TOLERANCE OF MAIZE TO ATRAZINE AND TERBUTHYLAZINE APPLIED POST-EMERGENCE

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Contents

Table of abbreviations

Abbreviation or Symbol	Explanation
DF	Degrees of freedom
F-Values	F-Values of analysis of variance
CV	Coefficient of variation
%	Percent
R^2	R –square
MS	Mean Squares
SDM	Shoot dry mass
SFM	Shoot fresh mass
RDM	Root dry mass
H	Herbicides
T	Temperature
LAI	Leaf area index
C	Cultivars
S	Stage
V/M	Volume per mass

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ABSTRACT

Recent reports of damage to maize seedlings where atrazine or atrazine/terbuthylazine was applied post-emergence again raised the issue of maize tolerance to triazines. The potential of atrazine or atrazine/terbuthylazine to injure maize when applied post-emergence was investigated under controlled conditions. Factors considered were growth stage, surfactants, cultivar tolerance, temperature and crop recovery from herbicide injury. The first experiment was conducted to determine the effect of atrazine and terbuthylazine on maize seedlings, and the second one was carried out to identify whether stage of maize development has an influence on atrazine or terbuthylazine phytotoxicity. In Experiment 1, herbicide treatments were applied at the 2-leaf stage of cultivar PAN 6043, and in Experiment 2 at the 2-leaf and 4-leaf stages. The third experiment was carried out to determine cultivar tolerance to atrazine or atrazine/terbuthylazine. The three cultivars used were CG 4526, PAN 6043 and PAN 6140. The fourth and fifth experiments were carried out in growth chambers where the role of temperature and cultivars were investigated. The temperature levels were 20/15 and 30/25 °C (day/night). Herbicide and temperature treatments were applied at the 2-leaf stage of cultivars CG 4526, CAN 3891, PAN 6043 and PAN

6140. Finally, an experiment was conducted to investigate the recovery of maize seedlings from the initial atrazine/terbuthylazine phytotoxicity. The cultivar PAN 6043 was used and the herbicide rates were the recommended and double the recommended rate. The rates of the herbicides and associated activity enhancers were 2.5 L ha^{-1} and 1 L ha^{-1} respectively. The following products were used: Atrazine 500 SC (485 g L^{-1} atrazine) + BP Agripon (940 g L^{-1} mineral oil); Gesaprim Super 600 SC (291 g L^{-1} atrazine and 291 g L^{-1} terbuthylazine) + AG Penetrex (363 g L^{-1}); Gesaprim Super 600 SC (291 g L^{-1} atrazine and 291 g L^{-1} terbuthylazine) + Complement (363 g L^{-1}).

Maize seedlings were tolerant when atrazine or atrazine/terbuthylazine was applied at the 4-leaf stage, but significant damage was caused when application was done at the 2-leaf stage. No significant differences were found between surfactants. Significant differences occurred with cultivars at specific temperatures. At the low temperature regime (20/15 °C), CG 4526 showed shoot dry mass (SDM) reduction of > 40 %, while the growth of PAN 6140 was reduced by less than 10 % by both herbicides. However, with an increase in temperature, CG 4526 was the most tolerant. It showed < 17 % reduction in SDM while others showed > 30 % reduction. It is suggested that the tolerance of maize (specifically at the two-leaf stage) to atrazine or atrazine/terbuthylazine is dependent on cultivar and temperature.

Although initial phytotoxic symptoms were visible several weeks after spraying, the growth inhibition of the atrazine/terbuthylazine-treated maize plants was transient. At five weeks after application, leaf area index (LAI) had recovered 100 % and 95 % at the recommended and double the recommended rate, respectively. Recovery in SDM and root dry mass (RDM) were similar, with 100 % recovery reached after eight weeks following application.

It was found that although growth stage plays an important role in the tolerance of maize seedlings when atrazine or atrazine/terbuthylazine is applied post-emergence, surfactants, cultivars, and temperature also have an influence on the tolerance of maize seedlings towards these herbicides.

Introduction

The s-triazine herbicide group dominates broadleaf weed control in maize in South Africa and most other countries. Of them, atrazine (2-chloro-4-ethylamino-6-isopropylamono-s-triazine) is cited as one of the most affordable, most widely used (Reinhardt, 1993; Rocha & Walker, 1993). It is also considered to be the safest herbicide in maize (Smit & Nel, 1977; Marshall *et al.*, 1982; Malan *et al.*, 1984; Nel & Reinhardt, 1984; Le Court De Billot, 1985; Le Court De Billot & Nel, 1985; Malan *et al.*, 1986; Reinhardt, 1993; 1994; Mersie *et al.*, 1998).

Despite all its positive attributes, atrazine phytotoxicity had been reported from the field during the 1981/82 and 1982/83 seasons by a number of farmers in large areas of the then western Transvaal and the northern and north-western Orange Free State (Le Court De Billot, 1985; Le Court De Billot *et al.*, 1986; and Malan *et al.*, 1986; Reinhardt, 1993). Stand losses ranging from 14 to 29 % and typical chlorosis symptoms were reported. Eventual yield loss was most probably due to stand losses (Malan *et al.*, 1984; Malan *et al.*, 1986). After several problem-free growing seasons, farmers in parts of the Free State in the 1997/98 season reported the same kind of damage that was seen during those earlier seasons. The most apparent difference between the most recent and historical events is that, in the case of the former situation, atrazine or atrazine/terbuthylazine was applied post-emergence, in combination with various activity enhancers. In earlier years the damage was associated with atrazine applied pre-emergence.

Since the first incidents of damage to maize, several factors were mentioned as possible explanations for the lack of selectivity of atrazine. Malan *et al.* (1986) mentioned temperature as a possible factor that led to reduced detoxification of atrazine in the maize plant. He reported that atrazine-treated maize plants died when kept under cold, wet conditions for 40h after the herbicidal spray was applied. Le Court De Billot (1985) attributed this lack of selectivity of atrazine to differential maize cultivar tolerances and Smit *et al.* (1979), Ehlers *et al.* (1988) and Reinhardt (1993) to soil and nutrition factors. In spite of fairly exhaustive research conducted in South Africa, mainly by the aforementioned researchers, the identification of factors or their interactions which could satisfactorily explain the extent of the damage encountered in the early 1980s have eluded us to this day.

Although problems of atrazine contamination of water resources have placed its continued use in jeopardy (Bowman, 1988; Grob & Li, 1989; Wood *et al.*, 1991; Sorenson *et al.*, 1993; Reinhardt, 1993), and despite the public's increasing awareness and interest about the behaviour of pesticides in general (Riley, 1991; Webber, 1991; Reinhardt, 1993; Walker, 1994), correct application of the herbicide at recommended or even lower dosages still present food producers in developing countries with great benefits.

In light of the above, a series of experiments were carried out to investigate atrazine activity on maize to try and explain the recent damage reported in the field. The broad aims were to identify factors that influence the selectivity of atrazine or atrazine/terbuthylazine in maize, and also to study the recovery ability of the maize

plants from triazine phytotoxicity. Knowledge about these factors should contribute towards the elimination of future problems of this nature. The following factors known to affect crop tolerance received specific attention: growth stage, surfactants, cultivars, temperature and crop recovery from herbicide injury.

Chapter 1

Literature review

1. Background to the triazines

This chemical group was synthesised by Pearlman and Banks in 1948 but was introduced as a herbicide by J.R. Geigy S.A. (now Norvartis) in the 1950`s (Fletcher & Kirkwood, 1982). Shortly after those major research efforts, world-wide field trials were carried out for producing the outstanding selective herbicidal properties of the s-triazines (Le Court De Billot, 1985). Talbert & Fletchall (1964) and Sikka & Davis (1965) reported their relative long persistence as compared to other herbicides, thus posing a risk to subsequent sensitive crops.

The group includes herbicides such as atrazine, simazine, prometon, propazine, and terbuthylazine to mention but a few. They are said to control a wide variety of mainly broadleaf weeds most effectively at the seedling stage (Muzik, 1970).

Of all the s-triazines, simazine was introduced first, followed by atrazine for which Sirons *et al.* (1973) and Le Court De Billot (1985) remarked on its dominance in controlling broadleaf weeds in South Africa and worldwide. For the purposes of this study only atrazine and terbuthylazine will be discussed.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is cited as the most important herbicide in the summer grain producing regions of South Africa (Nel & Reinhardt, 1984; Le Court De Billot *et al.*, 1989; Grob & Li, 1989), with many citing

it as the most widely used herbicide in maize production (Scot & Grogan, 1969; Smit & Nel, 1977; Le Court De Billot & Nel, 1985; Le court De Billot, 1985; Le Court De Billot *et al.*, 1986; Reinhardt, 1993) to control annual weeds in maize and sorghum (Shimabukuro & Swanson, 1969; Shimabukuro *et al.*, 1970).

Terbuthylazine (2-t-butylamino-4-chloro-6-ethylamino-1,3,5-triazine) was registered in 1966 for pre-emergence use in vineyards, citrus and apple orchards, and in sorghum. It provides good control of annuals and such perennials as *Cirsum arverse*, *Agropyron repens* and *Convolvulus* spp. In vineyards, its use reportedly does not result in detectable residues in the grapes (Niehuss, 1991).

2. Site of action

Fletcher & Kirkwood (1982) intimated that the inhibition of photosynthesis by triazines is well documented and has been demonstrated by many workers using a variety of techniques. Audus (1976) found that this inhibition of photosynthesis is associated with a reduction in the amounts of glucose, fructose, and/or sucrose in plants, which is what one would expect from a photosynthesis inhibitor. He further indicated that general growth inhibition and foliar chlorosis in some species treated with triazines are positively correlated and have assumed that they are causally linked to blockage of photosynthesis. Malan *et al.* (1984) stated that s-triazine herbicides act as inhibitors of the Hill-reaction at the reducing side of Photosystem II.

Several scientists have reported the effect of atrazine on chloroplasts. Audus (1976) investigated the effect of atrazine on mature, fully expanded primary leaves of bean

(*Phaseolus vulgaris* L.). He found that the chloroplasts were markedly changed in the light in contrast to those kept in the dark, which did not show any changes. Malan *et al.* (1984) indicated that mesophyll chloroplasts of maize lines sensitive to atrazine were damaged (broken, rendering them non-functional).

Generally, triazine herbicides are known to affect the synthesis of chlorophyll and other pigments in addition to affecting the light and dark reactions of photosynthesis (Muzik, 1970). Audus (1976) reported other effects of atrazine, such as the stopping of cambial activity and a reduction in the thickness of cell walls of sieve and tracheae elements in the plant stems.

Audus (1970) attributed the complete (barley) or partial (maize) inhibition of net photosynthesis to an inhibition of the photosynthetic reactions in which Fletcher & Kirkwood (1982) suggested that a stomatal closure mechanism is the determining factor in the selectivity of atrazine to maize.

