g., in cuticle development and rooting pattern). Consequently, results obtained indoors may not truly reflect the field situation.

The methodology followed in the present study was designed to match results generated in the field with those obtained previously under controlled conditions. The aim was to compare the relationships found between the bioactivity of atrazine and selected soil properties during a particular growing season in the field with those relationships which were obtained in previous bioassays (Nel & Reinhardt, 1984; Ehlers *et al.*, 1987, 1988; Nel, Reinhardt & Ehlers, 1988).

Materials & Methods

The bioactivity of atrazine was investigated in ten field trials, situated in eight districts of the summer grain region of South Africa. Soil was selected on which no atrazine had been applied during the previous three years. Soils used differed with respect to organic matter content, clay content, CEC, relative P-reversion and soil pH levels (Table 22).

No fertilizers were applied as soil analyses indicated that all trial sites had adequate soil nutrient reserves. All soils were irrigated to field capacity before seedbed preparation. Seedbeds were fine and firm, with no stubble on the soil surface.

Commercial atrazine (Gesaprim® 500 FW) applications of 0, 0.031, 0.062, 0.125, 0.25 and 0.5 kg ai ha^{-1} were made. A field sprayer mounted on bicycle wheels, which delivered 200 L ha^{-1} at 300 kPa, was used for these applications. A 2.7 m spray boom with five flat fan nozzles was used on 5.4 x 10 m plots. Weeds were hand-hoed when necessary. Treatments were replicated five times in a randomized block design. The precise positions of individual plots were demarcated with marker beacons. This was done to ensure that subsequent persistence experiments (see Chapter 5) were conducted on precisely the same plots.

Oats (*Avena sativa* L. cv SWK 001) was used as the test species. The same cultivar was employed in the bioassay conducted by Ehlers *et al.* (1988) in a glasshouse. Seed were treated with thiram, a broad spectrum fungicide. A plant density of approximately

300 000 plants ha^{-1}, in rows 900 mm apart, was used. Atrazine was applied directly after oats were planted.

Atrazine bioactivity was evaluated by harvesting the shoots of the plants, 35 days after planting, in five randomly distributed 2 m rows per net plot (4.4 x 8 m). Data were expressed as percent damage, i.e. percentage reduction in shoot dry mass compared to the untreated controls. Analyses of variance and regression analyses were performed on these data.

The relationships between atrazine bioactivity and selected soil properties were evaluated by means of correlation studies. Simple correlation coefficients (r values) were determined across herbicide rates. Since the order of importance of soil properties in the prediction of atrazine bioactivity and persistence could conceivably change in accordance with initial herbicide rates, separate regression analyses were also performed at individual rates to ascertain whether the order of importance of relationships was rate-dependent. It was found that herbicide rate did not influence the relative importance of relationships, and therefore these data are not presented. The significance of differences between r^2 values of the regression analyses were determined through pairwise comparisons between r^2 values according to the procedure of Bonferroni (Krishnaiah, 1984). It was considered inappropriate to perform multiple regression analyses (more than one soil variable in the model), since the number of soils, and thus the data base, was limited.

Table 22 Some chemical and physical properties of soils

Locality	% Clay	% C	CEC $cmol^{(+)}\ kg^{-1}$	$pH(H_2O)$	P-reversion $mg\ kg^{-1}$	Clay mineral content (%)		
						Kaolinite	Montmor.	Illite
Bapsfontein A	34	1.12	37	5.6	54	70	15	10
Bapsfontein B	27	0.76	34	6.4	75	75	15	5
Ermelo	16	0.79	8	5.3	82	65	-	10
Kroonstad	7	0.15	10	5.8	119	65	5	-
Nelspruit	4	0.29	2	6.8	170	40	-	10
Pretoria	20	0.50	24	6.7	151	72	3	-
Standerton	8	0.74	12	6.6	122	20	35	5
Ventersdorp	9	0.23	3	6.1	135	65	-	15
Warmbad A	29	0.47	59	7.7	83	10	80	-
Warmbad B	52	0.53	55	7.9	51	-	85	-

Results & Discussion

Results of the bioassays conducted at 10 sites are shown in Figure 3. The Atrazine rate x Soil interaction was significant. This can be explained by the relatively high atrazine bioactivity where rates of 0.125, 0.25 and 0.5 kg ai ha^{-1} were applied on the Kroonstad and Nelspruit soils. Application of these rates at Kroonstad caused growth reduction that ranged from 77 to 100%, whilst maximum damage at Nelspruit was already observed at 0.125 kg atrazine ha^{-1}. The same three rates were less phytotoxic in the other soils. The least variation in bioactivity amongst soils was observed at the lowest (0.031 kg ai ha^{-1}) atrazine rate used.

The low organic matter and clay contents of the Kroonstad, Nelspruit and Ventersdorp soils probably accounted for the relatively high atrazine bioactivity in them. According to the postulation of Smit *et al.* (1980, 1981), high P-reversion values would be indicative of low sorption capacities for atrazine in soils. Their hypothesis is based on the assertion that atrazine may be adsorbed to positive charge on the (Al.Fe.OH) component through free electrons on parts of the herbicide molecule. This is in contrast to the generally accepted view that the adsorption of atrazine to soil colloids involve sorption of atrazine cations on only negative sites on clay and organic matter colloids (Weber, 1991a). The significant positive correlation between P-reversion and herbicide bioactivity found in the present study (Table 23) ostensibly supports the theory of Smit *et al.* (1980, 1981) that atrazine may bind to sites with positive charge by means of free electrons which concentrate at N atoms in the molecule. However, it must be stressed that a significant correlation between a soil variable and atrazine

activity does not reveal much about the nature of the adsorption mechanism involved.

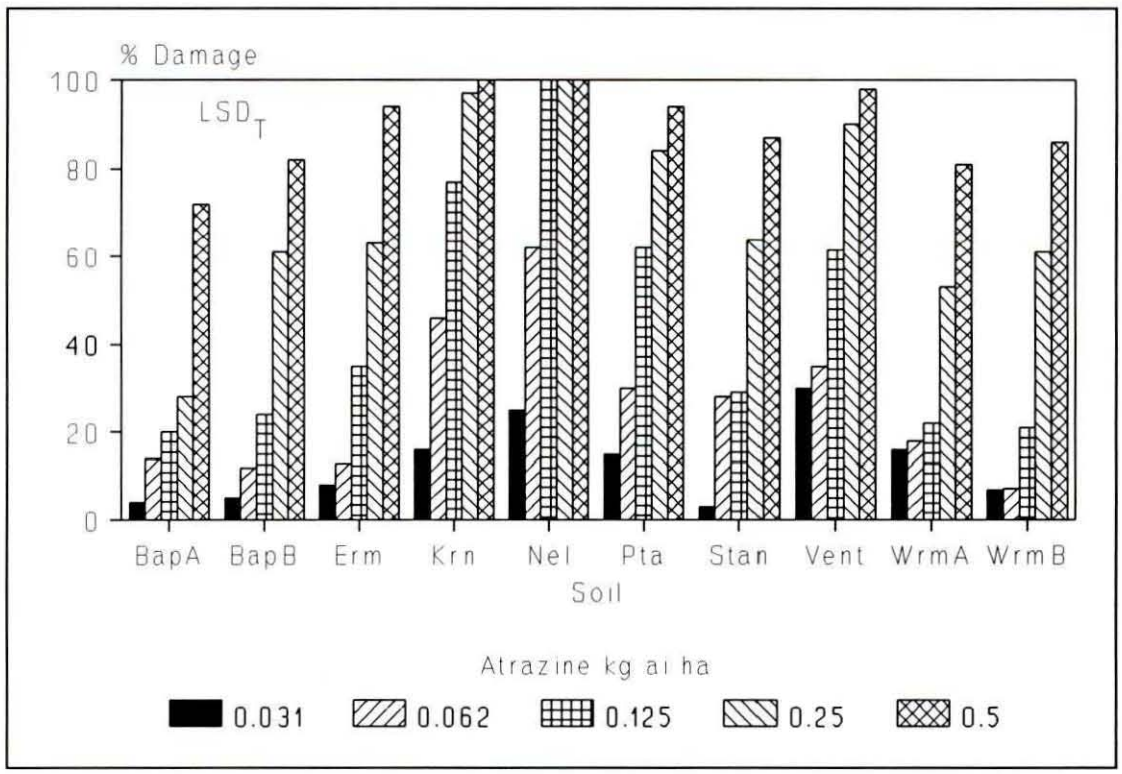

Figure 3 Percent reduction in shoot dry mass of oats treated with five atrazine rates at ten sites (ANOVA in Table 22A; data appear in Table 2B)

The relationships between atrazine bioactivity and the soil properties listed in Table 22 were evaluated by means of regression analyses. Simple correlation coefficients and r^2 values appear in Table 23. The order of importance of the soil properties for predicting atrazine bioactivity was as follows: P-reversion $\geq$ % C $>$ total clay % $\geq$ CEC $>$ pH. The order of importance was not dependent on herbicide rate. Both P-

reversion and organic matter content were better predictors than total clay content. Cation exchange capacity and soil pH were significantly less important than these three characteristics. These results correspond with those reported locally by: (a) Smit *et al.* (1981) - oats (cv Santon) the test plant in a glasshouse study, (b) Nel & Reinhardt (1984) - oats (cv SWK 001) in a glasshouse, (c) Ehlers, Reinhardt & Nel (1987) - soybeans [*Glycine max* (L.) Merr. cv Hutton] in a field study, (d) Ehlers *et al.* (1988) - oats (cv SWK001) in a glasshouse, and (e) Nel, Reinhardt & Ehlers (1988) - grain sorghum [*Sorghum bicolor* (L.) Moench cv NK 222] in a glasshouse.

In the bioassay with grain sorghum, rates of atrazine (0.5-4.0 kg ai ha^{-1}) were more representative of field application rates (Nel *et al.*, 1988). With grain sorghum being a moderately tolerant crop to atrazine, and oats and soybeans very sensitive, less herbicide was applied in experiments with oats (0.1-0.4 mg atrazine kg^{-1}) and soybeans (0.062-0.5 kg atrazine ha^{-1}). The relationships between atrazine bioactivity and certain soil properties could conceivably have been different at the much higher atrazine rates used in the grain sorghum study, but the order of importance for the above-mentioned five soil characteristics was found to be similar to those reported for the other test species.

The characteristics of the total of 56 soils used by Nel & Reinhardt (1984), Ehlers *et al.* (1987, 1988), and Nel *et al.* (1988) covered a wide spectrum with total clay ranging from 4 to 55%, organic matter 0.08 to 1.6% C (with only seven above 1% C), CEC 1.3 to 59 cmol(+) kg^{-1}, P-reversion 5 to 199 mg P kg^{-1}, and soil pH 4.2 to 7.9. These

soils were from various soil forms, the predominant clays being kaolinite (in about 53% of soils) and montmorillonite (about 21% of soils). In the remainder, these two clay minerals were represented in fairly equal proportions. According to Harrison *et al.* (1976) and Smit *et al.* (1981) the relationship between atrazine bioactivity and clay percentage in predominantly kaolinitic soils is relatively weak. In the present study, kaolinite predominated in all but the two Warmbad soils where montmorillonite was the main clay mineral component (Table 22).

Table 23 Simple correlation coefficients (r) and r^2 values to describe the relationships between atrazine bioactivity and selected soil properties

Variable in model	r	r^2
% Organic C	-0.71*	0.51**a**
CEC	-0.60*	0.36**a**
% Clay	-0.62*	0.38**a**
pH(H_2O)	-0.10	0.01**b**
P-reversion	+0.74*	0.55**a**

Coefficient of determination (r^2.100) = % variation in bioactivity explained by variable in model.
*Significant at P=0.05.
[a,b]Values followed by the same letter do not differ significantly at P=0.05.

Since recommendations for the application of atrazine in South Africa are currently based solely on total clay in the soil, the present findings and those of Nel & Reinhardt (1984), Ehlers *et al.* (1987, 1988) and Nel *et al.* (1988) pointed to a need for additional criteria on which to base atrazine rate recommendations.

In studies conducted over many years at the University of Pretoria (Smit *et al.*, 1981; Ehlers *et al.*, 1987, 1988; Nel *et al.*, 1988), combinations of P-reversion, % C and % clay in multiple regression equations always predicted atrazine bioactivity better than any one of these three soil properties alone. From data of the field and glasshouse studies mentioned, Nel, Smit & Reinhardt (1989) derived a formula for calculating atrazine application rates in maize. The formula is basically a multiple regression equation with % clay, % organic carbon (C) and P-reversion the independent variables, and atrazine rate the dependent variable. According to the proposed formula, atrazine dosage for a particular soil can be determined as follows:

$$\textit{kg atrazine } ha^{-1} = 1.0 + 0.03(\textit{clay \%}) + 1.0(\textit{\% C}) - 0.005(\textit{P-reversion mg } kg^{-1})$$

It was proposed that this formula be added to application recommendations which appear on atrazine product labels (Nel *et al.*, 1989).

Results presented in this chapter have been published (Reinhardt, Ehlers & Nel, 1990).

SECTION B - PERSISTENCE STUDIES

CHAPTER 5

RESIDUAL BIOACTIVITY OF ATRAZINE AS AFFECTED BY CERTAIN SOIL PROPERTIES: FIELD STUDY

Introduction

Despite increased public concern about the presence of residues of persistent pesticides in the environment, the economic state of agriculture in South Africa is bound to dictate that atrazine remains an essential component of many crop production systems. Atrazine is one of the cheapest and most effective herbicides available. The time for which this herbicide persists in soil is of particular importance as this has already been shown to have serious implications for the safety of sensitive following crops both locally and overseas (Eagle, 1978; Caverley, 1983; Gottesbüren, Pestemer, Wang, Wischnewsky & Zhao, 1991).

In South Africa, the persistence of atrazine under field conditions is often longer than 12 months, which may seriously affect rotational crops. It is important that crop producers know if there are any limitations or restrictions in the sequence of crops that can be grown after using a persistent herbicide. Thus considerable information concerning crop sensitivity and herbicide persistence is required (Walker, 1987).

Bioactivity of herbicides at any stage after application to soil is closely associated with adsorption and degradation processes. In addition to the large number of edaphic factors, climatological conditions play a determining role in the eventual bioactivity of soil-applied herbicides (Walker & Barnes, 1981). Factors that cause degradation of atrazine are important in neutralizing residual phytotoxic effects on rotational crops exhibiting low tolerances to the herbicide.

Since the persistence of atrazine over long periods has not been researched in this country, a series of field trials were conducted to compare the persistence of the herbicide in different soils. The aim was to determine relationships between the bioactivity of atrazine and selected soil properties at certain time intervals (maximum one year) after atrazine application in the field.

Materials & Methods

The initial bioactivity of atrazine, which was investigated in ten field trials, was reported in Chapter 4. In the present study, atrazine persistence was monitored on seven of those trial sites, six and twelve months after herbicide application. The original plots were reseeded six months (182 days) and again twelve months (365 days) after the initial planting on day 0. The position of plant rows were offset 250 mm in subsequent plantings to avoid planting directly on previous rows. This was done to minimise the already small effect that uptake by previous plants would have had on the concentration of herbicide residues in the soil. Procedures for establishing and maintaining the test species were similar to those described in the previous chapter.

Atrazine bioactivity was assessed by measuring dry mass of shoots, 35 days after each planting, in five randomly distributed 2 m rows per nett plot (4.4 x 8 m). Percent damage was calculated as the percentage reduction in shoot dry mass compared to the unsprayed controls. Analyses of variance and regression analyses were performed. Atrazine bioactivity was correlated with selected soil properties. Simple correlation coefficients were determined across herbicide levels. Separate regression analyses were also performed at individual rates to ascertain whether the order of importance of relationships was rate-dependent. The significance of differences between r^2 values were determined through pairwise comparisons between these values according to the procedure of Bonferroni (Krishnaiah, 1984). The limited number of soils employed in the study precluded the determination of multiple correlation coefficients (R values) for models containing more than one soil variable.

Results & Discussion

Bioactivity after 182 days

Results for the reduction in growth that was caused by five atrazine rates at seven localities in bioassays initiated 182 days after atrazine application appear in Figure 4. The Atrazine rate x Locality interaction was significant. In the five soils where bioactivity was clearly discernable, activity was still positively related to the atrazine rates applied six months previously. Bioactivity caused by residues of the 0.25 and 0.5 kg atrazine ha^{-1} rates applied six months earlier was significantly lower in both the Bapsfontein A and Ermelo soils compared with the other soils. Bioactivity induced by those two herbicide rates was significantly lower at Bapsfontein A than at Bapsfontein B after six months (Figure 4), despite an insignificant difference in initial activity

(Figure 3, Chapter 4). Differences in the organic matter content and pH of these two soils might explain the difference in atrazine persistence on these trial sites which were only 50 m apart. The organic matter content of Bapsfontein A soil (1.12% C) was higher than that of Bapsfontein B (0.76% C), while the pH of the former soil (pH 5.6) was lower than that of the latter (pH 6.4). Anderson *et al.* (1980) states that availability of atrazine for uptake by plants is negatively correlated with the organic matter content of soil. They also established that the persistence of the herbicide increases by a constant time period for every unit increase in the soil pH. It is unlikely that climatic factors were involved in the variation in atrazine persistence between the Bapsfontein A and B sites because of their proximity to one another.

The purported roles of organic matter and soil pH suggested above are supported by similar atrazine persistence in the Warmbad A and B soils (Figure 4). These soils had similar organic matter content and pH characteristics (Table 22) and were only about 100 m apart.

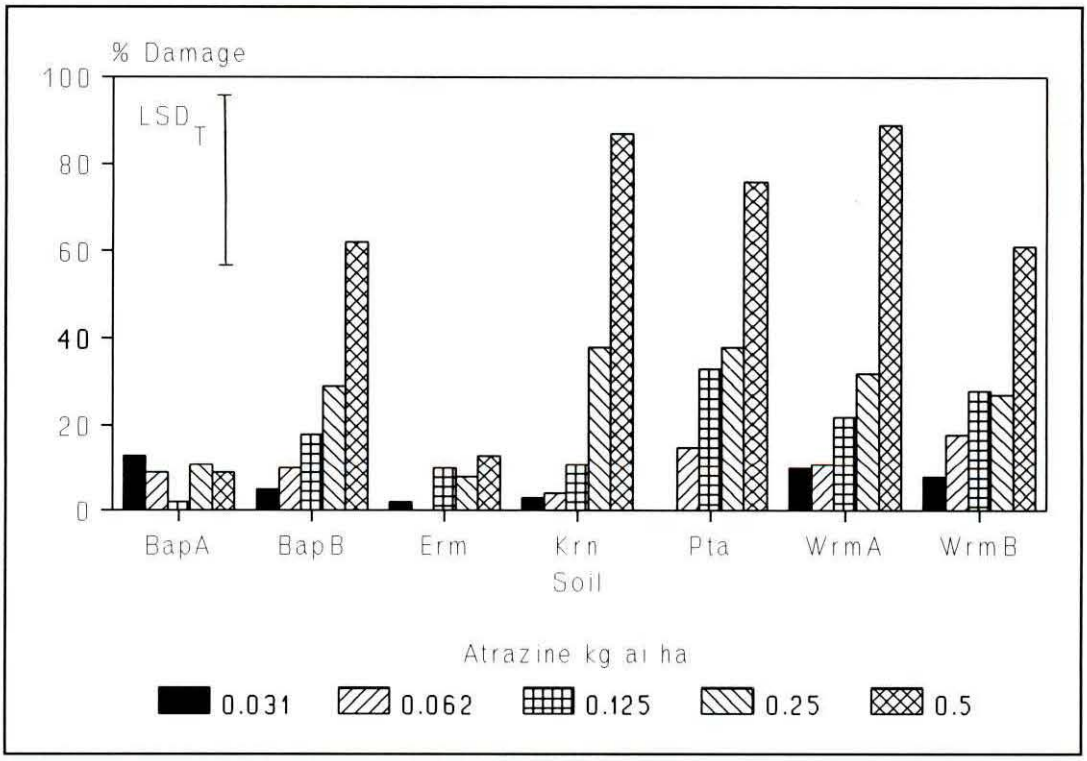

Figure 4 Percent damage to oats (% reduction in shoot dry matter yield) caused by atrazine at seven localities, 182 days after application (ANOVA in Table 23A; data in Table 3B)

Correlation coefficients to describe the relationships between atrazine bioactivity, 182 days after application, and the soil properties appear in Table 24. The relative importance of the soil properties on the prediction of bioactivity was as follows: % C > pH > P-reversion > CEC > total clay %. Organic matter content gave the best prediction of bioactivity (r = -0.59). Whereas six months previously soil pH was only weakly associated with bioactivity, it was now ranked second to % C, with an r value of +0.49. Clay content (r = +0.14) was now the poorest predictor of atrazine bioactivity (Table 24).

The above-mentioned order of importance of relationships was found at the three highest initial atrazine rates, namely 0.125, 0.25 and 0.5 kg ha^{-1}. The orders of importance at 0.031 and 0.062 kg atrazine ha^{-1} differed from this one and also from each other. This inconsistency was most likely due to the low residual herbicide activity at the two lowest atrazine rates (Figure 4).

A negative correlation between organic matter content and atrazine bioactivity has been found by many researchers, *inter alia* by Talbert & Fletchall (1965), Weber *et al.* (1969), and Anderson *et al.* (1980). The role of P-reversion is not as well documented, but this soil variable has been shown in the previous chapter and by Smit *et al.* (1980, 1981) and Ehlers *et al.* (1987, 1988) to be positively correlated with atrazine bioactivity.

The relatively strong positive relationship between pH and residual atrazine bioactivity suggests that phytotoxic residues were more readily available for uptake by plants at high compared to low soil pH levels. The stronger relationship found between soil pH and bioactivity in this study than in previous work (Chapter 4; Ehlers *et al.*, 1987, 1988) suggests another role for soil pH apart from its effect on adsorption, namely that soil pH also determines the rate of hydrolysis of the compound. The adsorption of various triazines has been shown to decrease as the soil pH increased (Colbert *et al.*, 1975; Harris & Hurle, 1979; Weber & Whitacre, 1982; Appleby, 1985). This would explain the positive correlations found between pH and atrazine bioactivity. Also, chemical hydrolysis of atrazine to inactive hydroxyatrazine occurs more readily at low pH levels than under high pH conditions (Armstrong *et al.*, 1967; Gamble & Khan,

1985). This implies that at high pH levels, atrazine molecules, although perhaps not strongly held on colloids, would be afforded protection against hydrolysis.

Granted that hydrolysis is a major route for the dissipation of atrazine, and that soil pH governs the rate of hydrolysis, this soil property can be expected to be a more important predictor of the persistence than the short-term bioactivity of the herbicide. Through lack of enough levels for the time factor, the present study was not ideal for assessing the importance of soil variables in the prediction of atrazine persistence. The approach followed in Chapter 9 was specifically aimed at linking degradation rates to selected soil properties.

Table 24 Simple correlation coefficients (r) and r^2 values to describe the relationships between atrazine bioactivity and selected soil properties 182 and 365 days after application

	Day 182		Day 365	
Variable in model	r	r^2	r	r^2
% Organic C	-0.59*	0.35**a**	+0.07	0.01**a**
CEC	+0.19	0.03**b**	-0.18	0.03**a**
% Clay	+0.14	0.02**c**	+0.29	0.08**a**
pH(H_2O)	+0.43*	0.19**ab**	-0.16	0.02**a**
P-reversion	+0.37*	0.14**ab**	-0.27	0.07**a**

Coefficient of determination (r^2.100) = % variation in bioactivity explained by variable in model.
*Significant at P=0.05.
[a,b]Values followed by the same letter do not differ significantly at P=0.05.

Bioactivity after 365 days

The results are illustrated in Figure 5. Only the main effect for soil was significant. Atrazine, or biologically active residues of the herbicide, were only detected in the Bapsfontein B, Pretoria and Warmbad A soils. Dissipation of atrazine was virtually complete in the other soils. Although not significant, apparent stimulation of growth occurred in some of the soils where atrazine bioactivity was negligible (Figure 5). The phenomenon of growth stimulation by sub-lethal triazine herbicide concentrations is well documented (Ashton & Crafts, 1981).

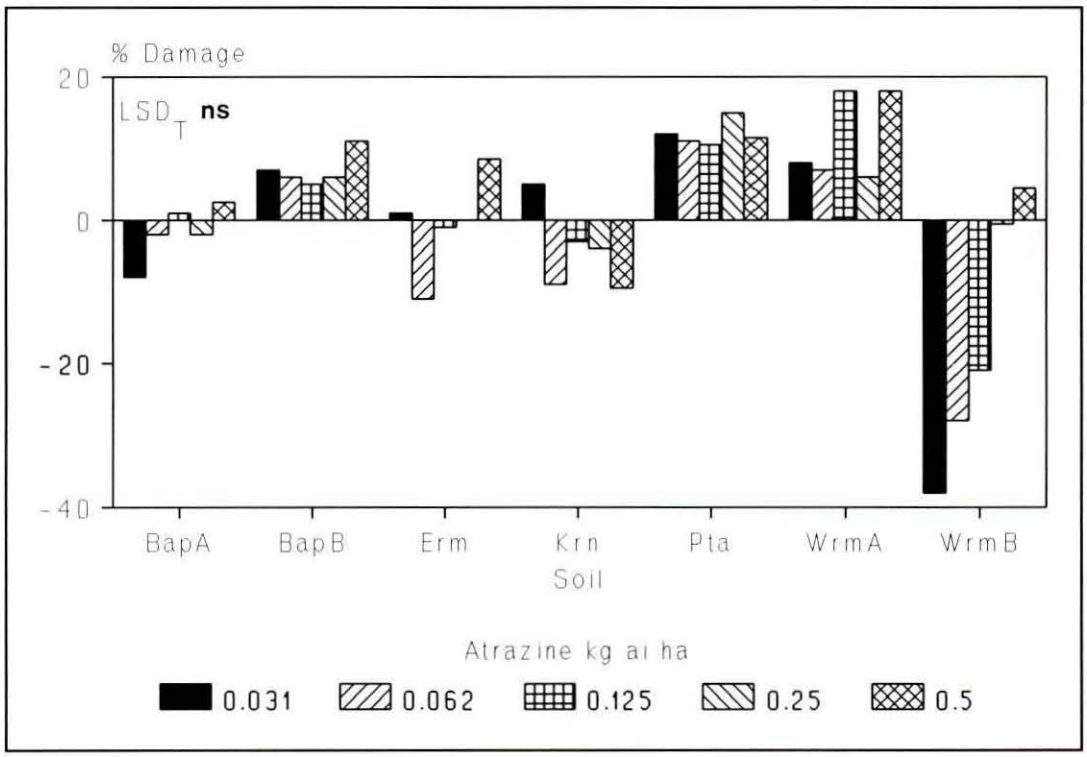

Figure 5 Percent damage to oats (% reduction in shoot dry matter yield) caused by atrazine at seven localities, 365 days after application (ANOVA in Table 24A; data appear in Table 4B)

The relationships between atrazine bioactivity and the soil properties were extremely poor at 365 days after herbicide application (Table 24). This is ascribed to the lack of atrazine bioactivity in most of the soils.

In conclusion, residues of atrazine applied at the relatively low rates used in this study persisted for at least six months in most of the soils tested and up to twelve months in a few soils. Persistence at two localities was found to vary over relatively short distances. This implies that persistence was more closely linked to soil characteristics than to climatic factors. Recropping periods that are specified for follow-up crops do not distinguish between soil types. Results presented above suggest that recropping periods could be refined. It is envisaged that recropping periods might be either shortened or extended, depending on whether the herbicide dissipation rate was high or low. The applicability of the waiting period that is specified for two atrazine-sensitive crops commonly grown in rotation with maize was assessed in work that is reported in the next chapter.

The work reported in this chapter has been published (Reinhardt et al., 1990).

CHAPTER 6

RESIDUAL EFFECT OF ATRAZINE ON FIELD-GROWN DRY BEANS AND SUNFLOWER

Introduction

In the previous field study it was shown that organic matter content, soil pH and P-reversion accounted for 35%, 19% and 14%, respectively, of the variation in atrazine bioactivity measured six months after its application. Also, it was found that the dissipation of atrazine varied considerably from soil to soil and from one locality to another. The persistence of a compound is bound to be variable because it is not an intrinsic characteristic of a chemical, and soil and climatic factors greatly influence the processes of herbicide dissipation and the availability of the herbicide to the following crop (Hurle & Walker, 1980; Duffy, 1991).

Reports by Haigh & Ferris (1991) and Wood *et al.* (1991) attest to the universality of problems caused by excessive persistence of atrazine. In South Africa, carry-over of atrazine occasionally causes injury to susceptible crops grown in rotation with maize. To minimize the possibility of injury to rotational crops, recropping intervals with wide safety margins are specified on the product label. However, this restricts the choice of follow-up crops. In addition, fixed recropping periods fail to reflect the wide extremes in soils and climatic conditions in South Africa. Lack of flexibility in recropping options are exacerbated when a response to changing market trends or forced recropping is demanded. Also, despite long recropping intervals, injury to susceptible

crops does occur. Ideally labels should reflect local differences with the view to vary the recropping interval on the basis of soil and climatic factors which govern the dissipation of atrazine.

The aims of the present study were to evaluate the applicability of current waiting periods by growing dry beans and sunflower subsequent to atrazine application in maize, and to compare the sensitivity of these crops to atrazine on diverse soils.

Materials and Methods

Trials were conducted under dry land conditions. Eight of a total of eleven experiments were successfully completed at six sites. Certain characteristics of the soils at these sites appear in Table 25. Atrazine was applied pre-emergence in maize during the 1987/88 growing season. Dry beans (cv Teebus) and sunflower (cv SO 222) were first seeded on the original plots during the 1988/89 season. The two follow-up crops were subsequently only grown again during the 1989/90 season (i.e. 24 months after treatment) at localities where significant yield reductions occurred the previous season.

Atrazine was applied at each site at the rate recommended for use in maize, and at both higher and lower rates than the one prescribed. Herbicide rate increments were 0.2 kg ha^{-1} for light soils, 0.3 kg ha^{-1} for medium soils, and 0.4 kg ha^{-1} for heavy soils. Six atrazine rates were used in each experiment. The atrazine rates applied at each site appear in Table 26.

Table 25 Certain characteristics of soils at different localities

Locality	Clay %	Organic C %	pH H_2O	CEC cmol(+) kg^{-1}	P-reversion mg P kg^{-1}	Clay mineral content (%) Kaolinite	Montmorillonite	Illite
Bapsfontein A	37	0.84	5.8	7.7	35	70	15	10
Bapsfontein B	29	0.69	6.4	5.4	55	75	15	5
Carletonville	23	0.48	5.7	4.2	150	75	10	10
Delmas	17	0.57	7.2	3.9	75	83	15	-
Kroonstad	19	0.36	5.6	4.8	75	70	15	10
Pretoria A	25	0.42	6.4	4.6	105	70	-	15
Pretoria B	41	0.66	6.2	10.5	85	83	12	-
Vryheid	53	2.04	5.5	15.8	25	80	5	5
Warmbad	35	0.50	7.8	26.6	69	10	80	-

Weed control efficacy was evaluated as part of a study (not reported on in detail here), which was conducted to refine atrazine application rates in maize (Nel *et al.*, 1989). In that study, maize yield on particular sites did not differ significantly between plots treated with different atrazine rates. The maize cultivar grown at each site and mean grain yield on plots treated with atrazine were the following: Carletonville - cv PNR 6549: 4.5 ton ha^{-1}; Delmas - PPPxK64R: 3.8 ton ha^{-1}; Pretoria A - cv PNR 6528: 5.3 tons ha^{-1}; Pretoria B - cv PNR 6528: 6.0 ton ha^{-1}; Bapsfontein A - cv PNR 6528: 5.1 ton ha^{-1}; Bapsfontein B - cv PNR 6528: 5.3 ton ha^{-1}; Kroonstad -cv PNR 6528: 4 ton ha^{-1}; Vryheid - cv PNR 6528: 4.7 ton ha^{-1}; Warmbad - cv PNR 542: 5.6 ton ha^{-1}.

Except at the Vryheid site where *Digitaria sanguinalis* (L.) Scop. (resistant to atrazine) was the main weed species, weed spectra at the other trial sites were dominated by various broadleaf weeds and the grass species *Eleusine indica* L. Gaertn. which were all adequately controlled (90-100% control) by the recommended atrazine rate applied at particular sites. A field sprayer which delivered 200 L ha^{-1} at 300 kPa was used to apply atrazine. A 2.7 m spray boom with five flat fan nozzles was used on 5.4x7.0 m plots. Treatments were replicated five times in a randomized block design. Untreated control strips of 4 m width were situated between replicates. There were no weed control measures in maize apart from that provided by the application of atrazine. In the recropping (persistence) experiments, shallow tillage (100 mm) with a hand-directed rotovator between rows and hand-hoeing within rows were used to eliminate interference from weeds in all plots.

Dry beans and sunflower were planted in alternate rows on the same plots. Seeding densities of approximately 100 000 and 37 000 plants ha^{-1}, in rows 900 mm apart, were used at each planting for dry beans and sunflower, respectively. At the second planting of these crops (i.e. during 1989/90), positions of plant rows were shifted 250 mm (the maximum allowable distance within the confines of plots treated with atrazine the previous season) to avoid seeding on previous rows. Broadcast application of fertilizer [300 kg 3:2:1 (25%) ha^{-1}] was made for each experiment, prior to seedbed preparation. A side-dressing application of N, equivalent to 50 kg N ha^{-1}, was made on individual plots about 6-8 weeks after planting.

Test crop response to atrazine residues was evaluated through plant counts and seed yield in two randomly selected 2 m rows per net plot (4.4x5 m). These data were also expressed as percentage damage relative to untreated controls in order to compare atrazine persistence between localities. The only reasonable comparison of persistence between localities can be made at the atrazine rates which are recommended for use in maize. The other rates were not deliberately chosen for the present persistence study, but rather to assess weed control efficacy in that crop in a preceding study which was aimed at refining atrazine application rates in maize.

Table 26 Atrazine rates applied in maize at different localities

Locality	Atrazine rate (kg ai ha^{-1})[a]					
Bapsfontein A	2.0	<u>2.4</u>	2.8	3.2	3.6	4.0
Bapsfontein B	1.8	<u>2.0</u>	2.2	2.4	2.6	2.8
Carletonville	1.4	1.6	1.8	<u>2.0</u>	2.2	2.4
Delmas	1.2	1.4	<u>1.6</u>	1.8	2.0	2.2
Kroonstad	1.2	1.4	<u>1.6</u>	1.8	2.0	2.2
Pretoria A	1.4	1.6	1.8	<u>2.0</u>	2.2	2.4
Pretoria B	1.8	2.2	2.6	<u>3.0</u>	3.4	3.8
Vryheid	2.6	<u>3.0</u>	3.4	3.8	4.2	4.6
Warmbad	1.8	2.1	<u>2.4</u>	2.7	3.0	3.3

[a]Underlined values are the recommended atrazine rates for particular soils.

Results and Discussion

The persistence of recommended amounts of atrazine at various trial sites are shown by the data for percent reduction in yield of dry beans (Table 27) and sunflower (Table 28) recorded 12 and 24 months after treatment (m.a.t.). The original yield and stand data that were recorded for both test crops, at all the herbicide rates initially applied in maize, appear in Appendix B in the following tables: Table 5B (dry beans: 12 m.a.t. - yield data), Table 6B (dry beans: 12 m.a.t. - stand data), Table 7B (sunflower: 12 m.a.t. - yield data), Table 8B (sunflower: 12 m.a.t. - stand data), Table 9B (dry beans: 24 m.a.t. - yield and stand data), Table 10B (sunflower: 24 m.a.t. - yield data), and Table 11B (sunflower: 24 m.a.t. - stand data).

The effect of locality on the reduction in yield caused by residues of recommended atrazine rates that were applied in maize was significant for sunflower only, at both 12 m.a.t. and 24 m.a.t. (Tables 27 & 28). Except at the Warmbad site, the reduction in

dry bean yield 12 m.a.t. was less than 10% (Table 27). In contrast, sunflower suffered considerably more damage in terms of yield than dry beans at most of the trial sites at the same stage after atrazine application. This greater susceptibility of sunflower compared to dry beans was again found at the subsequent planting 24 m.a.t. (Table 28). These results confirm the difference in susceptibility to atrazine that was established for the same dry bean and sunflower cultivars in an experiment reported in Chapter 3.

Table 27 Percent reduction in seed yield of dry beans and sunflower on plots treated with recommended atrazine rates 12 months previously (ANOVA for sunflower data in Table 25A[1])

	Yield reduction (%)	
Soil	Dry beans	Sunflower
Bapsfontein A	2	17
Bapsfontein B	2	15
Carletonville	7	21
Delmas	6	16
Krooonstad	3	-[a]
Pretoria A	1	13
Pretoria B	1	-2
Vryheid	9	1
Warmbad	100[b]	100[b]
LSD_T (P=0.05)	ns	10

[1]ANOVA not conducted for dry beans due to insignificant percentages damage in yield.
[a]Sunflower ignored at Kroonstad due to unsatisfactory seedling emergence in all plots.
[b]Data for Warmbad were not analyzed statistically. The LSD-value given here does not apply to Warmbad data.

