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TOLERANCE OF MAIZE GENOTYPES TO SELECTED HERBICIDES

by

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Submitted in partial fulfillment of the requirements for the degree PhD

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List of abbreviations

ai: active ingredient

ha: hectare

mg: milligram

g: gram

kg: kilogram

mm: millimeter

cm: centimeter

m: metre

ml: millilitre

L: litre

cm^{-3}: cubic centimetre

SDM: shoot dry mass

RDM: root dry mass

VIR: visual injury rating

Spp: species

LSD: Least Significant Difference test

CV: coefficient of variation

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ABSTRACT

Maize damage has been observed from time to time in many parts of South Africa where registered herbicides were applied. Differential cultivar tolerance to certain herbicides was identified as an important factor in many cases where herbicide selectivity was inadequate. A study was done to investigate the existence of differential tolerance of maize genotypes to selected herbicides. Several maize genotypes were screened for tolerance to selected herbicides, both in the greenhouse and in the field. Maize genotypes demonstrated significant differences in their tolerance to all herbicides. Some genotypes were severely injured by certain herbicides while others were not affected at all by the same herbicide. This suggests that maize genotypes should be screened for tolerance to herbicides in order to reduce crop injury by using only appropriate ones for specific genotypes. Generally, hybrids were more tolerant to herbicides than inbreds, indicating

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that screening may be most necessary in seed production, where inbreds are used, than in commercial production where only hybrids are used. Tolerance of maize to herbicides was more variable to metazachlor than to other herbicides, and metazachlor also injured more genotypes than other herbicides. This was probably due to differences in herbicide mechanism of action. Shoot or root dry mass reduction of some of the maize genotypes occurred without visual injury symptoms, thus suggesting that visual injury may not reliably indicate susceptibility or tolerance to herbicides. The degree of correspondence of herbicide effects on maize in the greenhouse and the field was determined. Comparison of results from the greenhouse and the field showed that there is positive correlation between herbicide effects in the greenhouse and in the field. There was generally good correspondence of major parameters, such as shoot dry mass and injury symptoms, in the greenhouse and in the field. Similarly these parameters were positively correlated with the grain yield obtained from the field. It appears that shoot dry mass and visual injury symptoms could be good predictors of the yield. This indicates that reliable data could be generated through quicker screening at greenhouse level. A total of four herbicides, metazachlor, dimethenamid, acetochlor and the combination atrazine / metolachlor / terbuthylazine, had significant correlations while only two, flufenacet and acetochlor + atrazine/sulcotrione, had no significant correlations for major parameters with the yield. This indicates that the correlation of data was herbicide-dependent. The influence of temperature on maize tolerance to alachlor, metazachlor and metolachlor was investigated. Results showed that low temperatures reduce the tolerance of maize to these herbicides. This could mean that low temperature may reduce the selectivity of these herbicides. Fluctuating temperature conditions of 10°C at night and 35°C during the day, which are found in some maize producing areas, did not affect maize tolerance to the herbicides. The possibility of improving metazachlor tolerance in maize was also investigated. Evidence provided for possible gene effects on the tolerance of metazachlor indicates that maize tolerance to the herbicide could be improved by crossing tolerant

parents. The results suggest that it may be possible to improve metazachlor tolerance by crossing appropriate parent lines with dominant genes for tolerance to metazachlor. Ultrastructural changes in the maize seedling root and shoot cells caused by metazachlor were investigated. In susceptible genotypes root cell nucleoli were found to be abnormally large, empty and more abundant than those in untreated control plants. In susceptible plants the chromatids appeared disorganised in cell nucleoli, and both the nuclear and plasma membranes showed signs of disintegrating. There were more and larger vacuoles in the herbicide-susceptible plants. Leaf cells from the susceptible plants had more empty vacuoles and more chloroplasts with generally disorganised content. The bundle sheath chloroplast membranes were dilated in susceptible plants, and the orientation of the grana was disrupted. In the herbicide-tolerant plants, the ultrastructure was not different from that of all the untreated plants. The established differential tolerance of maize to herbicides necessitates the screening of all genotypes to all registered herbicides in order to recommend specific herbicides for certain maize genotypes. Due to the large number of genotypes that would require screening, techniques that yield reliable data quickly have obvious merit. Pot experiments under controlled conditions, which could be selected to promote herbicide bioactivity, are likely to provide data with which the best possible predictions on the risk of herbicide damage in the field could be made. Based on this requirement, environmental factors that should be considered for greenhouse work are: soil with low adsorptive capacity, soil water content close to the field capacity level, and cool temperatures. When screening for herbicide tolerance, the use of herbicide rates in excess of the recommended rate could obviate the need for special environmental conditions, since all the aforementioned factors basically promote the accumulation of higher than usual amounts of herbicide at the site of action in the plant. Therefore, the use of at least a 2X-herbicide rate in screening experiments is advised.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important food crops of the world. It is ranked third in importance among the world's cereals exceeded only by wheat and rice (Purseglove, 1972), while in sub-Saharan Africa it is the most important food crop. Maize originated from Mexico and is currently grown on all continents under a more diverse range of climates than any other crop (Weatherwax & Randolph, 1955; Purseglove, 1972; Van Rensburg, 1996). It is mainly used as a staple human food, livestock feed and as a raw material for many industrial products (Purseglove, 1972; Mungoma, 1995).