3. Uptake and metabolism of atrazine

Fletcher & Kirkwood (1982), Stephens (1982) and Nel & Reinhardt (1984) indicated that atrazine is both foliar- and soil-acting through being taken up both by leaves of emerged weeds and the roots of weed seedlings emerging after spraying. Ashton & Monaco (1992) indicated that its translocation is acropetal in the xylem and that it accumulates in leaves of susceptible as well as tolerant plants.

In tolerant plants, atrazine is reported to be inactivated through three metabolic pathways, namely, hydroxylation, N-dealkylation and conjugation (Eastin *et al.*, 1963; Malan *et al.*, 1981; Malan *et al.*, 1984; Nel & Reinhardt, 1984;). Muzik (1978) attributed triazine selectivity to the transformation of the molecule by resistant plants, partly by the removal of a chlorine atom. Shimabukuro & Swanson, (1969) and Shimabukuro *et al.* (1970) indicated that glutathione conjugation occurs primarily in the leaves of plants when atrazine is foliarly applied, whereas detoxification by hydroxylation predominates when atrazine is absorbed through the roots.

Susceptible plants' light reaction of photosynthesis is inhibited by triazines and this is linked to a decline in carbon dioxide fixation (Nel & Reinhardt, 1984). They also postulated that stomatal closure on sensitive plants causes transpiration inhibition, owing to an accumulation of carbon dioxide in the stomatal regions. Furthermore, both Nel & Reinhardt (1984) and Le Court De Billot (1985) indicated photosynthesis as the process most sensitive to atrazine, with the chlorophyll and carotene contents of plants decreasing in light when disintegration of chloroplasts occur. As a consequence, leaves become chlorotic (Malan *et al.*, 1984; Malan *et al.*, 1986; Le Court De Billot, 1985; Le Court De Billot *et al.*, 1986), which according to Audus (1970), later turns necrotic as the effect progresses. The leaf symptoms commonly associated with atrazine phytotoxicity are veinal chlorosis of the lower leaves decreasing in intensity at subsequent leaves (Le Court De Billot, 1985).

4. Basis of selectivity

Herbicides are chemicals that kill plants; but if they killed all plants, they would be of very limited value to agriculture. Thus, it is the ability of herbicides to kill certain plants without appreciable harm to others that makes them useful, and this characteristic is called selectivity (Anderson, 1983). Ashton & Monaco (1992) and Klingman *et al.* (1982) indicated that a herbicide is selective to a particular crop only within certain limits. These limits are determined by a complex interaction between plants, the herbicide itself and the environment - with Anderson (1983) indicating that selectivity is not an absolute but a relative characteristic as it is dependent on many interrelated factors.

This makes it apparent that three aspects are working interrelatedly in bringing about the concept of selectivity, namely, the role of the plant (plant factors), the role of the herbicide itself and the role of the environment (climatic and edaphic factors).

4.1. Role of the plant

4.1.1. Stage of plant growth

Plant growth stage plays a very important role as far as the activity of certain herbicides is concerned. Anderson (1983) indicated that plants are basically most susceptible to foliar-applied herbicides when they are in the seedling stage, and that susceptibility tends to decrease as the plants grow older. However, certain plants get killed only at specific growth stages. For example, wild oat plants are killed by foliar application of barban only if the herbicide is applied at the 1.5- to 2-leaf stage (until

the third leaf first appears); similarly diclofop only kills them when applied during the 1.5- to 4-leaf stage of growth (Fletcher & Kirkwood, 1982).

4.1.2. Growth rate

The growth rate of the plant has a pronounced effect on their reaction to some herbicides. In general, fast growing plants are more susceptible than are slow growing plants. Ashton & Monaco (1992) reported exceptions to this rule stating stress as the cause. They indicated that a normally tolerant crop can be damaged by an otherwise non-injurious herbicide treatment, and conversely, a normally susceptible plant whose growth has been limited by stress conditions can be more tolerant to herbicide injury.

4.1.3. Cultivars

According to Le Court De Billot & Nel (1985) and Le Court De Billot (1986), differential cultivar response has been shown to exist for a number of maize cultivars and other crops to various herbicides as indicated in Table 1.

Table 1: A summary of differential crop and cultivar responses to atrazine and other herbicides (Le Court De Billot, 1985)

Crop	Herbicide	Reference
maize	atrazine	Hamilton (1964)
		Anderson (1964)
		Palmer & Grogan (1965)
		Eastin (1971)
		Voges & Nel (1973)
		Le Court De Billot (1979)
maize	alachlor	Narsaiah & Harvey (1977)
		Francis & Hamill (1980)
maize	butylate	Wright & Rieck (1973)
maize	cyanazine	Le Court De Billot & Nel (1979)
maize	2,4- D	Rossman & Staniforth (1949)
maize	trifluralin	Davis, Abernath & Wiese (1978)
cotton	atrazine	Abernath, Keeling & Ray (1979)
cucumber	atrazine	Werner & Putman (1980)
flax	atrazine	Comstock & Anderson (1968)
grain sorghum	atrazine	Burnside & Wicks (1972)
rapeseed	atrazine	SouzaMachado & Bandeen (1982)

Malan *et al.* (1984) reported on atrazine-susceptible maize lines, and confirmed these differential cultivar responses. However, Jensen (1982) and Arntzen *et al.* (1982) assumed that the differential response might be due to differences in uptake, translocation, metabolism or alterations at the site of the herbicide action. For example, Le Court De Billot (1985) reported a maize mutant low in benzoxazinone which is a catalyst for one of the three degradation processes occurring in maize, that was particularly susceptible to atrazine.

4.1.4. Growth points

Exposure, or lack thereof, of the growth points (buds and region of cell elongation) of plants to contact herbicide sprays are often vital to herbicide-plant selectivity. Grasses have the initial growing points located at the base of the plant and is protected by the surrounding leaves whilst the growth points of broad-leaf plants are directly exposed to the chemical spray (Ashton & Monaco, 1992). This difference in exposure can be used to full advantage in the selective control of broad-leaved weeds growing amongst grass crop plants (Fletcher & Kirkwood, 1982).

4.1.5. Inheritance

The genetic complement of the plant determine its capacity to respond to its environment. These responses vary from genus to genus, but, within a genus, plant reactions to a given herbicide tend to be similar (Ashton & Monaco, 1992). This inherited ability differs, with poor herbicide selectivity resulting either from incorrect application or adverse environmental conditions. Resistance is the inherited ability of a weed to survive a rate of herbicide which would normally give effective control

(Moss & Clarke, 1993). With the 1,2,3-triazine herbicides, resistance will involve either a reduction in the concentration of the herbicide at the binding site or a change in the molecular structure of the binding site. Resistant biotypes to triazines have an altered structure and composition of a thylakoid membrane protein. The gene which encodes the binding site protein mutates so that the nucleotide sequence of the chloroplast DNA of a resistant biotype differs from a susceptible one by a single amino acid substitution. Therefore, photosynthesis in the former biotype can not be inhibited. Examples of such resistant weeds are redroot pigweed (*Amaranthus thunberghii*), common ragweed (*Ambrosia sp.*), common groundsel (*Senecio vulgaris*), etc.

4.2. The role of the herbicide

4.2.1. Concentration / Herbicide dosage

The dosage may determine whether the herbicide inhibits or stimulates metabolism and growth of the plant. Anderson (1983) indicated that the amount of the chemical absorbed is a critical factor with regard to its subsequent activity in the plants. He further reported that herbicides like s-triazines, which were first introduced as soil sterilants, were lethal to almost every crop at high dosages of about 20 to 40 kg or more per ha, but were found to be selective at dosages of 2.5 to 5 kg per hectare in alfalfa (*Medicago sativa* L.) and cotton (*Gossypium hirsutum*).

Malan *et al.* (1986) indicated differential loss of fresh mass accumulation of roots and shoots at 1.25 L ha^{-1} and 5 L ha^{-1} atrazine-treated maize plants. The growth reduction in shoots or roots amounted to 20 to 30 % and 50 to 60 % respectively. The same

principle apply to foliar-applied herbicides. When applied at comparatively high dosages, they are toxic to most plants, i.e. both weeds, and crops but selectivity may be achieved by applying a suitable low dosage (Ashton & Monaco, 1992).

4.2.2. Herbicide formulation

The formulation of the herbicide is vital in determining whether it is selective or not with regard to a given species. Stephens (1982) indicated that herbicide selectivity can be increased by the use of specific protectants or antidotes, several of which are in use in maize and sorghum crops where they make it possible to use a herbicide giving some control of tropical nutsedges.

In addition, Anderson (1983) indicated that selectivity might be through the use of granular formulations of herbicides for the control of non-emerged weeds among crop plants. Such selectivity is based on the prevention of foliar retention and absorption of the herbicide by the emerged plants while channelling the herbicide to the soil where it becomes available for absorption from the soil by emerging weed seedlings. The most striking example is the granular form that permits the herbicide to ``bounce off'' the crop and fall to the soil. However, the inclusion of surfactants as ingredients of the formulation generally results in a reduction or loss of selectivity of foliar-applied herbicides (Klingman, 1961).

4.2.3. Surfactants

The name is derived from "surface active agents" which are chemicals that reduce surface tension of water or increase its wettability. The WSSA (Weed Science Society

of America) defines them as materials "that facilitate and accentuate the emulsifying, dispersing, spreading, wetting or other surface-modifying properties of liquids".

According to Valkenberg (1967) a pure herbicidal compound placed on a plant surface would exhibit only a small percentage of its potential herbicidal activity. Cottrell (1987) reported that the activity of a foliage-applied herbicide must ultimately depend on the concentration of the active ingredient that reaches the sites of action together with the effect of the herbicide on the biochemical mechanisms taking place at these sites. He further indicated that a number of interrelated factors might influence its uptake and movement, including the efficiency of cuticle retention and penetration, as well as translocation which could only be accomplished by the inclusion of the surface-active agent.

Klingman (1961) reported that surfactants generally intensify the action of the herbicide to such an extent that in certain cases selectivity of the herbicide is lost. This was intimated by Cottrell (1967) who found that surfactants are used at low concetrations but can increase the activity of the herbicidal spray, hence largely reflecting reductions in surface or interfacial tension of the spray solution.

There are three types of surfactants, namely, anionic (ionizes in water, and these groups lend a large amount of water solubility to a molecule), cationic and non-ionic (there are no ionizable polar groups but the molecule is comprised of hydrophilic and lipophilic segments). According to Valkenberg (1967), the biological activity of a herbicide is affected by whatever other chemicals the herbicide contacts and of all the

chemicals that affect activity, surfactants have the greatest impact. He further indicated that they function primarily by altering solubility relationships which may either enhance or decrease efficacy. The effectiveness of the compound may be influenced five- or ten-fold (Valkenberg, 1967), which in certain cases results in selectivity being lost (Klingman, 1961).