Table 28 Percent reduction in seed yield of dry beans and sunflower on plots treated with the recommended atrazine rates 24 months previously (ANOVA for sunflower data appears in Table 26A[1])

	Yield reduction (%)	
Soil	Dry beans	Sunflower
Bapsfontein a	-	0
Bapsfontein B	-	1
Carletonville	-	1
Delmas	-	1
Pretoria A	-	-1
Warmbad	38[1]	29
LSD_T (P=0.05)	-	5

[1]Data for dry beans were analyzed for the Warmbad site only (Data in Table 9B; ANOVA in Table 31A). Trials conducted only where more than 10% damage was recorded the previous season. Dash (-) denotes that a trial was not conducted.

The persistence of residues of atrazine rates recommended in maize varied considerably between localities (Tables 27 & 28). Dry bean yield was not reduced significantly by the recommended atrazine rates, 12 m.a.t. on soils in which the kaolinite clay mineral predominated (Table 27). At that stage, however, herbicide residues had reduced sunflower yield by between 13 and 21% at five of the seven trial sites. Damage in terms of yield was negligible only at Pretoria B and Vryheid. Although a lower recommended rate of atrazine was applied at the Pretoria A site (2 kg ai ha^{-1}) than the rate prescribed for Pretoria B (3 kg ai ha^{-1}), residues caused significantly more damage at the former site (Table 27). As the two sites are only 200 m apart, soil factors may have been more important than climatic factors in the determination of atrazine persistence at the Pretoria locality. This trend was not observed at Bapsfontein where

the two trials were about 50 m apart, possibly because the recommended atrazine rates and soil properties at these sites differed less than at the two sites of the Pretoria locality.

Atrazine persisted longest in the Warmbad soil (80% montmorillonite clay; pH 7.8). Twelve months after application of the recommended atrazine rate in maize, residues of all atrazine rates applied the previous season caused total failure of both follow-up crops on this soil (Table 27). Most dry bean and sunflower seedlings died within two weeks after emergence. Dry bean and sunflower plants of the second planting (24 m.a.t.) reached maturity on the Warmbad soil, but with substantial yield loss (Table 28). Residual activity in this soil was no longer detectable in subsequent bioassays conducted in a glasshouse 36 months after atrazine application. Soil samples taken from two soil layers (0-200 mm and 200-400 mm) in plots initially treated with the maximum atrazine rate (3.3 kg ai ha^{-1}) were bioassayed.

Results indicate that the dry bean cultivar Teebus could have been cropped with relatively low risk 12 months after application of atrazine on most of the soils (except at Warmbad), whilst the growing of sunflower constituted a higher risk in most of the soils (Table 27). Therefore the current waiting period of 18 months recommended for both crops was justified for sunflower only, but then only on the kaolinitic soils and not on the montmorillonite soil. Recropping intervals for both crops should be longer than 24 months for soils in which the rate of atrazine degradation is restricted, e.g. in montmorillonite soils with a neutral pH. This finding is in contrast with the computer

simulation of the persistence of atrazine that was reported by Walker (1991) for the same Warmbad soil. Walker (1991) used the model of Walker & Barnes (1981), with weather and soil data inputs which were collected during the first 12 months at the Warmbad trial site, to predict that phytotoxic atrazine residues would persist for 8 to 12 months in this soil.

According to Weber (1972), the montmorillonite clay mineral has a greater propensity for binding atrazine than kaolinite. Walker +23 others, (1983) found for simazine, as Allen & Walker (1983) did for metribuzin, that increased adsorption in heavier soils presumedly protected the compounds from degradation. In contrast, apparent adsorption-catalyzed degradation of atrazine and other chlorotriazines have been reported by Armstrong & Chesters (1968) and Hance (1979). Probably the most plausible explanations for the extended persistence of atrazine in the montmorillonite soil is the expected high degree of chemical stability of the compound in this relatively high pH soil (Armstrong *et al.*, 1967), as well as limited leaching in this soil type.

It can be expected that different crops and even cultivars are bound to respond differently to atrazine residues in soil. Mennega *et al.* (1990a,b) found that sunflower was less tolerant than dry beans to atrazine. They reported differences in the tolerance of local dry bean lines and cultivars, amongst them cv Teebus, to atrazine. In tests with ten sunflower cultivars including cv S0 222, at atrazine rates four times lower than those used in the tests with dry beans, Mennega *et al.* (1990a) demonstrated equally high sensitivity amongst cultivars.

Lack of day-to-day, on-site rainfall data, and no information on how it affected the leaching behaviour of atrazine in a particular soil, precludes speculation about the influence that rainfall had on atrazine persistence. Rainfall and temperatures recorded at the nearest weather station to each locality, from after atrazine application in maize until dry beans and sunflower were first established, appear in Tables 12B-18B (Appendix B). Although the total monthly rainfall at some localities was apparently either lower or higher than the long-term normal for certain months, the total amount of rain recorded for approximately one year after atrazine application did not deviate much from the long-term average. Mean daily maximum and minimum temperatures showed even less divergence from the long-term averages.

Results demonstrate that the single recropping interval (18 months) specified for both dry beans and sunflower are not based on the relative atrazine tolerance of these crops, at least not for the cultivars tested. Refinement of recropping intervals should at least be based on variation in soil characteristics known to influence atrazine persistence. Information on rainfall and temperatures that prevail after atrazine application would further increase the predictability of its persistence, provided the relationships between persistence and these weather factors are adequately defined. Computer simulation models that incorporate modules for the prediction of the responses of susceptible crops to atrazine residues, as well as routines which simulate herbicide dissipation, would probably be the best way to assess recropping risks. This topic is discussed in more detail in Chapter 9B. The effects of soil type, soil water content and temperature on the persistence of atrazine were demonstrated in a subsequent experiment.

CHAPTER 7

DEGRADATION OF ATRAZINE IN SOILS AT DIFFERENT WATER LEVELS AND TEMPERATURES

Introduction

Results of the field studies reported in Chapters 5 & 6 illustrated the variability in the rate of atrazine degradation in different soils. Many researchers have tried to predict persistence by describing the relationships between environmental factors and herbicide persistence in soil. However, establishing exact relationships are difficult because the rate at which a herbicide degrades is influenced by several soil and weather factors (Harris *et al.*, 1969; Hurle & Lang, 1981; Walker, 1987; Weber, 1991a).

According to Walker (1989), appreciable variability in herbicide degradation rate between soils necessitates extensive field testing at different sites and in different years. For this reason, comparison of herbicide degradation in different soils is often made in the laboratory where factors such as temperature and soil moisture can be carefully controlled.

The aims of the present laboratory investigation was to substantiate the noted variation in atrazine persistence between soils in the field, and to determine the combined effects of temperature and soil water on the degradation rate of the herbicide. In contrast to the two previous field studies, the methodology employed here negated the effect of leaching.

Materials & Methods

The degradation rate of atrazine (Gesaprim® 500 FW) was measured over a 60-day period in a fine-textured soil and a coarse-textured soil incubated under different temperature and soil water conditions in walk-in Conviron® growth cabinets. The heavier soil contained mostly montmorillonite clay (80%) and had a total clay content of 55%, 0.7% organic C, 48 cmol(+) kg^{-1} CEC, and a pH(H_2O) of 7.3. The coarse-textured soil contained mainly kaolinite clay, with 7% total clay, 0.34% C, 0.8 cmol(+) kg^{-1} CEC, and a pH(H_2O) of 5.4. Atrazine was applied to both soils at 1 and 2 mg kg^{-1} on day 0 by thoroughly mixing a pre-determined volume of a 50 mg atrazine L^{-1} solution into the soil. Each pot contained 0.5 kg air-dried and sifted (3 mm sieve) soil. Residual atrazine concentration was measured in soil samples 0, 30 and 60 days after application by means of high pressure liquid chromatography (HPLC). On day 0, ten pots from each of the 1 and 2 mg atrazine kg^{-1} treatments were randomly selected for determination of initial atrazine concentrations. At days 30 and 60 after application all treatment combinations were analyzed.

Potted soil was incubated in the dark in three growth chambers at temperature regimes of 30/16 °C, 30/8 °C and 16/8 °C on a 12/12 hour basis. Soil water levels were attained by applying half the required volume of water to the surface of the soil in each pot, prior to herbicide application, and the rest thereafter. Pots were weighed regularly and water added to ensure that original weights were maintained. Soil water contents of about 1% (air-dried soil), the water content at field capacity (13% and 26% for the coarse- and fine-textured soils, respectively) and 2x field capacity were maintained by weighing pots on alternate days and replenishing water lost through evaporation.

Water loss over, for example, an 8-day period was minimal due to the pots having been sealed in plastic bags immediately after the initial waterings.

Pots in the growth chambers were arranged according to a completely randomized design. Two soil samples from each treatment combination were analyzed. Separate analyses of variance were performed for day 30 and day 60 data.

For HPLC determination, soil samples were extracted according to the methods described by Mattson, Kahrs & Murphy (1970) and Sirons, Frank & Sawyer (1973), which were modified by extracting soil samples (50 g) for two hours with acetonitrile:water 9:1 (v/v; 150 ml). The acetonitrile:water:soil slurry was centrifuged at 5 000 r.p.m. for 5 minutes. The supernatant was filtered through a Whatman no 5 filter paper. A Millipore teflon filter disc (0.5 μm pore diameter) was used to filter 5 ml of this filtrate. The final filtrate was stored at -5°C until analyzed.

The final filtrate was used as such for analysis. A Spectra-Physics model SP 8000B equipped with a data system, autosampler and a 10 mm lightpath length flow-through cell fixed wavelength detector (254 nm) was used. A 10 μl sample loop and an Altex ODS 250x2.6 mm column were used. The instrument was operated isocratically at 1 ml min^{-1} with methanol:water (80:20). Apostolides *et al.* (1982) found that the HPLC technique described above was more suitable for determining atrazine concentrations similar to those encountered in this study than either thin layer chromatography, spectrophotometry in the ultraviolet region, or gas chromatography with a flame ionization detector or a nitrogen phosphorous detector.

Results and Discussion

Chemical analyses showed that initial (day 0) concentrations in both soils were close to the intended levels of 1 and 2 mg atrazine kg^{-1} (Tables 19B & 20B). Percentages atrazine remaining in the soils at day 30 are shown in Table 29, and data for day 60 in Table 30. Percentage data will be discussed.

The second-order soil by atrazine by water interaction was significant for the day 30 (Table 29) and day 60 data (Table 30). The rate of degradation was not significantly influenced by an increase in water content from f.c. to twice that amount. At both stages after atrazine application, degradation was significantly faster in the coarse-textured soil than in the fine-textured soil (Tables 29 & 30).

Table 29 Effect of temperature and soil water on percent atrazine remaining 30 days after application to two soils (ANOVA in Table 33A; original data appear in Table 19B)

		Temperature regime (day/night)											
		30/16 °C			30/8 °C			16/8 °C			Mean		
	Atrazine	Soil water content											
Soil	(mg kg$^{-1)}$	0	fc	2xfc	0	fc	2xfc	0	fc	2xfc	0	fc	2xfc
		% atrazine			% atrazine			% atrazine			% atrazine		
Sand	1	94	76	71	92	72	74	93	85	81	93	78	75
	2	97	65	64	96	63	67	98	82	79	97	70	70
Clay	1	99	92	95	103	87	93	97	101	96	100	93	95
	2	100	95	96	100	97	96	98	95	98	99	96	97
LSD_T (P=0.05)											Soil x Atrazine x Water = 6		

Table 30 Effect of temperature and soil water on percent atrazine remaining 60 days after application to two soils (ANOVA in Table 34A; original data appear in Table 20B)

		Temperature regime (day/night)											
		30/16 °C			30/8 °C			16/8 °C			Mean		
	Atrazine	Soil water content											
Soil	(mg kg^{-1})	0	fc	2xfc	0	fc	2xfc	0	fc	2xfc	0	fc	2xfc
		% atrazine			% atrazine			% atrazine			% atrazine		
Sand	1	86	45	45	82	60	51	94	70	64	87	58	53
	2	90	45	45	93	53	49	95	66	64	93	55	53
Clay	1	96	73	71	96	74	63	94	80	76	95	76	70
	2	97	78	76	98	79	75	98	86	81	98	81	77
LSD_T (P=0.05)											Soil x Atrazine x Water = 6		

Half-lives for atrazine can be inferred from the data in Table 30. In the sandy loam soil it appears that the half-life of atrazine was somewhat less than 60 days at 30/16°C with water content at the field capacity value. At 60 days after atrazine application to the clay soil at least 75% of the initial amount was still detectable, thus implying a halflife well beyond 60 days.

Soil characteristics were clearly determining the persistence of atrazine in the two soils. The assertion of Allen & Walker (1983) and Walker, *et al.* (1983) that increased adsorption provides protection against degradation, ostensibly explains the longer persistence of atrazine in the montmorillonite clay soil (Tables 29 & 30). In addition, atrazine molecules are highly stable under neutral pH conditions (Armstrong *et al.*, 1967). The clay soil had a near neutral pH (pH 7.3). However, the potential role of the high adsorptive capacity of the montmorillonite clay in this soil would presumably be partially negated by the fact that atrazine exists in the molecular form at high pH (Weber & Whitacre, 1982). In the neutral state, atrazine molecules would be relatively weakly bonded to negatively charged colloids which predominate under high pH conditions. In contrast to clay minerals, organic matter has both hydrophilic and lipophilic characteristics (Weber, 1991a), and therefore is likely to adsorb both atrazine molecules and cations. It is also possible that atrazine molecules can be temporarily trapped inside the expandable layers of montmorillonite clay. It seems likely that soil pH and adsorption on organic matter were the main determinants of the rate of atrazine degradation in the clay soil. Typical features of local montmorillonite soils include pH levels near or above pH 7 and relatively high clay and organic matter contents.

Soil water content and temperature both influenced the degradation of atrazine. The big differences in the magnitude of soil water levels, in contrast to the order differences for temperature, probably resulted in water content appearing to be more important than temperature. In laboratory experiments with many pesticides, Briggs (1983) and Walker & Allen (1984) reported a 2 to 2.5 fold increase in half-life with a 10°C decrease in temperature and a 1.5 to 2.5 fold increase in half-life if soil moisture is reduced by a factor of two. Grover (1965) contended that adsorption of *s*-triazines on the hydrophillic adsorptive sites of clay minerals, under high soil water conditions, will be insignificant because atrazine molecules would be desorbed from these sites by the overwhelming number of highly polar water molecules. Little or no degradation of atrazine occurred in air-dry soil, probably because both chemical and biological degradation processes occur in aqueous medium.

Results presented above suggest that weather conditions can have a marked influence on rates of atrazine loss in the field. In addition, the combination of high soil pH levels and adsorption on organic matter could be conducive to excessive persistence, and therefore, injury to sensitive follow-up crops. It is recognized that leaching is involved in the dissipation of atrazine from soil in the field. This aspect was considered in a subsequent investigation aimed at evaluating a bioassay technique for monitoring the movement of the herbicide, or its phytotoxic residues, in soil.

The work reported in this chapter has been published (Reinhardt & Nel, 1993).

CHAPTER 8

DISSIPATION AND MOBILITY OF ATRAZINE

Introduction

Previous chapters contain citations of numerous reports on the influence of climatic and edaphic factors on the persistence of atrazine in soil. The methodology in own experiments involved measurement of atrazine residues by biological means in bioassays (Chapters 5 & 6), as well as residue determination by means of chemical analysis in the absence of plants (Chapter 7).

Bioassays have the advantage that they measure only the plant-available residue fraction in soil. Their disadvantage is that they are non-specific, i.e. a particular test species may be affected by a wide range of herbicides. In contrast, the detection methods used for analytical determination of residues have greater specificity and require less time to produce results (Duffy, 1991). Results from chemical analyses are however more difficult to interpret since the amount detected is not necessarily available for uptake by plants. Analytical methods are therefore ideally suited to herbicide degradation studies in which the effect of herbicide residues on crops is not the main objective.

Walker (1989) states that degradation is only one component of the complex which determines pesticide persistence and activity in soil. Leaching is another important avenue for loss (dissipation) of herbicides in the field. In the present study the role of leaching in the dissipation of atrazine was assessed. The primary aim was to quantify

phytotoxic atrazine residues in different layers of a sandy clay loam soil by means of bioassays.

Materials & Methods

Bioassays with oats (cv SWK 001) as test species were conducted in parallel in the field and in the glasshouse with a sandy clay loam soil of the Hutton form with 22% total clay content, 0.31% organic C, P-reversion 117 mg kg^{-1}, and soil pH(H_2O) 6.3. The soil was from a cultivated field which had not been treated with herbicides during the last three years. A broadcast application of 37.5 kg N ha^{-1}, 25 kg P ha^{-1}, and 12.5 kg K ha^{-1} in the form of fertilizer [3:2:1 (25) + Zn] applied at a rate of 300 kg ha^{-1} was made three months before the experiment commenced.

Field bioassays

Six adjacent plots, each 4 m (width) by 8 m (length), were laid out on a fallow field. Each plot was divided into two sub-plots (each 4x4 m). One set of sub-plots served as controls, and were therefore not treated with atrazine. All plots denoted for treatment, except the day -1 plot (i.e. one day prior to application), were treated on day 0 with a single atrazine rate of 0.25 kg ha^{-1} (0.5 L Gesaprim® 500 FW ha^{-1}). A small plot sprayer which delivered 151 L water ha^{-1} at 300 kPa was used to apply the herbicide. On designated days (i.e. -1, 1, 30, 60, 90 and 120 days after atrazine application), four rows of oats were hand-seeded with a spacing of 500 mm x 40 mm on one half (2x4 m) of each sub-plot. The remaining 2x4 m section of each sub-plot was not seeded, and was kept free of weeds by hand-weeding. Soil samples for bioassays in the glasshouse were taken on these plant-free sections. On sub-plots seeded with oats, ten

neighbouring plants were harvested (15 days after emergence) at three randomly selected positions in a plant row. Three rows in every sub-plot were harvested this way. Plants were cut at ground level and oven-dried for determination of dry mass.

Bioassays in the glasshouse

Determination of leaching

Soil samples were collected from the 0-100 mm, 100-200 mm and 200-300 mm layers of the soil profile at days -1, 30, 60, 90 and 120 after application of atrazine. Only the top soil layer was sampled on day 1, since leaching of atrazine beyond that depth was not expected within one day after application. As atrazine could conceivably have been leached beyond the 200-300 mm zone by day 120, the 300-400 mm layer was sampled at that stage only. Sixty 50 mm diameter soil samples were taken with a field sampler from each soil layer in the unplanted sub-plots (2x4 m) at appointed intervals. Samples from a specific layer were combined before being air-dried and sifted (3 mm sieve). One kilogram of each sample was added to 1 L (100x100x100 mm) polyethylene pots lined with plastic bags to prevent drainage. Three pots (replicates) were prepared for each sampling depth at each stage after application of atrazine. Pots were arranged according to a completely randomized design. Eight ungerminated oats (cv SWK 001) seeds were planted in each pot, and plants thinned to six after emergence. Temperatures (day/night) in the glasshouse were maintained at 27/17°C on a 12/12 hour basis, without supplementary lighting. Pots were watered daily, and weighed on alternate days to bring the soil water content to 75% of the field capacity value (17% m/m). The nutrient solution of Nitsch (1972) was used to eliminate possible disparity in nutrients across soil samples by adding 0.05 L of the solution biweekly to the soil in all pots.

Plants were harvested fifteen days after emergence for determination of top growth dry mass.

Dose-response curves

Transformation of the measured residual activities (i.e. % reduction in dry matter yield relative to controls) into herbicide concentrations required comparison of these activities with those obtained in dose-response bioassays, which were performed concurrently with bioassays conducted on soil samples from the field experiment, under identical conditions in the glasshouse. Soil from the 0-200 mm layer in control plots was used to obtain dose-response curves. For this purpose, herbicide concentrations of 0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mg atrazine kg^{-1} were established in potted soil. Treatments were replicated three times. The growth response (dry matter yield) of the test plant to different atrazine rates was expressed as percent reduction in growth relative to growth in the control treatment (0 atrazine), and plotted against herbicide rate. Dose-response curves were obtained for days 1, 30, 60 and 90 after atrazine application in the field.

The procedure for estimating the amount of phytotoxic atrazine residues in different soil layers at specific intervals in the field was similar to that employed by Streibig (1988). Non-linear regression analysis of the data from the dose-response bioassays was carried out in order to obtain dose-response curves at certain intervals after herbicide application in the field. Residual atrazine concentrations in soil samples taken from the field at certain intervals were estimated by means of a single equation that described the relationship between test plant response (% reduction in dry matter yield) and atrazine

rate. The equation employed is given in the next section under the sub-heading *Glasshouse bioassays*.

Results and Discussion

Field bioassays

Damage caused by 0.25 kg atrazine ha^{-1} applied in the field was assessed visually at each stage after application by comparing the general growth of plants on treated plots with that of plants on the adjacent control plots. Concurrently, damage was determined by measuring the top growth dry mass at set intervals after herbicide application. Visual assessments indicated that residual atrazine caused 0%, 95%, 80%, 70%, 30% and 0% damage to the test plants at days -1, 1, 30, 60, 90 and 120, respectively. At corresponding stages after atrazine application, concurrent plant dry mass measurement showed that damage caused by residues was -2%, 86%, 90%, 65%, 51% and 0%, respectively (Table 31). Plant fresh weight data corresponded even better with the visual ratings. Both fresh and dry mass data indicated that amount of residues available to the test plant dropped significantly between days 30 and 60, and again between the latter stage and day 90. Lethal quantities of herbicide residues apparently persisted until about 90 days after application (Table 31).

Table 31 Persistence of atrazine in the field at different time intervals after application (ANOVA in Table 37A)

Days after treatment	Freshweight	Dry mass
	% damage	% damage
-1	1.0	-2.2
1	95.3	86.6
30	87.0	90.0
60	63.5	65.5
90	48.5	51.4
120	0.3	0.04
LSD_T (P=0.05)	13.8	12.1

Glasshouse bioassays

As the dose-response curves for days 1, 30, 60 and 90 were well matched (Figure 6), data from the dose-response bioassays were combined to derive a single equation that could be used throughout. The amount of phytotoxic atrazine residues in different soil layers at certain intervals after application was calculated with the equation:

$$y = 23.2 + 47.2\ (\log(100x)) \qquad \text{------ 5}$$

[where y = % damage in oats dry mass); x = amount of atrazine in soil (mg kg^{-1})]

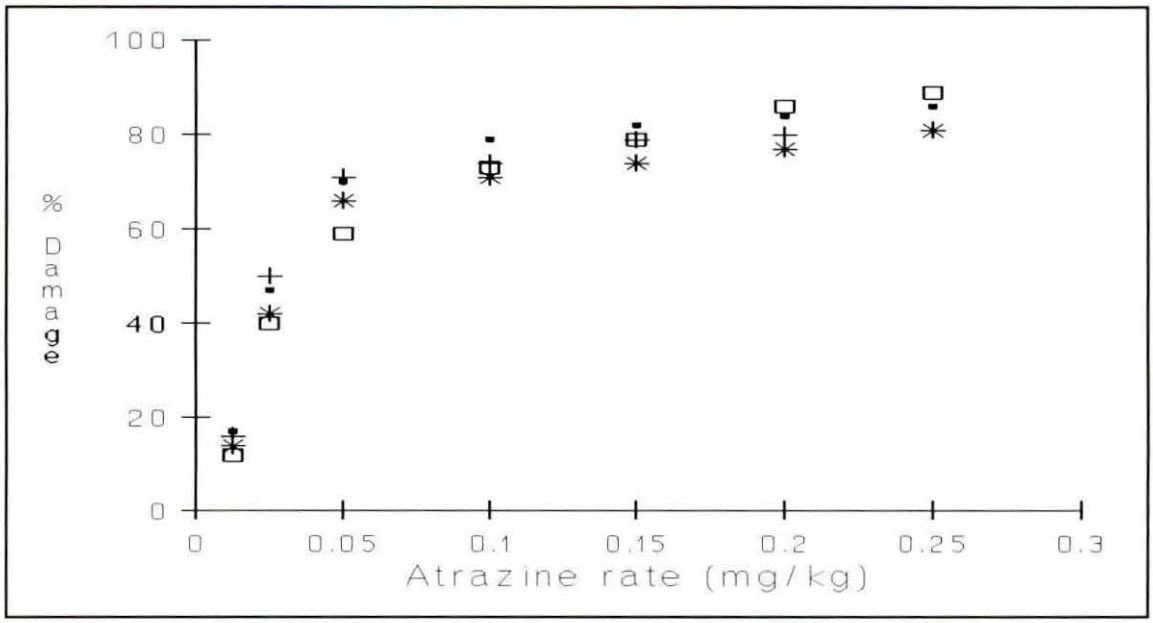

Figure 6 Dose-response curves derived from test plant response (percent reduction in top dry mass) to a range of atrazine rates in separate bioassays conducted in a glass-house at 1 (■), 30 (□), 60 (*) and 90 (+) days after atrazine application in the field

The estimated residual amount detected in the 0-100 mm soil layer was 0.177 mg atrazine ha^{-1} on day 1 after herbicide application (Table 32), which conforms well with the expected amount of about 0.179 mg kg^{-1}. The latter concentration was derived with expression [5] by taking into account the specific weight of the soil (i.e. 1.4 g cm^{-3}), and assuming that the atrazine applied in the field (i.e. 0.25 kg atrazine ha^{-1}) was distributed evenly in the top 100 mm soil layer.

$$x = \frac{0.25x10^6}{1.4x10^6}$$

[where x = mg atrazine kg^{-1} after application of 0.25 kg ai ha^{-1}]

Table 32 Percent damage caused by atrazine residues in soil sampled in different soil layers at certain stages after herbicide application, and the estimated amount of residue responsible for damage to the test plant (ANOVA's in Tables 38A & 39A)

	Damage (%) and estimated residue conc. (mg kg^{-1})									
	Soil layer (mm)									
Day	0-100		100-200		200-300		300-400		Mean	
	% damage	mg kg^{-1}	% damage	mg kg^{-1}	% damage	mg kg^{-1}	% damage	mg kg^{-1}	% damage	mg kg^{-1}
1	82	0.177	-	-	-	-	-	-	82	0.177
30	65	0.080	50	0.038	69	0.097	-	-	61	0.071
60	38	0.021	19	0.008	10	0.005	-	-	22	0.011
90	23	0.010	15	0.007	-2	0.003	-	-	12	0.007
120	3	0.004	17	0.007	9	0.005	22	0.010	13	0.007
$LSD_{T\ 0.05}$			**% damage**: Day x Depth = 15			**mg kg^{-1}**: Day x Depth = 0.033				

* Dash (-) denotes soil layer not monitored.

Thirty days after atrazine application in the field, concentrations causing reductions ranging from 50% to about 80% in the shoot dry mass of oats were detected in the 0-100 mm, 100-200 mm and 200-300 mm soil layers (Table 32; Figure 7a). The percentages damage recorded at that stage were transformed with the equation for the dose-response curves to 0.080, 0.038 and 0.097 mg atrazine kg^{-1} (in the 0-100 mm, 100-200 mm and 200-300 mm layers, respectively). This distribution pattern most likely resulted from 165 mm rainfall during the 30 day period after application (Figure 8). Disparity between the cumulative amount of atrazine residues present in the three soil layers monitored at day 30, and the amount applied at day 0, accentuate a restriction in the use of bioassays for quantifying herbicide residues. It is to be expected that the inherent biological variation in bioassays, and the multi-step methodology involved would make the procedure less accurate than chemical analysis. However, as pointed out earlier, bioassays allow plant-available residues to be estimated.

Beyond day 30, a further 180 mm of rainfall during the period ending on day 60 probably contributed to further reductions in the amount of atrazine residues in the respective layers to 0.021, 0.008 and 0.005 mg atrazine kg^{-1} (Table 32; Figure 7b). Results presented in Figures 7 & 8 indicate that atrazine leached quite rapidly in response to substantial rainfall. It is not only the total amount of rainfall which determines the leaching of a herbicide, but also the frequency and intensity of water received (Ammon, 1985).

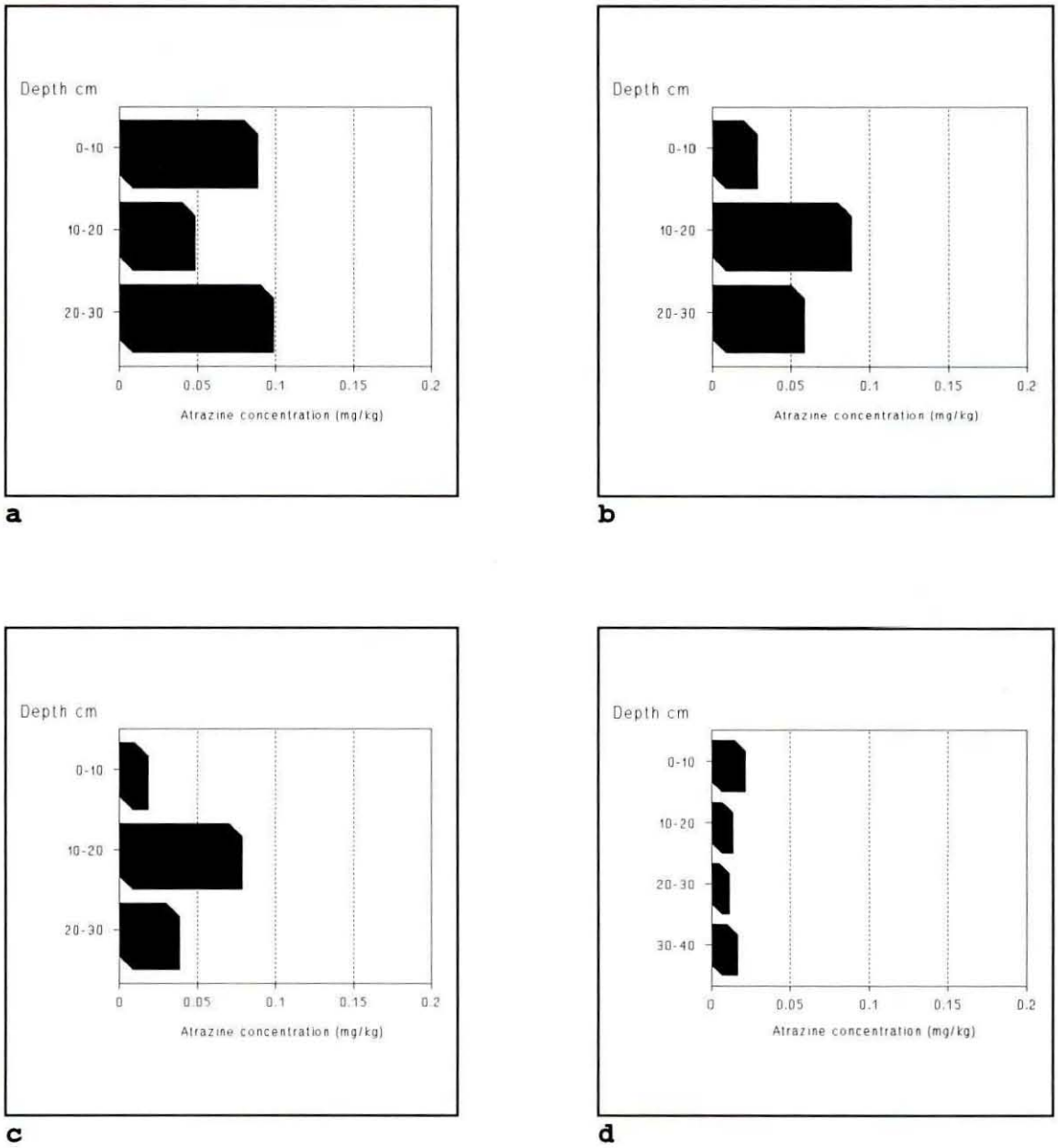

Figure 7 Leaching pattern of atrazine as depicted by estimated residual atrazine in different soil layers: (a) 30 days after treatment (d.a.t.); (b) 60 d.a.t.; (c) 90 d.a.t.; (d) 120 d.a.t.

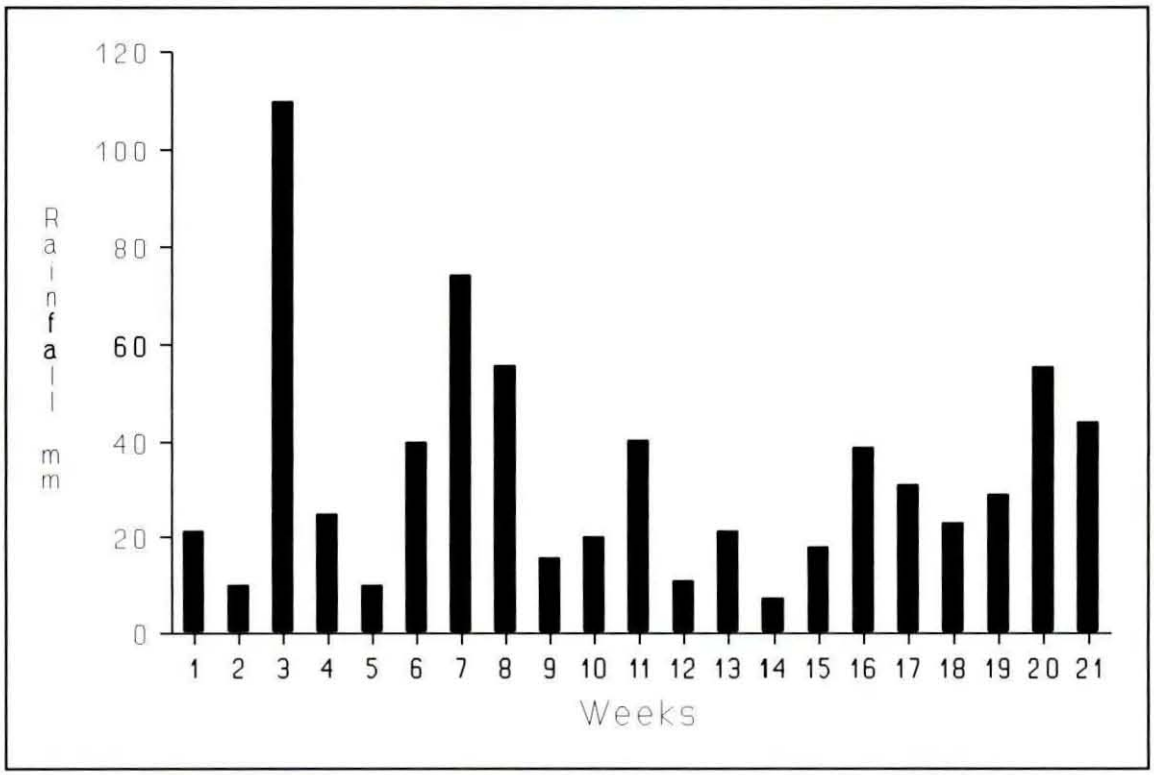

Figure 8 Total weekly rain and irrigation after atrazine application

Sampling on day 90 revealed a similar herbicide distribution pattern as that obtained 30 days previously (Figure 7c), but with reductions in residue concentration in all three soil layers tested (Table 32). On day 120, atrazine residues had decreased notably in the 100-200 mm and 200-300 mm soil layers from the levels detected 30 days previously, whilst little change in concentration was evident in the top soil layer (Table 24; Figure 7d). The upward movement of atrazine in capillary water in response to water evaporation from the soil surface as reported by Spencer & Cliath (1973) and Hubbs & Lavy (1990), could have been responsible for maintaining phytotoxic residues in the top soil layer for the duration of the experiment.

Phytotoxic atrazine residues (i.e. 5.6% of the amount applied) was also detected in the 300-400 mm soil layer at day 120 (Table 32; Figure 7d). From the results of previous bioassays it appears that atrazine residues might have been leached into the 300-400 mm layer earlier on in the experiment, due mainly to high rainfall during the 60 day period following herbicide application. The relatively low atrazine adsorption capacity of the soil (22% total clay of the kaolinite type; 0.31% C; pH 6.3) is expected to have contributed to the substantial leaching of the herbicide. According to Leonard *et al.* (1988), atrazine can be classified as moderately to slightly mobile in most soils. Dissipation of atrazine through leaching can be expected to accelerate under near neutral and alkaline soil conditions (McGlamery & Slife, 1965; Smit & Nel, 1977), particularly in soils with low adsorption capacities.

In the present study, application of a relatively small amount of atrazine (0.25 kg ha^{-1}) in the field made it possible to derive dose-response curves with a range of atrazine rates which elicited computable responses from the test plant. Experience gained in a previous study with the same atrazine rate and test plant on the same soil was useful for determining which herbicide rates to use in the present study. Without experience of the magnitude of a test plant's response to a given amount of a herbicide applied to a particular soil, the applicability of dose-response curves for quantifying herbicide residues could be disputed.

Ideally, dose-response curves should be obtained for each soil layer, since the availability of residues for plant uptake could conceivably be governed by differences

in certain soil properties (e.g. % clay, % C, nutrients and pH) down the soil profile. However, the number of observations required if dose-response curves for each soil layer at specific intervals after herbicide application are to be obtained is prohibitive. A collaborative study involving bioassays for monitoring the dissipation of metsulfuron-methyl and metribuzin in different soils was confronted with the same constraint (Krauskopf +25 others, 1991).

The bioassay technique proved useful for making projections of the amount of phytotoxic atrazine residues in different soil layers. It must be stressed, however, that the suitability of bioassays for quantifying atrazine residues could be tenuous under circumstances which differ from those that prevailed in the present study. There is agreement with the view of Krauskopf *et al.* (1991) that the technique does provide a relatively simple, time- and cost-effective procedure with which residual activities can be measured, thereby facilitating projections of the potential for sensitive follow-up crops to be injured.