Maize, like any other crop, has some production constraints and one of the major ones is weeds. If weeds are not properly controlled during the first 6-8 weeks, maize yields will be greatly reduced (Kasasian, 1971; Bhau & Singh, 1979; Akobundu, 1987). It is therefore imperative that weeds are properly controlled to achieve optimum yields.

In South Africa, herbicide use is very common and presently a number of herbicides, including those used in this study, are registered for use in maize (Vermeulen, *et al.*, 1998). This is because use of herbicides is the most practical way of controlling weeds for large-scale farming. Despite the significant contribution of herbicides to increased maize production and profitability to farmers, many cases of crop injury following herbicide application have been reported in South Africa, (Malan *et al.*, 1984; Le Court De Billot, 1985; Le Court De Billot, *et al.*, 1986; 1990; Van Biljon, 1991; Allemann, 1993; Reinhardt, 1993; Kanyomeka & Reinhardt, 2000). Alachlor and metolachlor have been reported to cause injury to crops (Reinhardt & Nel, 1986; Van Biljon, 1991). Even atrazine, a herbicide considered to have the largest safety margin in the crop, has been reported to cause injury to maize from time to time (Le Court De Billot, 1985; Reinhardt, 1993). Similarly, in other parts of the world, differential tolerance of crop cultivars, including those of maize, to herbicides has been noted in cases of crop injury (Randall *et al.*, 1995; Van Wychen, *et al.*, 1999; Grey

et al., 2000; Pauley *et al.*, 2000; Bussan, *et al.*, 2001; Cavero *et al.*, 2001; Blair-Kerth *et al.*, 2001; O'Sullivan *et al.*, 2001).

Of great concern is that inbreds and new cultivars appear to be less tolerant to herbicides than hybrids and older cultivars respectively (Le Court De Billot, 1985). This implies that screening for tolerance to herbicides should be incorporated in crop cultivar development programmes. All these differences in crop tolerance to herbicides have been attributed to differences in genetic composition, though other factors such as environment also affect herbicides' selectivity (Klingman & Ashton, 1982; Zimdahl, 1993). Temperature is one environmental factor that has been known to influence some herbicides', e.g., alachlor and metolachlor (Mulder *et al.*, 1978; Reinhardt & Nel, 1986). Crop injury is often reported when low temperatures prevail after herbicide application. This is especially true for pre-emergence herbicides such as the acetanilides where the rate of emergence of seedlings is negatively correlated with herbicide uptake by the emerging plant parts. In addition, low temperatures would impede the metabolism of herbicides in the plant system. Because the behaviour of so many of the herbicides registered in maize are affected in this way it is important to understand the influence of temperature on maize tolerance.

Screening maize genotypes for tolerance to all, or at least the important, registered herbicides is important. Knowledge of maize tolerance to specific herbicides should reduce the risk of crop injury through the ability to recommend "safe" or "low-risk" herbicides for seed and commercial maize production fields. Obtaining information on the pattern of herbicide tolerance would enable maize breeders to select for this character and to produce cultivars with increased tolerance. Availability of information on the tolerance of genotypes may prevent litigation or may be a defense in the event of litigation. This could also help to develop farmers' confidence in the seeds since they would know that what they are buying has been tested for tolerance to commonly used herbicides.