4.3. Environmental factors

According to Le Court De Billot & Nel (1985) and Reinhardt & Nel (1992), the relative patterns of some maize cultivars may alter with differing environmental and soil conditions. They went on to mention some of these factors, namely, temperature and nutrient status. Nel & Reinhardt (1984), Reinhardt & Nel (1986) and Reinhardt (1993) regarded soil as the complex medium in which biological, chemical and physical factors such as micro-organisms, texture, pH are important variables as they interact to determine the degradation rate of a particular compound. Jennies *et al.* (1977) confirmed that under field conditions, a plant's response is influenced, among other things by climatic conditions as well as soil factors.

4.3.1. Temperature

Temperature affects herbicide activity in various ways, often interrelated with other environmental factors. Reinhardt (1993) indicated that temperature affects the bioactivity of atrazine by influencing its adsorption in the soil, absorption by plants, and ultimate fate in plants. The temperature at which a plant is growing has a considerable influence on the rate of its physiological and biochemical reactions. The selectivity of various plants to herbicides also varies as the temperature differentially

affects their life processes. The effect of temperature on these processes is often expressed as the Q_{10}- coefficient. An Q_{10} of 10 means that the rate of physiological processes are doubled with an increase of 10 °C. Most reactions of herbicides that influence plant growth are chemical in nature. Therefore, a change from 15°C to 25°C may result in the doubling of the herbicidal effect. Malan *et al.,* (1986) identified temperature as the main contributing factor to atrazine-induced growth inhibition and decreased atrazine hydroxylation.

Le Court De Billot (1985) and Le Court De Billot *et al.* (1985) reported that the temperature which plants are exposed to might change the relative tolerance of at least some cultivars. In their study, some cultivars that were the most tolerant under a high temperature regime were the most sensitive under a low temperature regime. Apart from those for which their tolerance changed with temperature, there also were others that retained their relative tolerance positions under both temperature regimes.

Although for some of the cultivars, tolerance was not affected by temperature, the conclusion is still valid that an increase in ambient temperature, within the range 10 to 30 °C, enhances the phytotoxicity of s-triazine herbicides. Although this enhanced phytotoxicity is often attributed to increased herbicide uptake, Penner (1971) and Marshall *et al.* (1982) suggested that increased herbicide transport from root to shoot is involved.

Apart from an increase in temperature, Malan *et al.* (1986) intimated that a drastic change in temperature shortly after atrazine application could change the tolerance of maize plants. He found that atrazine-treated maize plants died when kept under cold, wet conditions for 48h after treatment, followed by exposure to high light intensity and high temperature.

4.3.2. Light

Light intensity is cited as one of the role players as far as enhanced phytotoxicity is concerned. Audus (1970) gave an example where he found increased terbutryne toxicity with an increase in light intensity from 11,000 to 22,000 lux. This was also confirmed by Malan *et al.* (1986) who indicated high light intensity as one of the factors that led to the death of atrazine-treated maize plants.

4.3.3. Soil moisture and nutrition

Generally, soil type (which will be discussed later) and amount of rainfall determine the actual location of the herbicide in the soil. The intensity with which the herbicide is held by the soil particles will strongly affect movement or lack of movement in the soil. Nel & Reinhardt (1984) indicated that atrazine adsorption increases with a decrease in the soil moisture content owing to the increase in atrazine concentration and the fact that the polar atrazine molecules can compete more effectively with fewer water molecules for sorption sites. Audus (1970) indicated a 1.5-fold increase in atrazine activity when moisture levels of 25 to 50 % of field capacity were raised to 75 to 100% of field capacity.

Some mineral elements have been linked to atrazine phytotoxicity on maize. Penner (1971) indicated growth reduction of maize seedlings through exposure to combinations of high atrazine and phosphorous concentration in the solution owing to increased respiration and reduced net photosynthesis. This was however, disputed by Reinhardt & Nel (1992) who indicated that high phosphorus concentration alone does not sensitise the plants to atrazine. They found significant growth inhibition where 310 and 403 mg P L^{-1} were used in combination with a 12:3 NH_4^+: NO_3^- N ratio.

4.3.4. Edaphic factors

Soil-applied herbicides are affected by several factors that include: (i) chemical decomposition, (ii) photochemical decomposition, (iii) microbial decomposition, (iv) plant uptake, (v) adsorption, (vi) volatility, and (vii) leaching (Ashton & Monaco, 1992). These are the factors that are involved in the withdrawal of the chemical from the soil solution, thereby rendering it temporarily unavailable for uptake by plants. Soil colloids, pH or both influence all of the above factors. Interactions between these two factors and climatological factors take place.

Soil colloids are microscopic (1 micron or less in diameter) inorganic or organic particles in the soil as defined by Ashton & Monaco (1992). Inorganic colloids are clay particles which could be divided into 1:1 and 2:1 clays (kaolinite and montmorillonite respectively). Though many researchers (Reinhardt *et al.*, 1990; Van Biljon, 1991) cited organic matter as the main constituent determining the leaching and bioactivity of soil-applied herbicides, and hence, soil organic matter should be used for making herbicide rate recommendations for pre-emergent herbicides (Strek

et al., 1990). High clay content also presents some influence on the availability and activity of soil-applied herbicides. It is due to those influences on herbicide adsorption that soils high in either of the two colloidal types require increased rates for a given level of herbicidal activity (Talbert & Fletchall, 1961; Ehlers *et al.*, 1987).

Alleman (1993) argued that high clay and organic matter amongst other things limit the biological activity of alachlor. However, clay content alone does not represent all the influence but clay mineral composition does play a determining role (Reinhardt & Nel, 1993 and Reinhardt, 1993). They indicated that 2:1 type clay, montmorillonite allows water and chemicals to enter the interlayer regions, thus affording this clay type much greater propensity for binding herbicides than kaolinite, a non-expanding 1:1 clay type. Smit & Nel (1977) indicated that, of the clay minerals, the 2:1 clay type minerals was the most determining factor in the degradation of atrazine in various soil types at a given temperature.

Cation exchange capacity plays a very important role in this difference between 2:1 and 1:1 clays. Smit & Nel (1977) found differences in activity of atrazine in two loamy sand soils (Pretoria and Glen loamy sand) at the same pH. The Pretoria loamy sand has very limited adsorptive capacity because of its low CEC and low surface area. In the Glen loamy sand, where 2:1 minerals that have high CEC and which could expand, dominated, availability of atrazine for absorption was reduced.

Apart from the above, Smit *et al.* (1979) indicated that the higher incidence of atrazine phytotoxicity on maize on red-brown and yellow-brown soils of the western maize

production regions of South Africa, compared to the soils of the Highveld, can possibly be attributed to the greater presence of the (Fe-Al-OH)-component in the latter soils. Soil pH also determines the adsorptive capacity of certain herbicides to soil colloids (Smit & Nel, 1977). Ashton & Monaco (1992) contend that basic herbicides such as the triazines can become cations at low pH and adsorb to soil particles. This according to them explains why s-triazine herbicidal activity is greater in high pH (basic) soils than in acidic soils.

Chapter 2

Tolerance of maize to atrazine or atrazine/terbuthylazine

Introduction

Though regarded as a very safe herbicide in maize (Smit & Nel, 1977; Nel & Reinhardt, 1984; Le Court De Billot, 1985; Reinhardt, 1993; Reinhardt, 1994), periodic damage caused by atrazine to maize have been reported from the field during the 1981/82 and 1982/83 seasons by a number of farmers in the former western Transvaal and the northern Orange Free State (Le Court De Billot, 1985 and Reinhardt, 1993).

Several investigations have been done to solve the problem, without much light being shed on what the cause could be. Numerous factors were mentioned as possible explanations for the lack of selectivity of atrazine in those specific regions at that time. Le Court De Billot (1985), Le Court De Billot & Nel (1985) and Le Court De Billot *et al.* (1986) cited differential cultivar responses, while Le Court De Billot (1978), Malan *et al.* (1986) and Reinhardt (1993) cited temperature (in particular a lowering in temperature) as the factors that were involved. Nel & Reinhardt (1984) implicated several soil factors, while Le Court De Billot (1985) and Reinhardt & Nel (1985) suggested that nutritional factors were also involved.

Nevertheless, similar kinds of damage was noted by some farmers in the Free State

in maize during the 1997/98-season where atrazine and terbuthylazine were applied post-emergence in combination with activity enhancers.

Two experiments were carried out using soil collected from the Hoopstad district in the Free State from where inadequate selectivity of the herbicides had been reported. The aim of these experiments were: (a) to determine the effect of atrazine and terbuthylazine on maize seedlings, (b) to identify whether maize growth stage has an influence on atrazine and terbuthylazine phytotoxicity, and (c) to determine whether a safe stage can be identified for the post-emergence application of atrazine or atrazine/terbuthylazine in combination with activity enhancers.

Experiment 1

Tolerance of maize to atrazine or atrazine/terbuthylazine applied in association with activity enhancers at the 2-leaf growth stage

Materials and Methods

In a preliminary experiment, where atrazine or atrazine/terbuthylazine was applied without activity enhancers, seedlings did not respond at all to both herbicide treatments. Therefore, in subsequent experiments, these treatments (herbicide/s alone) were not tested again.

A pot experiment was carried out in a greenhouse at the Hatfield experimental farm of the University of Pretoria. One maize cultivar was used, namely PAN 6043. Five

seeds were planted at a depth of 5 cm in 3 kg of a sandy soil contained in round plastic pots. The soil used consisted of 71.5% sand, 8.2% silt and 17.1% clay, with 0.4% organic matter and a pH of 6.5. Plastic bags were used to prevent contamination of the pots. Pots were weighed after every two days to return soil water content to the original levels (10 % field capacity). After emergence, plants were thinned to three per pot to reduce intra-species competition.

Atrazine Super 500 SC or Gesaprim 600 SC was foliarly applied at the two-leaf stage of maize seedlings.

The treatments* were:

1. Control (0 herbicide)

2. Atrazine 500 SC (495 g L^{-1} atrazine) + BP Agripon (940 g L^{-1} mineral oil)

3. Gesaprim Super 600 SC (291 g L^{-1} atrazine + 291 g L^{-1} terbuthylazine) + AG Penetrex (363 g L^{-1} mineral oil).

4. Gesaprim Super 600 SC (291 g L^{-1} atrazine + 291 g L^{-1} terbuthylazine) + Complement.

*[Rates for all treatments were 2.5 L ha^{-1} herbicide + 1.0 L ha^{-1} activity enhancer]

The application rates were calculated and applied on an area basis over the tops of the seedlings in pots placed in a 1 m^2 area. Prior to application all pots were watered with 100 ml of the Nitsch nutrient solution (1972). The herbicide formulations were from Atrazine Super 600, Gesaprim Super 600, BP Agripon, AG Penetrex and Complement.