As an alternative to direct measurements of the residual herbicide concentration in the root zone for defining the risk of carry-over, various computer simulation models are used to predict dissipation, as well as the likelihood of injury to sensitive following crops. A useful piece of input information is the half-life of a compound in a particular soil. Research reported in the following chapter was aimed at formulating a regression model based on the relationships between half-life of atrazine and selected soil properties.

CHAPTER 9

INFLUENCE OF SELECTED SOIL PROPERTIES ON THE PERSISTENCE AND HALF-LIFE OF ATRAZINE

Introduction

The persistence of herbicides and the availability of their residues for uptake by plants are important features of soil-applied compounds (Walker, 1991; Weber, 1991a,b). Many studies have therefore indirectly or directly focused on identifying compounds and conditions with which persistence problems are associated. Evidence in the literature and confirmation provided by work reported in previous chapters, identified soil pH as one of the important predictors of atrazine persistence. Soil pH exerts a strong influence on the adsorption and hydrolysis of atrazine. The bioavailability, mobility and stability of atrazine normally increase as soil pH increases.

Half-life values for herbicides are estimates of the length of time that a herbicide is present to exert an effect on plants. Weber (1991b) considers half-life values to be a relative index of the combined transformation processes involved in herbicide degradation. Leaching does not affect half-lives directly, but does contribute to the dissipation of a compound in soil. In the experiments reported on in this chapter, and glasshouse experiments reported earlier, leaching was eliminated. The objectives of the glasshouse experiments reported on here were: (a) to determine half-lives for atrazine in different soils by means of bioassays, and (b) to correlate atrazine half-life with selected soil properties.

A. Soil pH

Materials and Methods

Bioassays to assess the effect of soil pH on the persistence of atrazine were conducted in a glasshouse with soil samples of which essentially only the pH varied. Soil was collected at the Hatfield Experimental Farm of the University of Pretoria in plots of a long-term field trial which was conducted to evaluate the effects of soil acidity and liming on maize yield. Soil samples were taken from the top 200 mm soil layer in plots that had been treated annually with 2.25, 4.5 and 6.75 ton ha^{-1} pulverized calcitic lime (contains mainly $CaCO_3$ and less than 14% $MgCO_3$) for the past 15 years. Plots in which soil was collected were selected on the basis of pH(H_2O) levels that had been determined earlier for all plots. The aim was to obtain as wide a range of pH levels as possible within a limited number of soil samples. With the final selection of samples, six pH levels ranging from pH 4.8 to pH 7.0 were devised in a single soil. Selected properties of the six soil samples appear in Table 33.

Table 33 Selected properties of six samples of the loamy sand soil used in the study

Sample no.	Soil pH (H_2O)	% Clay	% C
1	4.8	20	0.31
2	5.3	18	0.28
3	5.5	22	0.33
4	5.7	19	0.34
5	6.3	18	0.29
6	7.0	18	0.29

The six soil samples were each treated with 0, 0.1 and 0.2 mg atrazine kg^{-1} on the day the experiment commenced, i.e. day 0. Pre-determined volumes of a 50 mg atrazine

L^{-1} solution were added to water, and a maximum of 30 cm^3 of this mixture was well mixed with the 500 g soil in each pot. Soil of control treatments (0 mg atrazine kg^{-1}) received 30 cm^3 water. Subsequently, additional water was added to attain 75% of the field capacity value of 17% (m/m). The soil water content at field capacity was determined gravimetrically. Pots were weighed immediately after watering to make allowance for subsequent replenishment of water losses through weighing. Soil was contained in plastic bags which prevented leaching.

Pots were allotted to each stage (days 0, 30, 60, 90 and 120 after treatment) at which a bioassay was to be done. The first bioassay was conducted on day 0, immediately after application of atrazine. Pots destined for bioassays at later stages were incubated in the dark at a constant 27/17°C (12/12 h day/night) temperature regime. Water loss during incubation was prevented by closing the plastic bags in which soil was held. Incubated pots were weighed weekly to check water loss, and when necessary, soil water content was adjusted with deionised water.

At each stage after atrazine application, pots destined for bioassaying were removed from incubation, and ten seeds of the test plant (oats cv SWK 001) were planted 20 mm deep in each pot. Those pots were moved to a glasshouse at a temperature regime of 27/17°C (12/12 h day/night regime) without supplemental lighting. Nutrients were supplied in the form of the nutrient solution of Nitsch (1972) which was applied in volumes of 50 cm^3 to all pots three times weekly. Plants were thinned to six in each pot after emergence and harvested fourteen days thereafter. Dry matter yield of the top growth was determined and expressed as percent damage (i.e. percent reduction in dry

matter relative to controls). Pots in the glasshouse and the adjoining darkroom were arranged in a completely random design with three replications. Standard analysis of variance was performed on percent damage data.

Use of a closed-system soil environment (leaching prevented) in the present experiment allowed the effects of leaching and soil pH to be separated. Another advantage of the procedure followed was the procurement of soil samples in which the pH was stabilised over several years. The use of a single soil in which essentially only the pH varied between samples (Table 33) afforded the rare opportunity to isolate the effect of this property from those of clay and organic matter content. It is unlikely that the slight variation in clay and organic matter contents between the soil samples used would have affected results.

The time span of this experiment (120 days) and the one reported on next (Experiment B - 150 days) was chosen in consideration of the conservation of biological activity in soils. Rates of atrazine degradation were shown to be markedly affected by the biological activity (biomass) of soils, with most rapid rates of loss occurring in fresh soil samples (Walker & Brown, 1981). It is generally accepted that prolonged incubation studies with pesticides should be avoided to conserve biological activity in soil (Anderson, 1987; Walker, 1989). Anderson (1987) concluded that incubation experiments with pesticides should be limited to a maximum duration of 90 days or until 50% of the biomass has been lost, whichever comes first.

Results and Discussion

Percentage reduction in dry mass of the test plant in response to atrazine, or its bioactive residues, over a 150-day period is presented in Table 34. Analysis of variance for percent reduction in dry mass detected significant interactions between soil pH, time and atrazine rate. The only effect that was not significant was the pH x Atrazine rate interaction, which indicated that the influence of soil pH on bioactivity resulting from application of either 0.1 or 0.2 mg atrazine kg^{-1} was similar.

The reduction in bioactivity of 0.1 mg atrazine kg^{-1} from day 0 to day 30 was significant at soil pH levels of 4.8, 5.3 and 5.5, but not at the higher pH levels of 5.7, 6.3 and 7.0 (Table 34). The time required for significant drops in activity of the 0.1 mg kg^{-1} rate to occur at the latter three pH levels increased with increasing soil pH. This trend was also evident for 0.2 mg atrazine kg^{-1}, with the difference that significant lowering of activity did not occur at either pH 6.3 or pH 7.0 at any stage after atrazine application.

The residual activity of atrazine at days 90 and 120 was not significantly different between pH levels of 4.8, 5.3 and 5.5 (Table 34). Residual activity at the latter pH level and pH 5.7 was similar at all stages after atrazine application. Initial differences in residual activity at pH 4.8, 5.3, 5.5 and 5.7 became progressively smaller from day 60 onward, and was eventually negligible at day 120. At days 90 and 120 the residual activity of both atrazine rates were significantly higher at pH 7.0 compared to activity at pH levels 4.8, 5.3, 5.5 or 5.7. At the same stages, differences in bioactivity between pH 6.3 and pH 7.0 were not significant.

Table 34 Effect of six soil pH levels on the residual activity of atrazine (based on percent reduction in dry mass of oats) in a sandy loam soil (ANOVA in Table 40A)

	Time (days after atrazine application)									
	0		30		60		90		120	
	Atrazine rate (mg kg^{-1})									
Soil pH (H_2O)	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2
	% damage		% damage		% damage		% damage		% damage	
4.8	74	83	19	62	1	13	0	14	-2	0
5.3	61	83	34	62	12	31	7	19	5	9
5.5	84	87	42	80	18	51	9	34	6	9
5.7	77	86	66	75	22	44	7	33	7	16
6.3	84	88	62	82	47	77	40	72	33	63
7.0	82	86	79	85	65	72	57	75	45	66
LSD_T (P=0.05)	pH x Time x Atrazine = 27									

Rough estimates of the half-lives of atrazine, based on comparison of the percentages reduction in dry mass on day 0 with those measured on specific days after application of 0.2 mg atrazine kg^{-1}, show that atrazine half-lives (x days) at different pH levels could be categorized as follows: $30 < x < 60$ at pH 4.8 and 5.3; $60 < x < 90$ at pH 5.5 and 5.7; $x > 120$ days at pH 6.3 and 7.0. As test plant response to atrazine rapidly declined below levels of 0.1 mg atrazine kg^{-1}, half-life estimates based on test plant response to residues of 0.1 mg kg^{-1} would be less accurate than approximations based on growth response to larger amounts of residues resulting from treatment with 0.2 mg atrazine kg^{-1}.

The effect of soil pH on the residual activity/persistence of atrazine first became apparent in the day 30 bioassay. This concurs with the findings of previous work in a glasshouse (Ehlers *et al.*, 1988) which indicated that the short-term bioactivity of atrazine (test species planted immediately after herbicide application) was not significantly influenced by soil pH. Results of the field study reported on in Chapter 4 also agree with the findings of Ehlers *et al.* (1988) as far as the activity of atrazine within 30 days of application is concerned. It was also shown in Chapter 5 that pH was an important predictor of atrazine bioactivity/persistence at 182 days after application. Results of the present study also appear to substantiate the extended persistence of atrazine that was found in the field in a montmorillonite clay type soil with pH 7.8 (see Chapter 6). In that soil, dissipation of atrazine would conceivably have been restricted by limited leaching due to the unique water-holding capacity of the soil type, and also by the high stability of atrazine at neutral pH levels (Armstrong *et al.*, 1967; Hiltbold

& Buchanan, 1977). Colbert *et al.* (1975) and Smit *et al.* (1979, 1980) reported that the adsorption and hydrolysis of atrazine generally decreased as soil pH was increased from relatively low pH to near neutral pH conditions.

B. Atrazine half-lives in 25 soils

Materials and Methods

Two sets of bioassays were conducted in a glasshouse.

Dose-response experiments

Experiments in the first grouping (Experiments I, II, III and IV) were conducted to obtain dose-response curves for each soil used. The equations that describe these best-fit curves (percent reduction in top growth dry mass plotted against atrazine concentration) were used for transforming residual bioactivity (expressed as percent reduction in top growth dry mass) in a soil into amount of bioactive residues present in that soil.

Differential dose thresholds for the test plant in different soils demanded that four ranges of atrazine rates be used for obtaining dose-response curves. Thus dose-response bioassays were grouped according to sets of soils treated with four ranges of rates (i.e. four experiments). The rates used appear in the sub-headings in Table 35. The choice of suitable atrazine rates (neither too high, nor too low) was based on preliminary experiments with some of the soils. A total of 34 soils were employed in these bioassays, but due to poor fit of data to dose-response curves and either too low

or too high bioactivity the data for 25 soils were eventually used. Certain key characteristics of those 25 soils appear in Table 35.

Table 35 Selected properties of the 25 soils used

Exp. I: Soils treated with the lowest range of atrazine rates (0, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, 0.256 mg kg^{-1})

	Soil properties				
Locality	Clay %	% C	pH(H_2O)	P-reversion (mg P kg^{-1})	CEC cmol(+) kg^{-1}
Colby	11	0.18	5.5	115	2.98
Fairdale	10	0.18	5.8	100	2.14
Nelspruit	4	0.29	6.8	170	2.62

Exp. II: Soils treated with the first intermediate range of atrazine rates (0, 0.0125, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 mg kg^{-1})

Locality	Clay %	% C	pH(H_2O)	P-reversion (mg P kg^{-1})	CEC cmol(+) kg^{-1}
Bethal	13	0.40	5.1	109	3.10
Bothaville	15	0.20	4.9	137	2.50
Ermelo A	9	0.38	4.8	92	1.80
Leeudstd. A	7	0.32	5.3	105	1.20
Leeudstd. B	8	0.40	7.1	93	3.00
Nylstroom	13	0.40	5.1	75	3.10
Pretoria A1	20	0.31	4.9	120	5.32
Pretoria A2	18	0.28	5.3	110	4.38
Pretoria A3	19	0.34	5.7	117	5.73
Pretoria A4	18	0.29	6.5	123	4.17
Pretoria A5	18	0.29	7.2	118	4.22
Warmbad A	35	0.50	7.8	83	26.60

Continued overleaf

Table 35 continued

Exp. III: Soils treated with the second intermediate range of atrazine rates (0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35 mg kg^{-1})

	Soil properties				
Locality	Clay %	% C	pH(H_2O)	P-reversion (mg P kg^{-1})	CEC cmol(+) kg^{-1}
Carletonville	21	0.83	5.3	130	5.31
Ermelo B	16	0.80	5.3	82	8.50
Morgenzon	21	0.47	6.4	65	12.48
Redhill	50	0.98	5.0	5	3.30
Vryheid	53	2.04	5.5	25	15.80

Exp. IV: Soils treated with the highest range of atrazine rates (0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg kg^{-1})

Potgietersrus	27	0.65	7.2	90	10.37
Pretoria B	41	1.06	5.9	70	14.11
Roodeplaat	17	0.41	6.9	40	12.22
Utrecht	27	1.18	5.8	115	11.33
Warmbad B	55	0.77	7.5	20	54.43

Bioassays for determining the degradation rates of atrazine

The second category of bioassays involved periodic measurement of bioactivity of atrazine residues in soils that were incubated for specific periods after treatment with 0.2 mg atrazine kg^{-1} on day 0. Except for those pots designated for bioassaying on day 0, all pots were incubated in the dark for specific intervals. The rate of 0.2 mg atrazine kg^{-1} was attained by mixing a pre-determined volume of a 50 mg atrazine L^{-1} stock solution with 500 g soil in each pot. Water only was added to the controls. Soil in pots were contained in plastic bags to prevent leaching of the herbicide out of the soil. Soils removed from incubation were thoroughly mixed before the test species was planted.

At specific intervals (30, 60, 90, 120 and 150 days after atrazine application), pots were removed from the incubator and bioassayed with oats (cv SWK 001). Atrazine half-life (days) in each soil were estimated by transforming the percentage reduction in top growth dry mass, which was recorded 0, 30, 60, 90, 120 and 150 days after herbicide application, to mg atrazine kg^{-1} soil by means of the logarithmic equation (see Appendix C) for each soil. This transformation involved entering the percentage reduction in dry mass (i.e. the dependent variable y) that was obtained for a certain soil, at a specific stage after application, in the dose-response equation for that soil, and solving the equation for mg atrazine kg^{-1} soil (i.e. x in the equation).

As bioactivity caused by 0.2 mg atrazine kg^{-1} varied from soil to soil, and from one stage of measurement to the next, it was necessary to dilute the amount of herbicide in

the incubated soils so as to ensure a measurable plant response. Dilution ratios of 100:0 (500 g treated soil:0 g untreated soil), 50:50 (250 g treated:250 g untreated) and 25:75 (125 g treated:375 g untreated) were brought about by mixing untreated soil with treated samples. The dilutions made it possible to avoid the insensitive extreme ends of dose-response curves when data (% reduction in growth) were transformed to herbicide amounts. The amounts of phytotoxic residues that were estimated with the 25:75 and 50:50 soil dilutions were transformed to full (100%) concentrations by multiplying the amounts estimated in the diluted soil by four and two, respectively.

Degradation rates in the 25 soils over the 150 day period were best described by the quadratic formula $y = ax^2 + bx + c$. Atrazine half-life in each of the 25 soils were estimated by means of these equations by calculating x (half-life in days) after inserting the value 0.1 (i.e. half the amount of atrazine applied) as the dependent variable (y). Regression analysis was performed on half-life data according to the Stepwise Procedure in the SAS programme, with the aim to identify variables (soil properties) for inclusion in a multiple regression model for the prediction of atrazine half-lives in soils. The real values of the soil variables (x), as well as their squares (x^2) were correlated with half-life data, in order to distinguish between linear and non-linear relationships.

General

Environmental conditions in the glasshouse were the same for both categories of bioassays. Water content of the soils in which the test plant was grown was adjusted

to 75% of the total water content per pot at field capacity by weighing pots on alternate days. The water content of the incubated soils was checked fortnightly. A volume of 30 cm^3 of the nutrient solution of Nitsch (1972) was applied to pots with test plants on days not designated for weighing of pots. A constant day/night temperature of 27/17°C (± 1°C) was maintained for a 12/12 h thermoperiod in a glasshouse. The same temperatures were maintained in the interconnected darkroom in which pots were incubated. Supplemental lighting was used to extend the daylight period in the glasshouse to a minimum of 12 hours. The growth period was 21 days, from seeding until harvesting of seedling top growth. The dry mass yield in each pot was calculated on a per plant basis (six plants per pot). Data were expressed as percent damage, i.e. percent reduction in shoot dry mass compared to untreated controls. Pots were arranged according to a completely randomized design in all the experiments. All treatments were replicated three times.

Results and Discussion

Logarithmic regression lines gave the best fit for dose-response data presented in Table 21B. The dose-response curves for the 25 soils are illustrated in Figures 1C-25C in Appendix C (p. 250). The amounts of phytotoxic atrazine residues that were estimated by means of the dose-response equations on days 0, 30, 60, 90, 120 and 150 after treatment are presented in Table 36. Analysis of variance of residual concentration data detected a significant Soil x Time (days) interaction. The atrazine concentration in most soils on day 0 was predicted fairly accurately, considering the inherent variation encountered in bioassays.

Table 36 Estimated amounts of atrazine, or its phytotoxic residue(s), which were available to the test plant at specific intervals after application (ANOVA in Table 45A)

	Number of days after atrazine application					
Locality	0	30	60	90	120	150
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Bethal	0.167	0.150	0.069	0.073	0.020	0.013
Bothaville	0.226	0.161	0.052	0.035	0.042	0.001
Carletonville	0.207	0.163	0.119	0.036	0.034	0.026
Colby	0.238	0.170	0.068	0.085	0.031	0.009
Ermelo A	0.161	0.124	0.080	0.025	0.009	0.000
Ermelo B	0.176	0.152	0.116	0.104	0.061	0.028
Fairdale	0.162	0.160	0.086	0.079	0.055	0.003
Leeudrgstd. A	0.149	0.165	0.093	0.031	0.043	0.006
Leeudrgstd. B	0.224	0.161	0.141	0.064	0.050	0.030
Morgenzon	0.217	0.168	0.091	0.079	0.031	0.033
Nelspruit	0.167	0.171	0.108	0.122	0.064	0.067
Nylstroom	0.163	0.156	0.069	0.072	0.019	0.009
Potgietersrus	0.177	0.189	0.167	0.110	0.112	0.062
Pretoria A1	0.190	0.171	0.061	0.061	0.045	0.009
Pretoria A2	0.167	0.133	0.047	0.013	0.035	0.010
Pretoria A3	0.227	0.133	0.121	0.089	0.026	0.025
Pretoria A4	0.222	0.146	0.142	0.046	0.052	0.011
Pretoria A5	0.192	0.190	0.137	0.089	0.088	0.015
Pretoria B	0.162	0.174	0.147	0.062	0.071	0.038
Redhill	0.185	0.170	0.065	0.081	0.045	0.008
Roodeplaat	0.206	0.179	0.116	0.095	0.071	0.050
Utrecht	0.224	0.163	0.152	0.051	0.067	0.022
Vryheid	0.168	0.179	0.121	0.086	0.074	0.025
Warmbad A	0.209	0.161	0.156	0.106	0.122	0.056
Warmbad B	0.193	0.157	0.162	0.166	0.067	0.090
LSD_T (0.05)			Soil x Days = 0.069			

The rate of degradation of atrazine in each of the 25 soils is illustrated in Figures 9-33. The r^2-values shown in Figures 9-33 were invariably significant at the 5% level. Differential degradation rates between soils can be best judged from the half-life data presented in Table 37.

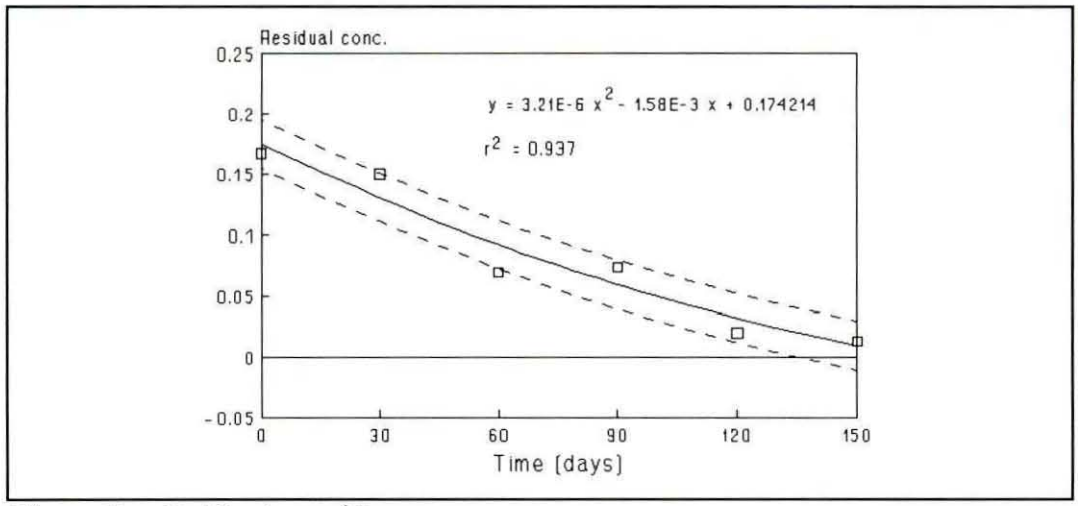

Fig. 9 Bethal soil

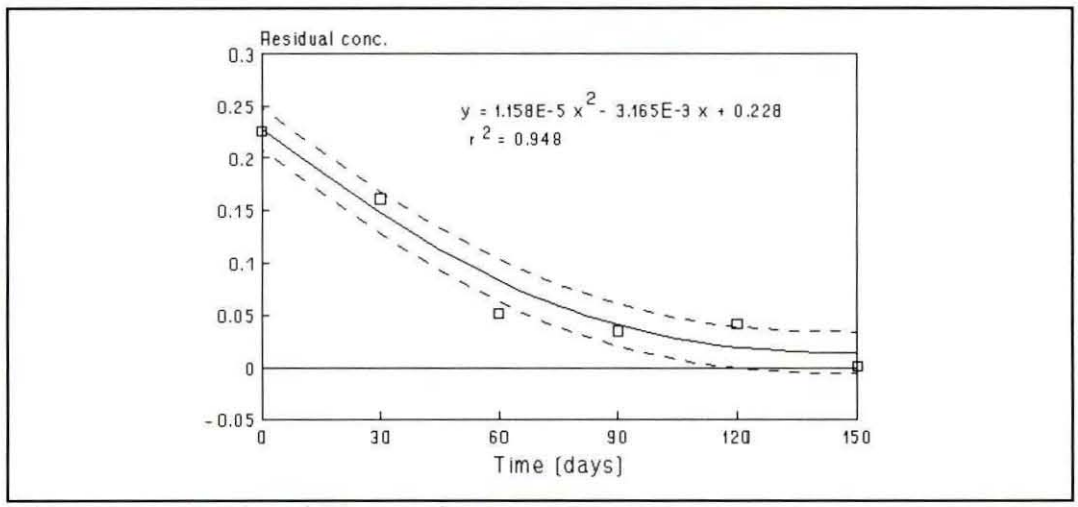

Fig. 10 Bothaville soil

Figure 9 & 10 Rate of atrazine degradation in the Bethal and Bothaville soils (Broken lines denote quadratic relationships for upper and lower 95% confidence limits)

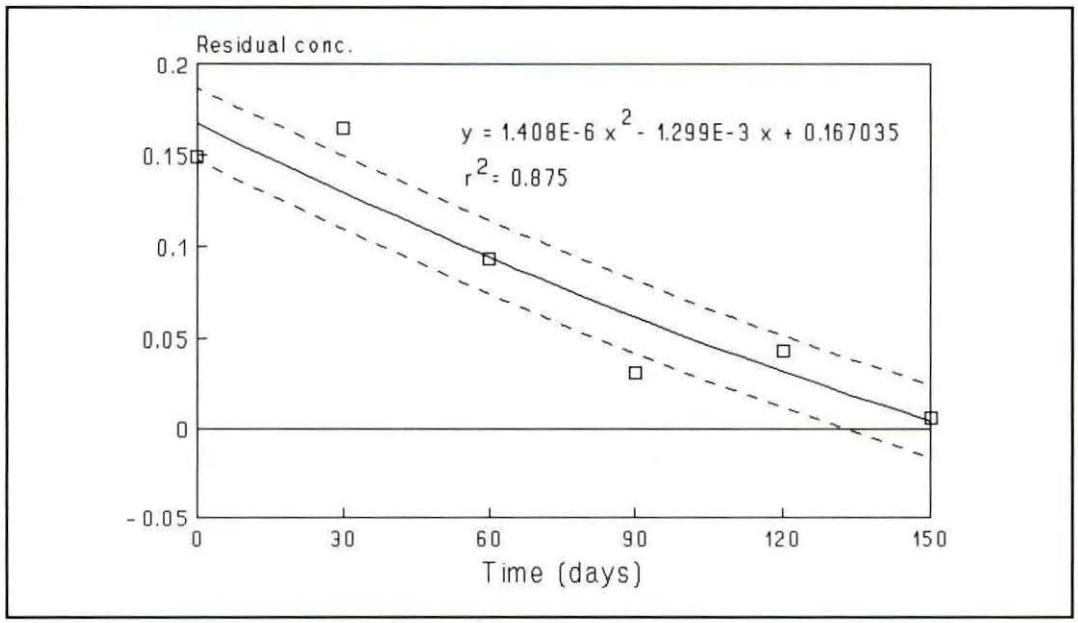

Fig. 11 Leeudrgst. A soil

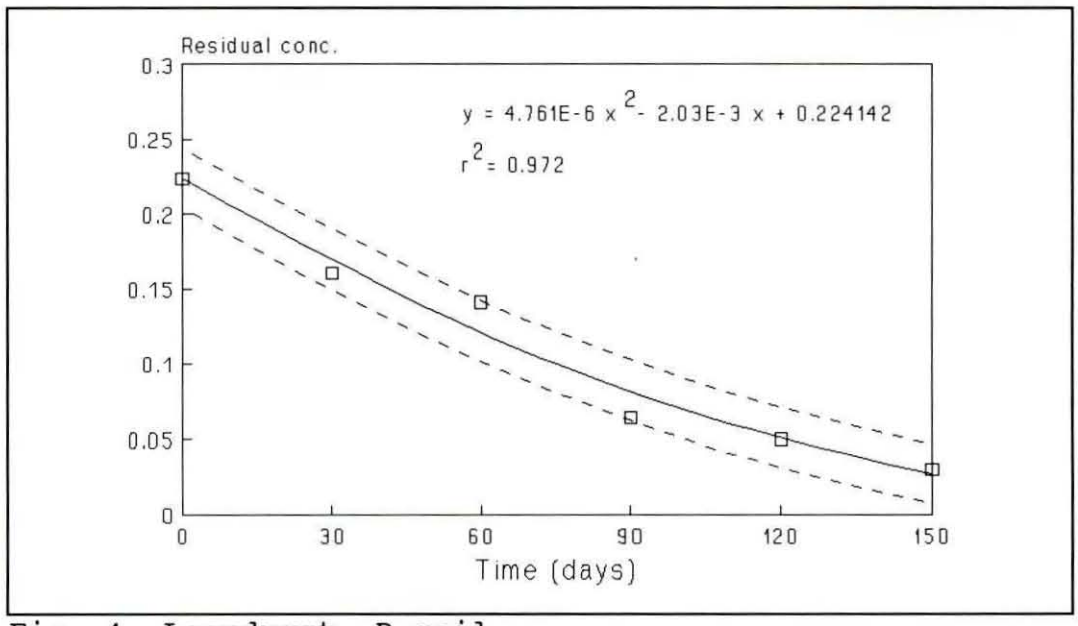

Fig. 4 Leeudrgst. B soil

Figures 11 & 12 Rate of atrazine degradation in the Leeudoringstad A and Leeudoringstad B soils

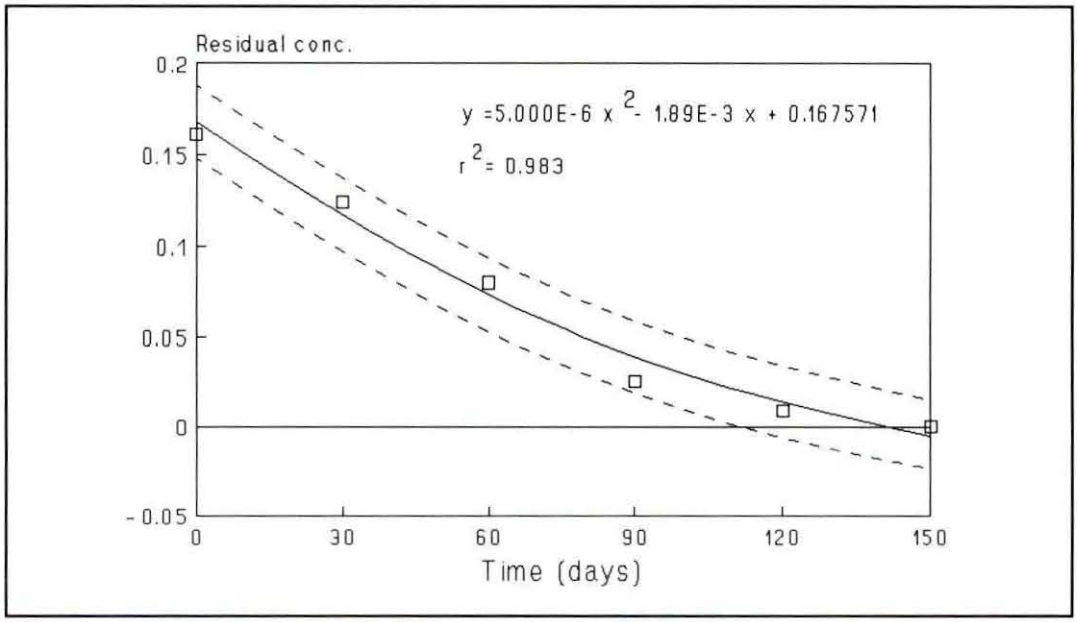

Fig. 13 Ermelo A soil

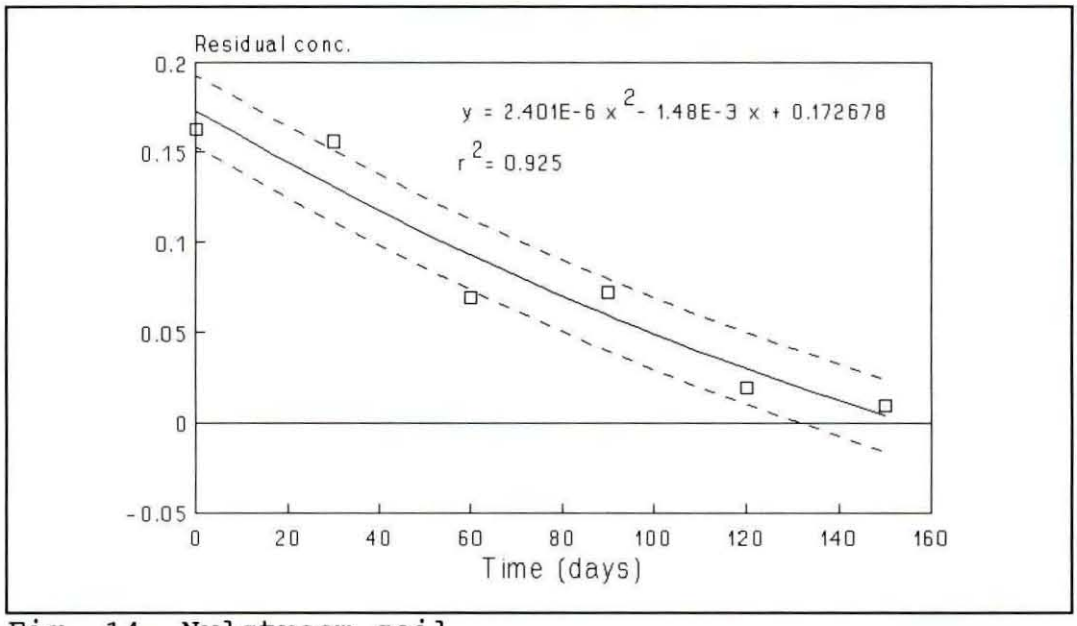

Fig. 14 Nylstroom soil

Figures 13 & 14 Rate of atrazine degradation in the Ermelo A and Nylstroom soils

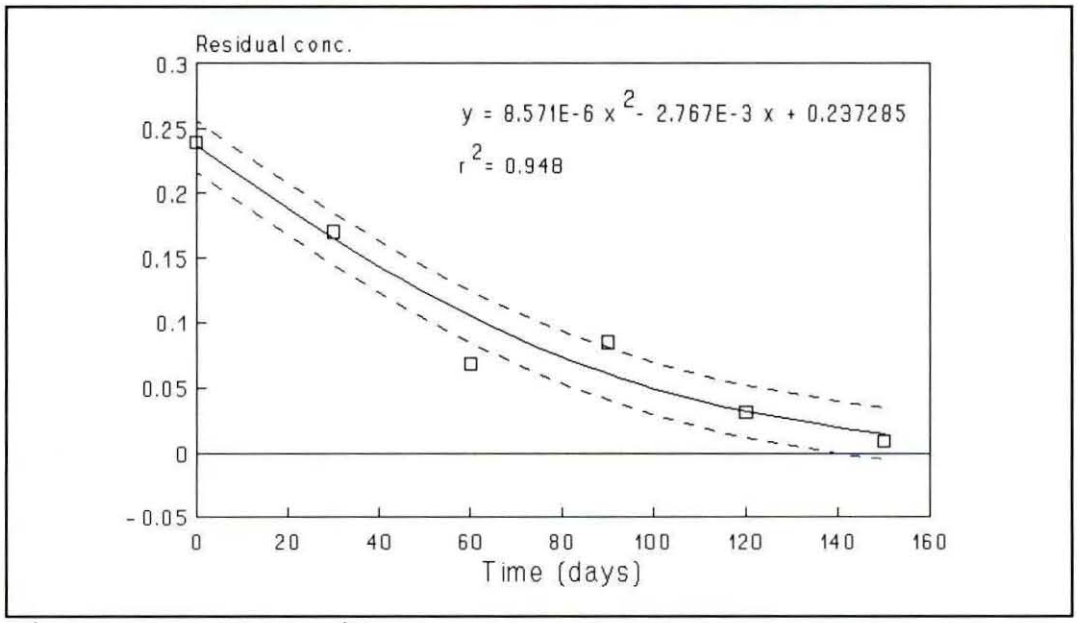

Fig. 15 Colby soil

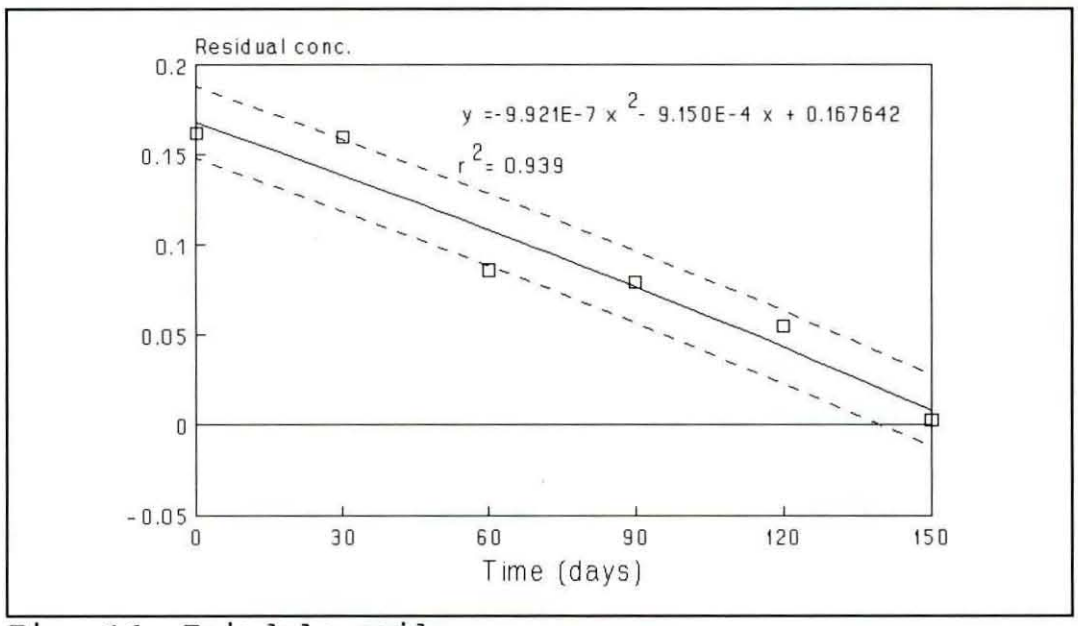

Fig. 16 Fairdale soil

Figures 15 & 16 Rate of atrazine degradation in the Colby and Fairdale soils

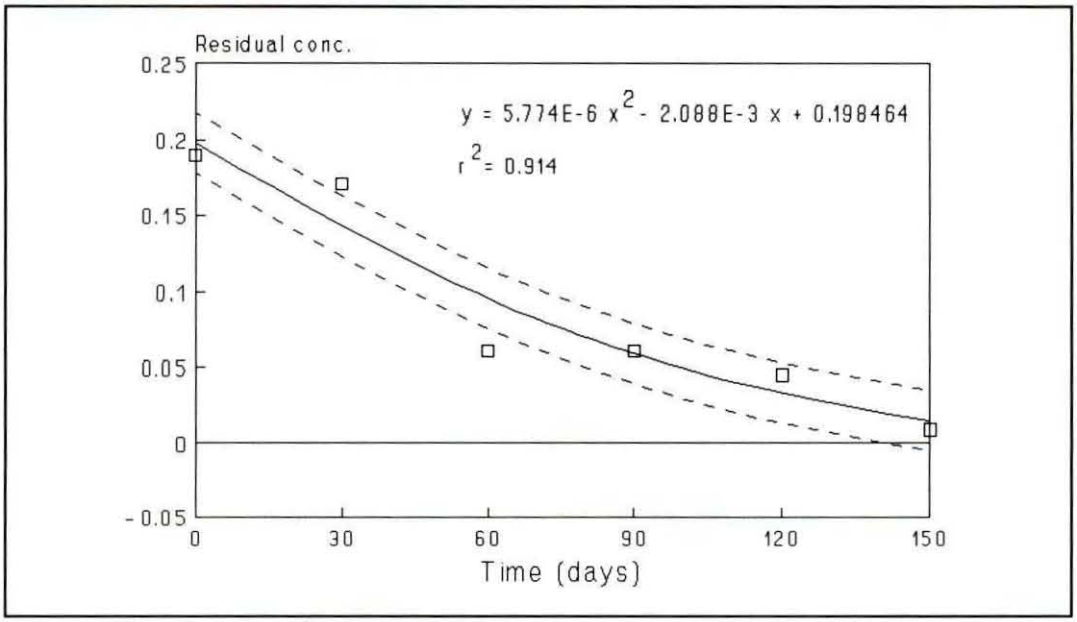

Fig. 17 Pretoria A1 soil

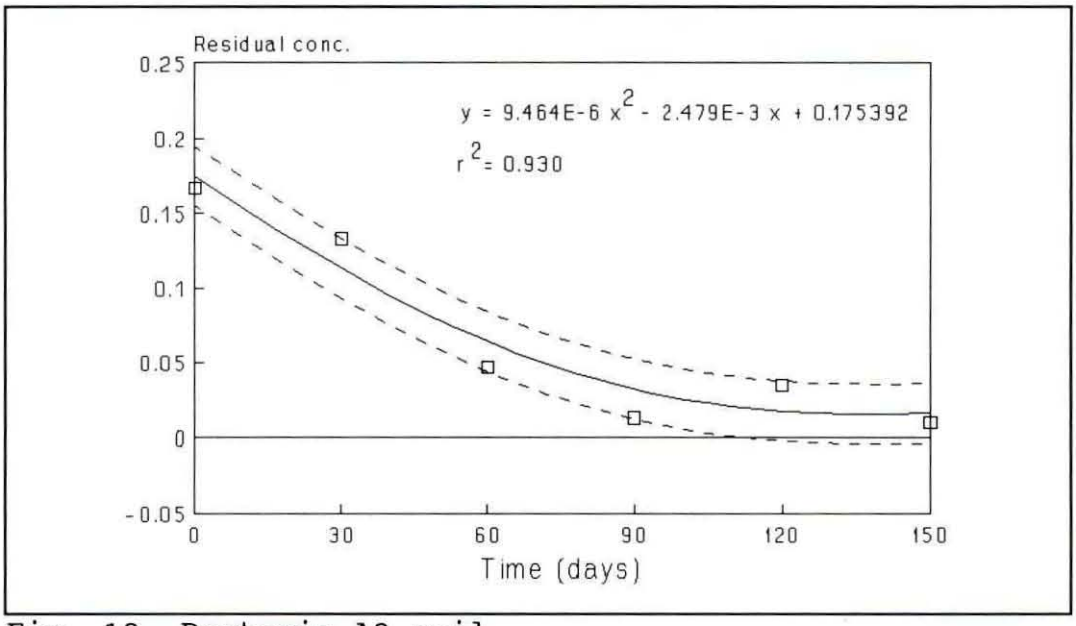

Fig. 18 Pretoria A2 soil

Figures 17 & 18 Rate of atrazine degradation in the Pretoria A1 and Pretoria A2 soils