In South Africa, few maize genotypes have been screened for tolerance to registered herbicides. Currently, regulatory authorities and the relevant seed and chemical companies have not reached consensus as to who should be responsible for screening new cultivars for tolerance to registered herbicides. Agrochemical companies may argue that it is almost impossible to assume this responsibility because of the large number of new maize cultivars that enter the market every year, and many are phased out from time to time. Generally, they only test the efficacy of their products on a few crop cultivars for the purpose of herbicide registration. An official agreement between the seed and agrochemical industries is long overdue in South Africa to avoid losses in terms of crop yield and/or liability cases as a result of herbicide use. Seed companies in their breeding programmes routinely screen material for crop performance traits, and it is only recently that two seed companies based in South Africa introduced a systematic screening programme for herbicide tolerance. It is this latter initiative that made this investigation possible.

The objectives of this study were:

1. To determine the tolerance of maize inbreds and hybrids to certain important herbicides under greenhouse and field conditions;
2. To appraise the correlation between greenhouse and field data for herbicide effects on maize genotypes;
3. To assess the influence of temperature on the tolerance of maize genotypes to alachlor, metazachlor and metolachlor;
4. To discern the pattern of inheritance for maize tolerance to metazachlor; and
5. To induce and note ultrastructural changes caused by metazachlor in root and leaf cells of maize seedlings.

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CHAPTER 1

GENERAL LITERATURE REVIEW

The literature cited in this chapter is mostly general information on differential tolerance of maize to herbicides, screening techniques and the herbicides used in the study. Literature that is specific to the study objectives is provided mainly in the respective chapters.

Terminology

Herbicides' effects on plants are variable depending on the type of herbicide, plant species, and environmental conditions. Plant response to herbicides range from stimulated growth, through temporary growth retardation and loss of vigor to irreversible growth inhibition and finally death. There are generally three terms that are used to categorise plants regarding their response to herbicides and these are: susceptibility, tolerance and resistance. Many authors have defined these terms to mean more or less the same (Le Baron & Gressel, 1982; Akobundu, 1987; Zimdahl, 1993). In this thesis the following definitions of these terms will be used:

Susceptibility (sensitivity) is a measure of the degree to which normal growth and development can be disrupted in a plant by a herbicide treatment. Susceptible plants will generally be killed or their growth inhibited when exposed to a particular herbicide at a dose at which the herbicide is used for weed control (Akobundu, 1987).

Tolerance refers to the plant's capacity to withstand a herbicide treatment at normal-use dose without injury or permanent damage to its growth and development. Tolerant plants are not

affected or injured by a herbicide at normal-use doses (Akobundu, 1987). These plants have a site of herbicide action, and would depend on prevention of uptake, limited translocation, and/or increased breakdown of a herbicide in order to be tolerant. This is the natural variability of response to herbicides that exists within a species and can easily and quickly develop (Zimdahl, 1993).

Resistance refers to the ability of a plant to grow normally in spite of its exposure to an above- normal-use rate of a herbicide. In other words, a resistant plant is one that always survives and grows normally at the usually effective dose of a herbicide (Akobundu, 1987; Zimdahl, 1993) Resistant plants often lack a metabolic site of action for the herbicide (Arntzen *et al.*, 1982; Zimdahl, 1993). This is the major difference between resistant and tolerant plants.

Differential tolerance of maize to herbicides

Differential tolerance of maize, and other crops, as well as weed species are well documented. Many researchers have reported differential tolerance among crop genotypes to specific herbicides (Malan *et al.*, 1984; Landi *et al.*, 1989; Le Court de Billot *et al.*, 1990; Rowe *et al.*, 1990; Randall *et al.*, 1995; O'Sullivan, *et al.*, 1998; Van Wychen, *et al.*, 1999; Grey *et al.*, 2000; Kanyomeka & Reinhardt, 2000; Pauley *et al.*, 2000; Bussan *et al.*, 2001; Cavero *et al.*, 2001; Blair-Kerth *et al.*, 2001; O'Sullivan, *et al.*, 2001). A summary of some reported cases of differential tolerance to selected herbicides is presented in Table 1.1.

The remarkably wide tolerance of maize inbreds and hybrids to herbicides is mediated by interplay of herbicide uptake, translocation, metabolism, interaction of metabolism with translocation and active site sensitivity (Jensen, 1982; Arntzen *et al.*, 1982; Akobundu, 1987; Randall, *et al.*, 1995; Grey *et al.*, 2000). Factors that affect the efficacy (weed control) of

herbicides may also influence the tolerance of maize genotypes to herbicides (Jensen, 1982). For example, environmental and soil factors can affect the tolerance of maize genotypes to herbicides. Environmental factors could increase herbicide activity or uptake and can also reduce the crop's ability to metabolise the herbicide, and hence crop injury could occur (Ashton & Crafts, 1981; Akobundu, 1987; Butzen, 2000).