Pots were placed within the demarcated 1 m^2 area and the spray solution applied evenly over the area with a hand sprayer delivering 200 L ha^{-1} at 200 kPa. The trial was laid out as a completely randomised design with each treatment replicated four times.

Plants were harvested two weeks after herbicide treatment. They were cut at the soil surface and dried in an oven at 65 °C for two days. Shoot fresh mass (SFM) and shoot dry mass (SDM) were recorded. Data expressed as % of the relevant controls. Standard analysis of variance (ANOVA) was done to demonstrate atrazine effects on growth.

Results

The SDM data appear in Table 2. There were significant differences between herbicides. The SDM showed highly significant differences ($P>0.01$) in response to either atrazine or atrazine/terbuthylazine in association with activity enhancers (Table 2). The highest growth reduction of 61.8 % occurred at the Gesaprim Super 600 SC + Complement treatment. There were also significant differences between Atrazine 500 SC + BP Agripon and Gesaprim Super 600 SC + Complement while Gesaprim Super 600 SC + AG Penetrex was not significantly different to Atrazine 500 SC treatment. However, the difference could at least be attributed to the activity enhancer used (complement) since the same herbicide (Gesaprim Super 600 SC) showed no significant difference from the treatment where AG Penetrex was the activity enhancer (Table 2)

Table 2: Reduction in SDM of maize treated with mixtures of atrazine or atrazine/terbuthylazine with different activity enhancers (ANOVA in Table 2A)

Herbicide treatment	**% Reduction in SDM**
Control	0a
Atrazine 500 SC + BP Agripon	35.3b
Gesaprim Super 600 SC + AG Penetrex	51.8bc
Gesaprim Super 600 SC + Complement	61.8c

* Values followed by the same letter do not differ significantly (P=0.05)

Experiment 2

Tolerance of maize to atrazine or atrazine/terbuthylazine in association with activity enhancers at two growth stages

Materials and Methods

The same procedure as in the previous experiment was used. In this experiment the treatments* were:

1. Control (0 herbicide)
2. Atrazine 500 SC (495 g L^{-1} atrazine) + BP Agripon (940 g L^{-1} mineral oil)
3. Gesaprim Super 600 SC (291 g L^{-1} atrazine + 291 g L^{-1} terbuthylazine) + AG Penetrex (363 g L^{-1} mineral oil).

*[Rates for all treatments were 2.5 L ha^{-1} of herbicides + 1.0 L ha^{-1} activity enhancer)

The average temperature regime in the greenhouse was 28.6 ^{0}C (day) and 17.4 ^{0}C (night).

Atrazine Super 500 SC + BP Agripon and Gesaprim 600 SC + AG Penetrex were foliarly applied at two growth stages, i.e., at the 2-leaf and the 4-leaf stage.

Results

The effects of atrazine and atrazine/terbuthylazine on maize at two growth stages are shown in Table 3. There were highly significant differences between the two growth stages, and no significant differences between herbicides. Highly significant differences were found with reduction in fresh mass accumulation ranging from 7.4 to 48.7 (Atrazine 500 SC + BP Agripon) and 4.1 to 57.4 (Gesaprim Super 600 SC + AG Penetrex).

The results indicate that growth stage play an important role when atrazine or atrazine/terbuthylazine are applied post-emergence. Both herbicides at the 2-leaf stage caused >40 % reduction in SFM (Table 3). On average, only 5.7 % reduction in SFM occurred when the herbicides were applied at the 4-leaf stage

Table 3: Reduction in SFM of maize treated with mixtures of Atrazine or atrazine/terbuthylazine combined with an activity enhancer at two growth stages of the crop (ANOVA in Table 4A)

Herbicide treatment	% Reduction in SFM Growth stage	
	Two-leaf	**Four-leaf**
Control	0a	0a
Atrazine 500 SC + BP Agripon	48.7b	7.4a
Gesaprim Super 600 SC + AG Penetrex	57.4b	4.1a

*Values followed by the same letter are not significantly different at P=0.05

Discussion

Atrazine injury to maize in South Africa has been reported previously (Le Court De Billot, 1978; Marshall *et al.*, 1982; Nel & Reinhardt, 1984; Malan *et al.*, 1984; Le Court De Billot, 1985; Le Court De Billot & Nel, 1985; Reinhardt & Nel, 1986; Le Court De Billot *et al.*, 1986; Malan *et al.*, 1986; Reinhardt & Nel, 1992; Reinhardt, 1993; Nel *et al.*, 1995).

The order of injury found in the present study agrees with the findings by Malan *et al.*, 1984; 1986) who reported growth inhibition of between 20 to 60 % depending on the

rate of atrazine applied in nutrient solution (1.25 L ha^{-1} and 5 L ha^{-1} respectively). Though the herbicides were foliarly applied in the present study, and despite adsorption of at least part of the fraction reaching the soil, reduction of fresh mass accumulation of between 35 to 61.8 % occurred.

The findings that the younger seedlings (2-leaf stage) were more susceptible than the older ones (4-leaf stage) could be attributed to the increased penetration and absorption of the herbicides through the cuticle of the younger plants (Ashton & Monaco, 1992).

This supported the findings of Anderson (1983) who indicated that plants are basically most susceptible to foliar-applied herbicides when they are in the seedling stage. He further indicated that susceptibility tends to decrease as the plants grow older. Besides the fact that younger plants have more penetrable cuticles, they also have more metabolically active tissue than older plants (Ashton & Monaco, 1992), which should promote both uptake and translocation of herbicides in combination with activity enhancers. Therefore, it is suggested that atrazine or atrazine/terbuthylazine in combination with activity enhancers be applied at the 4-leaf-stage to avoid seedling phytotoxicity.

Chapter 3

Tolerance of three maize cultivars to atrazine or atrazine/ terbuthylazine in association with activity enhancers

Introduction

Different cultivars do not always react to a herbicide in the same way. Susceptibility and resistance within the same species has always been found with some of the tried and tested herbicides which are considered safe when applied at prescribed rates (Alleman, 1993).

Several scientists have reported this differential cultivar tolerance with a number of herbicides in numerous crop plants (Malan *et al.*, 1984; Le Court De Billot and Nel, 1985; Reinhardt & Nel, 1993). Werner & Putnam (1980) reported differential cultivar tolerance with cucumber to atrazine. They showed that Marketmore 70 cucumber was injured more extensively than P1 390244 by atrazine at 1.14 kg ha^{-1}. Le Court De Billot (1978) indicated that spraying maize (*Zea mays* L.) with the same herbicide resulted in a number of reports from the field of damage to various maize cultivars. He attributed this damage to a large diversity of maize genetic material. Le Court De Billot & Nel (1985) noted that this differential cultivar response has been shown to exist for alachlor, simazine, cyanazine and atrazine in maize. They observed that some maize lines were killed at field related rates of atrazine.

Reinhardt & Nel (1993) found differential cultivar tolerance to atrazine in crops such as cucumber (*Cucumis saliva* L.), dry beans (Phaseolus vulgaris L.), maize (*Zea mays* L.), soybeans (*Glysine max* L.) and sunflower (*Heliathus annus* L.). They suggested that factors which govern the amount of herbicide in the plant system and not the concentration of the herbicide in the growing medium, would determine the plant's response to the compound.

Most of the work on cultivar differences with respect to the tolerance of maize to atriazine has involved using either nutrient solution or different soil types as growth medium, and atrazine was always applied directly to that medium. The most recent reports of damage involved post-emergence applications of the triazines. Therefore, it was important to do an experiment with the herbicidal spray applied post-emergence. The objective of this experiment was to determine whether cultivars react differently to atrazine and atrazine/terbuthylazine.

Materials and Methods

The experiment was carried out in a phytotron at the Hatfield experimental farm of the University of Pretoria. Three maize cultivars were used, namely CG 4625, PAN 6043 and PAN 6140. Five seeds of each cultivar were planted 5 cm deep in 3 kg of a sandy soil in plastic pots. Plastic bags were used to prevent contamination of the pots. Soil from the Hoopstad area in the Free State was used Selected soil properties appear in Chapter 2). Pots were weighed every second day to return soil water content to the original level of 75 % of the field capacity level which was calculated as 10 % for the particular soil. After emergence, plants were thinned to three per pot to reduce intra-

species competition. The average greenhouse temperature was 29.5 °C (max) during the day and 18.9 °C (min) during the night.

The Atrazine 500 SC + BP Agripon and Gesaprim + AG Penetrex mixtures were applied at the 2-leaf stage using a hand sprayer delivering 200 L ha^{-1} at 200 kPa.

The treatments* were:

1. Control (0 herbicide)

2. Atrazine 500 SC (495 g L^{-1} atrazine) + BP Agripon (940 g L^{-1} mineral oil)

3. Gesaprim Super 600 SC (291 g L^{-1} atrazine + 291 g L^{-1} terbuthylazine) + AG Penetrex (363 g L^{-1} mineral oil).

*[Rates for all treatments were 2.5 L ha^{-1} herbicide + 1.0 L ha^{-1} activity enhancer)

The same procedures as in previous experiments were employed.

Results

Fresh and Dry Mass

The responses of the three cultivars to atrazine or atrazine/terbuthylazine in the greenhouse are illustrated in Table 4 and 5 respectively. The cultivar * herbicide interaction is significant. The growth parameters SFM and SDM showed highly significant differences ($P>0.01$) in response to atrazine or atrazine/terbuthylazine between cultivars.

Cultivars CG 4526 and PAN 6043 were most sensitive to both treatments. They showed SFM and SDM reductions of > 40% for both herbicide treatments, whereas

cultivar PAN 6140 was the least sensitive of the three, with injury ranging from 5 – 22 % at both herbicide treatments. There was no significant difference in SDM for PAN 6140 between the herbicides (Table 5).

Visual symptoms

Though plant height was not measured, the least sensitive cultivar, PAN 6140 showed no height difference as compared to the untreated control. In contrast, the least tolerant cultivars appeared unhealthy and plant height was reduced.

The leaf symptom commonly associated with atrazine phytotoxicity is veinal chlorosis of the lower leaves decreasing in intensity at subsequent leaves (Le Court De Billot, 1985). These symptoms were present at all three cultivars treated with atrazine or atrazine/terbuthylazine combination. They were very apparent on lower leaves as reported by Malan *et al.* (1986). The intensity of the symptoms differed greatly between cultivars, with PAN 6140 exhibiting very little phytotoxic symptoms. The other two cultivars (CG 4526 and PAN 6043) showed severe symptoms and their stems were thinner as compared to the untreated control.