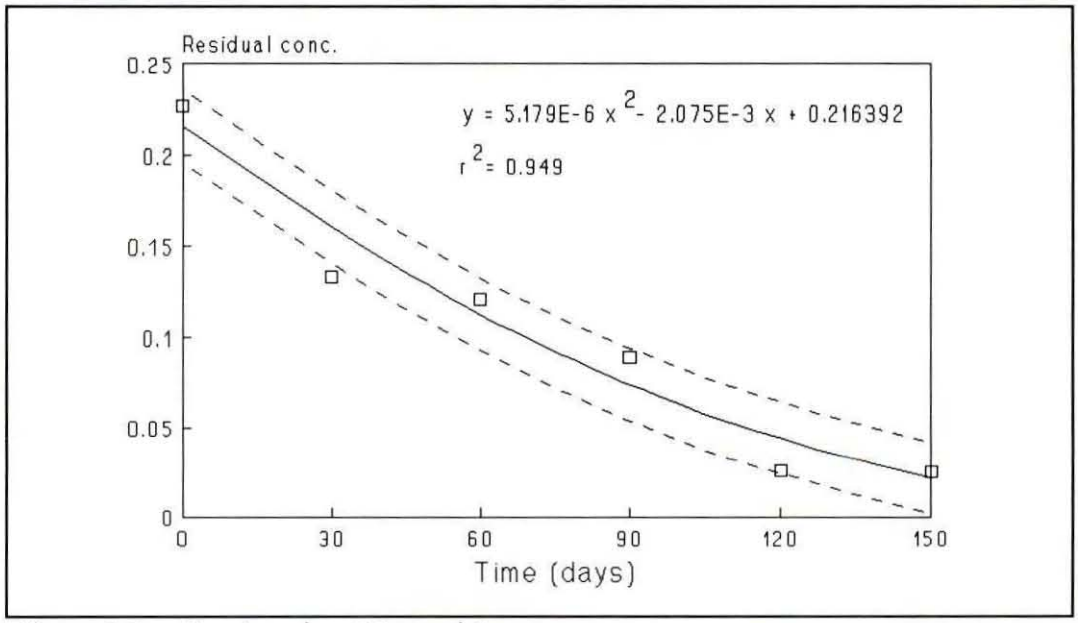

Fig. 19 Pretoria A3 soil

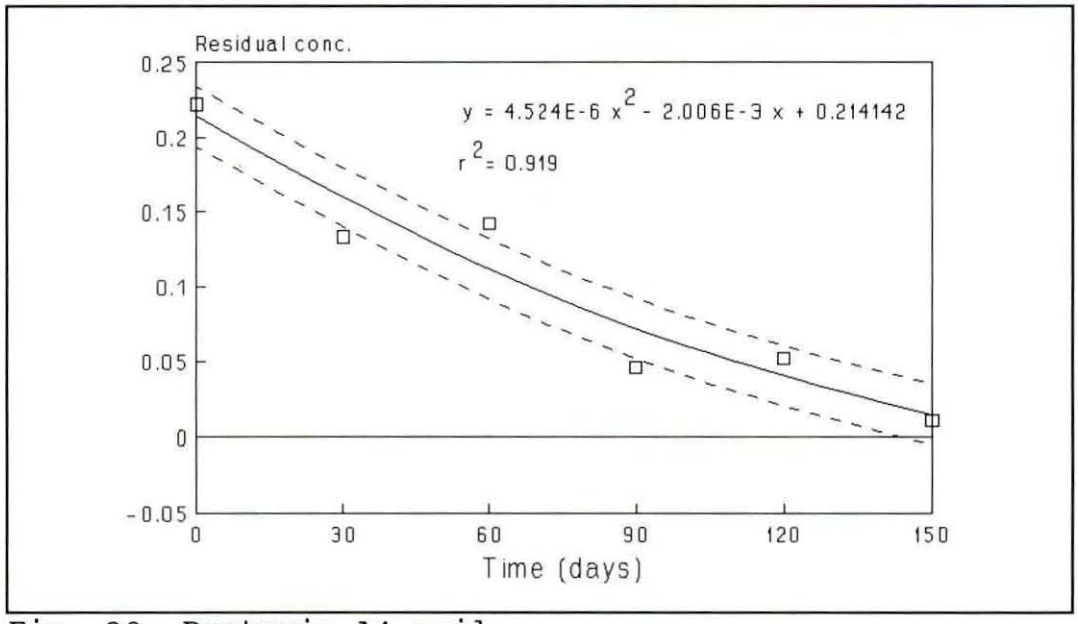

Fig. 20 Pretoria A4 soil

Figures 19 & 20 Rate of atrazine degradation in the Pretoria A3 and Pretoria A4 soils

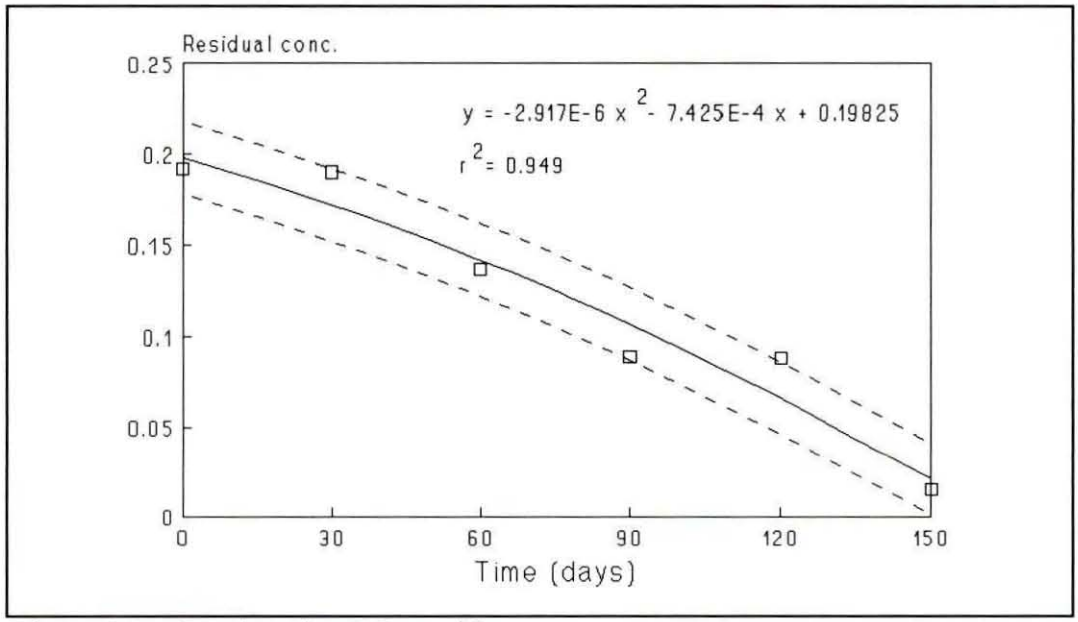

Fig. 21 Pretoria A5 soil

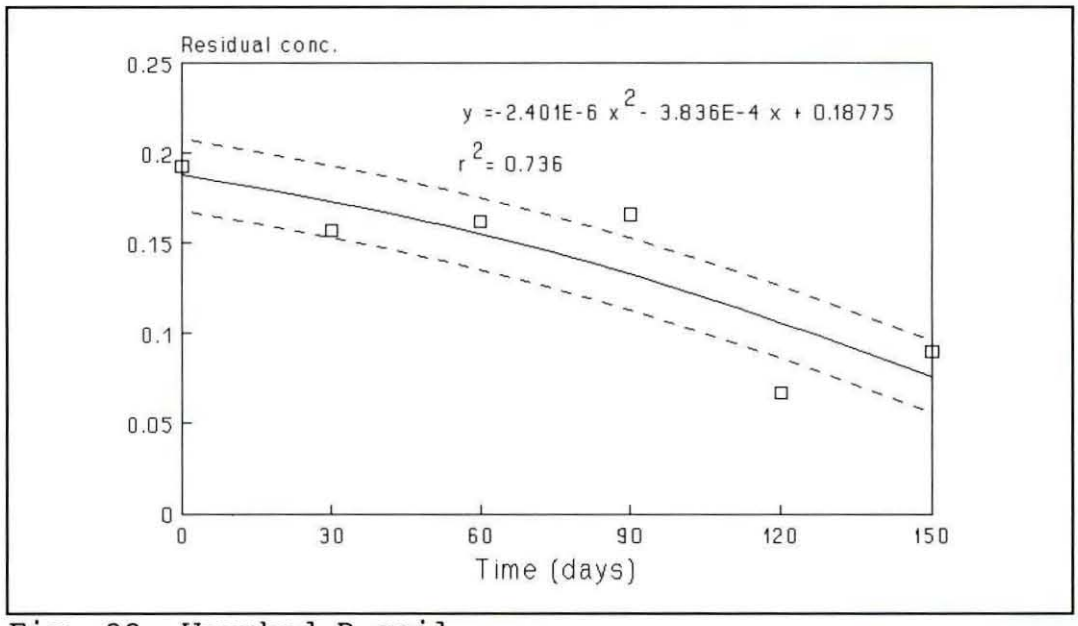

Fig. 22 Warmbad B soil

Figures 21 & 22 Rate of atrazine degradation in the Pretoria A5 and Warmbad B soils

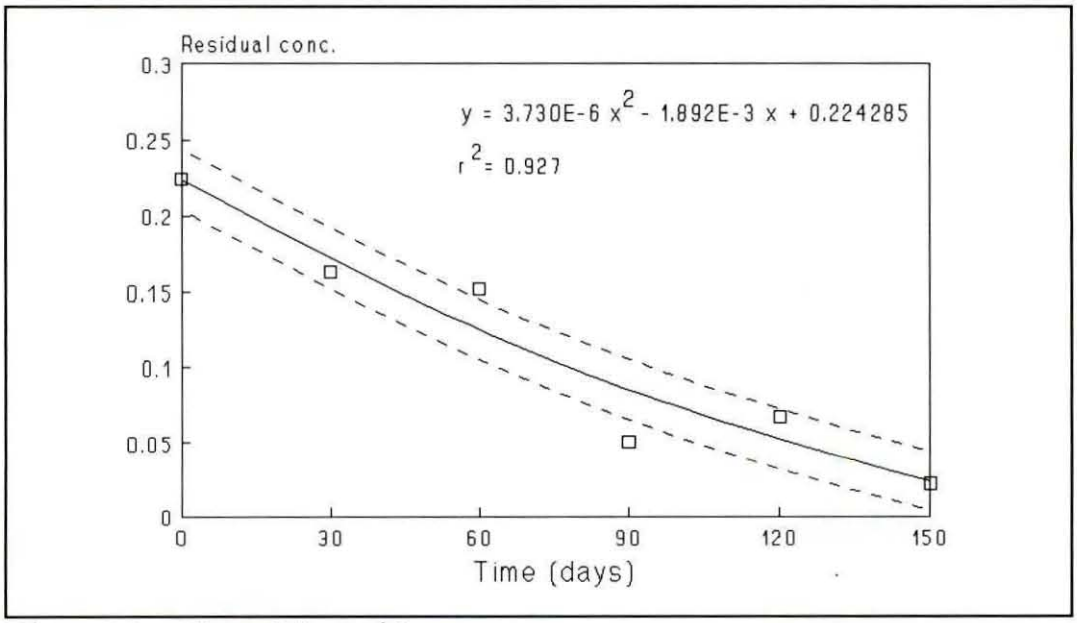

Fig. 23 Utrecht soil

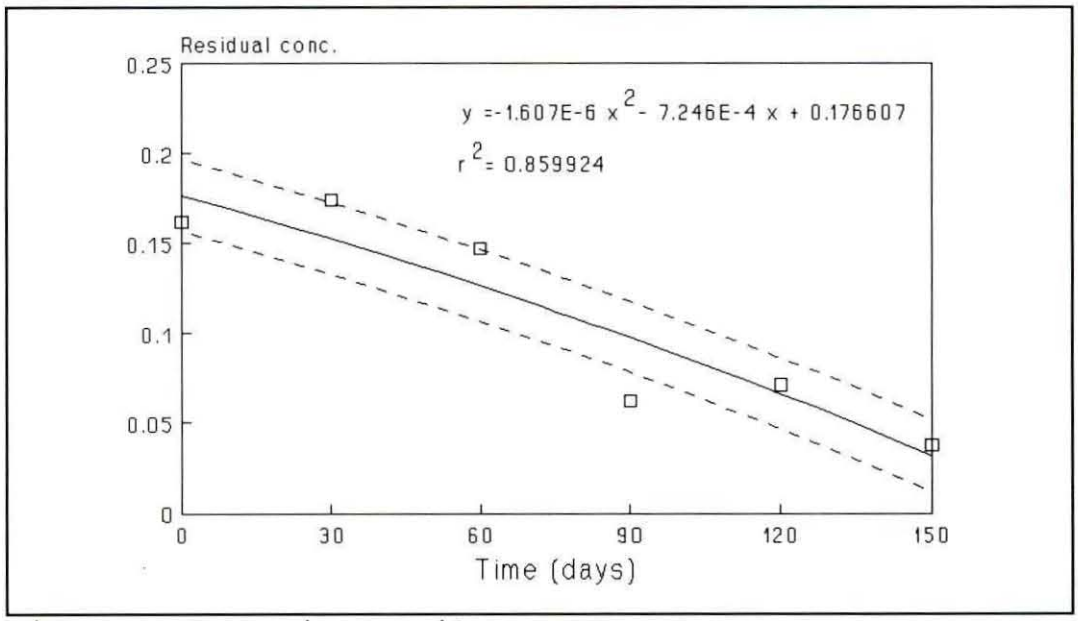

Fig. 24 Pretoria B soil

Figures 23 & 24 Rate of atrazine degradation in the Utrecht and Pretoria B soils

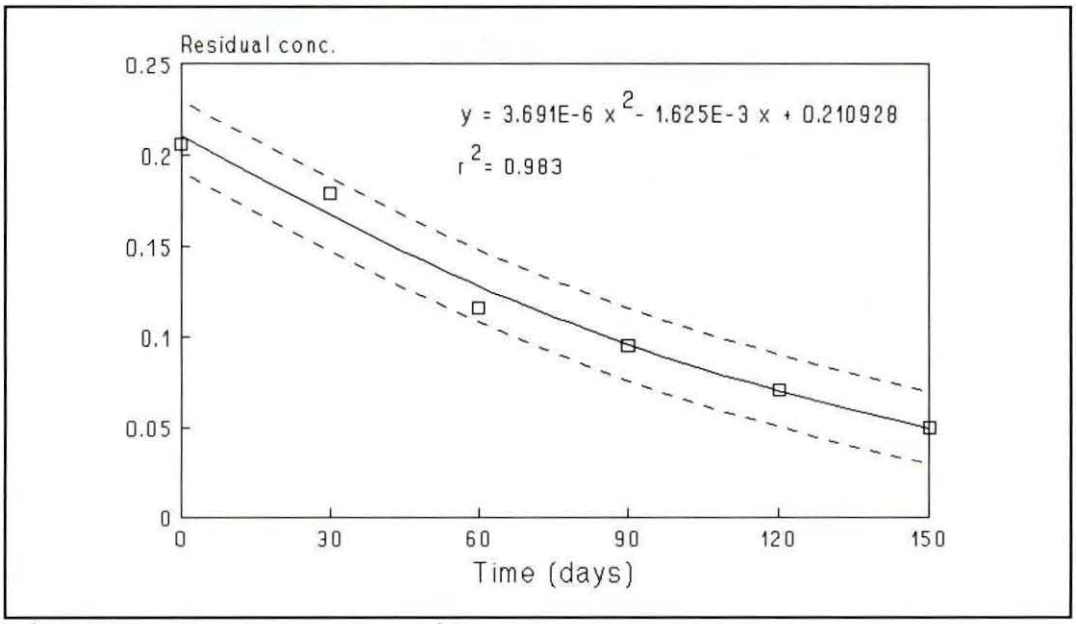

Fig. 25 Roodeplaat soil

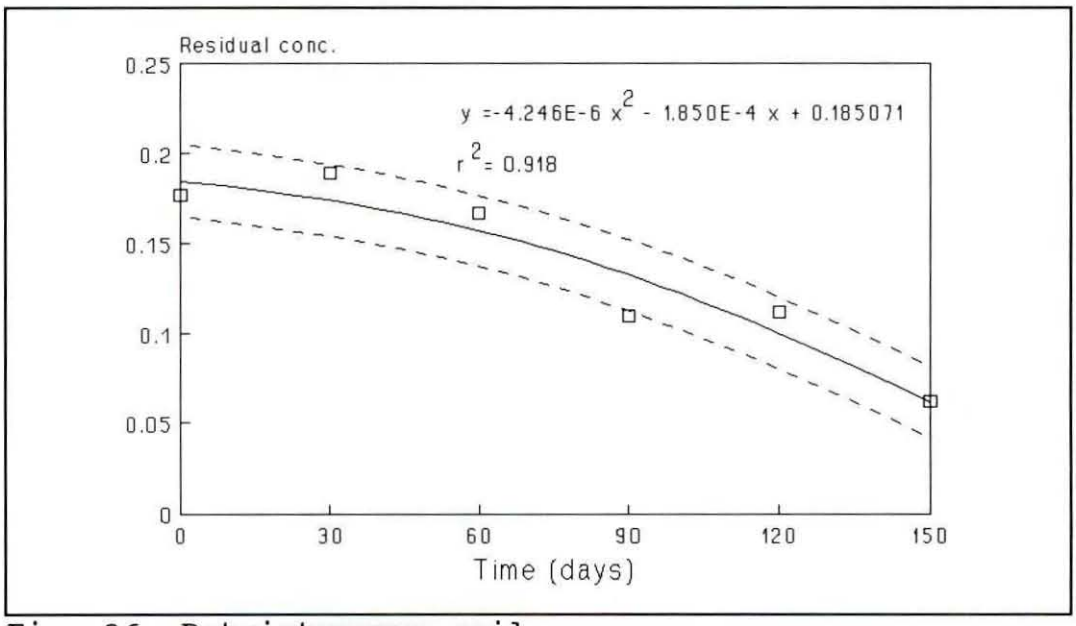

Fig. 26 Potgietersrus soil

Figures 25 & 26 Rate of atrazine degradation in the Roodeplaat and Potgietersrus soils

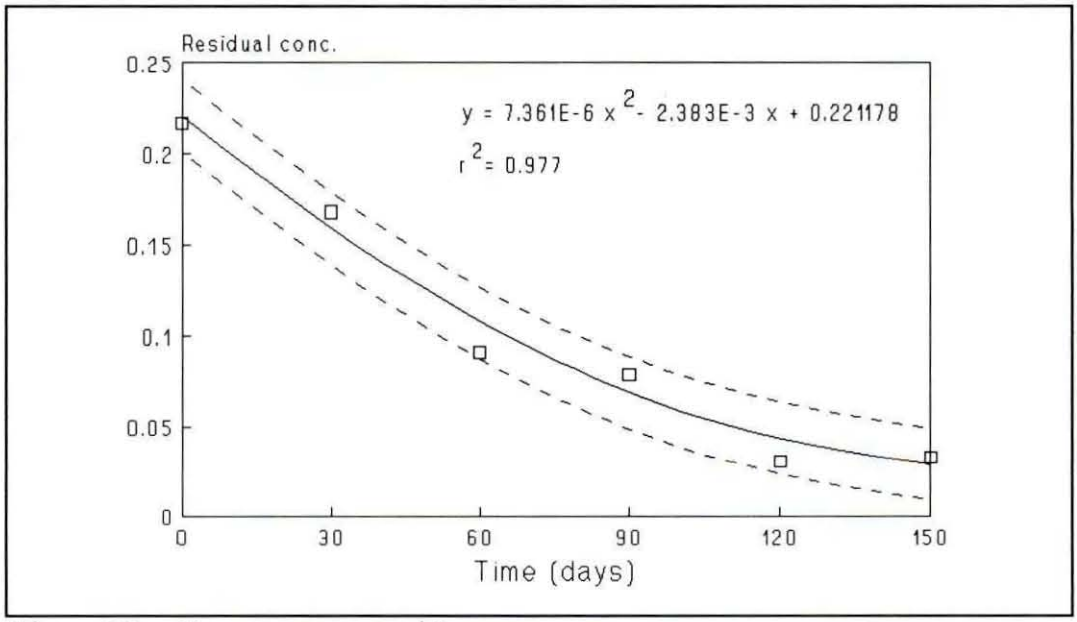

Fig. 27 Morgenzon soil

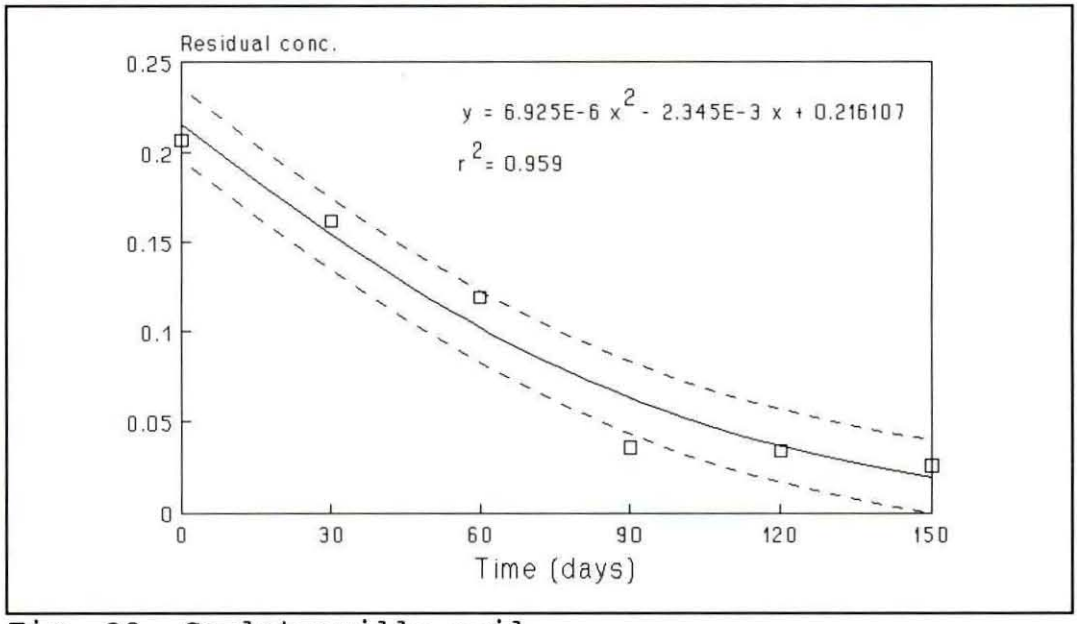

Fig. 28 Carletonville soil

Figures 27 & 28 Rate of atrazine degradation in the Morgenzon and Carletonville soils

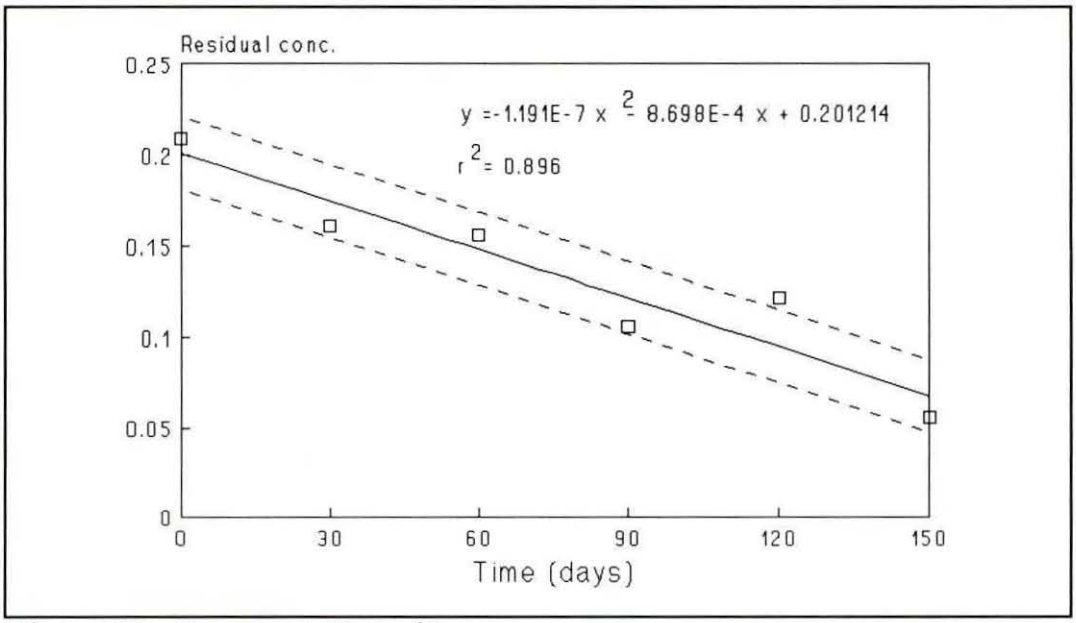

Fig. 29 Warmbad A soil

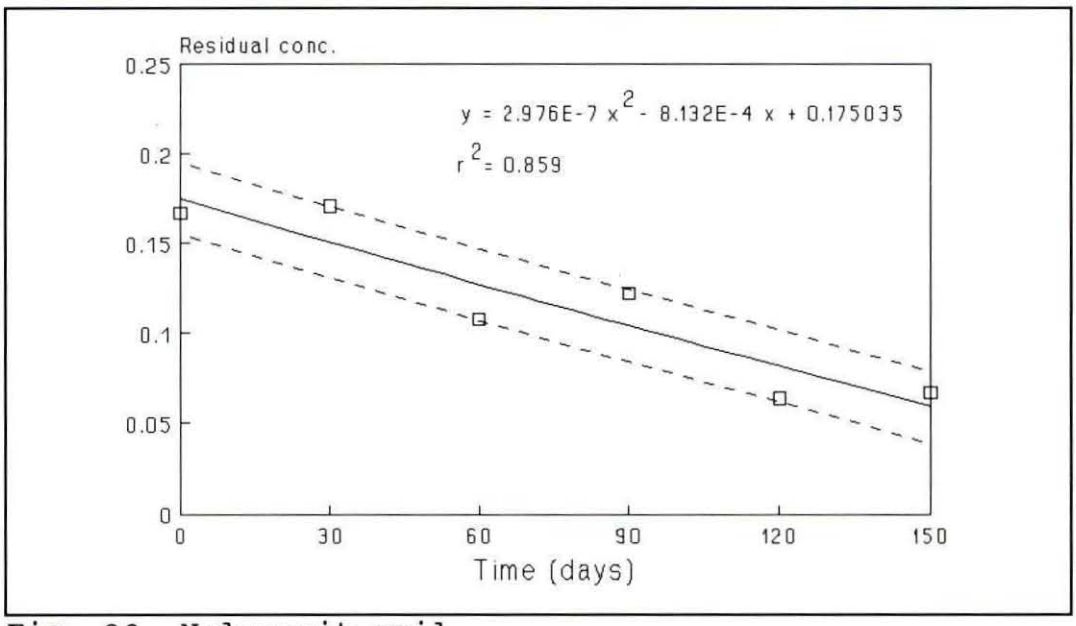

Fig. 30 Nelspruit soil

Figures 29 & 30 Rate of atrazine degradation in the Warmbad A and Nelspruit soils

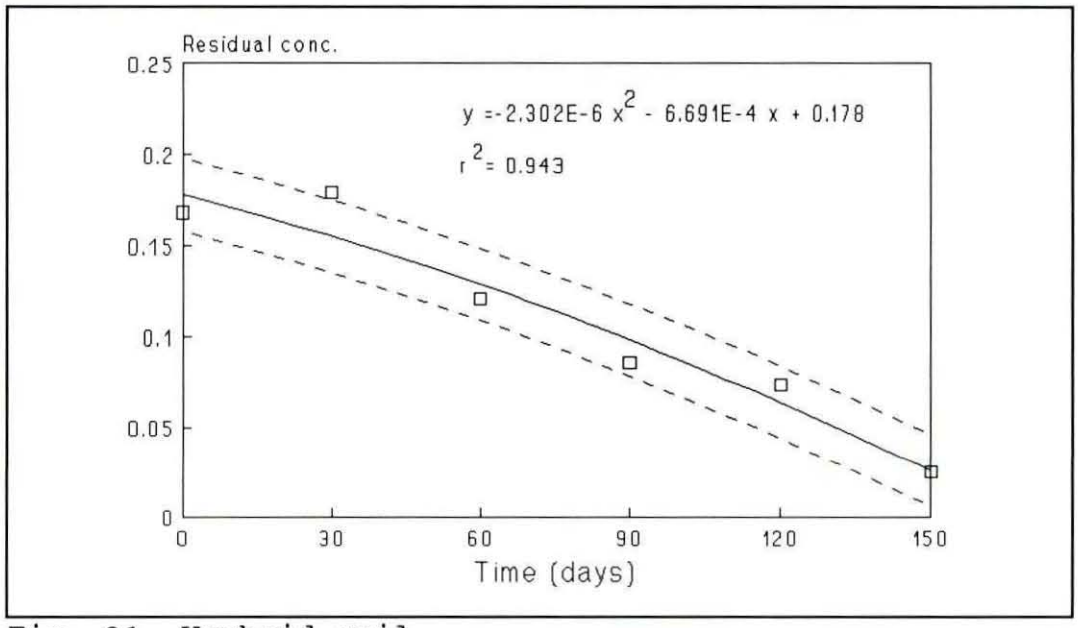

Fig. 31 Vryheid soil

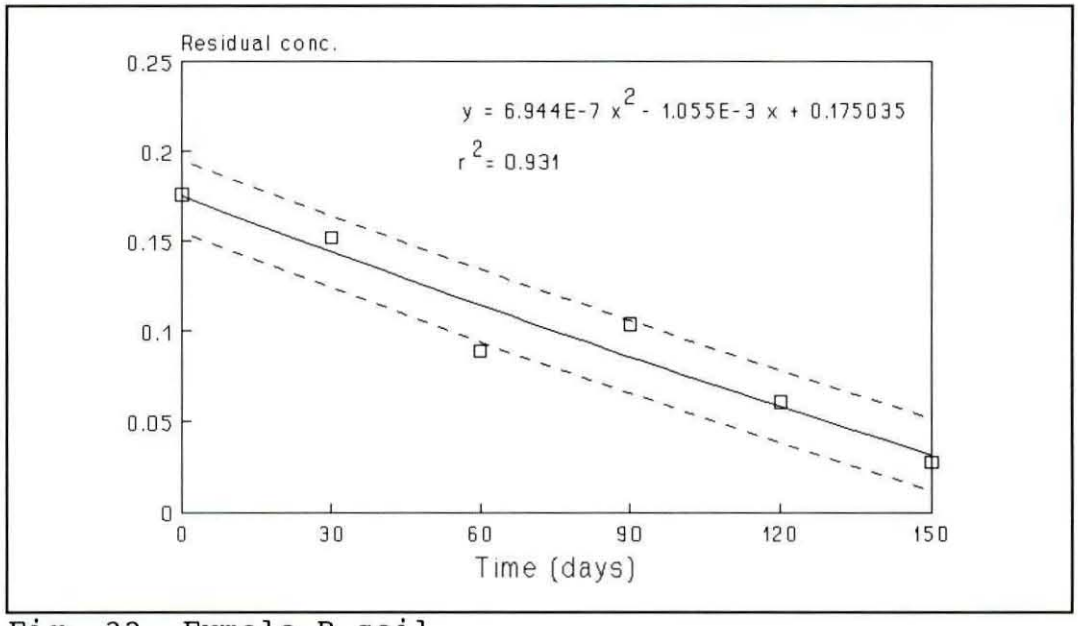

Fig. 32 Ermelo B soil

Figures 31 & 32 Rate of atrazine degradation in the Vryheid and Ermelo B soils

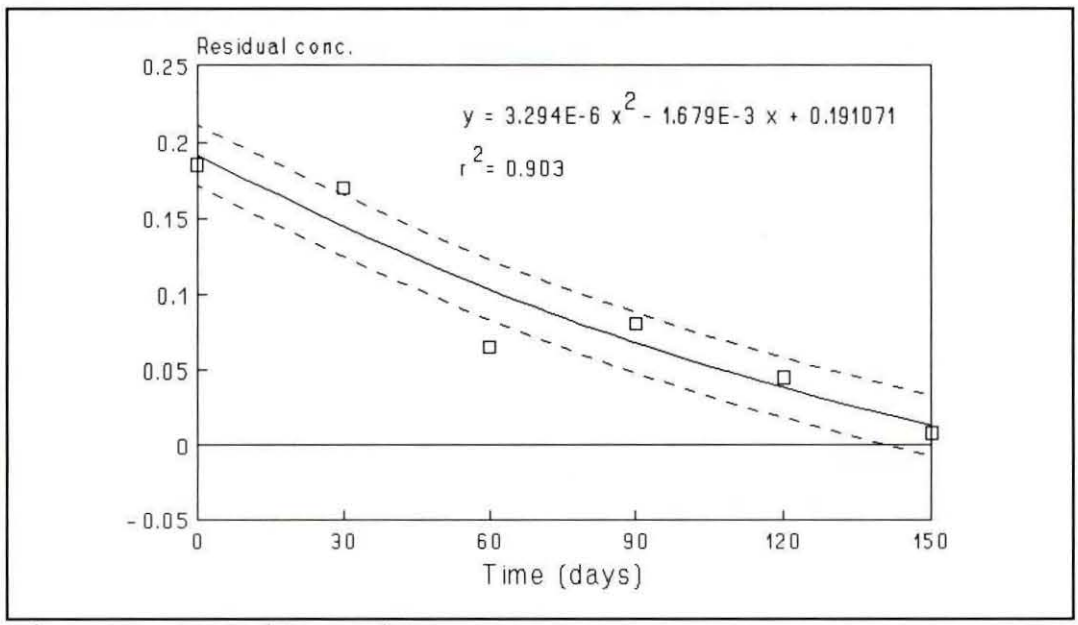

Fig. 33 Redhill soil

Figure 33 Rate of atrazine degradation in the Redhill soil

Considering the methodology employed, the quadratic regression lines fitted the data fairly well. The confidence bands indicate that the variation in the estimated half-life of atrazine can be substantial (Figures 9-33). The atrazine half-lives that were estimated by means of the quadratic formula for each soil (Figures 9-33) are given in Table 37.

Table 37 Atrazine half-lives extrapolated from Figures 9-33

Soil	Half-life (days)	Soil (cont.)	Half-life (days)	Soil (cont.)	Half-life (days)
Bethal	63	**Morgenzon**	78	**Redhill**	69
pH 5.1/0.40% C		pH 6.4/0.47% C		pH 5.0/0.98% C	
Bothaville	54	**Nelspruit**	107	**Roodeplaat**	102
pH 4.9/0.20% C		pH 6.8/0.29% C		pH 6.9/0.41% C	
Carletonville	63	**Nylstroom**	58	**Pretoria B**	86
pH 5.3/0.83% C		pH 5.1/0.40% C		pH 5.9/1.06% C	
Colby	75	**Potgietersrus**	102	**Utrecht**	81
pH 5.5/0.18% C		pH 7.2/0.65% C		pH 5.8/1.18% C	
Ermelo A	50	**Pretoria A1**	57	**Vryheid**	88
pH 4.8/0.38% C		pH 4.9/0.31% C		pH 5.5/2.04% C	
Ermelo B	85	**Pretoria A2**	50	**Warmbad A**	120
pH 5.3/0.8 % C		pH 5.3/0.28% C		pH 7.8/0.50% C	
Fairdale	71	**Pretoria A3**	71	**Warmbad B**	147
pH 5.8/0.18% C		pH 5.7/0.34% C		pH 7.5/0.77% C	
Leeudrgstd. A	64	**Pretoria A4**	75		
pH 5.3/0.32% C		pH 6.5/0.29% C			
Leeudrgstd. B	86	**Pretoria A5**	85		
pH 7.1/0.40% C		pH 7.2/0.29% C			

The half-lives that were determined for atrazine in different soils (Table 37) demonstrate the appreciable effect that soil type can have on rates of degradation. As all experimental conditions, except soil type, are considered to have been equal, the variation in atrazine half-life between soils is ascribed to differences in soil properties

which affect atrazine persistence. Results of the Stepwise Procedure that was used to identify the soil characteristics which give the best prediction of atrazine half-life appear in Table 38.

Table 38 Summary of the Stepwise Procedure that was used for determining the model which gives the best prediction of the dependent variable, atrazine half-life (The complete procedure is given in Table 46A)

	Variable (soil property)					
Step no.	Entered	Removed[1]	Partial R^2	Model R^2	F-value	Prob > F
1	$(pH)^2$		0.6939	0.6939	52.13	0.0001
2	% C		0.1331	0.8270	16.93	0.0005
3	$(CEC)^2$		0.0162	0.8432	2.17	0.1558
4		$(CEC)^2$	0.0162	0.8270	2.17	0.1558

[1]All variables left in the model are significant at the 5% level. No other variables met the required significance level for entry into the model.

The best predictor of atrazine half-life was the square of soil pH (partial $r^2=0.69$)(Table 38). This indicated that soil pH was not linearly correlated with the half-life of the herbicide. The next best predictor was soil organic matter (partial $r^2=0.13$). The Stepwise Procedure eliminated all other variables from the multiple regression model for prediction of atrazine half-life (Table 38). Thus the model giving the best prediction of half-life, with a model R^2-value of 0.8270, included only the square of soil pH and organic matter content (% C).

The multiple regression model which best described atrazine half-life in soil was the following:

$$y = -2.29 + 1.77x_1 + 20.81x_2$$

[where y = half-life in days; x_1 = [soil pH(H_2O)]^2; x_2 = % C]

The important role shown for soil pH (Table 38) substantiates those results reported in section A of this chapter and those presented in Chapter 5. It is abundantly clear that soil pH was the main determinant of atrazine persistence. This finding concurs with those of many authors who reported that the stability of atrazine against hydrolysis to inactive hydroxyatrazine increases progressively as soil pH increases to around neutral (Armstrong *et al.*, 1967; Jordan, Farmer, Goodin & Day, 1970; Best & Weber, 1974 and Hiltbold & Buchanan, 1977). The second-best predictor of persistence was soil organic matter content, which appears to confirm that adsorption on these colloids does provide some protection against degradation of atrazine. 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. The leaching of atrazine can be expected to be governed by its adsorption on soil colloids, especially organic matter, and therefore soil organic matter content would probably have been better correlated with atrazine half-life if the work had been conducted in the field. This view is confirmed by the equally important roles of soil pH and % C in the persistence of atrazine that was reported for the field study in Chapter 5.

It is suggested that the regression equation presented above could contribute towards categorizing atrazine half-lives in soils more accurately, e.g. in cases where published half-life categories are the only alternative sources of information. Walker (1987) states that the half-life concept is valuable in comparing loss rates in different situations, but that its use is often an oversimplification, and therefore its use to characterize dissipation rates in the highly variable field environment should be avoided. Walker (1987) does acknowledge that the only way to take account of soil type on degradation is by regression analysis, provided the data base from which equations are arrived is large enough. Granted that half-life data have limited value in forecasts of persistence if considered alone, it is suggested that their inclusion in existing simulation models might improve the prediction of, in this instance, atrazine persistence. Computer models that can advise on, for example, recropping intervals after atrazine use in maize would signify a tremendous improvement in the way recommendations are made in this country. In the light of the findings reported in Chapters 3 & 6, the most suitable models in the case of atrazine would be those which also take account of the differential growth response of susceptible crops to specific concentrations of the herbicide in different soils. In practical terms, validation of any model requires data from numerous field sites with variations in soil type and weather patterns. Unfortunately, such studies are often beyond the scope of individual research laboratories.

Some applications of the use of simulation models for the prediction of herbicide persistence have been described by Walker & Barnes (1981), Walker & Eagle (1983), Gottesbüren *et al.* (1991) and Walker (1991). Of these models, only the model of

Gottesbüren *et al.* (1991) contains a module which predicts the effects of herbicide residues on succeeding crops. Walker (1991) acknowledges that in addition to the stability of specific herbicides, information on the critical soil residue levels in terms of rotational crop safety is required for reliable risk assessments. Walker (1991) used a simple mathematical model (Walker & Barnes, 1981) to make generalized predictions of atrazine persistence in four soils in South Africa. Using a half-life of 45 days for atrazine, Walker (1991) estimated that the herbicide would persist from 8 to 12 months following application in spring. Past reports of damage caused by atrazine residues, and own results reported in Chapter 6 (Table 28) attest that atrazine persistence often exceeds 12 months. From the predictions made by Walker (1991) it appears that atrazine persistence in the Warmbad soil was underestimated. Predicted atrazine losses for Warmbad were similar to those for Kroonstad, but results reported in Chapter 6 showed that atrazine residues in the same Warmbad soil caused significant damage to sunflower and dry beans at both 12 and 24 months after atrazine application. In contrast, negligible damage occurred at Kroonstad at the 12 month stage. The weather and soil data used by Walker (1991) as inputs in the model of Walker & Barnes (1981) were data which were recorded during the first 12 months at the two trial sites. It is suggested that if the regression model presented here had been available to predict atrazine half-lives in the two soils, more accurate predictions of persistence might have ensued. The regression model estimates of the half-lives for atrazine in the Kroonstad (pH 5.6; 0.36% C) and Warmbad (pH 7.9; 0.53% C) soils are 36 and 119 days, respectively. The estimated half-life of atrazine in the Kroonstad soil corresponds with the value of 45 days (irrespective of soil type) that was used in the model of Walker &

Barnes (1981). However, their model underestimated persistence in the Warmbad soil, probably because the half-life input of 45 days was inaccurate for this soil.

Further demonstration of the worth of the regression model in the prediction of atrazine persistence is given in Table 39 where atrazine half-lives that were determined by Walker & Zimdahl (1981) in a laboratory incubation study are compared with those predicted by the regression model developed in own work.

Table 39 Half-lives for atrazine in three soils (adapted from a laboratory study by Walker & Zimdahl (1981)), and the half-lives as predicted with the regression model developed in own work

		Atrazine half-life (days)		
Soil % C	Soil pH	Laboratory[1]	Regr. model[2] (% C + square pH)	Regr. model[3] (square pH alone)
1.45	8.0	41-87	141	120
1.51	6.4	28-50	101	80
0.64	7.3	47-100	105	102

[1]Adapted from Walker & Zimdahl (1981). The lower values were determined at 25°C and 16.8% soil water content, and the upper values at 15°C and 17.9% soil water.
[2]Multiple regression model: $y = -2.29 + 1.77(pH)^2 + 20.81(\%\ C)$ - from Table 46B.
[3]Simple regression model: $y = 10.6 + 1.73(pH)^2$ - from Table 46B.

Atrazine half-life predicted by the multiple regression model (pH^2 + % C) for the soil with the lowest organic matter content (Table 39) closely matched the maximum value reported for that soil by Walker & Zimdahl (1981). Compared to the laboratory measurements, the multiple regression model overestimated atrazine half-life in the other soils. The predictions given by the model which contained only square pH

corresponded slightly better with the laboratory measurements of Walker & Zimdahl (1981) for all three soils (Table 39). It is not surprising that both the regression models employed in Table 39 were not consistent in their prediction of half-lives determined in an unrelated study, since it is unlikely that simple empirical models based on limited environmental factors will be applicable in a wide range of weather and soil conditions. The environmental conditions were not exactly the same in the two studies that are compared in Table 39. As shown in Chapter 7, and as has been extensively reported in the literature, the persistence of atrazine is affected by various soil (Hiltbold & Buchanan, 1977; Walker, 1987, 1991) and weather factors (Roeth, Lavy & Burnside, 1969; Walker & Zimdahl, 1981; Walker, 1987, 1991). The regression model is expected to be valid for different atrazine rates. Hiltbold & Buchanan (1977) provided evidence to support the theory of first-order kinetics in atrazine degradation. They found that degradation rates of 1.12, 2.24 and 3.36 kg atrazine ha^{-1} did not differ significantly.

Experimentally derived atrazine half-lives (Table 37) and those values predicted with the model for each of the 25 soils are compared in Table 40. Presentation of the data in Table 40 should not be regarded as an attempt to validate the model - it merely serves to illustrate the variation inherent in the techniques that were used. The methodology of the present study involved time-consuming and relatively inaccurate bioassays. Chemical analysis would have generated data quicker and more accurately. Nonetheless, it is proposed that bioassays could be useful in work of this nature, should the available facilities dictate that the technique be used.

Table 40 Comparison of atrazine half-lives, which were determined experimentally for 25 soils, with those values predicted with the multiple regression model

Soil organic matter (% C)	Soil pH(H_2O)	Atrazine half-life (days)	
		Measured	Predicted
0.40	5.1	63	52
0.20	4.9	54	44
0.83	5.3	63	64
0.18	5.5	75	54
0.38	4.8	50	46
0.80	5.3	85	63
0.18	5.8	71	60
0.32	5.3	64	53
0.40	7.1	86	95
0.47	6.4	78	79
0.29	6.8	107	86
0.40	5.1	58	52
0.65	7.2	102	102
0.31	4.9	57	46
0.28	5.3	50	52
0.34	5.7	71	62
0.29	6.5	75	78
0.29	7.2	85	95
0.98	5.0	69	62
0.41	6.9	102	90
1.06	5.9	86	81
1.18	5.8	81	81
2.04	5.5	88	93
0.50	7.8	120	115
0.77	7.5	147	113

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSIONS

More than three decades of research on atrazine have accumulated a wealth of data and yet occasional loss of selectivity (Le Court de Billot & Nel, 1985) and excessive persistence (Riley, 1991; Del Re *et al.*, 1991) are encountered. Decreased selectivity is associated with increased bioactivity, usually as a result of excess amounts of the herbicide at the site of action in plants. This type of damage to crops is virtually unpredictable, except where overdosing is known to have occurred. In contrast, potential damage due to carry-over of residues can be predicted by considering factors which influenced herbicide dissipation from soil during the period after its application until the next crop is planted. It is important for crop producers to know if there are any limitations or restrictions in the sequence of crops that can be grown after using atrazine. Therefore considerable information is required regarding crop sensitivity (e.g. residue concentration thresholds for different crops), herbicide stability, mobility, adsorption/desorption relationships and transfer between the different environmental compartments.

Research on factors implicated in apparent atrazine damage to maize during the 1981/82 and 1982/83 growing seasons was concluded with work on aspects of nutrient supply. Maize seedlings which showed symptoms of N, P, K, Ca and Mg deficiencies (Chapter 2) were actually affected less by atrazine than seedlings supplied with adequate amounts of nutrients, probably due to greater uptake of the herbicide by control plants in which

life processes proceeded normally. The finding of Sosnovaya & Merezhinskii (1979) that maize plants growing in a nutrient-deficient medium showed less tolerance to atrazine than plants supplied with optimal levels of nutrients was not substantiated. The observed tendency (statistically insignificant) of increased sensitivity to atrazine which was displayed by seedlings deficient in Mg could possibly be ascribed to the vital function of this element in the chlorophyll molecule. Atrazine inhibits electron transport during the light phase of photosynthesis (Fuerst & Norman, 1991), and therefore both insufficient Mg and high amounts of atrazine could conceivably have acted together in reducing maize seedling tolerance to atrazine. From the viewpoint of carry-over of atrazine this aspect warrants further investigation, since crops that are inherently susceptible to atrazine may show greater responses to the particular combination of atrazine and Mg than would maize.

The purported inhibiting effect of high P supply on photosynthetic CO_2-fixation (Claassens & Fölscher, 1985) was not substantiated in work reported in Chapter 2. High P levels in maize seedlings did not sensitize them to high levels of atrazine, as would be expected if a synergistic effect was involved. This is in contrast with the finding of Stolp & Penner (1973) that synergism between atrazine and high phosphorus caused increases in respiration and reductions in net photosynthesis of maize plants. It is improbable that nutrient imbalances of the order of those investigated in Chapter 2 could have played a role in the damage caused to maize in the field. The localized and isolated nature of that incident probably excludes both macro- and micronutrients as causative factors, and more likely points to a combination of soil and weather factors which caused phytotoxic amounts of atrazine to accumulate in maize seedlings.

Experiments conducted to evaluate the susceptibility of dry beans (cv Teebus), grain sorghum (NK 222), oats (SWK 001), soybeans (cv Forrest) and sunflower (cv SO 222) to atrazine showed that the species differed in their susceptibility to the herbicide. Also, results demonstrated that the herbicide threshold concentration for a particular species varied from soil to soil (Chapter 3). Thus the need to associate measured amounts of atrazine, or its phytotoxic residues, with responses of sensitive crops was highlighted. For carry-over risk assessment purposes the need for more information on the relative availability of atrazine residues in soils, as well as on species differences in susceptibility, have been stressed by Stalder & Pestemer (1980), Pestemer, Stalder & Eckert (1980) and Pestemer *et al.* (1983). Blanket atrazine threshold values for crops would have little or no value in deciding which crop to plant in soil containing the herbicide and/or its phytotoxic residues, since different amounts will be available to a particular crop in different soils. Threshold values determined under similar conditions (e.g. by using a single soil) should provide reliable orders of susceptibility for different species, or even cultivars.

Comprehensive research has been conducted locally on factors which influence the bioactivity of atrazine (Smit *et al.*, 1977, 1979, 1980; Nel & Reinhardt, 1984; Ehlers *et al.*, 1987, 1988), but its persistence in South African soils had not been extensively studied before the present investigation. The short-term (< 30 days) bioactivity study (Chapter 4) confirmed the results of previous work which indicated that organic matter content, clay content and P-reversion (in this order) were more important than soil pH in determining atrazine bioactivity. The subsequent persistence investigation (Chapter 5), which was conducted 182 days after atrazine application, showed that the order of

importance for variables changed with time: organic matter content > soil pH > P-reversion > clay content. Only the role of organic matter content remained constant. Probably the most significant result was the apparent importance of soil pH as a predictor of persistence, in contrast to its poor prediction of short-term bioactivity. The finding on the importance of soil pH on the persistence of atrazine corroborated results reported by Armstrong *et al.* (1967), Roeth *et al.* (1969), Best & Weber (1974), Hiltbold & Buchanan (1977), Smit *et al.* (1979, 1980) and Walker *et al.* (1983). Generally, it was reported that atrazine stability increased with increasing pH-levels. Adsorption on colloids such as organic matter apparently provides protection against degradation. Increasing half-lives with increased adsorption have been reported for atrazine (Burkhard & Guth, 1980). Moyer, Hance & McKone (1972) found lower degradation rates for atrazine in soil amended with activated charcoal.

The fixed recropping intervals that apply when atrazine is used in maize restrict recropping options, or crop choice in cases of forced recropping. Results reported in Chapter 6 showed that recropping intervals could be refined by considering soil properties which determine atrazine persistence. It was also shown that the present classification of crops for recropping purposes, according to their perceived sensitivity to atrazine, requires re-examination. Even cultivars within species may differ in tolerance to atrazine. Stalder & Pestemer (1980), Pestemer *et al.* (1980), Gottesbüren *et al.* (1991) showed that information on the relative susceptibility of rotational crops to herbicide residues, as well as the availability of residues for uptake by crops, would improve forecasts of recropping risks.

Chemical analysis by means of HPLC (Chapter 7) showed that the persistence of atrazine varied between two diverse soils, despite the exclusion of leaching as a factor. It was suggested that the relatively high pH and the high adsorptive capacity of the montmorillonite clay soil restricted the breakdown of atrazine in that soil. High atrazine stability at neutral pH (Hiltbold & Buchanan, 1977; Appleby, 1985) and apparent protection against degradation through adsorption on colloids (Burkhard & Guth, 1980) have been reported. The well reported roles of soil water content and temperature (Walker & Zimdahl, 1981; Walker & Allen, 1984; Walker, 1989) in the persistence of this herbicide were substantiated. Of the two weather factors, soil water content had the dominant effect, despite leaching being negated. The faster breakdown rate that was observed at the field capacity soil water level, compared to the rate in air-dry soil, suggests that processes which require water, e.g. desorption, hydrolysis and microbial degradation, were probably involved.

Chemical analysis makes detection of herbicide residues possible in all compartments of the environment, but the measured amounts still need to be linked to the responses of different crops (Gottesbüren *et al.*, 1991). The bioassay technique that is described in Chapter 8 could be used to estimate phytotoxic atrazine residues in soils, provided the residue concentrations are of such an order that an indicator species responds neither too strongly nor too weakly. In view of the perceived disadvantages of both techniques, a combination of bioassays and chemical analysis would be ideal for prediction of carry-over effects on sensitive crops.

Many computer simulation models are available for quantification of herbicide residues in soils, even at different depths, before the following crop is planted (Walker & Barnes, 1981; Gottesbüren *et al.*, 1991). Amongst the many information inputs required for these models to produce reliable estimates, is the half-life of a compound in soil. As half-life values that are used in models are usually gleaned from published data of other workers, predictability may be reduced. The aim of the work reported in Chapter 9 was to improve the situation for atrazine by at least taking into account the variability of its half-life in different soils. Results of the experiment in which the role of soil pH in atrazine persistence was investigated (Section A in Chapter 9) confirmed conjectures in previous experiments about the role of pH. It was found that atrazine persistence increases with increased soil pH. The subsequent experiment with 25 soils (Section B in Chapter 9) confirmed the importance of soil pH on the herbicide's persistence. Soil organic matter content was the most important predictor of the bioactivity (Chapter 4) and persistence (Chapter 5) of atrazine. In Chapter 9, organic matter content was not of prime importance in the prediction of atrazine persistence because leaching was negated in that investigation. An insignificant role for P-reversion was found in Chapter 9, in contrast to the findings in Chapters 4 & 5. The reasons for this are not obvious, and may remain unexplained until more about the mechanism involved in the purported interaction between the Al.Fe.OH-component of soil and atrazine molecules is known. The culminant multiple regression model, which was derived in the incubation study (Section B in Chapter 9) for the prediction of atrazine half-lives in soils will hopefully reduce reliance on categorized half-life values that are given in textbooks and other reference material.

Failure to establish the exact causes of the purported atrazine damage to maize as far back as 1981/82, illustrates the complexities involved in studies on the environmental fate and behaviour of the herbicide. Progress was made in the present study towards identification of factors which influence the bioactivity and persistence of atrazine in soil. Although work on the roles of essential macronutrients in the tolerance of maize to atrazine did not produce positive results, it may be worthwhile to investigate what effects the same treatments would have on the tolerance of normally susceptible crop species. Useful re-confirmation of the importance of certain soil properties on the bioactivity of atrazine was provided in field trials. The persistence of atrazine was studied for the first time in South Africa in both field and glasshouse experiments. With this work the need to refine recropping periods for susceptible crops which follow maize treated with atrazine was highlighted. In other persistence studies the dominating role of soil pH in the determination of atrazine persistence was conclusively established. A regression model which may improve the prediction of atrazine half-lives in soil was presented. It is foreseen that atrazine will remain an important component of weed management strategies in South Africa, and that the need for accurate prediction of its persistence in soil will prevail.

SUMMARY

BIOLOGICAL ACTIVITY AND PERSISTENCE OF ATRAZINE

1. Certain aspects of the phytotoxicity and availability of atrazine and its residues for uptake by plants were researched in this study. Bioassays with plants as indicators of the availability and phytotoxicity of atrazine were conducted in glasshouses and in the field. Chemical analysis for measurement of atrazine in aqueous medium and soil were done in three experiments. Emphasis was on the identification of environmental factors, particularly soil properties, which govern the bioactivity and persistence of atrazine and its phytotoxic residues.

2. The roles of certain essential macronutrients in the resistance of maize to atrazine was evaluated. Growth-retarding levels of N, P, K, Ca and Mg, individually or in certain combinations, had no significant effects on the crop species' resistance to atrazine. In a separate investigation it was found that the tolerance of maize seedlings to atrazine was neither influenced by high phosphorous (P) application in the root zone nor by relatively high P concentrations in shoots. Results indicate that high amounts of P containing fertilizers in the root zone of maize seedlings are unlikely to sensitize the plants to atrazine.

3. It was shown in pot experiments that atrazine threshold concentrations in soil for dry beans, grain sorghum, oats, soybeans and sunflower varied from species to species and from soil to soil. The differential sensitivity of these crops to atrazine infers that

recropping intervals recommended after atrazine use should be based more closely on differences in crop susceptibility. Further research is needed to relate known amounts of phytotoxic atrazine residues in soils to the response of different species, with a view to better assess the risk involved in growing a particular crop where atrazine carry-over occurred.

4. The strength of relationships between selected soil properties and the bioactivity of atrazine were investigated in a total of 30 field trials conducted over a one year period at ten trial sites. The initial bioactivity of atrazine which was assessed 35 days after application was best correlated with the organic matter content and P-reversion characteristics of the soils. Clay content and CEC were also important, but at lower levels of significance. These findings confirmed those of several previous investigations which were aimed at identifying predictors of the short-term activity of atrazine.

5. Six months after herbicide application in the trials mentioned above (point 4), organic matter content, soil pH and P-reversion predicted 35%, 19% and 14% of the variation in bioactivity, respectively. Both CEC and clay content were poor criteria at that stage. Organic matter content dominated as the best predictor of both the short-term (bioassay on day 0) and longer term (assayed on day 182) bioactivity of atrazine. Indications were that soil pH could also be an important predictor of atrazine persistence. Differences in persistence between trials in close proximity suggest that persistence of atrazine was more closely linked to soil characteristics than to climatic conditions. Results suggest that current waiting periods, which are recommended for specific crops grown after maize in which atrazine was used, can be refined by

distinguishing between soils.

6. Atrazine persistence was monitored at 12 and 24 months after application in maize at eight localities in order to evaluate the applicability of the single recropping period that is specified for dry beans and sunflower. The test crops were the dry bean and sunflower cultivars mentioned under point 3. Carry-over of phytotoxic atrazine residues, as judged from the extent of crop yield reduction, varied considerably from site to site. In a wide range of soils the sunflower cultivar was generally less tolerant to atrazine than the dry bean cultivar, thus confirming the relative susceptibility of these crops to atrazine (see point 3). With the exception of a montmorillonite soil in which significant carry-over occurred, no significant damage to the dry bean cultivar was incurred on soils in which kaolinite clay predominated. Results suggest that the current recropping intervals for crops which are sensitive to atrazine could be refined by assigning different recropping periods based on soil and species differences.

7. The influence of temperature and soil water content on the persistence of atrazine in a clay soil and a loamy sand soil was investigated under controlled conditions. Atrazine was measured by means of high pressure liquid chromatography. The half-life of atrazine in the loamy sand was reached after about 60 days, whilst at the same stage 70-75% of the applied atrazine remained in the montmorillonite clay soil. Virtually no degradation of atrazine occurred in air-dry soil. Degradation in both soils at field capacity soil water was significantly higher than in air-dry soil. Increases in soil water from field capacity to 2x field capacity had little or no effect on persistence. The lowest temperature regime slowed the rate of degradation of the herbicide in the light

soil only. In this experiment soil type and soil water content had greater effects on the degradation rate of atrazine than temperature.

8. A simple bioassay technique was used to study the residual activity of atrazine applied at 0.25 kg ai ha^{-1} in samples taken from different depths in a sandy clay loam soil. It was estimated by means of dose-response curves that 55% of the amount applied in the field was present in the 200-300 mm soil layer on day 30 after application. At days 60, 90 and 120 the percentages remaining in the 200-300 mm soil layer were about 3%, 2% and 3%, respectively. Estimates on day 120 indicated that 2% of the amount applied remained in the top (0-100 mm) soil layer, and 6% in the 300-400 mm layer. There was generally good correlation between visual and measured assessments of damage in the field. The bioassay technique could be convenient for estimating atrazine residues in the soil profile, especially to predict the potential for damage to sensitive follow-up crops.

9 (a). The relationship between soil pH and atrazine persistence already reported above was demonstrated again in an experiment where soil samples treated with atrazine were incubated for different intervals over a period of 120 days. Essentially only the pH(H_2O) levels of these samples were different. Atrazine persistence was shown to increase with increasing soil pH, thereby substantiating findings of increased stability of atrazine in high pH soils.

9 (b). Dose-response curves were obtained with the test plant oats (cv SWK 001) for 25 soils, and used to estimate amounts of atrazine, or its phytotoxic residues, in these

soils 0, 30, 60, 90, 120 and 150 days after application of 0.2 mg atrazine kg^{-1}. The rate of atrazine degradation in each soil was subsequently plotted (mg atrazine kg^{-1} against number of days after treatment). From the quadratic formulas of these relationships, atrazine half-lives were estimated, i.e. the number of days required for the applied amount to be halved. Regression analysis showed that the square of soil pH was the best predictor of atrazine persistence. Soil pH and organic matter content (% C) were the only variables which qualified for inclusion in a multiple regression equation for prediction of atrazine persistence. It is proposed that half-lives of atrazine in soils can be predicted with the following regression equation:

$$y = -2.29 + 1.77x_1 + 20.81x_2$$

where y = half-life in days; x_1 = [soil pH(H_2O)]^2; x_2 = % C

Results generated in this study should contribute to knowledge of the factors which govern the bioactivity and persistence of atrazine in soil, and therefore, hopefully improve the predictability of both phenomena.

OPSOMMING

BIOLOGIESE AKTIWITEIT EN NAWERKING VAN ATRASIEN

1. Sekere aspekte van die fitotoksisiteit en beskikbaarheid van atrasien en residue daarvan vir opname deur plante is ondersoek. Biotoetse met plante as indikatore van die bio-aktiwiteit van atrasien is in glashuise en op die land uitgevoer. Chemiese analise van grond- en watermonsters vir atrasien is in drie proewe gedoen. Die klem van die studie was op identifisering van omgewingsfaktore, en dan veral grondeienskappe, wat bepalend is by die bio-aktiwiteit en nawerking van atrasien en die fitotoksiese residue daarvan.

2. Die verdraagsaamheid van mieliesaailinge teenoor atrasien is in vloeistofmedium ondersoek. Tekorte aan N, P, K, Ca en Mg, afsonderlik of in sekere kombinasies, het by hoë atrasienkonsentrasies nie betekenisvolle verskille in die verdraagsaamheid van saailinge teenoor atrasien veroorsaak nie. In 'n afsonderlike ondersoek is die verdraagsaamheid van mieliesaailinge teenoor atrasien ook nie betekenisvol deur hoë P-toediening in die wortelsone of relatief hoë P-konsentrasies in die bogroei van plante beïnvloed nie. Dit blyk dat hoë P-voorsiening en hoë P-vlakke in mieliesaailinge nie die plante se verdraagsaamheid teenoor atrasien beïnvloed nie. Resultate dui daarop dat hoë konsentrasies P, wat in die vorm van kunsmis in die wortelsone van mielies teenwoordig is, nie aanleiding sal gee tot die beskadiging van die gewas deur atrasien nie.

3. Met potproewe is aangetoon dat atrasiendrumpelwaarde-konsentrasies in grond vir droëbone, graansorghum, hawer, sojabone en sonneblom van spesie tot spesie en van grond tot grond verskil. Die differensiële gevoeligheid van hierdie gewassoorte behoort meer akkuraat deur wagperiodes, wat na atrasiengebruik in mielies vir gevoelige soorte gestel word, weerspieël te word. Verdere navorsing is nodig om bekende hoeveelhede fitotoksiese atrasienresidue in grond met die reaksie van verskillende spesies in verband te bring, met die oog op beter evaluering van die risiko vir 'n gewassoort waar oordraging van atrasien voorgekom het.

4. Die sterkte van verwantskappe tussen geselekteerde grondeienskappe en die bio-aktiwiteit en nawerking van atrasien is in 'n totaal van 30 veldproewe by altesaam 10 lokaliteite ondersoek. Die bio-aktiwiteit van atrasien was 35 dae na toediening die beste met die grondeienskappe organiese materiaalinhoud (% C) en P-reversie gekorreleerd. Klei-inhoud en KUV was ook belangrik, maar by laer vlakke van betekenisvolheid. Hierdie bevindings het dié van verskeie vorige ondersoeke, wat geloods is om voorspellers van die aktiwiteit van atrasien te identifiseer, ondersteun.

5. Ses maande na onkruiddodertoediening in die proewe wat onder punt 4 bespreek is, het organiese materiaalinhoud, grond-pH en P-reversie onderskeidelik 35%, 19% en 14% van die variasie in bio-aktiwiteit voorspel. Beide klei-inhoud en KUV was swak kriteria van bio-aktiwiteit op daardie stadium. Organiese materiaalinhoud was dominant as voorspeller van beide die korttermyn bio-aktiwiteit (biotoets op dag 0) en langer termyn aktiwiteit (toetsing op dag 182) van atrasien. Aanduidings was dat grond-pH ook 'n belangrike voorspeller van nawerking kon wees. Verskille in verliese tussen

nabygeleë proewe dui daarop dat nawerking van atrasien sterker verband gehou het met die grondeienskappe as met weerstoestande. Resultate dui daarop dat huidige wagperiodes wat vir bepaalde gewasse aanbeveel word, verfyn kan word deur tussen gronde te onderskei op basis van grondeienskappe wat die nawerking van atrasien beïnvloed.

6. Atrasiennawerking na toediening in mielies is vir twee groeiseisoene gemonitor met dieselfde droëboon- en sonneblomkultivars wat hierbo onder punt 3 genoem is. Die doel was om die toepaslikheid van die wagperiode (18 maande) wat vir dié twee gewassoorte aanbeveel word te toets. Oordraging van fitotoksiese atrasienresidue, wat beoordeel is o.g.v. gewasopbrengsverliese, het tussen lokaliteite verskil. Sonneblom was 12 en 24 maande na toediening van atrasien op 'n verskeidenheid grondsoorte meer gevoelig as die droëbone. Dit het die relatiewe gevoeligheid van twee spesies bevestig (sien punt 3). Behalwe by 'n montmorillonietkleigrond is die droëbooncultivar op geeneen van die ander oorwegend kaolinietklei-gronde betekenisvol beskadig nie. Dit word voorgestel dat die wagperiodes vir alle gevoelige spesies verfyn kan word deur differensiële herplant-intervalle op grond- en spesieverskille te baseer.

7. Die invloed van temperatuur en grondwaterinhoud op die nawerking van atrasien is met 'n kleigrond en 'n leemsandgrond ondersoek. Atrasien is bepaal d.m.v. hoëdrukvloeistofchromatografie. Die halfleeftyd van atrasien is ongeveer 60 dae na toediening in die ligte grond bereik, terwyl 70-75% daarvan op dieselfde stadium in die montmorillonietkleigrond oor was. Min tot geen afbraak het in lugdroë grond plaasgevind. Verhoging van die grondwaterinhoud tot by veldkapasiteit het atrasienaf-

braak betekenisvol versnel. Verdere verhoging in die waterinhoud tot by 2x v.k. het nie 'n noemenswaardige invloed gehad nie. Die laagste temperatuurregime het afbraak slegs in die ligte grond betekenisvol vertraag. Grondwaterinhoud en -soort het in hierdie proef 'n groter effek op atrasiennawerking as temperatuur gehad.

8. 'n Eenvoudige biotoetstegniek is gebruik om die nawerking van atrasien, wat teen 0.25 kg ab ha^{-1} op die land toegedien is, in verskillende lae van die profiel van 'n sandkleileemgrond te bepaal. Met dosis-reaksiekurwes is geskat dat 55% van die toegediende atrasien in die 200-300 mm grondlaag, 30 dae na toediening, teenwoordig was. Teen dae 60, 90 en 120 was die persentasies residue in die 200-300 mm grondlaag respektiewelik ongeveer 3%, 2% en 3%. Skattings op dag 120 het daarop gedui dat 2% fitotoksiese residue in die boonste grondlaag teenwoordig was; en 6% in die 300-400 mm laag. Die biotoetstegniek kan toegepas word vir skatting van atrasienresidue in die grondprofiel, veral vir vasstelling van die potensiële risiko vir gevoelige opvolggewasse.

9 (a). Die relatief sterk verwantskap tussen grond-pH en atrasiennawerking wat alreeds hierbo gerapporteer is, is weer aangetoon in 'n eksperiment waar atrasienbehandelde grondmonsters op verskillende stadiums gedurende 'n totale inkubasieperiode van 120 dae vir atrasienaktiwiteit getoets is. Basies slegs die pH van die grondmonsters het verskil. Atrasiennawerking het toegeneem met toenemende grond-pH, en sodoende die bevindings van verhoogde atrasienstabiliteit in hoë pH gronde bevestig.

9 (b). Dosis-reaksiekurwes is met die toetsplant hawer (cv SWK 001) vir 25 gronde

opgestel vir skatting van die hoeveelhede atrasien, of die fitotoksiese residue daarvan, in elke grond 0, 30, 60, 90, 120 en 150 dae na toediening van 0.2 mg atrasien kg^{-1}. Die tempo van atrasienafbraak in elke grond kon vervolgens voorgestel word met die verwantskap tussen hoeveelheid atrasien en tyd (dae) na dodertoediening. Met hierdie kwadratiese formules is atrasienhalfleeftyd (d.i. aantal dae om 0.1 mg atrasien kg^{-1} te bereik) vir elke grond geskat. Regressie-analise het getoon dat die kwadraat van grond-pH die beste voorspeller van atrasiennawerking is. Organiese materiaalinhoud (% C) was die naasbeste voorspeller. Statisties beoordeel, het slegs $(pH)^2$ en % C vir insluiting in 'n meervoudige regressiemodel vir voorspelling van halfleeftyd gekwalifiseer. Dit word voorgestel dat die halfleeftyd van atrasien in grond met die volgende regressie-vergelyking geskat kan word:

$$y = -2.29 + 1.77x_1 + 20.81x_2$$

waar y = halfleeftyd in dae; $x_1 = [pH(H_2O)]^2$; x_2 = % C

Resultate wat gedurende hierdie studie gegenereer is behoort 'n bydrae tot kennis en voorspelling van die bio-aktiwiteit en nawerking van atrasien in grond te lewer.