Table 4: Shoot fresh mass (SFM) response of three maize cultivars to atrazine and atrazine/terbuthylazine combination (ANOVA in Table 5A)

Herbicide treatment	**% Reduction in SFM**		
	Cultivars		
	CG 4526	PAN 6043	PAN 6140
Control	0a	0a	0a
Atrazine 500 SC + BP Agripon	47.9c	53.2c	13.0a
Gesaprim Super 600 SC + AG Penetrex	57.9c	50.2c	22.1b

* Means followed by the same letter are not significantly different at P=0.05

Table 5: Shoot dry mass (SDM) response of three maize cultivars to atrazine and atrazine terbuthylazine combination (ANOVA in Table 6A)

Herbicide treatment	**% Reduction in SDM**		
	Cultivars		
	CG 4526	PAN 6043	PAN 6140
Control	0a	0a	0a
Atrazine 500 SC + BP Agripon	44.7b	45.9b	5.4a
Gesaprim Super 600 SC + AG Penetrex	58.9b	41.7b	9.4a

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

Discussion

The results of this experiment indicated that maize cultivars do react differently to atrazine or atrazine/terbuthylazine in association with activity enhancers. This differential cultivar tolerance to the triazines tested confirms the findings for atrazine as reported by Le Court De Billot & Nel (1979), Le Court De Billot & Nel (1985), Le Court De Billot *et al.* (1985), Malan *et al.* (1986). Two of the cultivars used showed SFM and SDM reduction of more than 40% at both treatments. Malan *et al.* (1984) also reported this magnitude of reduction, but with atrazine (5 L ha^{-1}) added in the nutrient solution.

Visual phytotoxicity symptoms, i.e. veinal chlorosis which were reported by Le Court De Billot (1985), Le Court De Billot & Nel (1985) and Malan *et al.* (1986) were also noted in the present study. Both treatments caused almost the same symptoms though atrazine/terbuthylazine seemed more injurious in terms of reductions in SFM and SDM.

The two cultivars CG 4526 and PAN 6043 showed severe veinal chlorosis, with PAN 6140 showing slight symptoms. There seems to be correlation between SFM or SDM and veinal chlorosis, which could lead one to conclude that veinal chlorosis may be a reliable measure of cultivar tolerance to atrazine or atrazine/terbuthylazine. However, under different environmental conditions, this association could change as indicated by Le Court De Billot (1985) and Le Court De Billot & Nel (1985). They found that the tolerance patterns of some maize cultivars change with differing environmental

conditions, whereas others maintain their positions in order of tolerance, irrespective of the environment.

The differential effect of atrazine or atrazine/terbuthylazine in association with activity enhancers on maize cultivars is probably related to the rate at which atrazine or atrazine/terbuthylazine are broken down in a particular cultivar's system. Inactivation of triazines in maize is controlled mainly by three main detoxification mechanisms, which are hydrolysis, N-dealkylation and gluthathione conjugation (Le Court De Billot, 1985; Reinhardt, 1993). The rate at which each of these mechanisms operates within the cultivar most probably determines the tolerance of that cultivar (Le Court De Billot, 1985).

Chapter 4

Tolerance of maize to atrazine or atrazine/terbuthylazine in association with activity enhancers under different temperature regimes

Introduction

The temperature of the environment in which a plant is growing has a considerable influence on the rates of its physiological and biochemical reactions (Klingman & Ashton, 1982; Alleman, 1993; Reinhardt, 1993). The selectivity of various plants to herbicides also varies as temperature differentially affects their herbicide metabolism processes. Reinhardt (1993) indicated that temperature affects atrazine bioactivity by influencing its adsorption in soil, absorption by plants, and eventual fate in plants.

Several researchers cited temperature as an important factor as far as herbicide bioactivity is concerned. Mulder & Nalewaja (1978), Marshall *et al.* (1982), Nel & Reinhardt (1984), Malan *et al.* (1984) indicated that an increase in ambient temperature, within the range 10 to 30 ^{0}C enhances the phytotoxicity of the s-triazine herbicides. Therefore, a change from 15 to 25 °C may result in doubling the effect of the herbicide.

Temperature fluctuation has been reported to cause atrazine phyototoxicity as well. Malan *et al.* (1986) reported the death of atrazine-treated maize plants when kept under cold, wet conditions for 48h after the herbicide was applied, followed by exposure to high light intensity and high temperature. Therefore, it appears that increased herbicide uptake at a higher temperature and/or reduced detoxification of

atrazine in the maize plant at a low temperature plays a role in maize tolerance to atrazine.

The objectives of this study were (a) to investigate the effects of temperature on the tolerance of maize to atrazine or atrazine/terbuthylazine, and (b) to determine the response of cultivars to atrazine or atrazine/terbuthylazine under different temperature regimes.

Experiment 1

Materials and Methods

The experiment was carried out at the phytotron at the University of Pretoria's experimental farm. Three maize cultivars were used, namely, CG 4526, PAN 6043 and PAN 6140. The soil used consisted of 71.5% sand, 8.2% silt and 17.1% clay, with 0.4% organic matter and a pH of 6.5.

The treatments* were:

1. Control (0 herbicide)
2. Atrazine 500 SC (495 g L^{-1} atrazine) + BP Agripon (940 g L^{-1} mineral oil)
3. Gesaprim Super 600 SC (291 g L^{-1} atrazine + 291 g L^{-1} terbuthylazine) + AG Penetrex (363 g L^{-1} mineral oil).

*[Rates of each treatment were 2.5 L ha^{-1} herbicides + 1.0 L ha^{-1} activity enhancer respectively]

After the herbicides had been applied, pots were placed in growth chambers where the temperatures were 20/15 or 30/25 °C (day/night) for 12/12h day/night periods.

The same procedure as in previous experiments was followed except for the above information.

Results

Cultivar and temperature influenced the tolerance of maize seedlings to atrazine or atrazine/terbuthylazine. The relative SDM responses of cultivars to atrazine and the atrazine/terbuthylazine combination under two temperature regimes are shown in Figures 1 and 2 respectively. The cultivar * temperature * herbicide interaction was significant. Cultivars responded differently to a particular herbicide at a certain temperature.

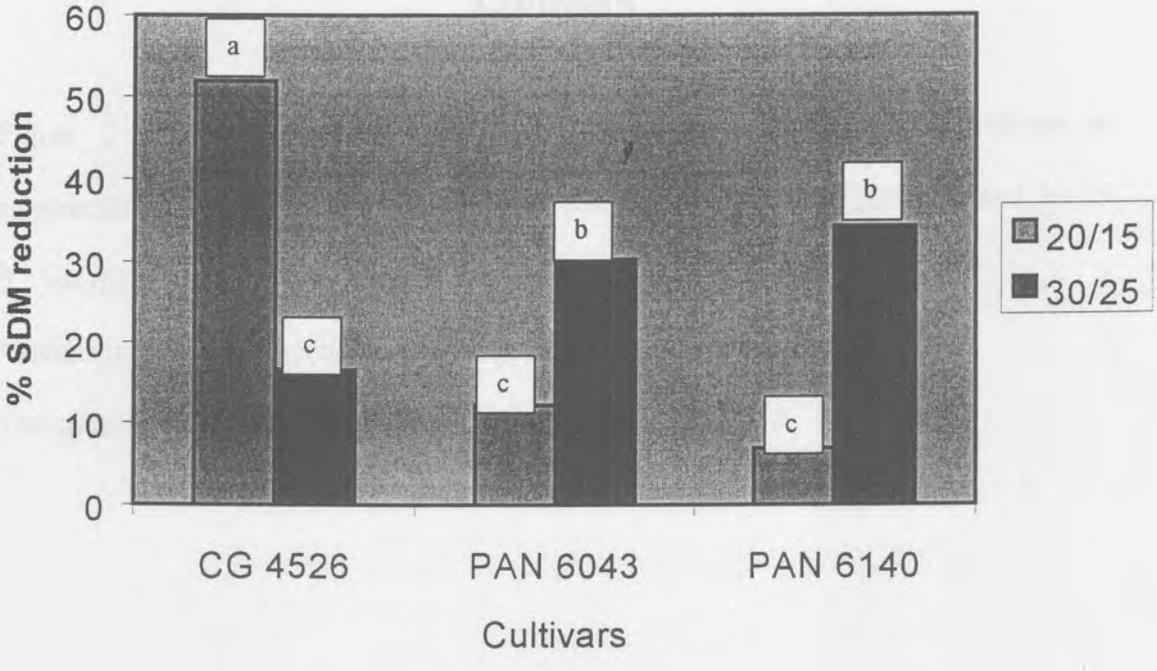

Figure 1: Reduction in SDM of maize treated with atrazine in combination with BP Agripon under two temperature regimes (20/15 ^{0}C and 30/25 ^{0}C -day/night)

* Means with the same letter do not differ significantly at P =0.05

The summary of the analysis of variance is given in Table 7A

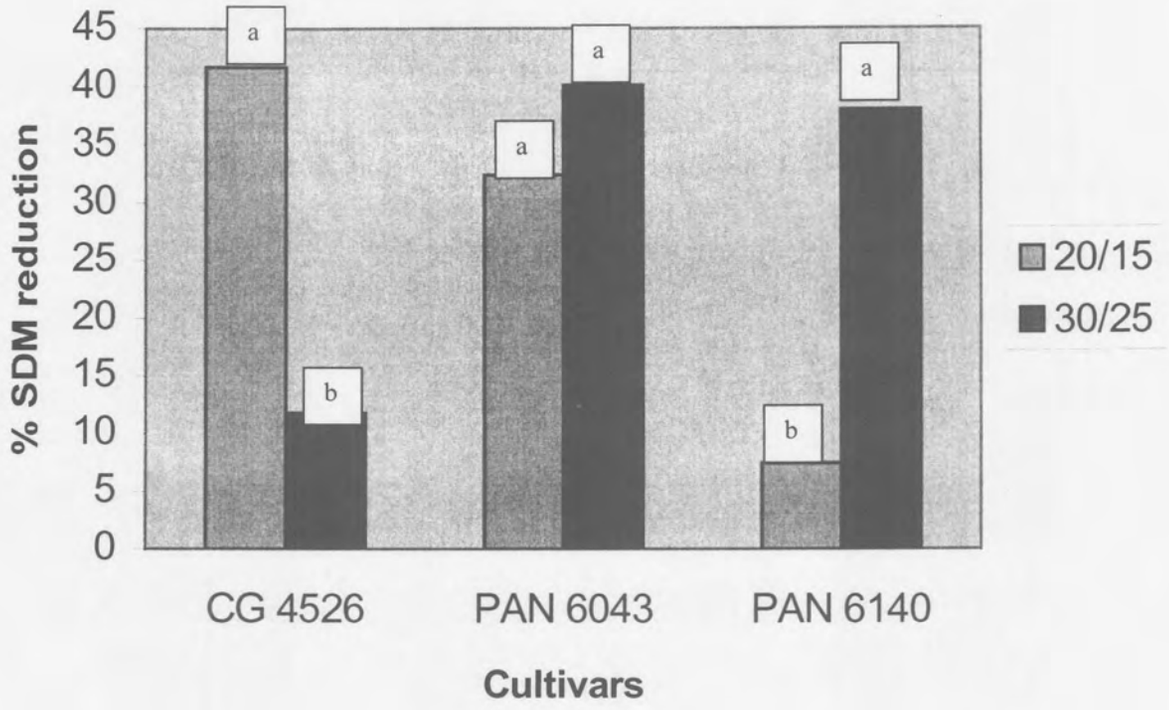

Figure 2: Reduction in SDM of maize treated with atrazine/terbuthylazine in combination with AG Penetrex under two temperature regimes (20/15 ^{0}C and 30/25 ^{0}C -day/night)

*Means with the same letter do not differ significantly at P =0.05

The summary of the analysis of variance is given in Table 7A

At the low temperature (20/15 ^{0}C), CG 4526 showed SDM reduction of >40 %, while the growth of PAN 6140 was reduced by less than 10 %. Also at the low temperature, atrazine caused 13% SDM reduction of PAN 6043 (Figure 1), but the atrazine/terbuthylazine combination resulted in 32 % SDM reduction (Figure 2). The general low phytotoxic response shown by some cultivars at the low temperature regime can probably in part be attributed to reduced uptake of the herbicides at this regime.

However, with an increase in temperature (30/25 ^{0}C), CG 4526 was the most tolerant cultivar, showing <17 % reduction in SDM while the others (PAN 6043 and PAN 6140) showed >30 % reduction. The latter two cultivars suffered almost a doubling or even tripling of phytotoxicity at the 30/25 °C temperature regime. However, PAN 6043 showed a non-significant difference between temperatures in response to the atrazine/tebuthylazine combination (Figure 2). The overall doubling and / or tripling of bioactivity could most probably be attributed to increased total atrazine or atrazine/terbuthylazine being taken up at the high temperature regime.

Experiment 2

Materials and Methods

The previous experiment was repeated. Apart from the substitution of cultivar CG 4526 with CAN 3891 and root dry matter (RDM) measured, the same procedure as in Experiment 1 was followed.

Results

The SDM response of cultivars to atrazine and atrazine/terbuthylazine are presented in Table 6. The temperature * herbicide interaction was significant at P= (0.05). There were no cultivar differences as were the case in the previous experiment.

Table 6. Reduction in SDM, averaged across cultivars, to atrazine or atrazine/terbuthylazine under two temperature regimes (ANOVA in Table 9A)

Herbicide treatment	**% Reduction in SDM** Temperature (^{0}C)	
	20/15	30/25
Control	0a	0a
Atrazine 500 SC +BP Agripon	22.9b	42.8c
Gesaprim Super 600 SC +AG Penetrex	15.3b	40.6c

* Means with the same letter do not differ significantly at P =0.05

The bioactivity of both herbicides, based on SDM reduction (Table 6), was nearly doubled at the high temperature regime. The SDM reduction did not exceed 23 % with both herbicides at the low temperature regime (20/15 °C day/night). However, at the higher temperature (30/25 °C day/night), SDM reduction of > 40 % was recorded.

Table 7: Reduction in RDM, averaged across cultivars, to atrazine or atrazine/terbuthylazine under two temperature regimes (ANOVA in Table 10A)

Herbicide treatment	**% Reduction in RDM**	
	Temperature (^{0}C)	
	20/15	30/25
Control	0a	0a
Atrazine 500 SC +BP Agripon	37.3c	63.9d
Gesaprim Super 600 SC +AG Penetrex	21.4b	66.8d

* Means with the same letter do not differ significantly at P =0.05

The RDM reduction caused by both herbicides at the low temperature regime was less than 38 %, in contrast with >66 % at the high temperature regime. Similar kinds of effects as in SDM occurred, though RDM showed to be more sensitive to atrazine or

atrazine/terbuthylazine. At the low temperature (20/15 ^{0}C), atrazine + BP Agripon differed significantly to atrazine/terbuthylazine + Penetrex. The results are presented in Table 7.

In this experiment maize tolerance towards both herbicides was consistently, significantly lower at the high temperature than at the low temperature. The overall increased response of maize to atrazine and atrazine/terbuthylazine at the high temperature regime can probably in part be attributed to more total atrazine and terbuthylazine being taken up under the high temperature regime.

Discussion

The tolerance of maize cultivars to atrazine or atrazine/terbuthylazine that was evident from the two experiments, confirms that the relative tolerance patterns of maize cultivars may alter with differing temperature conditions. All of the cultivars used had their tolerance pattern changed either with a decrease or increase in temperature.

The only cultivar which had its tolerance increased with an increase in temperature was CG 4526, whereas all the others (Pan 6043 and PAN 6140) had their tolerance decreased with an increase in temperature. None of the three cultivars retained its tolerance position at both temperatures.

The findings in this study support those of Nel & Reinhardt (1984) and Le Court De Billot (1985) who indicated that both high and low temperature may increase

Chapter 5

Recovery of maize from the effects of atrazine/terbuthylazine in association with an activity enhancer

Introduction

The ability of a crop or a specific cultivar to recover from herbicide injury may negate the effects of initial phytotoxicity (Le Court De Billot, 1985). This may lead to complete recovery of the crop without any effect on yield whatsoever. Shimabukuro & Swanson (1969) who reported of the ability of resistant plants to metabolize and detoxify atrazine rapidly also reported complete recovery of crops from herbicide injury. They regard this complete recovery as the basis of selectivity for higher plants.

Several scientists have stressed that phytotoxic effects on final yield should be taken into account when determining crop tolerance to herbicides. Jensen *et al.* (1979) and Van Ooschot & Van Leeuwen (1979) have used the ability of crops to recover from initial phytotoxicity as a measure of tolerance, with Le Court De Billot (1985) uncompromisingly stating that crop tolerance to herbicides ought to be measured by the phytotoxic effects on final yield.

Le Court De Billot (1985) reported 95 to 100 % recovery of grain sorghum after one-week exposure to atrazine. Malan *et al.* (1986) reported complete recovery of atrazine-treated maize, with some significantly outyielding the control plants. This according to the above authors might be explained by the efficiency of the detoxifying mechanisms of

herbicide toxicity in certain cases. However, in the present study most of the cultivars tolerance decreased with an increase in temperature.

The reduction in SDM and RDM increased significantly with an increase in temperature. This effect could be attributed to more total atrazine or terbuthylazine being taken up by the plant under the high temperature regime (30/25 ^{0}C day/night) (Le Court De Billot, 1985; Le Court De Billot & Nel, 1985).

This increase in phytotoxicity with an increase in temperature have been confirmed by Marshall *et al.* (1982) and Nel & Reinhardt (1984) who reported the enhancement of phytotoxicity of s-triazines herbicides with an increase in ambient temperature within the range of 10 to 30 °C. Of the two herbicides used in the present study, the atrazine/terbuthylazine combination was more injurious than atrazine alone.

In conclusion, differential cultivar tolerance reported under different temperature regimes in the present study could have been due to the differential influence of temperature on the rate of herbicide detoxification mechanisms (Le Court De Billot, 1985).

hydroxylation, conjugation and N-dealkylation present in the maize plant. Shimabukuro & Swanson (1969) and Shimabukuro *et al.* (1970) reported the recovery of sorghum with concomitant metabolism of atrazine which clearly implicating metabolism as an inactivating process. They indicated that metabolic inactivation of atrazine restored the photochemical activity in illuminated leaf discs of resistant sorghum plants. Chivinge & Mpofu (1990) reported initial crop phytotoxicity which did not have an adverse effect on maize grain yield with atrazine, prometon or a 1:3 mixture of metolachlor and terbuthylazine at the recommended rate and double the rate.

A greenhouse experiment was conducted to investigate whether maize recovers from atrazine/terbuthylazine phytotoxicity, and if they do, how long recovery takes.

Materials and Methods

The experiment was carried out at the phytotron at the University of Pretoria experimental farm. One maize cultivar was used *viz.* PAN 6043. Five seeds planted 5 cm in 5-kg soil in each pot. The soil used consisted of 71.5 % sand, 8.2 % silt and 17.1 % clay, with 0.4 % organic matter and a pH of 6.5. Plastic bags were used to prevent contamination of the pots. Pots were weighed after every two days to return soil water content to the original level (field capacity: 10 % v/m basis). After emergence, plants were thinned to three per pot to reduce intra-species competition. Herbicide treatments were applied at the 2-leaf stage.

The treatments* were:

1. 0 herbicide (control)

2. Gesaprim Super 600 SC (291 g L^{-1} atrazine & 291 g L^{-1} terbuthylazine) + AG Penetrex (363 g L^{-1} mineral oil).

3. Rates double that of Treatment 2 (5 L ha^{-1} atrazine/terbuthylazine).

*[Rates were 2.5 L ha^{-1} herbicides + 1.0 L ha^{-1} activity enhancer respectively]

Prior to herbicide application, all pots were watered with 100 ml of a nutrient solution to reduce intra-species competition. Nitsch nutrient solution (1972) was applied twice a week. Herbicide application was done on an area basis (m^2). Pots were put in the center of a marked 1 m^2 area. The treatments were applied evenly with a hand sprayer delivering 200 L ha^{-1} at 200 kPa.

Seedlings were harvested after two, five and eight weeks' exposure to herbicides. Plants were cut at the soil surface and dried in an oven at 65 °C for two days. The SDM, leaf area index (LAI) and RDM were recorded. Data were expressed as percentage reduction from the control.

Results

Leaf Area Index

Leaf area index (LAI) responses of maize to the atrazine/terbuthylazine combination at two dosages are illustrated in Figure 3. There were significant differences between the times of exposure to the herbicides. Both dosages (recommended and double the recommended) caused similar reductions in LAI after two weeks exposure to atrazine/terbuthylazine (34 and 42 % respectively). At that stage, damage was at a maximum and significantly greater than at any subsequent stage. After five and eight weeks' exposure, the rate of recovery did not differ significantly between the two herbicide rates. Recovery in LAI was 100 % with the recommended rate and about 95 % with double the recommended rate after five weeks exposure. At eight weeks after spraying, the recovery from initial injury caused by both herbicide rates was virtually 100 %.