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APPENDIX A

Contents: abbreviated analysis of variance (ANOVA) tables

Table 1A Analysis of variance of total dry mass (roots + shoots) of maize seedlings exposed to atrazine in aqueous medium in the NPK-experiment (Table 4, Chapter 2)

	Total dry mass			
Source	DF	MS	F value	PR > F
Nutrient (Ntr)	7	23.50	18.00	0.0001
Atrazine (A)	2	179.01	137.07	0.0001
Ntr x A	14	17.64	13.51	0.0001
Error	72	1.30		
Total	95			
C.V. (%)		25		
R^2		0.89		

Table 2A Analysis of variance of percent damage to maize seedlings exposed to atrazine in aqueous medium in the NPK-experiment (Table 4, Chapter 2)

	Total dry mass			
Source	DF	MS	F value	PR > F
Nutrient (Ntr)	7	1836.6	9.06	0.0001
Atrazine (A)	1	6506.7	32.09	0.0001
Ntr x A	7	264.9	1.31	0.2698
Error	48	185.9		
Total	63			
C.V. (%)		30		
R^2		0.70		

Table 3A Analysis of variance of leaf diffusive resistance of maize seedlings exposed to atrazine in aqueous medium in the NPK-experiment (Table 5, Chapter 2)

	Leaf diffusive resistance			
Source	DF	MS	F value	PR > F
Nutrient (Ntr)	7	2.98	3.09	0.0066
Atrazine (A)	2	103.29	106.92	0.0001
Ntr x A	14	0.49	0.51	0.9196
Error	72	0.96		
Total	95			
C.V. (%)		12		
R^2		0.77		

Table 4A Analysis of variance of percent atrazine remaining 0, 14 and 28 days after application of the herbicide in aqueous medium in the NPK-experiment (Figure 1, Chapter 2)

	Percent atrazine remaining in solution			
Source	DF	MS	F value	PR > F
Time (T)	2	33645.9	749.29	0.0001
Nutrient (Ntr)	7	270.1	6.02	0.0001
Atrazine (A)	1	12.3	0.28	0.6024
T x Ntr	14	159.8	3.56	0.0005
T x A	2	32.2	0.72	0.4924
Ntr x A	7	123.3	2.75	0.0176
T x Ntr x A	14	69.1	1.54	0.1334
Error	48	44.9		
Total	95			
C.V. (%)		9		
R^2		0.97		

Table 5A Analysis of variance of total dry mass of maize seedlings exposed to atrazine in aqueous medium in the CaMg-experiment (Table 6, Chapter 2)

	Total dry mass			
Source	DF	MS	F value	PR > F
Nutrient (Ntr)	3	10.05	3.86	0.0149
Atrazine (A)	2	168.49	64.71	0.0001
Ntr x A	6	15.06	5.79	0.0001
Error	48	2.60		
Total	59			
C.V. (%)		29		
R^2		0.78		

Table 6A Analysis of variance of percent damage to maize seedlings exposed to atrazine in aqueous medium in the CaMg-experiment (Table 6, Chapter 2)

	Total dry mass			
Source	DF	MS	F value	PR > F
Nutrient (Ntr)	3	3709.7	10.44	0.0001
Atrazine (A)	1	8342.3	23.47	0.0001
Ntr x A	3	476.0	1.34	0.2789
Error	32	355.4		
Total	39			
C.V. (%)		39		
R^2		0.64		

Table 7A Analysis of variance of leaf diffusive resistance of maize seedlings exposed to atrazine in aqueous medium in the CaMg-experiment (Table 7, Chapter 2)

	Leaf diffusive resistance			
Source	DF	MS	F value	PR > F
Nutrient (Ntr)	3	6.01	0.95	0.4269
Atrazine (A)	2	101.56	16.04	0.0001
Ntr x A	6	10.33	1.63	0.1666
Error	36	6.33		
Total	47			
C.V. (%)		32		
R^2		0.55		

Table 8A Analysis of variance of percent atrazine remaining 0, 14 and 28 days after application in aqueous medium in the CaMg-experiment (Figure 2, Chapter 2)

	Percent atrazine remaining in solution			
Source	DF	MS	F value	PR > F
Time (T)	2	31432.5	615.6	0.0001
Nutrient (Ntr)	3	482.2	9.45	0.0001
Atrazine (A)	1	402.9	7.89	0.0072
T x Ntr	6	128.3	2.51	0.0339
T x A	2	101.9	2.00	0.1469
Ntr x A	3	19.6	0.38	0.7649
T x Ntr x A	6	5.2	0.10	0.9958
Error	48	51.1		
Total	71			
C.V. (%)		12		
R^2		0.96		

Table 9A Analysis of variance of total dry mass (roots + shoots) of maize seedlings exposed to different P-levels and atrazine in aqueous medium (Table 9, Chapter 2)

	Dry mass (g plant^{-1})			
Source	DF	MS	F value	PR > F
Phosphorus (P)	5	0.12	1.73	0.1360
Atrazine (A)	2	75.97	1070.45	0.0001
P x A	10	0.09	1.35	0.2161
Error	90	0.07		
Total	107			
C.V. (%)		14		
R^2		0.96		

Table 10A Analysis of variance of leaf diffusive resistance of maize seedlings exposed to different P-levels and atrazine in aqueous medium (Table 9, Chapter 2)

	Leaf diffusive resistance (s cm^{-1})			
Source	DF	MS	F value	PR > F
Phosphorus (P)	5	258.1	7.88	0.0001
Atrazine (A)	2	6177.3	188.57	0.0001
P x A	10	75.9	2.32	0.0177
Error	90	32.7		
Total	107			
C.V. (%)		26		
R^2		0.83		

Table 11A Analysis of variance of total dry mass (roots + shoots) of maize seedlings exposed to atrazine in nutrient solutions containing different combinations of P and NH_4^+:NO_3^- -N ratios (Table 10, Chapter 2)

	Dry mass (g plant^{-1})			
Source	DF	MS	F value	PR > F
P/NH_4^+:NO_3^- (Ntr)	4	2.05	85.12	0.0001
Atrazine (A)	2	4.77	197.91	0.0001
Ntr x A	8	0.09	3.57	0.0011
Error	105	0.02		
Total	119			
C.V. (%)		12		
R^2		0.87		

Table 12A Analysis of variance of photosynthetic CO_2 fixation tempo of maize seedlings exposed to atrazine in nutrient solutions containing different combinations of P and NH_4^+:NO_3^- -N ratios (Table 11, Chapter 2)

	CO_2-fixation tempo (mg m^{-2} s^{-1})			
Source	DF	MS	F value	PR > F
P/NH_4^+:NO_3^- (Ntr)	4	0.120	3.45	0.0154
Atrazine (A)	2	3.954	113.78	0.0001
Ntr x A	8	0.095	2.72	0.0154
Error	45	0.035		
Total	59			
C.V. (%)		19		
R^2		0.85		

Table 13A Analysis of variance of percent P in shoots of maize seedlings exposed to atrazine in solutions containing different combinations of P and NH_4^+:NO_3^- -N ratios (Table 10, Chapter 2)

	Percentage P in shoots			
Source	DF	MS	F value	PR > F
P/NH_4^+:NO_3^- (Ntr)	4	0.991	62.23	0.0001
Atrazine (A)	2	0.898	56.39	0.0001
Ntr x A	8	0.034	2.13	0.0981
Error	15	0.016		
Total	29			
C.V. (%)		11		
R^2		0.96		

Table 14A Analysis of variance of grain yield of maize exposed to different fertilizer (3:2:1 25%) and atrazine rates in the field (Table 13, Chapter 2)

	Grain yield (ton ha^{-1})			
Source	DF	MS	F value	PR > F
Replicate (R)	2	1.05	0.54	0.3675
Fertilizer (F)	4	2.22	1.62	0.2607
Atrazine (A)	6	3.24	9.59	0.0005
F x A	24	0.36	1.65	0.0692
Error a (A x R)	12	0.34		
Error b (F x R)	8	1.37		
Error c (A x F x R)	48	0.22		
Total	104			
C.V.(%) Main plots		31		
C.V.(%) Sub-plots		18		
R^2		0.71		

Table 15A Analysis of variance[1] of percent reduction in growth caused by atrazine to dry beans and sunflower in a glasshouse (Table 15, Chapter 3)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	2	26	0.5	0.5888
Crop (C)	1	50577	1049.7	0.0001
Soil (S)	8	38410	797.2	0.0001
Atrazine (A)	9	19770	410.3	0.0001
C x S	8	2133	44.3	0.0001
C x A	9	673	13.9	0.0001
S x A	72	1067	22.2	0.0001
C x S x A	72	318	6.6	0.0001
Error	355	49		
Total[2]	536			
C.V. (%)		20		
R^2		0.97		

[1]ANOVA conducted on data from Experiment I.
[2]Number of missing values = 3 (unsatisfactory emergence at three separate treatment combinations).

Table 16A Analysis of variance[1] of percent reduction in growth caused by atrazine to oats and soybeans in a glasshouse (Table 16, Chapter 3)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	2	39	1.4	0.2546
Crop (C)	1	68431	2394.2	0.0001
Soil (S)	8	13178	461.1	0.0001
Atrazine (A)	9	18518	647.9	0.0001
C x S	8	438	15.3	0.0001
C x A	9	620	21.7	0.0001
S x A	72	346	12.1	0.0001
C x S x A	72	588	20.6	0.0001
Error	356	29		
Total[2]	537			
C.V. (%)		11		
R^2		0.97		

[1]ANOVA conducted on data from Experiment I.
[2]Number of missing values = 2 (unsatisfactory emergence at two separate treatment combinations).

Table 17A Dry beans alone: analysis of variance of percent growth reduction caused by atrazine (Table 17, Chapter 3)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	2	45	0.85	0.4281
Soil (S)	8	14752	278.40	0.0001
Atrazine (A)	9	7041	132.87	0.0001
S x A	72	723	13.65	0.0001
Error	174	53		
Total[1]	265			
C.V. (%)		29		
R^2		0.96		

[1]Four missing values were recorded due to unsatisfactory emergence at four separate treatment combinations.

Table 18A Oats alone: analysis of variance of percent reduction in growth caused by atrazine (Table 18, Chapter 3)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	2	73	2.45	0.0886
Soil (S)	9	16569	554.71	0.0001
Atrazine (A)	9	11312	378.72	0.0001
S x A	81	527	17.65	0.0001
Error	198	30		
Total	299			
C.V. (%)		10		
R^2		0.98		

Table 19A Soybeans alone: analysis of variance of percent reduction in growth caused by atrazine (Table 19, Chapter 3)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	2	23	0.81	0.4484
Soil (S)	8	6720	234.46	0.0001
Atrazine (A)	9	7230	252.25	0.0001
S x A	72	445	15.52	0.0001
Error	176	28		
Total[1]	267			
C.V. (%)		15		
R^2		0.97		

[1]Two missing values were recorded due to unsatisfactory emergence at two separate treatment combinations.

Table 20A Sunflower alone: analysis of variance of percent reduction in growth caused by atrazine (Table 20, Chapter 3)

	Percent reduction in seed yield			
Source	DF	MS	F value	PR > F
Replicate	2	89	2.32	0.1010
Soil (S)	9	24275	627.54	0.0001
Atrazine (A)	9	16056	415.06	0.0001
S x A	81	682	17.65	0.0001
Error	192	53		
Total[1]	293			
C.V. (%)		13		
R^2		0.98		

[1]Six missing values were recorded because of unsatisfactory emergence at six separate treatment combinations.

Table 21A Analysis of variance of percent reduction in growth caused by atrazine to grain sorghum (Table 21, Chapter 3)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	2	24	0.90	0.4104
Soil (S)	8	6981	260.25	0.0001
Atrazine (A)	8	13345	497.45	0.0001
S x A	64	338	12.63	0.0001
Error	160	26		
Total	242			
C.V. (%)		13		
R^2		0.97		

Table 22A Analysis of variance of percent reduction in growth caused by atrazine to the test plant oats, 35 days after herbicide application (Figure 3, Chapter 4; data given in Table 2B)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	4	101.3	0.87	0.4852
Locality (L)	9	6450.4	55.16	0.0001
Atrazine (A)	4	45356.8	387.89	0.0001
L x A	36	570.3	4.88	0.0001
Error	181	116.9		
Total[1]	234			
C.V. (%)		22		
R^2		0.92		

[1]Two replicates at locality no.8, and one at locality no. 10 were discarded due to unsatisfactory plant stand.

Table 23A Analysis of variance of percent reduction in growth caused by atrazine/atrazine residues to the test plant oats, 182 days after herbicide application (Figure 4, Chapter 5; data given in Table 3B)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	4	840.5	2.77	0.0301
Locality (L)	6	2852.4	9.40	0.0001
Atrazine (A)	4	12972.3	42.84	0.0001
L x A	24	936.7	3.09	0.0001
Error	125	303.4		
Total[1]	163			
C.V. (%)		74		
R^2		0.71		

[1]Two replicates were discarded at locality no. 4 due to unsatisfactory plant stand. In addition, one missing value was recorded.

Table 24A Analysis of variance of percent reduction in growth caused by atrazine/atrazine residues to the test plant oats, 365 days after herbicide application (Figure 5, Chapter 5; data given in Table 4B)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Replicate	4	2372.5	4.62	0.0016
Locality (L)	6	2401.7	4.68	0.0002
Atrazine (A)	4	527.6	1.03	0.3957
L x A	24	279.7	0.54	0.9572
Error	126	513.4		
Total[1]	164			
C.V. (%)		50		
R^2		0.33		

[1]Ten missing values were recorded because data from one replicate at each of the no. 4 and no. 6 localities were discarded due to unsatisfactory plant stand.

Table 25A Analysis of variance of percent reduction in sunflower yield caused by residues of the recommended rates of atrazine applied 12 months previously (Table 27, Chapter 6)

	Percentage yield reduction			
Source	DF	MS	F value	PR > F
Replicate	4	14	0.6	0.6360
Soil	6	396	18.2	0.0001
Error	24	22		
Total	34			
C.V. (%)		40		
R^2		0.82		

Table 26A Analysis of variance of percent reduction in sunflower yield caused by residues of the recommended rates of atrazine applied 24 months previously (Table 28, Chapter 6)

	Percentage yield reduction			
Source	DF	MS	F value	PR > F
Replicate	4	4	0.7	0.6083
Soil	5	676	116.1	0.0001
Error	20	6		
Total	29			
C.V. (%)		45		
R^2		0.96		

Table 27A Analysis of variance of percent reduction in seed yield of dry beans caused by residues of all atrazine rates applied 12 months previously (Data appear in Table 5B)

	Percent reduction in seed yield			
Source	DF	MS	F value	PR > F
Replicate	4	22.99	1.48	0.2089
Locality (L)	7	543.01	35.04	0.0001
Atrazine (A)	5	174.48	11.26	0.0001
L x A	35	33.11	2.14	0.0007
Error	182	15.49		
Total[1]	233			
C.V. (%)		78		
R^2		0.68		

[1]Six missing values were recorded - five of which were due to only four replicates being used at Kroonstad as a result of flooding in that part of the trial at one stage.

Table 28A Analysis of variance of percent reduction in plant stand of dry beans caused by residues of all atrazine rates applied 12 months previously (Data appear in Table 6B)

	Percent reduction in stand			
Source	DF	MS	F value	PR > F
Replicate	4	27.38	3.16	0.0154
Locality (L)	7	17.77	2.05	0.0512
Atrazine (A)	5	10.72	1.24	0.2936
L x A	35	5.74	0.66	0.9251
Error	182	15.49		
Total[1]	233			
C.V. (%)		192		
R^2		0.23		

[1]Six missing values were recorded (see footnote Table 27A).

Table 29A Analysis of variance of percent reduction in seed yield of sunflower caused by residues of all atrazine rates applied 12 months previously (Data appear in Table 7B)

	Percent reduction in seed yield			
Source	DF	MS	F value	PR > F
Replicate	4	30.1	1.31	0.2703
Locality (L)	6	2629.4	114.15	0.0001
Atrazine (A)	5	742.8	11.26	0.0001
L x A	30	42.9	1.86	0.0077
Error	162	23.0		
Total[1]	207			
C.V. (%)		38		
R^2		0.85		

[1]Two missing values were recorded as a result of unsatisfactory plant emergence in two plots.

Table 30A Analysis of variance of percent reduction in plant stand of sunflower caused by residues of all atrazine rates applied 12 months previously (Data appear in Table 8B)

	Percent reduction in stand			
Source	DF	MS	F value	PR > F
Replicate	4	29.58	1.04	0.3858
Locality (L)	6	1 071.20	37.84	0.0001
Atrazine (A)	5	254.99	9.01	0.0001
L x A	30	40.79	1.44	0.0788
Error	162	28.31		
Total[1]	207			
C.V. (%)		86		
R^2		0.66		

[1]Two missing values were recorded as a result of unsatisfactory emergence.

Table 31A Analysis of variance of percent reduction in seed yield and plant stand of dry beans caused by residues of atrazine that was applied 24 months previously at Warmbad (Data appear in Table 9B)

	Seed yield			
Source	DF	MS	F value	PR > F
Replicate	4	8.33	0.63	0.6494
Atrazine	5	621.63	46.91	0.0001
Error	15	13.25		
Total[1]	24			
C.V. (%)		10		
R^2		0.98		
	Plant stand			
Source	DF	MS	F value	PR > F
Replicate	4	115.27	1.58	0.2283
Atrazine	5	1296.14	17.74	0.0001
Error	16	73.07		
Total[2]	25			
C.V. (%)		38		
R^2		0.88		

[1]Five missing values recorded for yield data as a result of flooding across replicates on one side of the trial.
[2]Four missing values recorded because plants in only one of the plots mentioned above could still be counted.

Table 32A Analysis of variance of percent reduction in seed yield and plant stand of sunflower caused by residues of atrazine that was applied 24 months previously (Data for yield and stand are given in Table 10B and Table 11B, respectively)

	Seed yield			
Source	DF	MS	F value	PR > F
Replicate	4	6.6	1.7	0.1535
Locality (L)	5	4933.0	1277.6	0.0001
Atrazine (A)	5	30.3	7.8	0.0001
L x A	25	31.0	8.0	0.0001
Error	136	3.9		
Total[1]	175			
C.V. (%)			36	
R^2			0.98	
	Plant stand			
Source	DF	MS	F value	PR > F
Replicate	4	80.6	2.79	0.0288
Locality (L)	5	91.9	3.18	0.0095
Atrazine (A)	5	83.0	2.88	0.0168
L x A	25	21.7	0.75	0.7931
Error	137	28.9		
Total[2]	176			
C.V. (%)			25	
R^2			0.31	

[1]Four missing values were recorded - three as a result of unsatisfactory emergence and one due to bird damage.
[2]Three missing values were recorded.

Table 33A Analysis of variance of percent atrazine remaining in two soils 30 days after incubation under different soil water and temperature levels (Table 29, Chapter 7)

	Percent atrazine in soil 30 d.a.t.			
Source	DF	MS	F value	PR > F
Soil (S)	1	4712.6	492.28	0.0001
Soil water (W)	2	1352.1	141.24	0.0001
Temperature (T)	2	205.2	21.44	0.0001
Atrazine (A)	1	9.0	0.94	0.3379
S x W	2	33.3	3.47	0.0417
S x T	2	100.5	10.49	0.0003
S x A	1	77.1	8.05	0.0074
W x T	4	86.3	9.01	0.0001
A x W	2	33.3	3.47	0.0417
A x T	2	3.4	0.36	0.7030
S x W x T	4	15.1	1.58	0.2010
S x W x A	2	91.9	9.60	0.0005
S x T x A	2	26.9	2.81	0.0735
S x W x T x A	8	15.5	1.62	0.1546
Error	36	9.6		
Total	71			
C.V. (%)		3		
R^2		0.96		

Table 34A Analysis of variance of percent atrazine remaining in two soils 60 days after incubation under different soil water and temperature levels (Table 30, Chapter 7)

	Percent atrazine in soil 60 d.a.t.			
Source	DF	MS	F value	PR > F
Soil (S)	1	4736.8	569.38	0.0001
Soil water (W)	2	6381.4	767.04	0.0001
Temperature (T)	2	650.3	78.16	0.0001
Atrazine (A)	1	147.3	17.71	0.0002
S x W	2	443.1	53.26	0.0001
S x T	2	234.4	28.17	0.0001
S x A	1	98.0	11.78	0.0015
W x T	4	101.1	12.16	0.0001
A x W	2	15.2	1.83	0.1753
A x T	2	2.4	0.28	0.7558
S x W x T	4	21.1	2.54	0.0568
S x W x A	2	73.8	8.87	0.0007
S x T x A	2	4.5	0.54	0.5869
S x W x T x A	8	11.1	1.33	0.2605
Error	36	8.3		
Total	71			
C.V. (%)		4		
R^2		0.98		

Table 35A Analysis of variance of amount of atrazine remaining in two soils 30 days after incubation under different soil water and temperature levels (Data given in Table 19B)

	Atrazine remaining in soil 30 d.a.t.			
Source	DF	MS	F value	PR > F
Soil (S)	1	1.1526	666.60	0.0001
Soil water (W)	2	0.3363	194.49	0.0001
Temperature (T)	2	0.0458	26.47	0.0001
Atrazine (A)	1	13.860	8015.49	0.0001
S x W	2	0.1821	105.29	0.0001
S x T	2	0.0311	17.97	0.0001
S x A	1	0.2255	130.45	0.0001
W x T	4	0.0173	9.98	0.0001
A x W	2	0.0725	41.95	0.0001
A x T	2	0.0054	3.13	0.0560
S x W x T	4	0.0048	2.76	0.0425
S x W x A	2	0.0717	41.48	0.0001
S x T x A	2	0.0164	9.46	0.0005
S x W x T x A	8	0.0040	2.30	0.0423
Error	36	0.0017		
Total	71			
C.V. (%)		3		
R^2		0.99		

Table 36A Analysis of variance of amount of atrazine remaining in two soils 60 days after incubation under different soil water and temperature levels (Data appear in Table 20B)

	Atrazine remaining in soil 60 d.a.t.			
Source	DF	MS	F value	PR > F
Soil (S)	1	1.1704	774.56	0.0001
Soil water (W)	2	1.4603	966.39	0.0001
Temperature (T)	2	0.1421	94.06	0.0001
Atrazine (A)	1	11.3129	7486.50	0.0001
S x W	2	0.1286	85.09	0.0001
S x T	2	0.0486	32.17	0.0001
S x A	1	0.2426	160.59	0.0001
W x T	4	0.0219	14.46	0.0001
A x W	2	0.1871	123.81	0.0001
A x T	2	0.0126	8.31	0.0011
S x W x T	4	0.0036	2.35	0.0724
S x W x A	2	0.0547	36.21	0.0001
S x T x A	2	0.0026	1.74	0.1893
S x W x T x A	8	0.0027	1.78	0.1128
Error	36	0.0015		
Total	71			
C.V. (%)		3		
R^2		0.99		

Table 37A Analysis of variance of fresh and dry mass of test plants seeded in the field at various stages after atrazine application (Table 31, Chapter 8)

	Fresh mass of top growth			
Source	DF	MS	F value	PR > F
Day	5	5086.6	199.73	0.0001
Error	12	25.5		
Total	17			
C.V. (%)		10		
R^2		0.98		
	Dry mass of top growth			
Source	DF	MS	F value	PR > F
Day	5	5040.9	260.54	0.0001
Error	12	19.4		
Total	17			
C.V. (%)		9		
R^2		0.99		

Table 38A Analysis of variance of percent reduction in top growth dry mass caused by atrazine to oats in soil samples taken from different soil layers at 1, 30, 60, 90 and 120 days after herbicide application (Table 32, Chapter 8)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
Day (D)	4	6133.2	322.01	0.0001
Layer (L)	3	422.1	22.16	0.0001
D x L	6[1]	470.4	24.70	0.0001
Error	36	19.0		
Total	49			
C.V. (%)		14		
R^2		0.98		

[1]Data for six treatment combinations (day 1/100-200 mm; day 1/200-300 mm; day 1/300-400 mm; day 30/300-400 mm; day 60/300-400 mm; day 90/300-400 mm) were not available for analysis because only certain soil layers were monitored at specific intervals.

Table 39A Analysis of variance of the estimated concentration of atrazine in soil samples taken from different soil layers 1, 30, 60, 90 and 120 days after herbicide application in the field (Table 32, Chapter 8)

	Estimated atrazine concentration			
Source	DF	MS	F value	PR > F
Day (D)	4	0.0219	229.81	0.0001
Layer (L)	3	0.0005	5.65	0.0028
D x L	6[1]	0.0009	9.26	0.9196
Error	36	0.0001		
Total	49			
C.V. (%)		29		
R^2		0.97		

[1]Data for six treatment combinations (day 1/100-200 mm; day 1/200-300 mm; day 1/300-400 mm; day 30/300-400 mm; day 60/300-400 mm; day 90/300-400 mm) were not available because only certain soil layers were monitored at specific intervals.

Table 40A Analysis of variance of percent reduction in top growth dry mass of oats caused by atrazine residues at different soil pH levels (Table 34, Chapter 9)

	Percent reduction in top growth dry mass			
Source	DF	MS	F value	PR > F
pH	5	10170	157.28	0.0001
Day (D)	4	20830	322.14	0.0001
Atrazine (A)	1	13187	203.95	0.0001
pH x D	20	676	10.45	0.0001
pH x A	5	120	1.86	0.1064
D x A	4	448	6.94	0.0001
pH x D x A	20	230	3.56	0.0001
Error	120	64		
Total	179			
C.V. (%)		17		
R^2		0.95		

Table 41A Analysis of variance of percent damage caused to oats by the lowest range of atrazine rates used to obtain dose-response curves for three soils (Data in Table 21B)

	Percent damage in top growth dry mass			
Source	DF	MS	F value	PR > F
Soil (S)	2	1310.2	80.7	0.0001
Atrazine (A)	7	11216.0	691.1	0.0001
S x A	14	177.6	10.9	0.0001
Error	48	16.2		
Total	71			
C.V. (%)		9		
R^2		0.99		

Table 42A Analysis of variance of percent damage caused to oats by the first intermediate range of atrazine rates used to obtain dose-response curves for 12 soils (Data in table 21B)

	Percent damage in top growth dry mass			
Source	DF	MS	F value	PR > F
Soil (S)	11	685	33.40	0.0001
Atrazine (A)	7	27223	1327.17	0.0001
S x A	77	71	3.48	0.0001
Error	192	20		
Total	287			
C.V. (%)		8		
R^2		0.98		

Table 43A Analysis of variance of percent damage caused to oats by the second intermediate range of atrazine rates used to obtain dose-response curves for five soils (Data in Table 21B)

	Percent damage in top growth dry mass			
Source	DF	MS	F value	PR > F
Soil (S)	4	2092	51.46	0.0001
Atrazine (A)	6	12755	313.75	0.0001
S x A	24	301	7.42	0.0001
Error	70	41		
Total	104			
C.V. (%)		17		
R^2		0.97		

Table 44A Analysis of variance of percent damage caused to oats by the highest range of atrazine rates used to obtain dose-response curves for five soils (Data in Table 21B)

	Percent damage in top growth dry mass			
Source	DF	MS	F value	PR > F
Soil (S)	4	962	39.12	0.0001
Atrazine (A)	6	18617	756.72	0.0001
S x A	24	104	4.21	0.0001
Error	70	25		
Total	104			
C.V. (%)		9		
R^2		0.98		

Table 45A Analysis of variance of estimated amounts of residual atrazine which were available to the test plant at certain intervals after application of the herbicide (Table 36, Chapter 9)

	Concentration (mg kg^{-1})			
Source	DF	MS	F value	PR > F
Soil (S)	24	0.0068	22.37	0.0001
Days (D)	5	0.3063	1002.47	0.0001
S x D	120	0.0015	5.04	0.0001
Error	300	0.0003		
Total	449			
C.V. (%)		17		
R^2		0.95		

Table 46A Stepwise procedure for dependent variable (atrazine half-life)

Variable entered in sequence	Variable	Parameter estimate	Standard error	F-value	Prob > F
$[pH]^2$	Intercep	10.6015	9.1	1.36	0.2560
(r^2=0.6938)	$[pH]^2$	1.7368	0.2	52.13	0.0001
% C	Intercep	-2.2907	7.6	0.09	0.7678
(R^2=0.8270)	% C	20.8124	5.0	16.93	0.0005
	$[pH]^2$	1.7752	0.2	91.96	0.0001
$[CEC]^2$	Intercep	2.9517	8.2	0.13	0.7248
(R^2=0.8431)	% C	19.0224	5.0	14.04	0.0012
	$[pH]^2$	1.6246	0.2	61.35	0.0001
	$[CEC]^2$	0.0060	0.0	2.17	0.1558
Removed: **$[CEC]^2$**					

APPENDIX B

Table 1B Composition of the Nitsch (1972) nutrient solution used in certain pot experiments

Combination[1]	Salt	Concentration g 10 L^{-1}
A	KNO_3	610
	KH_2PO_4	310
B	$MgSO_4.7H_2O$	610
	$(NH_4)_2SO_4$	310
C	$Ca(NO_3)_2.4H_2O$	2440
	$EDTA.Na_2Fe$	60
D	KCL	6.1
	H_3BO_3	6.7
	$MnSO_4.H_2O$	3.8
	$ZnSO_4.7H_2O$	0.61
	$(NH_4)_6Mo_7O_{24}.4H_2O$	6.1
	$CuSO_4.5H_2O$	0.31
	H_2SO_4	0.31 cm^3

[1]A, B, C and D were made up separately to 10 L using deionised water. Once dissolved, these combinations were combined and made up to 2219 L using deionised water.

Table 2B Percent reduction in the top growth dry mass of the test plant oats at 10 sites, 35 days after atrazine application (ANOVA in Table 22A)

	Atrazine rate (kg ai ha^{-1})					
Locality	0.031	0.062	0.125	0.250	0.500	Mean
	%	%	%	%	%	
Bapsftn. A	4.4	13.8	19.9	28.5	72.2	27.7
Bapsftn. B	5.5	12.1	23.9	60.2	81.9	36.7
Ermelo	8.8	13.2	34.8	62.0	94.4	39.0
Kroonstad	16.4	46.1	77.4	97.4	99.7	67.4
Nelspruit	24.9	62.9	99.8	100.0	100.0	77.5
Pretoria	15.5	30.7	63.0	85.1	94.4	57.8
Standerton	1.3	18.5	19.3	64.2	87.4	38.1
Ventersdorp	30.2	35.0	62.4	90.7	97.9	63.2
Warmbad A	16.3	19.2	23.8	53.3	82.6	39.0
Warmbad B	8.0	8.2	23.0	62.6	88.2	38.0
LSD_T (0.05)	Locality x Atrazine rate = 27.3					

Table 3B Percent reduction in the top growth dry mass of the test plant oats at seven sites, 182 days after atrazine application (ANOVA in Table 23A)

	Atrazine rate (kg ai ha^{-1})					
Locality	0.031	0.062	0.125	0.250	0.500	Mean
	%	%	%	%	%	
Bapsftn. A	13.2	8.8	1.5	11.2	9.0	9.0
Bapsftn. B	5.8	9.8	17.4	30.0	62.2	25.0
Ermelo	2.2	0.0	10.4	8.8	12.8	6.8
Kroonstad	3.4	4.4	21.4	38.2	87.2	30.9
Pretoria	-3.8	14.8	33.6	38.6	75.8	31.8
Warmbad A	10.3	10.3	24.3	36.0	85.7	33.3
Warmbad B	8.2	18.0	29.2	27.8	62.2	29.1
LSD_T (0.05)	Locality x Atrazine rate = 43					

Table 4B Percent reduction in the top growth dry mass of the test plant oats at seven sites, 365 days after atrazine application (ANOVA in Table 24A)

	Atrazine rate (kg ai ha^{-1})					
Locality	0.031	0.062	0.125	0.250	0.500	Mean
	%	%	%	%	%	
Bapsftn. A	-7.3	-1.2	0.8	-1.2	2.1	-1.4
Bapsftn. B	6.7	5.8	4.6	5.9	11.0	6.8
Ermelo	0.3	-10.9	-1.0	-0.0	8.5	-0.6
Kroonstad	4.5	-9.4	-3.4	-4.0	-9.7	-4.4
Pretoria	11.6	11.1	10.5	15.6	11.3	12.0
Warmbad A	8.1	7.1	18.5	6.5	18.4	11.7
Warmbad B	-37.4	-27.8	-21.3	-0.6	4.4	-16.5
LSD_T (0.05)			Locality = 22			

Table 5B Dry bean yield and percentage reduction in yield 12 months after atrazine application in maize (ANOVA for percentage data appears in Table 27A; data for recommended rate only are presented in Table 27, Chapter 6)

Locality	Atrazine rate number[3]												
	Control	1		2		3		4		5		6	
	kg[1]	%[2]	kg	%	kg	%	kg	%	kg	%	kg	%	kg
Carltnv.	0.483	4	0.466	0	0.481	9	0.442	4	<u>0.465</u>	3	0.468	8	0.447
Baps. A	0.520	0	0.518	2	<u>0.511</u>	5	0.495	7	0.485	7	0.485	6	0.487
Baps. B	0.478	-1	0.483	1	<u>0.475</u>	4	0.461	1	0.472	-2	0.485	-2	0.486
Vryheid	0.232	3	0.224	10	<u>0.210</u>	10	0.208	17	0.192	19	0.188	21	0.183
Pta. A	0.748	-2	0.766	-2	0.760	1	0.744	-2	<u>0.764</u>	-3	0.772	0	0.747
Pta. B	0.808	0	0.808	-1	0.817	1	0.800	1	<u>0.802</u>	-1	0.819	2	0.791
Delmas	0.279	2	0.273	4	0.268	5	<u>0.264</u>	8	0.258	11	0.249	17	0.231
Krnstad.	0.729	1	0.723	2	0.716	3	<u>0.705</u>	5	0.695	8	0.674	9	0.664

[1]Kilogram seed per 4 m row section. Formula for transformation to ton ha^{-1} = 111.1[25(kg seed 4 m^{-1})]/1000.
[2]Percent reduction in yield relative to control (0 atrazine).
[3]Different atrazine rates were used in each trial. Rates used appear in Table 26, Chapter 6. Underlined values were measured at the recommended rate for each locality.

<u>NB</u> The trial at Warmbad was terminated when 90-100% of seedlings died soon after emergence on all plots treated with atrazine.

Table 6B Dry bean stand and percentage reduction in stand 12 months after atrazine application in maize (ANOVA for percentage data appears in Table 28A)

Locality	Atrazine rate number[3]											
	Control	1		2		3		4		5		6	
	no.[1]	%[2]	no.	%	no.	%	no.	%	no.	%	no.	%	no.
Carletnv.	47	0	47	-2	48	-2	48	0	47	0	47	0	47
Baps. A	45	0	45	0	45	4	43	2	44	0	45	2	44
Baps. B	40	0	40	3	39	5	38	0	40	0	40	3	39
Vryheid	42	0	42	2	41	2	41	0	42	0	42	0	42
Pta. A	37	3	36	3	36	0	37	3	36	3	36	3	36
Pta. B	35	0	35	0	35	-3	36	0	35	0	35	0	35
Delmas	32	0	32	3	31	0	32	3	31	3	31	3	31
Krnstad.	33	0	33	0	33	-3	34	3	32	0	33	3	32

[1]Number of plants per 4 m row section monitored.
[2]Percent reduction in stand relative to control.
[3]Different atrazine rates were used at each locality. Rates used are given in Table 26 (Chp. 6). Underlined values were measured at recommended rates for each locality.

Table 7B Sunflower yield and percentage reduction in yield 12 months after atrazine application in maize (ANOVA for percentage data appears in Table 29A; percentage data for recommended rates only appear in Table 27, Chapter 6)

Locality	Atrazine rate number[3]												
	Control	1		2		3		4		5		6	
	kg[1]	%[2]	kg	%	kg	%	kg	%	kg	%	kg	%	kg
Carltnv.	1.293	14	1.117	18	1.063	21	1.025	21	1.016	25	0.972	37	0.818
Baps. A	1.328	10	1.190	17	1.103	19	1.078	21	1.050	23	1.022	35	0.858
Baps. B	1.526	10	1.373	15	1.296	14	1.305	17	1.270	13	1.321	18	1.247
Vryheid	1.340	0	1.340	1	1.330	2	1.315	1	1.326	1	1.327	5	1.274
Pta. A	2.107	13	1.824	11	1.881	13	1.844	16	1.770	20	1.683	21	1.666
Pta. B	1.458	-6	1.540	-7	1.558	-3	1.505	3	1.411	-1	1.467	4	1.395
Delmas	1.261	12	1.106	15	1.073	19	1.027	16	1.064	17	1.043	24	0.961

[1]Kilogram seed per 4 m row section. Formula for transformation to ton ha^{-1} = 111.1[25(kg seed 4 m^{-1})]/1000.
[2]Percent reduction in yield relative to control (0 atrazine).
[3]Different atrazine rates were used in each trial. Rates used appear in Table 26, Chapter 6. Underlined values were measured at the recommended rate for each locality.

NB The trial at Warmbad was terminated when 90-100% of seedlings died soon after emergence on all plots that had been treated with atrazine. Sunflower was not monitored at Kroonstad due to unsatisfactory emergence which was clearly not linked to atrazine damage.

Table 8B Sunflower stand and percentage reduction in stand 12 months after atrazine application in maize (ANOVA for percentage data appears in Table 30A)

Locality	Atrazine rate number[3]												
	Control	1		2		3		4		5		6	
	no.[1]	%[2]	no.	%	no.	%	no.	%	no.	%	no.	%	no.
Carletnv.	16.0	6	15.0	13	14.0	15	13.6	16	13.4	18	13.2	26	11.8
Baps. A	14.2	9	12.8	6	13.4	17	11.8	14	12.2	18	11.6	18	11.6
Baps. B	15.2	0	15.2	1	15.0	1	15.0	0	15.2	3	14.8	11	13.6
Vryheid	14.2	0	14.2	0	14.2	-1	14.4	0	14.2	1	14.0	4	13.6
Pta. A	14.4	7	13.4	3	14.0	3	14.0	3	14.0	8	13.2	6	13.6
Pta. B	13.6	3	13.2	3	13.2	3	13.2	3	13.2	0	13.6	6	12.8
Delmas	12.4	2	12.2	2	12.2	3	12.0	3	12.0	2	12.2	6	11.6

[1]Number of plants per 4 m row segment monitored.
[2]Percent reduction in stand relative to control.
[3]Different atrazine rates were used at each locality. Rates used are given in Table 26 (Chp. 6). Underlined values were measured at recommended rates for each locality.