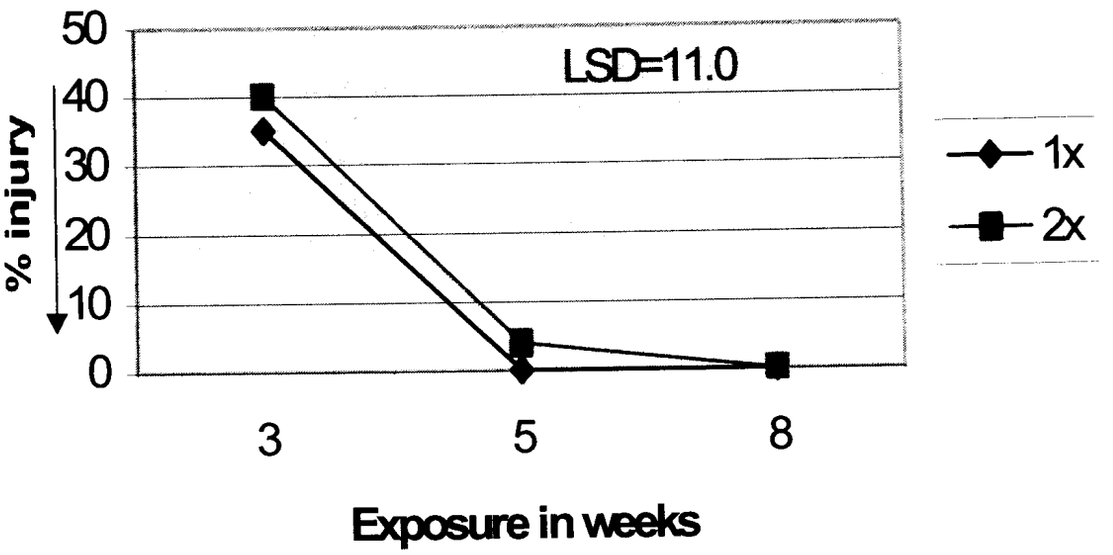

Figure 3: LAI recovery after exposure to atrazine/terbuthylazine after eight weeks (1X (2.5 L ha^{-1}, and 2X =5 L ha^{-1})

The summary of the analysis of variance is given in Table 11A

Shoot dry mass

The recovery in SDM of maize after exposure to atrazine/terbuthylazine after two weeks, five weeks and eight weeks is illustrated in Figure 4. With both rates the SDM reduction increased from 41 to 42 % (2.5 L ha^{-1}) and 43 to 63 % (5 L ha^{-1}) during the period between week two and five. However, there was a significant increase in SDM reduction at double the recommended rate compared to the recommended rate. After eight weeks exposure, both rates showed

< 4 % SDM reduction, which indicates almost complete recovery.

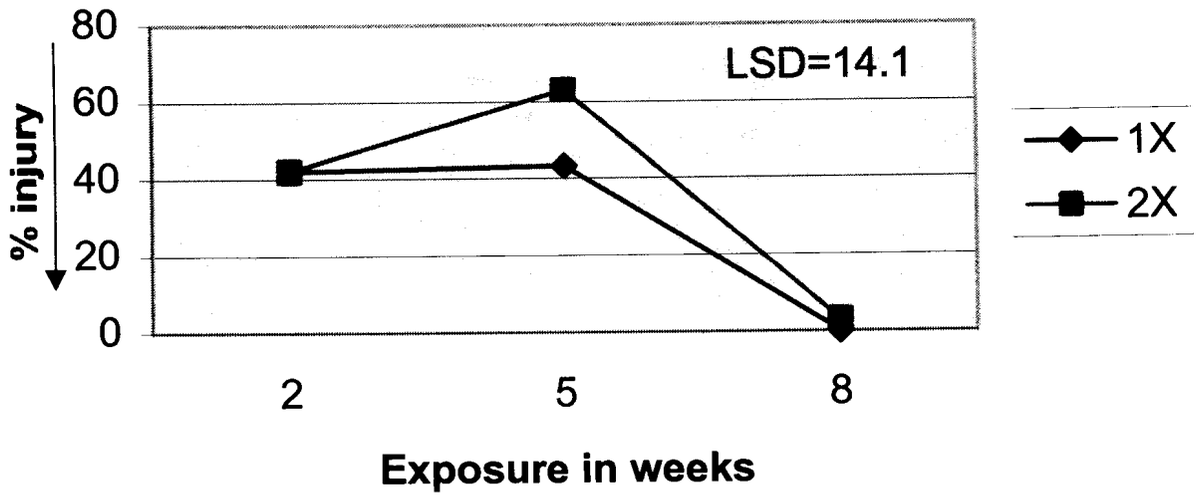

Figure 4: SDM recovery of maize to atrazine/terbuthylazine after eight weeks exposure (1X =2.5 L ha^{-1}, and 2X =5 L ha^{-1})

The summary of the analysis of variance is given in Table 12A

Root dry mass (RDM)

The RDM responses of maize after exposure to atrazine/terbuthylazine for two, five and eight weeks are illustrated in Figure 5. There were significant differences in RDM reduction at the recommended rate between two and five weeks' exposure whereas non-significant differences were noted between this periods at double the recommended rate at 2.5 L ha^{-1} or 5 L ha^{-1}. After eight weeks exposure, RDM showed 100 % recovery of maize roots to the atrazine/terbuthylazine in association with AG Penetrex.

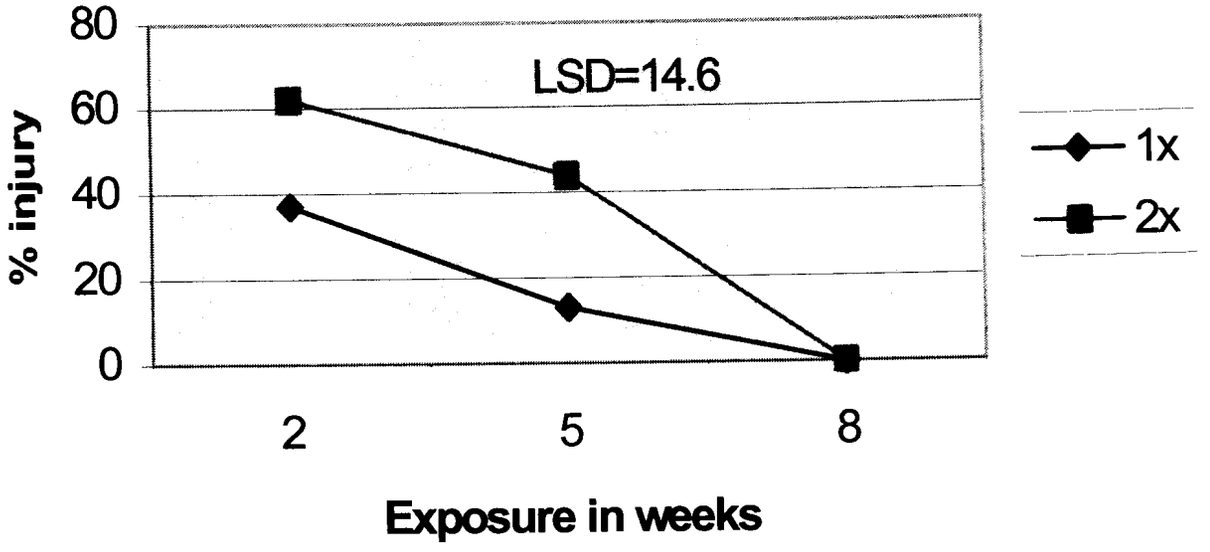

Figure 5: RDM recovery of maize to atrazine/terbuthylazine after eight weeks (1X= 2.5 L ha^{-1}, and 2X= 5 L ha^{-1})

The summary of analysis of variance is given in Table 13A

Discussion

Several scientists working with a wide range of herbicides have reported similar growth achieved in this experiment. Le Court De Billot (1985) reported 100 % recovery of grain sorghum, after one and two week's exposure to atrazine. He demonstrated that differential cultivar recovery might also occur following atrazine phytotoxicity.

This study showed that atrazine-treated maize plants, irrespective of the degree of initial phytotoxicity, could recover fully from injury within eight weeks of initial application of atrazine/terbuthylazine, as shown in Figure 3, 4 and 5.

The findings of this study confirms the one by Malan *et al.* (1986) who reported that atrazine-treated maize plants eventually significantly outyielded untreated ones. The results of this study suggest that recovery from atrazine/terbuthylazine phytotoxicity might be explained by the efficiency of the detoxifying mechanisms of dyhdroxylation, conjugation and N-dealkylation present in the maize plants as reported by Malan *et al.* (1986).

They also suggested that crop tolerance could also be judged by the way a particular crop or cultivar recovers from injury that was caused by a particular herbicide.

Chapter 6

General Discussion and Conclusions

This study was initiated primarily as a result of the occurrence of maize injury from atrazine or atrazine/terbuthylazine in association with activity enhancers in the field. These field cases led to the suspicion that the stage of application, activity enhancers, cultivar differences and temperature are the important factors in the occurrence of the reported field injury. The recovery of maize from herbicide damage was also investigated.

Since the first reported damage to maize in 1981/82, some of the aforementioned factors and others were cited as possible explanations for the lack of selectivity of atrazine. Malan *et al.* (1986) mentioned temperature, Le Court De Billot, (1985) cultivar differences and the stage of application, and lastly, soil and nutrition factors (Smit *et al.*, 1988; Reinhardt, 1993).

An important finding that emerged from this study is that maize seedlings are very susceptible to foliar-applied atrazine or atrazine/terbuthylazine in association with activity enhancers at the two-leaf stage and even younger. This most probably could be attributed to the fact that younger plants have more penetrable cuticles, underdeveloped wax layers and a greater relative amount of metabolically active tissue than older plants (Ashton & Monaco, 1992). Therefore, it is suggested that atrazine or atrazine/terbuthylazine not be applied before the four-leaf stage to avoid seedling phytotoxicity.

Differential cultivar responses, which was reviewed by Le Court De Billot (1985) with numerous South African cultivars, was also investigated in the present study. Although Le Court De Billot (1985) found that South African maize in general is tolerant to atrazine, some cultivars did show some degree of susceptibility. The percentages reduction of SDM obtained in the present study (> 40 % reduction) with two cultivars (PAN 6043 and PAN 6140) indicate the potential for susceptibility of maize to atrazine or atrazine/terbuthylazine as indicated in Chapter 3. Furthermore, the results of this study indicate that one should make calculated decisions when choosing cultivars where atrazine or atrazine/terbuthylazine might be used.

Another important finding of the study was the confirmation that the relative tolerance of maize cultivars may be altered with temperature changes as previously reported by Nel & Reinhardt (1984) and Le Court De Billot (1985). They indicated that both high and low temperature might increase herbicide toxicity in certain cases. In the present study, the herbicide injury could have been due to the differential influence of temperature on the rate of herbicide detoxification mechanisms, and/or the action of the activity enhancers, thereby altering cultivar tolerance under certain conditions. Nevertheless, the growing of cultivars that appear to be the most tolerant to atrazine or atrazine/terbuthylazine in those areas where injury most frequently occurs could possibly reduce the risk of injury.

Although growth stage, cultivar differences and temperature identified as important aspects of this study, seedling recovery from herbicide injury should not be ignored as several scientists suggested that the ability of the crop to recover be considered as a measure of tolerance. Jensen *et al.* (1979) and Le Court De Billot (1985), amongst

others, intimated this. The interesting finding is that within five weeks exposure to atrazine/terbuthylazine, LAI had recovered completely from the initial phytotoxicity of the herbicide (as indicated in Chapter 5). The recommended and double the recommended rates were used in this experiment. The cultivar used in experiments showed no less than 30 % SDM reduction in all experiments, but recovered completely within eight weeks after exposure to atrazine/terbuthylazine. This suggests that recovery from initial atrazine/terbuthylazine injury can be used as a measure of tolerance as previously proposed by several scientists.