Table 9B Dry bean yield and percentage reduction in yield 24 months after atrazine application in maize at Warmbad (ANOVA for percentage data appears in Table 31A)

Atrazine rate	Yield (4 m row segment)		Plants (4 m row segment)	
kg ai ha^{-1}	%[1]	kg	%[1]	no.
0	-	0.197	-	38.0
1.8	23	0.151	11	33.8
2.1	21	0.155	4	36.6
2.4[2]	38	0.121	10	34.2
2.7	42	0.114	41	22.2
3.0	48	0.103	35	24.6
3.3	50	0.098	18	31.2
LSD_T (0.05)		0.015		15.7
CV%		6		25

[1]Percent reduction in yield or stand compared to the controls (0 atrazine). ANOVAS for percentage data appear in Table 31A.
[2]Recommended herbicide rate = 2.4 kg ai ha^{-1}.

NB Warmbad was the only site at which significant damage to dry beans was observed 12 months previously.

Table 10B Sunflower yield and percentage reduction in yield 24 months after atrazine application in maize (ANOVA for percentage data appears in Table 32A; percentage data for recommended rates only are given in Table 28, Chapter 6)

Locality	Atrazine rate number[3]												
	Control	1		2		3		4		5		6	
	kg[1]	%[2]	kg	%	kg	%	kg	%	kg	%	kg	%	kg
Carltnv.	1.200	1	1.192	0	1.195	2	1.180	1	1.188	1	1.187	0	1.200
Baps. A	1.050	2	1.032	0	1.046	0	1.047	0	1.053	1	1.037	0	1.051
Baps. B	1.265	0	1.260	1	1.247	1	1.251	1	1.256	1	1.247	1	1.257
Pta. A	1.800	-2	1.839	-2	1.833	-1	1.816	-1	1.815	1	1.789	0	1.806
Delmas	1.560	2	1.524	0	1.555	1	1.546	0	1.555	-1	1.572	0	1.563
Warmbad	1.419	29	1.007	28	1.024	29	1.008	38	0.872	40	0.845	41	0.843

[1]Kilogram seed per 4 m row section. Formula for transformation to ton ha^{-1} = 111.1[25(kg seed 4 m^{-1})]/1000.
[2]Percent reduction in yield compared to control (0 atrazine).
[3]Different atrazine rates were used in each trial. Rates used appear in Table 26, Chapter 6. Underlined values were measured at the recommended rate for each locality.

Table 11B Sunflower stand and percentage reduction in stand 24 months after atrazine application in maize (ANOVA for percentage data appears in Table 32A; percentage data for recommended rates only appear in Table 28, Chapter 6)

Locality	Atrazine rate number[3]												
	Control	1		2		3		4		5		6	
	no.[1]	%[2]	no.	%	no.	%	no.	%	no.	%	no.	%	no.
Carletnv.	14.0	3	13.6	0	14.0	0	14.0	3	13.6	0	14.0	0	14.0
Baps. A	14.0	0	14.0	0	14.0	3	13.6	3	13.6	0	14.0	0	14.0
Baps. B	14.0	3	13.6	0	14.0	0	14.0	3	13.6	0	14.0	3	13.6
Pta. A	14.0	0	14.0	0	14.0	6	13.2	3	13.6	0	14.0	3	13.6
Delmas	14.0	0	14.0	0	14.0	0	14.0	6	13.2	0	14.0	3	13.6
Warmbad	14.0	1	13.9	3	13.6	6	13.2	14	12.0	7	13.0	4	13.4

[1]Number of plants per 4 m row segment monitored.
[2]Percent reduction in stand relative to control.
[3]Different atrazine rates were used at each locality. Rates used are given in Table 26 (Chp. 6). Underlined values were measured at recommended rates for each locality.

Table 12B Rainfall and mean daily maximum and minimum temperatures recorded at Kroonstad for the period after atrazine application on 27 November 1987 until the seeding of dry beans and sunflower on 3 December 1988 (Chapter 6)

		Temperature (°C)[b]	
Period	Rainfall[a] (mm)	Max.	Min.
27 Nov-31 Dec 1987	56	29	15
Jan. 1988	17 (99)	32 (28)	16 (15)
Feb.	89 (81)	28 (27)	17 (15)
Mar.	220 (96)	27 (26)	15 (13)
Apr.	126 (55)	22 (22)	9 (9)
May	15 (19)	21 (20)	3 (5)
Jun.	12 (6)	16 (16)	-1 (1)
Jul.	1 (6)	19 (17)	-1 (0)
Aug.	1 (17)	22 (20)	2 (2)
Sept.	45 (33)	23 (24)	7 (8)
Oct.	152 (67)	24 (26)	10 (11)
Nov.	93 (80)	26 (27)	12 (13)
30 Nov-3 Dec	23	25	13
Total rainfall	850 (632)		

[a]Long-term average for the monthly total appears in parenthesis.
[b]Long-term mean daily maximum and minimum temperatures appear in parenthesis.
[c]Long-term yearly rainfall (1 Jan-31 Dec) appears in parenthesis.

Table 13B Rainfall and mean daily maximum and minimum temperatures recorded at Vryheid for the period after atrazine application on 23 November 1987 until the seeding of dry beans and sunflower on 5 December 1988 (Chapter 6)

		Temperature (°C)[b]	
Period	Rainfall[a] (mm)	Max.	Min.
23 Nov-31 Dec 1987	90	27	17
Jan. 1988	95 (133)	27 (26)	16 (15)
Feb.	95 (138)	25 (25)	16 (15)
Mar.	96 (95)	24 (25)	16 (15)
Apr.	66 (37)	20 (23)	12 (12)
May	3 (10)	22 (22)	9 (10)
Jun.	30 (25)	18 (19)	7 (7)
Jul.	23 (7)	20 (19)	6 (6)
Aug.	75 (24)	22 (21)	9 (8)
Sept.	20 (45)	22 (22)	11 (10)
Oct.	135 (106)	21 (23)	11 (11)
Nov.	93 (100)	21 (23)	12 (12)
30 Nov-5 Dec	51	22	14
Total rainfall	872 (869)		

[a]Long-term average for the monthly total appears in parenthesis.
[b]Long-term mean daily maximum and minimum temperatures appear in parenthesis.
[c]Long-term yearly rainfall (1 Jan-31 Dec) appears in parenthesis.

Table 14B Rainfall and mean maximum and minimum temperatures recorded at Carletonville for the period after atrazine application on 1 December 1987 until the seeding of dry beans and sunflower on 6 December 1988 (Chapter 6)

		Temperature (°C)[b]	
Month	Rainfall[a] (mm)	Max.	Min.
Dec. 1987	110 (109)	27 (27)	18 (14)
Jan. 1988	45 (120)	29 (27)	16 (14)
Feb.	50 (78)	27 (27)	16 (14)
Mar.	113 (79)	26 (25)	14 (12)
Apr.	32 (56)	22 (23)	9 (8)
May	30 (12)	21 (20)	3 (3)
Jun.	13 (7)	17 (17)	0 (0)
Jul.	2 (3)	19 (18)	-1 (0)
Aug.	2 (7)	22 (20)	3 (2)
Sept.	113 (19)	27 (24)	8 (7)
Oct.	84 (71)	24 (25)	11 (10)
Nov.	46 (89)	25 (26)	13 (13)
30 Nov-6 Dec	8	23	14
Total rainfall[c]	648 (665)		

[a]Long-term average for the monthly total appears in parenthesis.
[b]Long-term mean daily maximum and minimum temperatures appear in parenthesis.
[c]Long-term yearly rainfall (1 Jan-31 Dec) appears in parenthesis.

Table 15B Rainfall and mean daily maximum and minimum temperatures recorded at Delmas for the period after atrazine application on 1 December 1987 until the seeding of dry beans and sunflower on 6 December 1988 (Chapter 6)

		Temperature (°C)[b]	
Period	Rainfall[a] (mm)	Max.	Min.
Dec. 1987	86 (91)	26 (26)	13 (13)
Jan. 1988	89 (94)	28 (27)	15 (14)
Feb.	48 (75)	26 (26)	14 (13)
Mar.	77 (80)	26 (25)	13 (11)
Apr.	25 (28)	22 (23)	8 (7)
May	2 (4)	21 (21)	2 (2)
Jun.	18 (6)	17 (17)	-1 (0)
Jul.	7 (1)	19 (18)	-1 (-1)
Aug.	0 (5)	21 (20)	1 (1)
Sept.	55 (42)	21 (37)	7 (7)
Oct.	81 (72)	23 (24)	10 (10)
Nov.	20 (103)	24 (25)	11 (11)
30 Nov-6 Dec	7 (91)	25 (26)	13 (13)
Total rainfall[c]	515 (607)		

[a]Long-term average for the monthly total appears in parenthesis.
[b]Long-term mean daily maximum and minimum temperatures appear in parenthesis.
[c]Long-term yearly rainfall (1 Jan-31 Dec) appears in parenthesis.

Table 16B Rainfall and maximum and minimum temperatures recorded at Pretoria for the period after atrazine application on 10 November 1987 and the seeding of dry beans and sunflower on 15 November 1988 (Chapter 6)

		Temperature (°C)[b]	
Period	Rainfall[a] (mm)	Max.	Min.
10 Nov-30 Nov 1987	54	26	15
Dec. 1987	84 (110)	28 (28)	18 (16)
Jan. 1988	64 (133)	30 (28)	18 (17)
Feb.	37 (77)	28 (27)	17 (17)
Mar.	138 (84)	27 (26)	16 (16)
Apr.	78 (49)	24 (23)	12 (12)
May	0 (11)	22 (21)	9 (8)
Jun.	9 (4)	18 (18)	7 (4)
Jul.	3 (2)	20 (19)	4 (4)
Aug.	0 (6)	23 (21)	9 (7)
Sept.	34 (21)	25 (25)	11 (11)
Oct.	70 (69)	25 (26)	13 (14)
1 Nov-15 Nov	23	26	14
Total rainfall[c]	594 (668)		

[a]Long-term average for the monthly total appears in parenthesis.
[b]Long-term mean daily maximum and minimum temperatures appear in parenthesis.
[c]Long-term yearly rainfall (1 Jan-31 Dec) appears in parenthesis.

Table 17B Rainfall and maximum and minimum temperatures recorded at Bapsfontein for the period after atrazine application on 13 November 1987 until the seeding of dry beans and sunflower on 16 November 1988 (Chapter 6)

		Temperature (°C)[b]	
Period	Rainfall[a] (mm)	Max.	Min.
13 Nov-30 Nov 1987	53	24	12
Dec. 1987	105 (102)	27 (26)	14 (13)
Jan. 1988	100 (139)	29 (27)	15 (14)
Feb.	47 (108)	27 (26)	14 (13)
Mar.	129 (89)	26 (25)	13 (12)
Apr.	77 (38)	22 (23)	9 (8)
May	2 (5)	21 (20)	4 (4)
Jun.	20 (14)	17 (17)	1 (1)
Jul.	4 (6)	19 (18)	2 (1)
Aug.	0 (6)	21 (24)	5 (4)
Sept.	69 (34)	23 (23)	9 (7)
Oct.	70 (76)	24 (25)	9 (10)
1 Nov-16 Nov	170	25	8
Total rainfall[c]	846 (739)		

[a]Long-term average for the monthly total appears in parenthesis.
[b]Long-term mean daily maximum and minimum temperatures appear in parenthesis.
[c]Long-term yearly rainfall (1 Jan-31 Dec) appears in parenthesis.

Table 18B Rainfall and the mean daily maximum and minimum temperature recorded at Towoomba for the period after atrazine application on 3 December 1987 until the seeding of dry beans and sunflower on 10 December 1988 (Chapter 6)

		Temperature (°C)[b]	
Period	Rainfall[a] (mm)	Max.	Min.
3 Dec-31 Dec 1987	161	29	17
Jan. 1988	39 (113)	31 (29)	17 (17)
Feb.	88 (87)	29 (29)	17 (16)
Mar.	107 (76)	28 (28)	16 (14)
Apr.	43 (37)	25 (26)	12 (11)
May	0 (6)	24 (23)	6 (6)
Jun.	63 (5)	20 (20)	3 (3)
Jul.	0 (2)	22 (21)	2 (3)
Aug.	1 (4)	25 (24)	6 (5)
Sept.	36 (16)	27 (27)	10 (10)
Oct.	133 (61)	27 (29)	13 (13)
Nov.	18 (102)	28 (29)	14 (15)
1 Dec-10 Dec	21	28	16
Total rainfall[c]	710 (634)		

[a]Long-term average for the monthly total appears in parenthesis.
[b]Long-term mean daily maximum and minimum temperatures appear in parenthesis.
[c]Long-term yearly rainfall (1 Jan-31 Dec) appears in parenthesis.

Table 19B Effect of temperature and soil water on amount of atrazine (mg kg^{-1}) remaining 30 days after application to a loamy sand and a clay soil (Chapter 7) - ANOVA in Table 35A

		Temp. (day/night)								
		30/16 °C			30/8 °C			16/8 °C		
		Soil water								
Soil type	Atrazine rate (mg kg^{-1})	0	fc	2xfc	0	fc	2xfc	0	fc	2xfc
		mg kg^{-1}			mg kg^{-1}			mg kg^{-1}		
Loamy sand	1	0.94	0.76	0.71	0.92	0.72	0.74	0.93	0.85	0.81
	2	1.95	1.30	1.28	1.93	1.27	1.35	1.96	1.63	1.58
Clay	1	1.00	0.92	0.95	1.03	0.87	0.93	0.97	1.01	0.96
	2	2.00	1.91	1.93	2.00	1.95	1.93	1.96	1.91	1.96
$LSD_T(0.05)$		Atrazine x Soil x Temp. x Water=0.33								

Table 20B Effect of temperature and soil water on amount of atrazine (mg kg^{-1}) remaining 60 days after application to a loamy sand and a clay soil (Chapter 7) - ANOVA in Table 36A

		Temp. (day/night)								
		30/16 °C			30/8 °C			16/8 °C		
		Soil water								
Soil type	Atrazine rate (mg kg^{-1})	0	fc	2xfc	0	fc	2xfc	0	fc	2xfc
		mg kg^{-1}			mg kg^{-1}			mg kg^{-1}		
Loamy sand	1	0.86	0.45	0.45	0.82	0.60	0.51	0.94	0.70	0.64
	2	1.81	0.90	0.91	1.87	1.06	0.99	1.91	1.33	1.29
Clay	1	0.96	0.73	0.71	0.96	0.74	0.63	0.94	0.80	0.76
	2	1.95	1.57	1.53	1.96	1.59	1.50	1.96	1.72	1.62
$LSD_T(0.05)$		Atrazine x Soil x Water=0.08								

Table 21B Dose-response of the test plant (percent reduction in top growth dry mass of oats) to different ranges of atrazine rates applied to a total of 25 soils (ANOVA for lowest range of rates in Table 41A; ANOVA for 1st intermediate range of rates in Table 42A; ANOVA for 2nd intermediate range of rates in Table 43A; ANOVA for highest range of rates in Table 44A)

Exp. I: Lowest range of atrazine rates

	Atrazine rate (mg kg^{-1})								
Soil	0.002	0.004	0.008	0.016	0.032	0.064	0.128	0.256	Mean
Colby	4	7	15	42	82	89	89	89	52
Fairdale	4	11	13	25	67	75	89	88	46
Nelspruit	3	7	14	19	43	58	75	82	38
LSD_T (P=0.05)	Soil x Atrazine rate = 13								

Exp. II: First intermediate range of atrazine rates

	Atrazine rate (mg kg^{-1})								
Soil	0.0125	0.025	0.05	0.075	0.10	0.125	0.15	0.20	Mean
Bethal	8	20	44	61	75	77	83	82	56
Bothaville	16	35	51	68	77	78	80	83	61
Ermelo A	10	30	44	69	74	79	84	84	59
Leeudoringst. A	19	53	64	69	81	79	84	84	67

Continued overleaf

Table 21B cont.	0.0125	0.025	0.05	0.075	0.10	0.125	0.15	0.20	Mean
Leeudoringst. B	19	32	49	60	74	77	80	81	59
Nylstroom	9	20	45	54	61	73	80	82	53
Pretoria A1	7	14	38	62	69	80	82	82	54
Pretoria A2	5	16	45	59	68	79	83	83	55
Pretoria A3	7	20	40	58	71	78	83	84	55
Pretoria A4	9	26	41	52	69	76	80	82	54
Pretoria A5	4	13	38	52	70	79	82	82	53
Warmbad A	4	9	24	43	56	67	73	81	45
LSD_T (P=0.05)				Soil x Atrazine rate = 16					

Exp. III: Second intermediate range of atrazine rates

	Atrazine rate (mg kg^{-1})							
Soil	0.025	0.05	0.10	0.15	0.20	0.25	0.30	Mean
Carletonville	3	8	33	56	68	90	91	50
Ermelo B	4	-5	2	22	55	64	65	30
Morgenzon	-1	7	23	45	56	89	89	44

Continued overleaf

Table 21B continued

Redhill	1	12	22	43	56	66	65	38
Vryheid	7	7	21	18	29	43	56	26
LSD_T (P=0.05)				Soil x Atrazine rate = 19				

Exp. IV: Highest range of atrazine rates

	Atrazine rate (mg kg^{-1})							
Soil	0.025	0.05	0.10	0.15	0.20	0.30	0.40	Mean
Potgietersrus	-4	21	44	66	89	90	90	57
Pretoria B	4	10	27	42	65	81	83	45
Roodeplaat	8	37	45	64	93	93	94	62
Utrecht	1	8	38	56	77	90	91	52
Warmbad B	4	4	27	58	79	85	86	49
LSD_T (P=0.05)				Soil x Atrazine rate = 15				

APPENDIX C

Contents: Dose-response curves for 25 soils (Chapter 9, Section B)

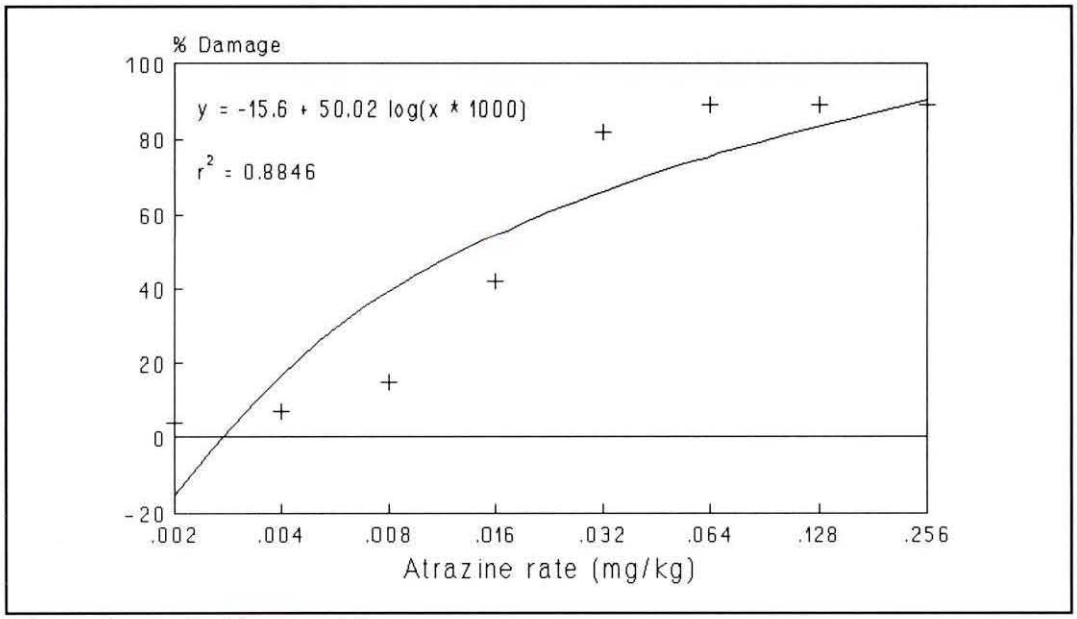

Fig. 1c Colby soil

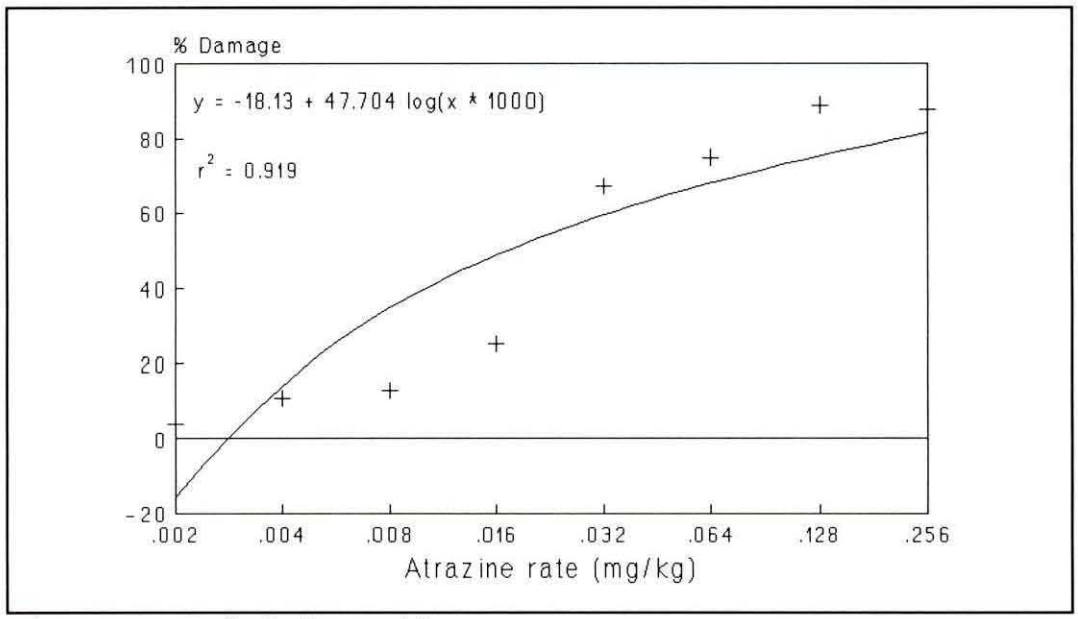

Fig. 2c Fairdale soil

Figures 1C & 2C Dose-response to atrazine in the Colby and Fairdale soils

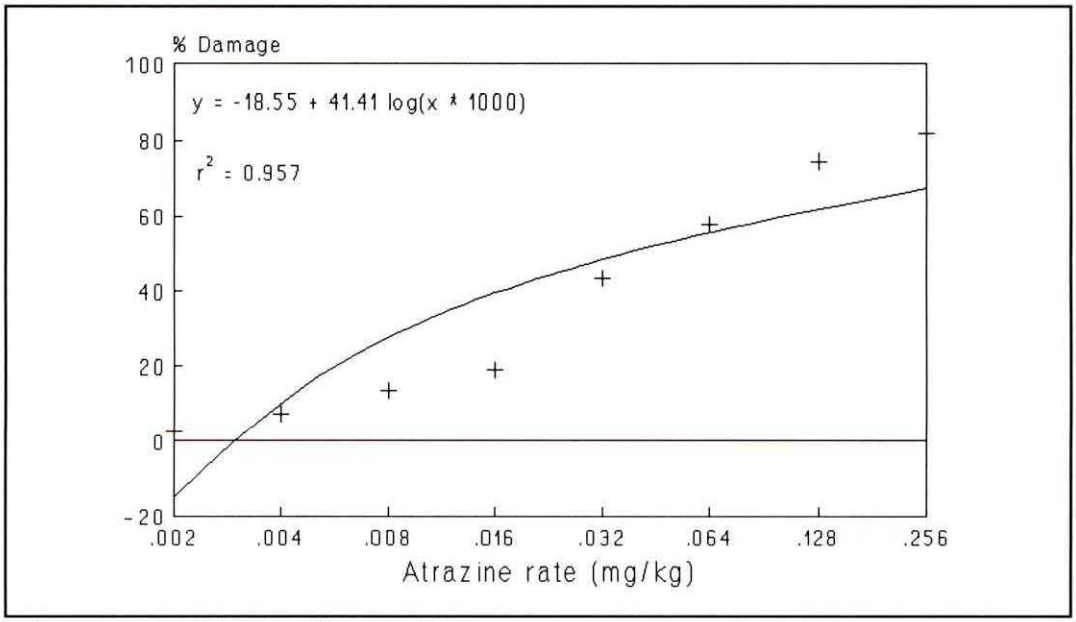

Fig. 3c Nelspruit soil

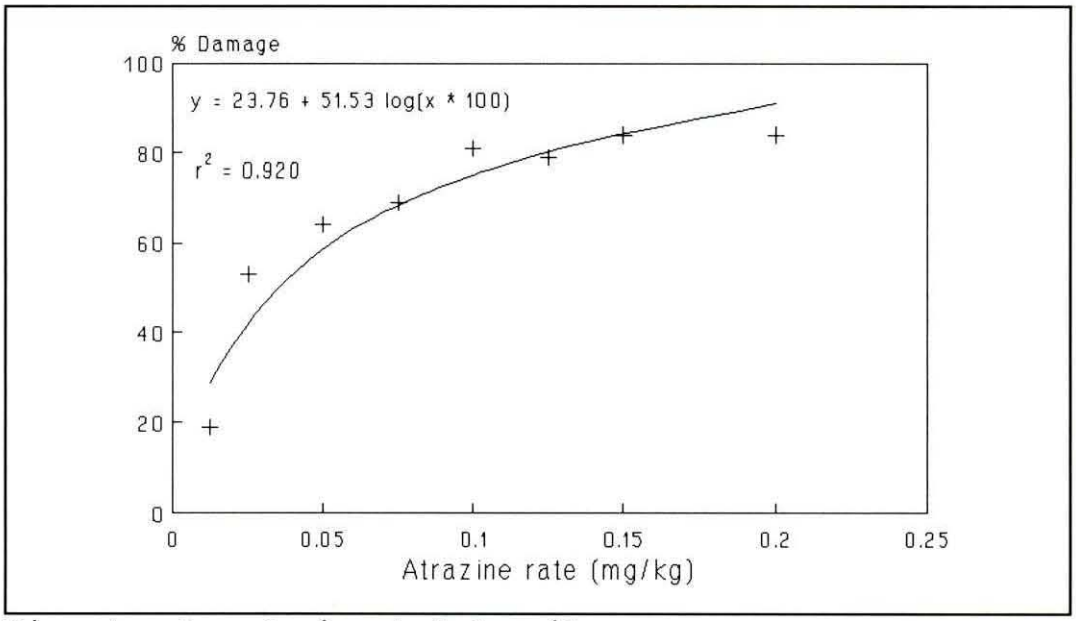

Fig. 4c Leeudoringstad A soil

Figures 3C & 4C Dose-response to atrazine in the Nelspruit and Leeudoringstad A soils

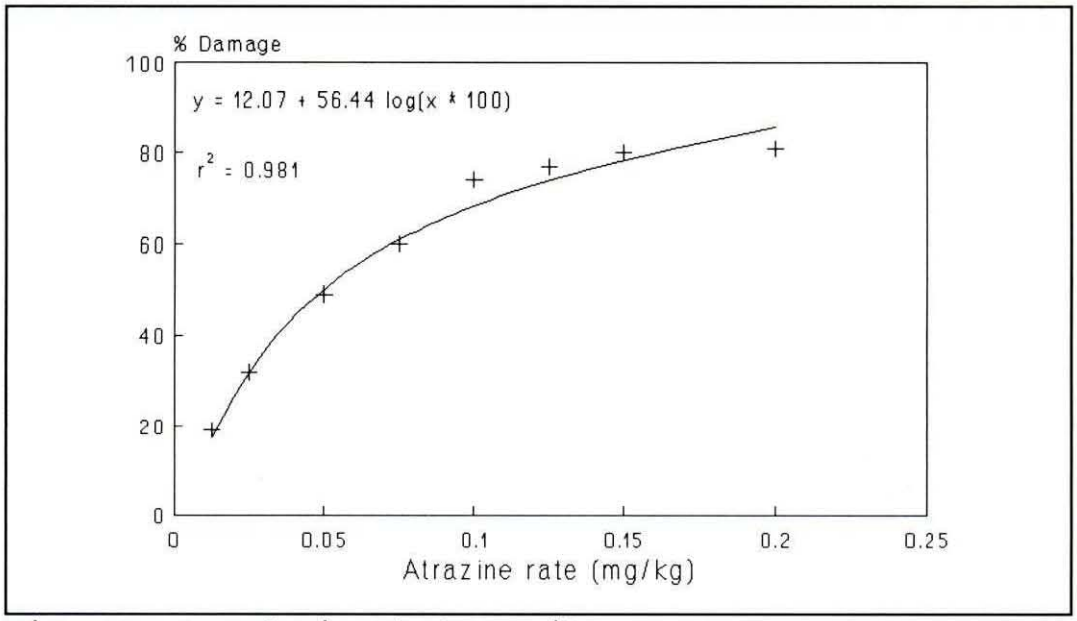

Fig. 5c Leeudoringstad B soil

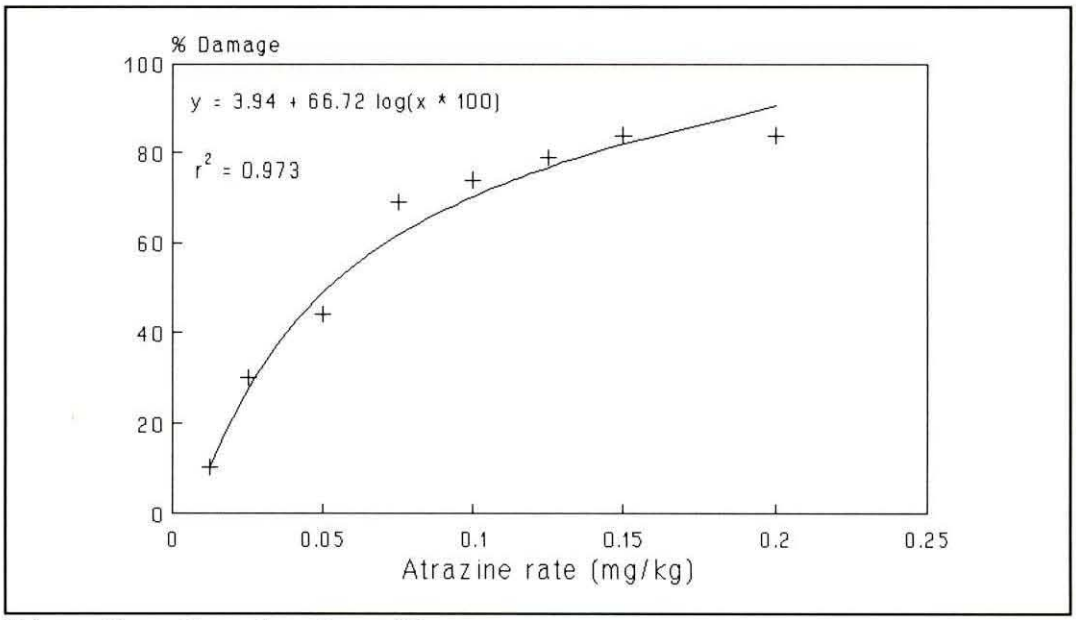

Fig. 6c Ermelo A soil

Figures 5C & 6C Dose-response to atrazine in the Leeudoringstad B and Ermelo A soils

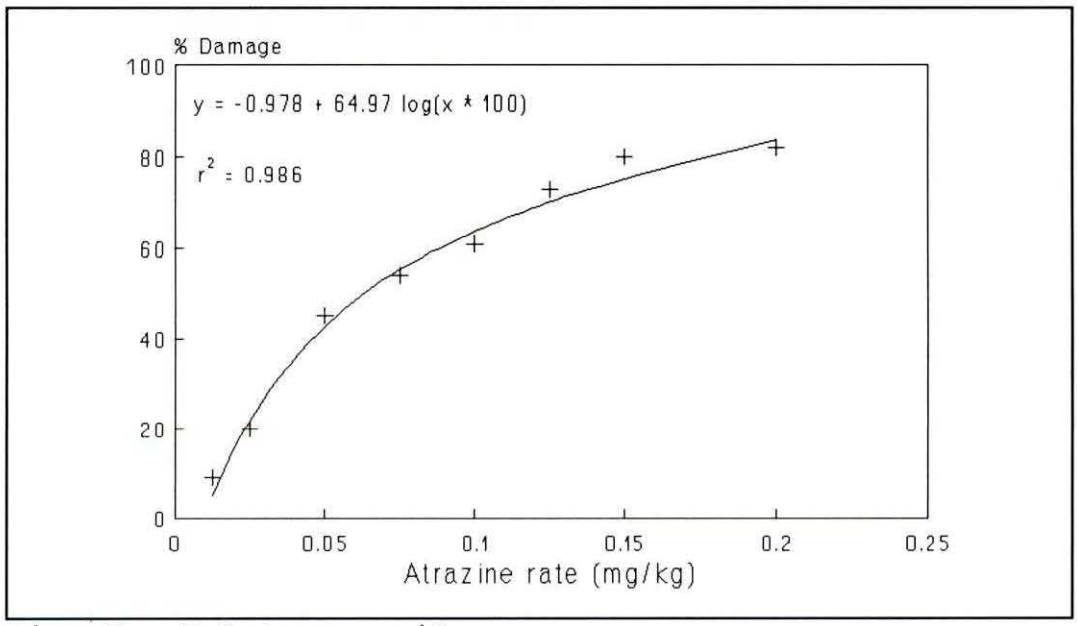

Fig. 7c Nylstroom soil

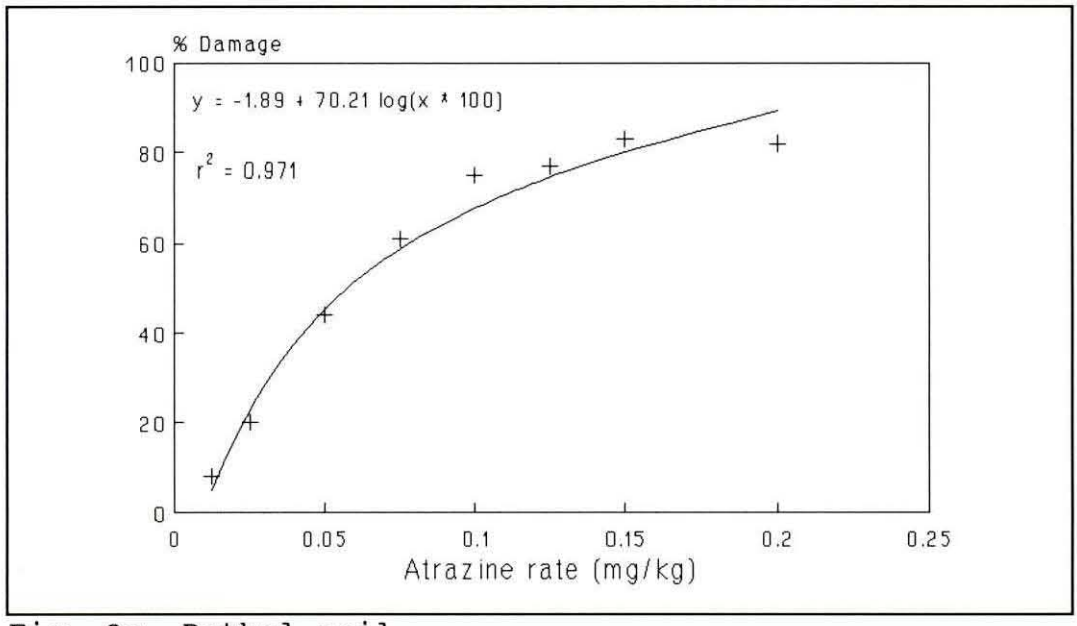

Fig. 8c Bethal soil

Figures 7C & 8C Dose-response to atrazine in the Nylstroom and Bethal soils

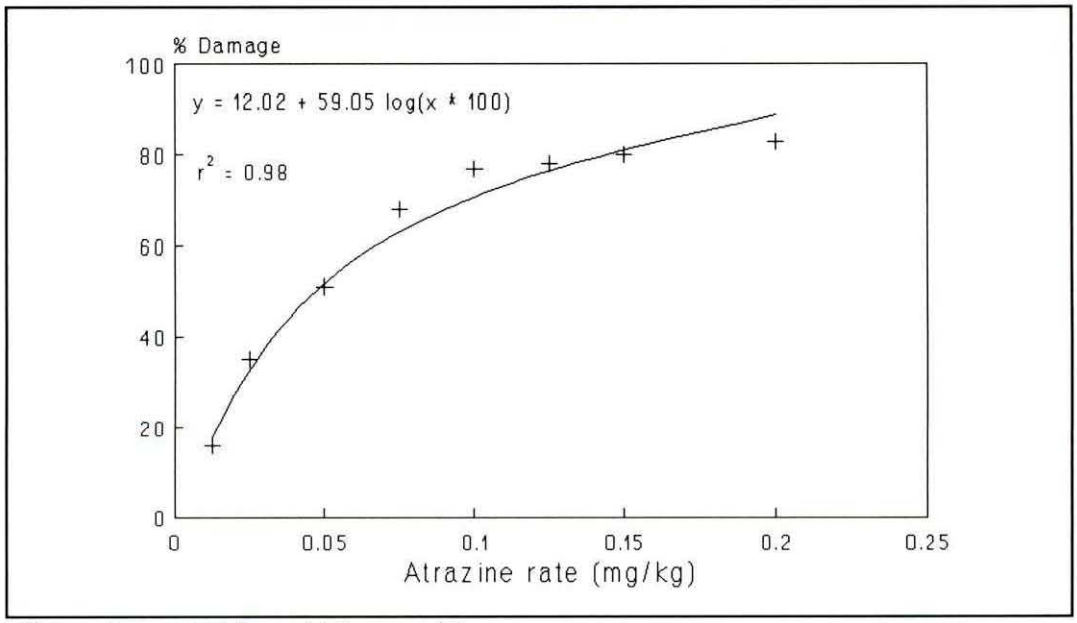

Fig. 9c Bothaville soil

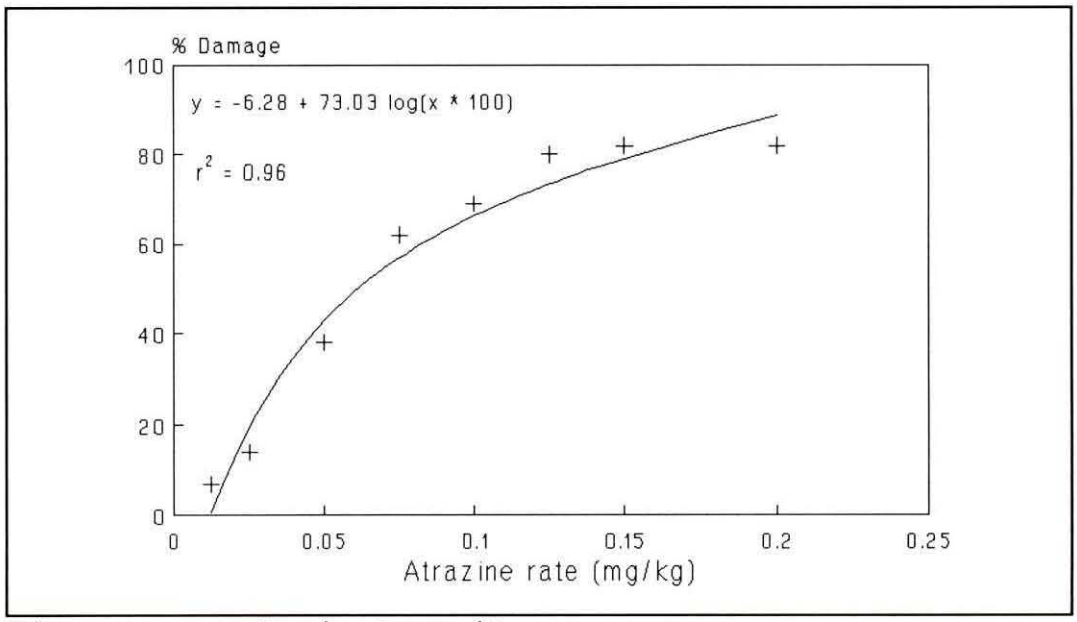

Fig. 10c Pretoria A1 soil

Figures 9C & 10C Dose-response to atrazine in the Bothaville and Pretoria A1 soils

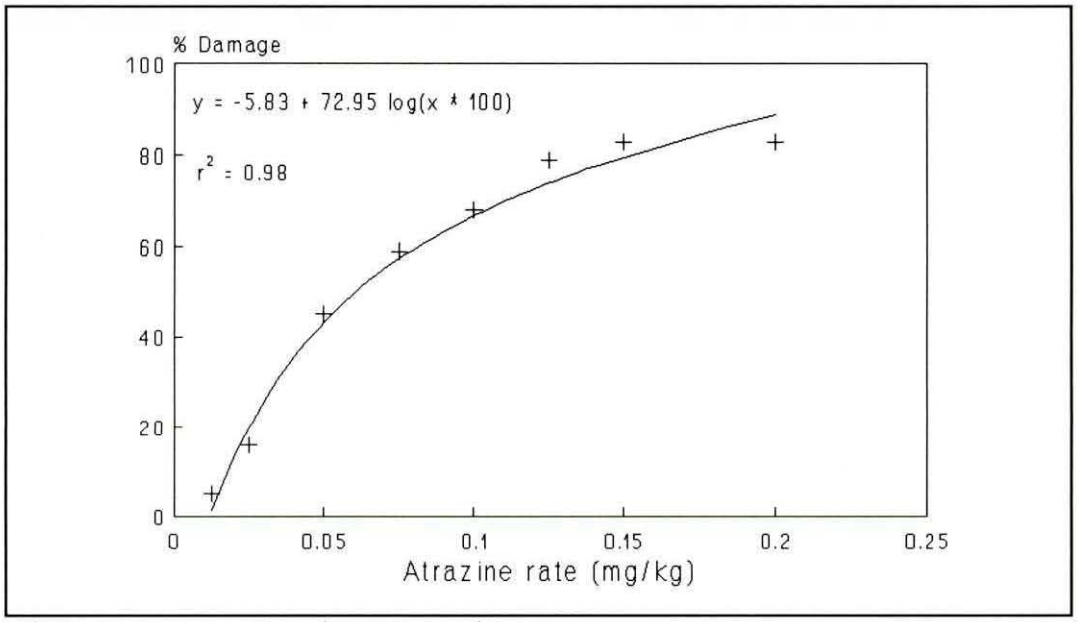

Fig. 11c Pretoria A2 soil

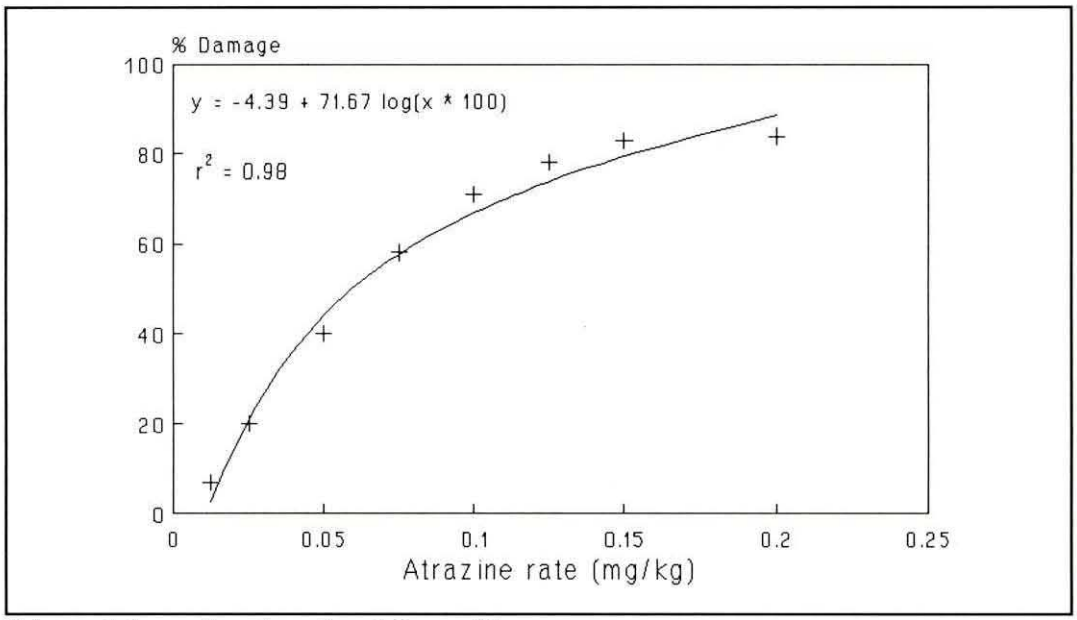

Fig. 12c Pretoria A3 soil

Figures 11C & 12C Dose-response to atrazine in the Pretoria A2 and Pretoria A3 soils

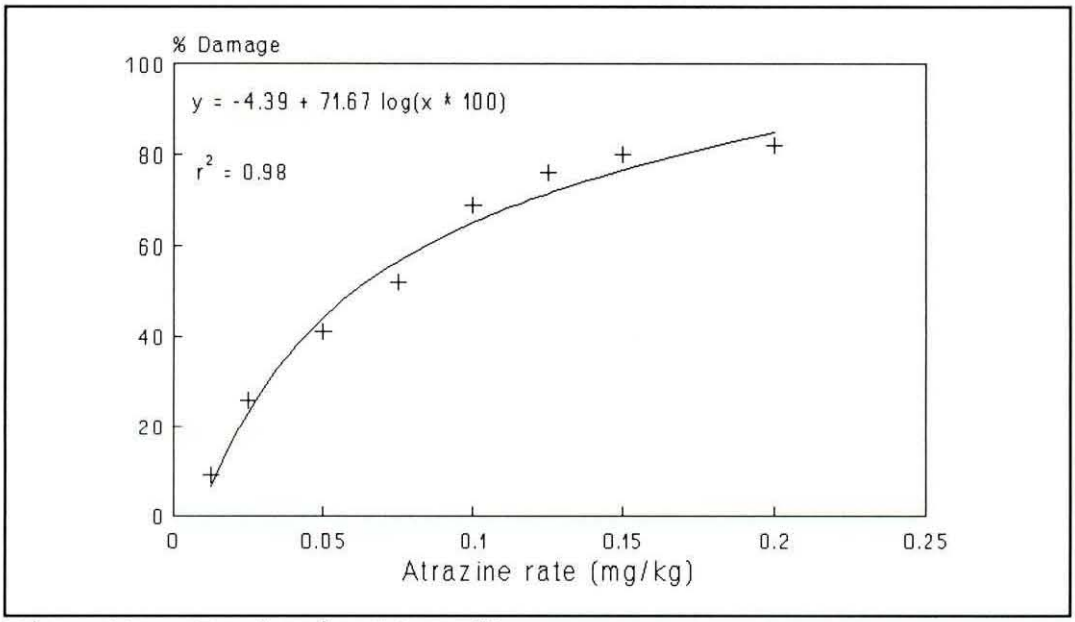

Fig. 13c Pretoria A4 soil

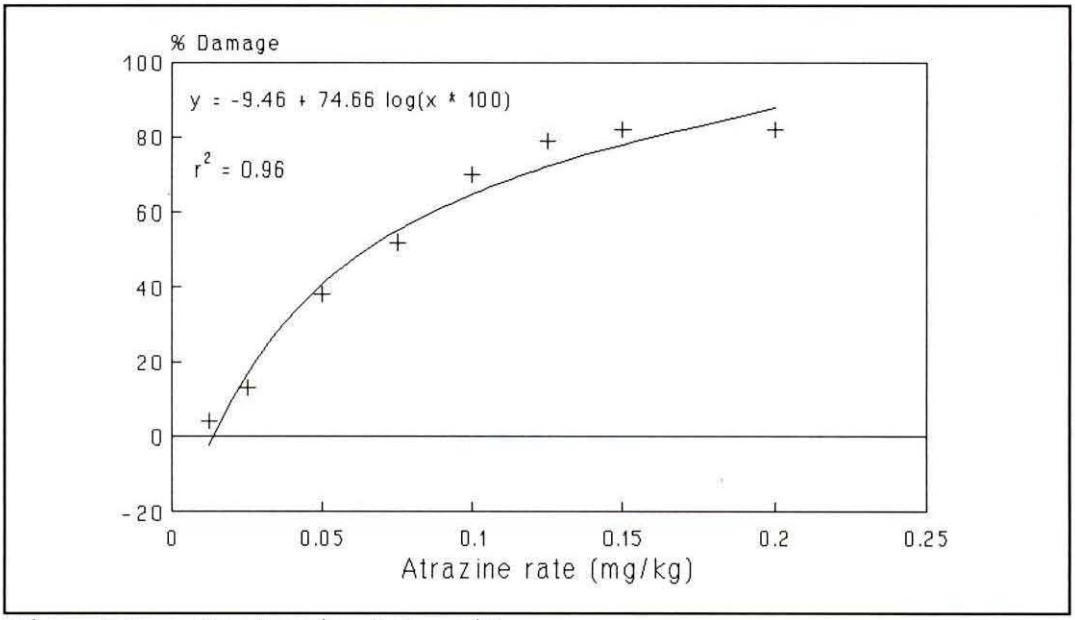

Fig. 14c Pretoria A5 soil

Figures 13C & 14C Dose-response to atrazine in the Pretoria A4 and A5 soils

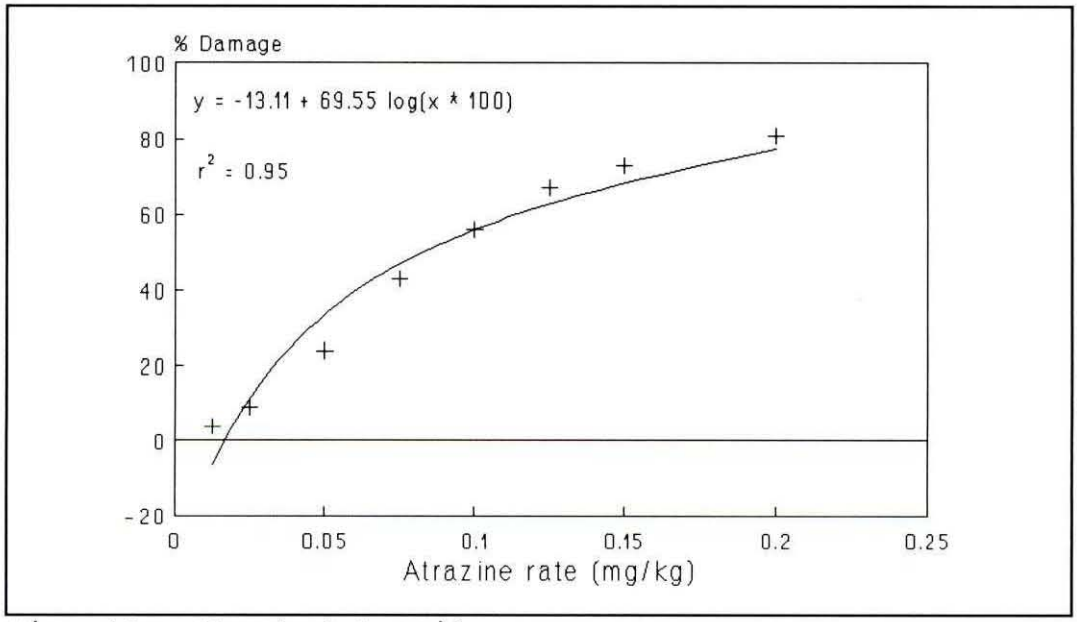

Fig. 15c Warmbad A soil

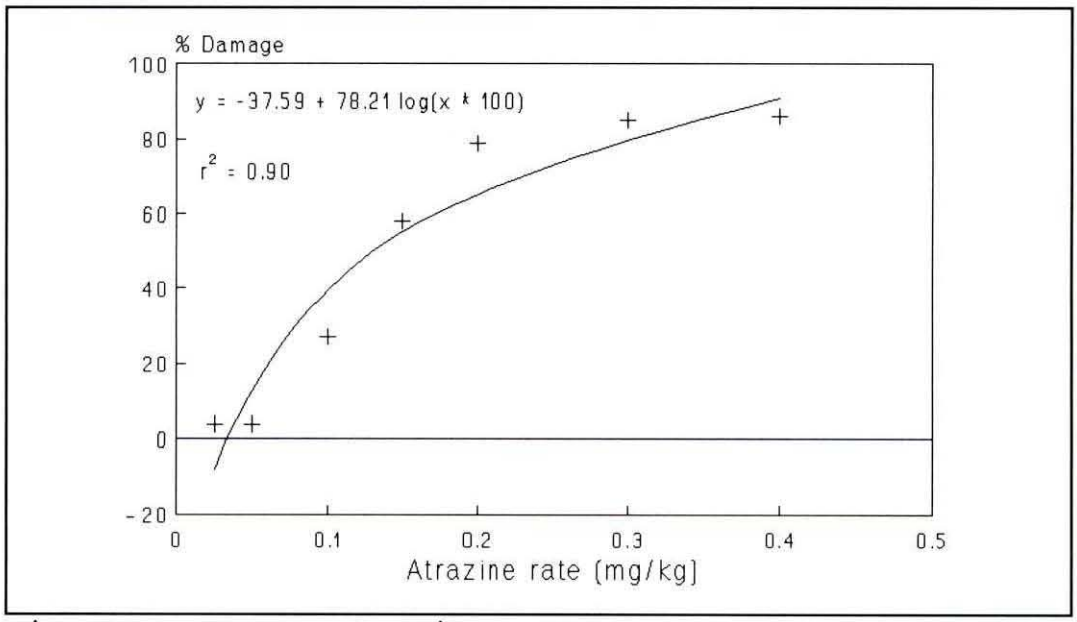

Fig. 16c Warmbad B soil

Figures 15C & 16C Dose-response to atrazine in the Warmbad A and Warmbad B soils

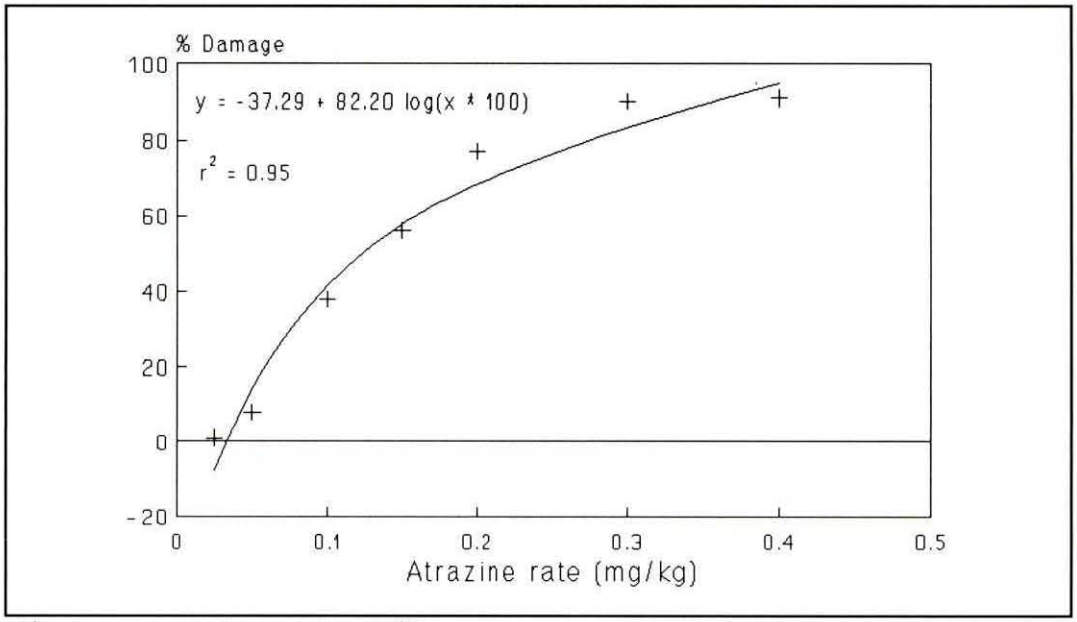

Fig. 17c Utrecht soil

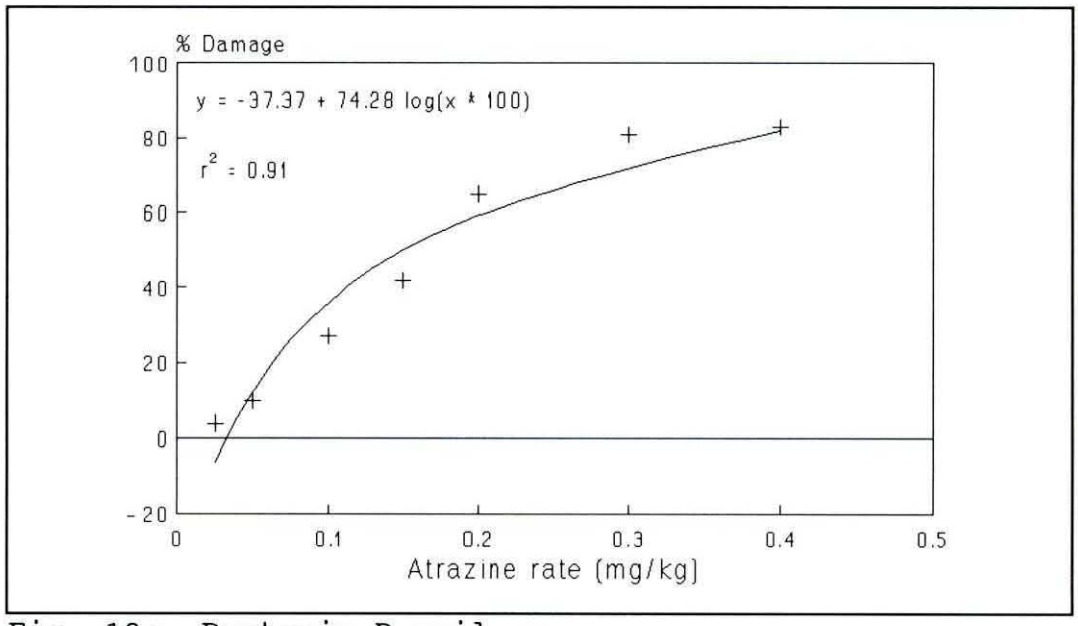

Fig. 18c Pretoria B soil

Figures 17C & 18C Dose-response to atrazine in the Utrecht and Pretoria B soils

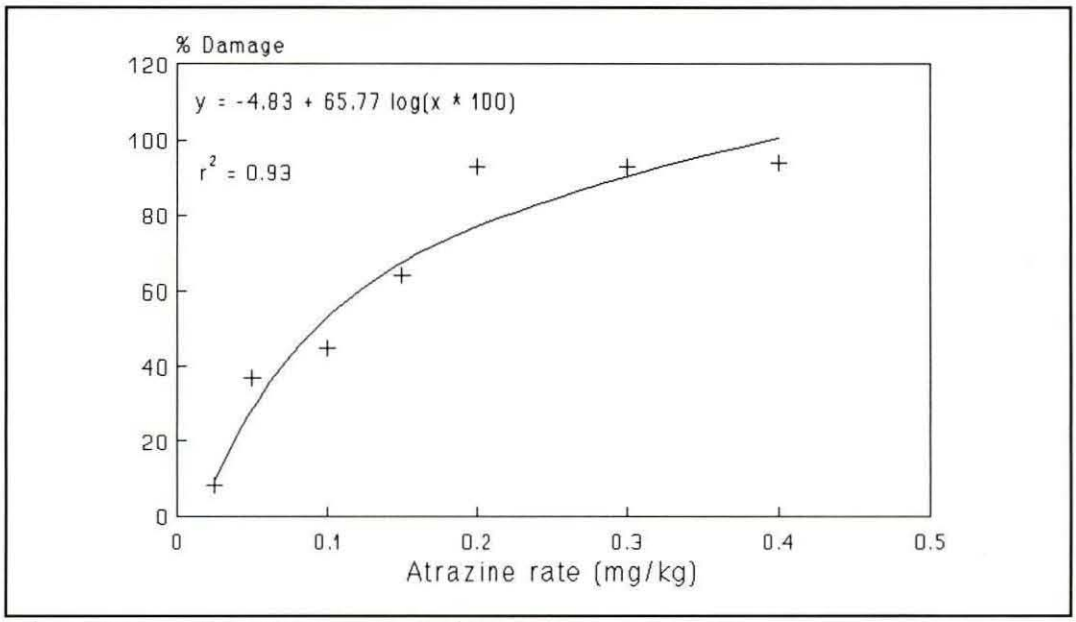

Fig. 19c Roodeplaat soil

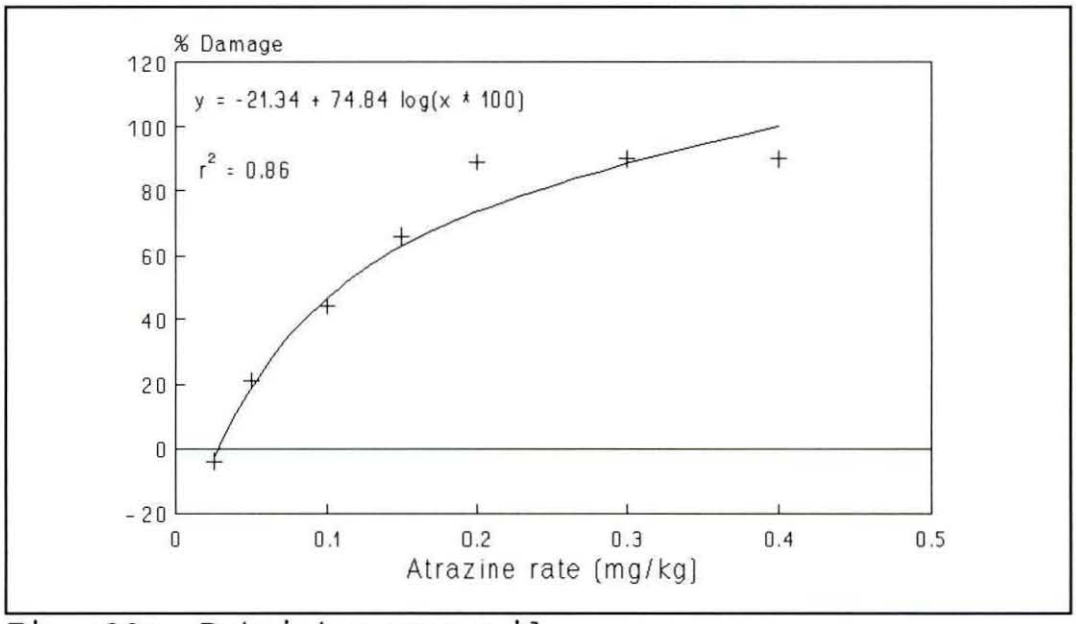

Fig. 20c Potgietersrus soil

Figures 19C & 20C Dose-response to atrazine in the Roodeplaat and Potgietersrus soils

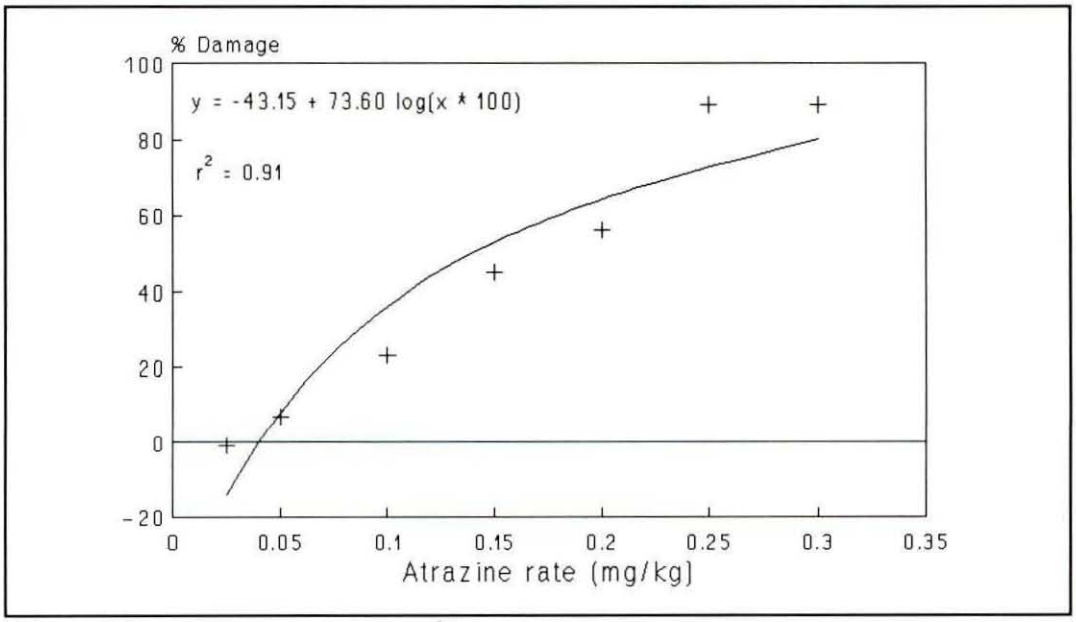

Fig. 21c Morgenzon soil

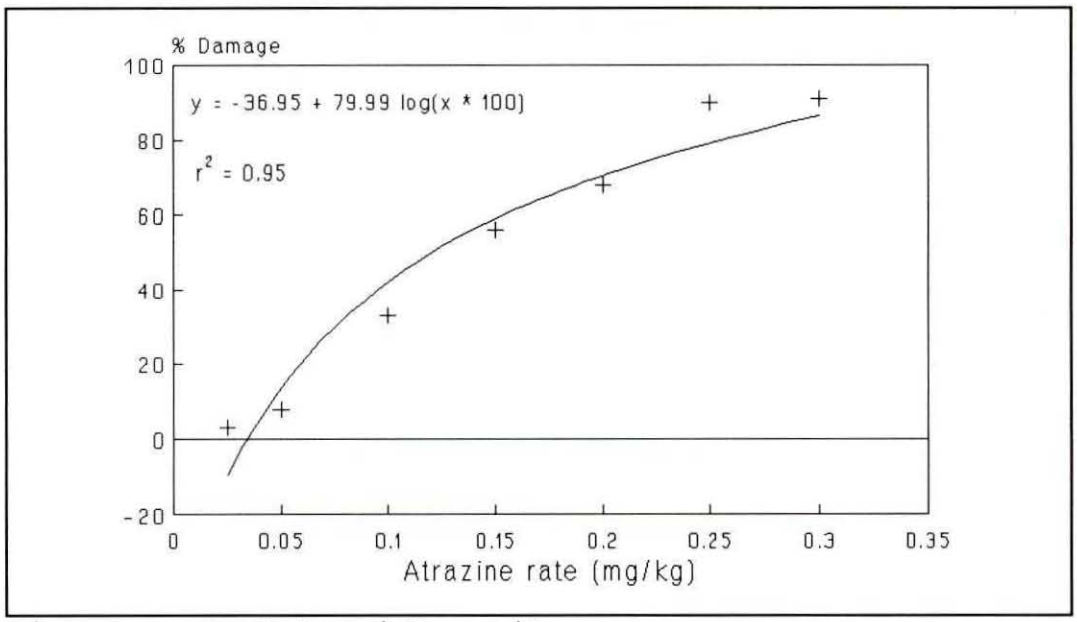

Fig. 22c Carletonville soil

Figures 21C & 22C Dose-response to atrazine in the Morgenzon and Carletonville soils

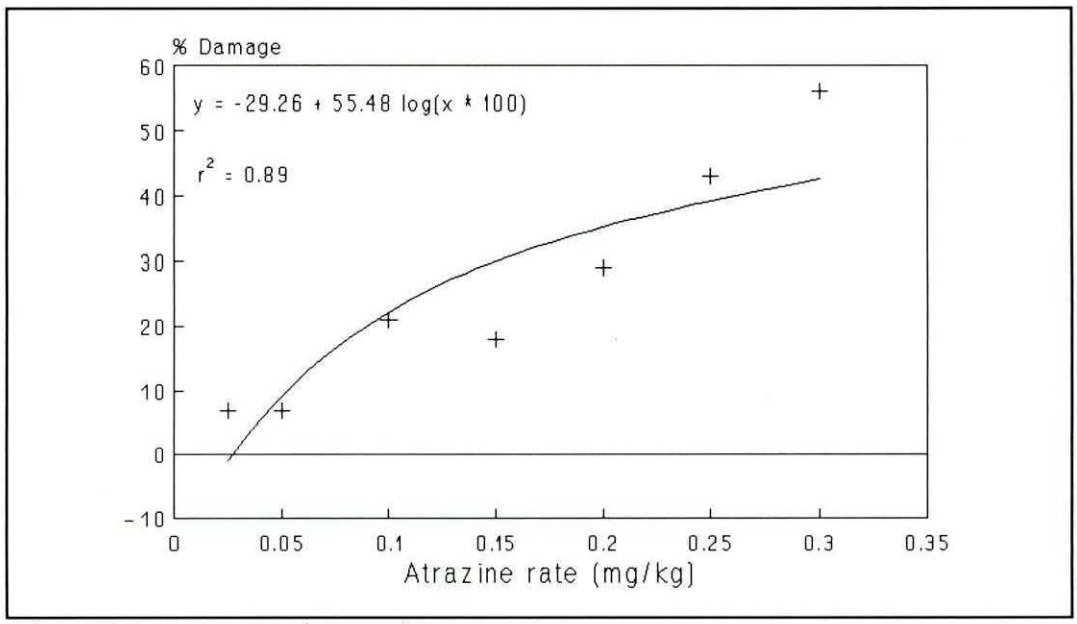

Fig. 23c Vryheid soil

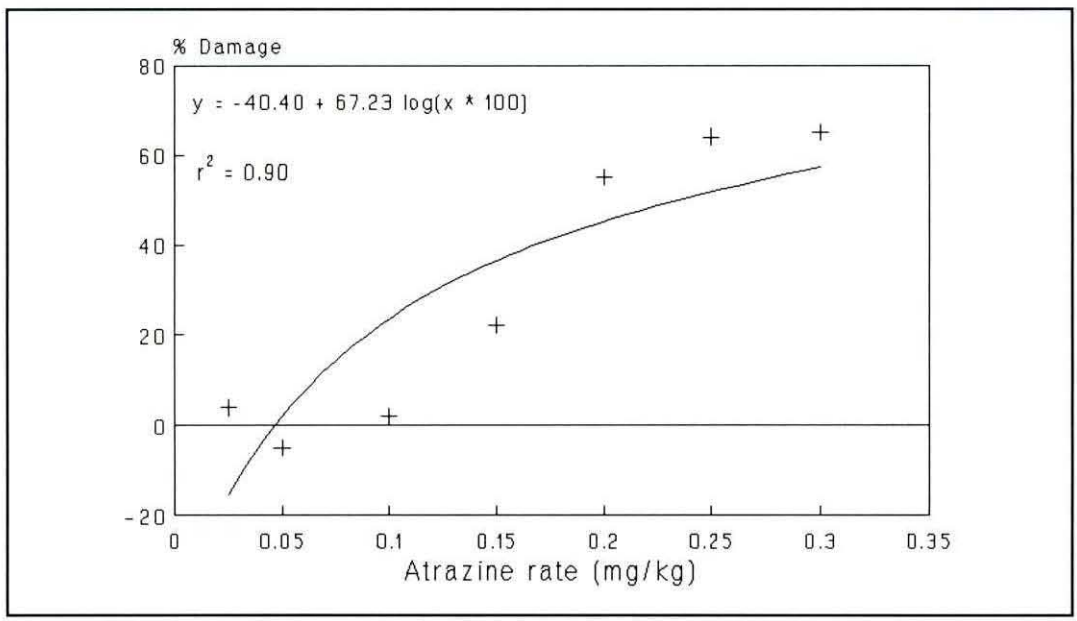

Fig. 24c Ermelo B soil

Figures 23C & 24C Dose-response to atrazine in the Vryheid and Ermelo B soils

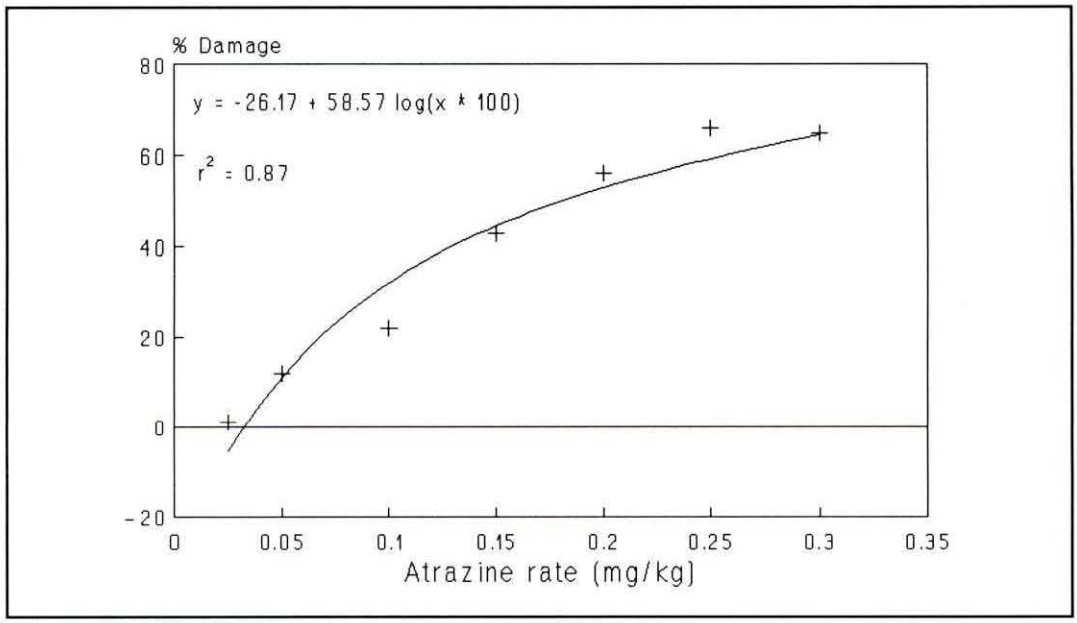

Fig. 25c Redhill soil

Figure 25C Dose-response to atrazine in the Redhill soil