Evidence provided by this study suggests that it is very difficult to establish exactly what combination of factors brings about maize injury to the herbicide. Growth stage, cultivar differences and temperature have all been shown to affect tolerance. However, recovery ability from herbicide injury does affect tolerance rankings (Le Court De Billot, 1985). Although specific explanations for the roles of these factors have been proposed in their specific sections, growth stage and/or stage of application, and temperature are probably the major factors which influenced the most recent maize injury with atrazine or atrazine/terbuthylazine in the field.

Summary

Recent reports of damage to maize seedlings where atrazine or atrazine/terbuthylazine was applied post-emergence again raised the issue of maize tolerance to triazines.

The tolerance of maize to atrazine or atrazine/terbuthylazine was tested in pots under controlled conditions. The aspects investigated included growth stage, surfactants, cultivar tolerance, temperature and the recovery ability of maize seedlings from herbicide injury. In preliminary studies, it was found that atrazine or atrazine/terbuthylazine causes veinal chlorosis and reduction in SDM. In Experiment 1, maize seedlings were found to be susceptible to atrazine or atrazine/terbuthylazine at an early growth stage (2-leaf stage and or even younger). This could be attributed to the fact that maize seedling cuticles at the 2-leaf stage are more penetrable by atrazine or atrazine/terbuthylazine than at older stages (4-leaf stage).

In subsequent experiments, cultivar responses were investigated to establish whether cultivars exhibit differential tolerance. It was found that cultivars react differently to atrazine or atrazine/terbuthylazine under specific environmental conditions. Two of the three cultivars used showed appreciable susceptibility. Due to the differential cultivar response found in this study, a comparison of tolerance was done under different temperature regimes. The tolerance patterns of cultivars changed, with none of the cultivars retaining its position at both temperatures used. Two of the cultivars (PAN 6340 and 6140) had their tolerance increased with an increase in temperature.

In an experiment conducted to determine whether maize seedlings can recover from atrazine/terbuthylazine exposure, where both recommended and double the

recommended herbicidal rates were used, it was found that maize seedlings could recover completely within eight weeks exposure to atrazine/terbuthylazine. The tolerance of atrazine or atrazine/terbuthylazine was discussed in the light of the stage of application, differential cultivar tolerance, surfactants, temperature and the recovery ability from the herbicide treatment.

Implications for small-scale farmers

Weeds cause drastic yield losses in crop production, especially in developing countries where it is estimated that 25 % of crop yields are lost despite control measures (Koch, 1992). Akobundu (1987) indicated that uncontrolled weed growth causes yield loss of 40 to 60% in maize in the tropics and that weeds such as *Rottboeliia concinchinensis* and *Striga hermonthica* can cause total crop loss if they are not controlled. Weeds reduce crop yield by interfering with crop growth through interference which include competition with crops for water, nutrients and light; reduce quality of harvested agricultural products because of the presence of weeds in harvests; interfere with harvest operations and increase cost of harvesting by hampering picking maize by hand. Weeds could also serve as hosts for many plant diseases and pests, and could pose a threat to farm size expansion considering labor, time and energy needed to weed the field.

Most of the activities of people in rural communities are directed to weed control, which is very tedious and laborious due to practices such as hoeing, hand pulling and hand slashing. These manual weeding continues to require over 50% of farmers time, leaving him or his family with little time for either recreation, creative thinking or other activities. The subsistence nature of small-scale farming is therefore largely due to the presence of weeds and the absence of improved methods of controlling them (Akobundu, 1987).

This study provides valuable information on atrazine and terbuthylazine as regards their selectivity in maize. Since maize is the primary crop in most African countries,

and because these herbicides are relatively cheap and effective compared to others, resource-poor farmers are likely to consider them. Moreover, herbicides generally save time and give the farmer an opportunity to extend his energy to other equally important activities. These farmers would prefer to use post-emergence applications, since it will give them the opportunity to judge whether chemical weed control is warranted. Akobundu (1987) indicated that maize is susceptible to competition from annual weeds during the first 6-8 weeks after planting. The application of atrazine or atrazine/terbuthylazine at the 4-leaf stage (approximately 4 weeks after planting) could give satisfactory results. Moreover, proper labeling of cultivar packages on these herbicides through the working together of chemical and seed companies could help farmers to carefully select cultivars if ever they consider using these herbicides. Knowledge on application techniques, choice of cultivars and, most importantly, application of these herbicides at the appropriate stage of maize development, should benefit all farmers that have to cope with weeds.

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APPENDIX

Tables of the analysis of variance

Table 1A: Tolerance of maize to atrazine or atrazine/terbuthylazine in association with activity enhancers at the 2-leaf stage

Source	DF	MS	F-value	PR>F
H	3	3024.10	11.32	0.0003
Error	16	267.09		
Total	19			
CV (%)	48.4			
R^2	0.68			

Table 2A: Tolerance of maize to atrazine or atrazine/terbuthylazine in association with activity enhancers at the 2-leaf stage (Shoot dry mass data appear in Table 2)

Source	DF	MS	F-value	PR>F
H	3	3791.67	19.58	0.0001
Error	16	193.69		
Total	19			
CV (%)	37.6			
R^2	0.79			

Table 3A: Tolerance of maize to atrazine or atrazine/terbuthylazine in association with activity enhancers at two growth stages

Source	DF	MS	F-value	PR>F
S	1	12970.4	46.09	0.0001
H	2	3702.5	13.16	0.0003
S x H	2	3085.5	10.96	0.0008
Error	18	281.4		
Total	23			
CV (%)	35.15			
R^2	0.84			

Table 4A: Tolerance of maize to atrazine or atrazine/terbuthylazine in association with activity enhancers at two growth stages (Shoot fresh mass data appear in Table 3)

Source	DF	MS	F-value	PR>F
S	1	5960.8	124.89	0.0001
H	2	2295.9	48.11	
S x H	2	1557.82	32.64	
Error	18	47.73		
Total	23			
CV (%)	35.17			
R^2	0.94			

Table 5A: Tolerance of three maize cultivars to atrazine or atrazine/terbuthylazine in association with activity enhancers (Shoot fresh mass data appear in Table 4)

Source	DF	MS	F-value	PR>F
C	2	2140.21	11.77	0.0002
H	2	6724.3	36.99	0.0001
C x H	4	587.7	3.23	0.0273
Error	27	101.8		
Total	35			
CV (%)	49.64			
R^2	0.80			

Table 6A: Tolerance of three maize cultivars to atrazine or atrazine/terbuthylazine in association with activity enhancers (Shoot dry mass data appear in Table 5)

Source	DF	MS	F-value	PR>F
C	2	2992.27	13.51	0.0001
H	2	47.85.88	21.61	0.0001
C x H	4	833.61	3.76	0.0147
Error	27	221.42		
Total	35			
CV (%)	64.97			
R^2	0.76			

Table 7A: Tolerance of three maize cultivars to atrazine or atrazine/terbuthylazine in association with activity enhancers under different temperature regimes (Shoot dry mass data appear in Figure 1 and 2)

Source	DF	MS	F-value	PR>F
C	2	233.59	1.14	0.3278
H	2	5872.38	28.62	0.0001
T	1	72.66	0.35	0.5543
C x H	4	314.02	1.53	0.2064
C x T	2	2714.45	13.23	0.0001
H x T	2	18.56	0.09	0.9137
C x H x T	4	715.49	3.49	0.0132
Error	54	205.16		
Total	71			
CV (%)	79.74			
R^2	0.66			

Table 8A: Tolerance of three maize cultivars to atrazine or atrazine/terbuthylazine in association with activity enhancers under two temperature regimes

Source	DF	MS	F-value	PR>F
C	2	513.32	2.67	0.0781
H	2	6103.05	31.80	0.0001
T	1	54.84	0.29	0.5952
C x H	4	403.70	2.10	0.0930
C x T	2	1817.91	9.47	0.0003
H x T	2	13.53	0.07	0.9320
C x H x T	4	556.83	2.90	0.0301
Error	54	191.93		
Total	71			
CV (%)	75.77			
R^2	0.66			

Table 9A: Tolerance of three maize cultivars to atrazine or atrazine/terbuthylazine in association with activity enhancers under two temperature regimes (Shoot dry mass data appear in Table 6)

Source	DF	MS	F-value	PR>F
C	2	85.36	0.69	0.5058
H	2	7182.49	58.08	0.0001
T	1	7493.97	60.59	0.0001
C x H	4	100.04	0.81	0.5249
C x T	2	385.59	3.12	0.0523
H x T	2	2542.30	20.56	0.0001
C x H x T	4	611.16	4.94	0.0018
Error	54	123.67		
Total	71			
CV (%)	57			
R^2	0.82			

Table 10A: Tolerance of three maize cultivars to atrazine or atrazine/terbuthylazine in association with activity enhancers under two temperature regimes (Root dry mass data appear in Table 7)

Source	DF	MS	F-value	PR>F
C	2	118.16	0.40	0.6733
H	2	18198.04	61.38	0.0001
T	1	10367.46	34.97	0.0001
C x H	4	93.04	0.31	0.8675
C x T	2	105.64	0.36	0.7019
H x T	2	3125.06	10.54	0.0001
C x H x T	4	47.97	0.16	0.9567
Error	54	296.48		
Total	71			
CV (%)	54.52			
R^2	0.77			

Table 11A: Recovery ability of maize seedlings to the effects of atrazine/terbuthylazine (Leaf area index data appear in Figure 3)

Source	DF	MS	F-value	PR>F
S	2	4849.63	17.27	0.0001
H	2	2362.31	8.41	0.0006
S x H	4	999.28	3.56	0.0111
Error	63	280.84		
Total	71			
CV (%)	182.84			
R^2	0.51			

Table 12A: Recovery ability of maize seedlings to the effects of atrazine/terbuthylazine (Shoot dry mass data appear in Figure 4)

Source	DF	MS	F-value	PR>F
S	2	11902.32	28.60	0.0001
H	2	8056.93	19.36	0.0001
S x H	4	3330.65	8.00	0.0001
Error	63	416.21		
Total	71			
CV (%)	106.50			
R^2	0.67			

Table 13A: Recovery ability of maize seedlings to the effects of atrazine/terbuthylazine (Root dry mass data appear in Figure 5)

Source	DF	MS	F-value	PR>F
S	2	10318.35	23.39	0.0001
H	2	6584.98	14.93	0.0001
S x H	4	2953.89	6.70	0.0001
Error	63	441.09		
Total	71			
CV (%)	137.97			
R^2	0.62			