Hot and humid conditions could promote the uptake of herbicides by maize, especially growth regulators such as 2,4-D. Under those conditions the plant probably cannot metabolise the herbicide effectively (Ashton & Crafts, 1981; Butzen, 2000). Maize emerging under cool and wet conditions is vulnerable to herbicide injury from soil-applied herbicides. This exposes the emerging maize seedlings to prolonged contact with the herbicide in the soil, and therefore, may promote more herbicide absorption than the normal amounts. Also, under these conditions, the plant may not effectively metabolise the herbicide, resulting in the plant being unable to detoxify the herbicide absorbed (Butzen, 2000). If these conditions prevail after planting and herbicide application, the chloroacetamide herbicides, for example metazachlor, metolachlor and alachlor, may injure maize (Butzen, 2000).

Table 1.1 A summary of some reported differential crop tolerances to specific herbicides

Crop	Herbicide	Nature of tolerance	Reference
Maize	Atrazine	Differential metabolism among genotypes	Andersen (1964), Eastin (1971), Le Court De Billot & Nel (1985), Le Court De Billot *et al.*, (1990)
Maize	Alachlor	Differential metabolism among genotypes	Niccum (1970), Ashley (1972), Narsaiah & Harvey (1977), Francis & Hamill (1980), Mellis *et al.* (1982)
Maize	Metolachlor	Differential metabolism among genotypes	Rowe *et al.*, (1990)
Maize	2,4-D	Differential translocation among genotypes	Gauvrit & Gaillardon (1991)
Cotton	Atrazine	Differential metabolism among genotypes	Abernathy *et al.*, (1979)
Soybean	Metribuzin	Differential metabolism among genotypes	Hagood *et al.*, (1980)
Sorghum	Atrazine	Differential metabolism among genotypes	Burnside & Wicks (1972)

Many other factors, however, could result in crop injury. Herbicide misapplication, such as spraying at the wrong crop stage, poor weather conditions, overlapping spray patterns, directly spraying into the whorl of the maize plant, deep planting, and crust formation during crop emergence are just some of the factors which may reduce crop tolerance, and hence cause crop injury.

Crop tolerance to herbicides has been extensively studied and found to be under genetic control (Grogan *et al.*, 1963; Edwards *et al.*, 1976; Francis & Hamill, 1980; Faulkner, 1982; Souza Machado, 1982; Souza Machado & Bandeen, 1982; Le Court De Billot *et al.*, 1990; Van Wychen *et al.*, 1999). The nature of inheritance of herbicide tolerance in crops varies from one herbicide to another. Generally, single or multiple genes control inheritance. Le Court De Billot *et al.*, (1986) reported polygenic inheritance and the presence of additive gene effects in a study on inheritance of atrazine tolerance in maize. Strong additive effects in inheritance of diclofop-methyl tolerance in maize have been reported (Geadelman & Andersen, 1977). However, non-additive gene effects in the inheritance of alachlor tolerance in maize were reported (Francis & Hamill, 1980). Cytoplasmic influence in crop tolerance to herbicides has been reported (Rao & Fleming, 1978; Souza Machado & Bandeen, 1982). For example, cytoplasmic influence on the response of maize to butylate was found (Rao & Fleming, 1978). This means that inheritance of herbicide tolerance is herbicide-specific and should be determined for each herbicide.

A well-established pattern of inheritance of a herbicide provides valuable information for breeding cultivars that are tolerant to specific herbicides. Faulkner (1982) discussed the breeding of herbicide-tolerant cultivars by conventional methods. Although there are several

reported possibilities for achieving improved crop tolerance to herbicides through breeding, some researchers have found no meaningful gains from this approach. Butzen (2000) reported that breeding for tolerance to herbicides does not achieve appreciable results. *De Felice (2000, personal communication), is of the opinion that no breeding programme has succeeded in improving the tolerance of maize cultivars to herbicides. Perhaps this is the reason why most, if not all, maize breeding programmes are not specifically aimed at improving herbicide tolerance. Also, the fact that maize cultivars usually do not last many years in the market does not warrant relatively expensive herbicide tolerance testing in a breeding programme.

Screening techniques

Various techniques to assess the effects of herbicides on plants exist. These could involve laboratory, greenhouse or field screening. Truelove & Hensley (1982) reviewed these techniques and indicated that they involve treating plants or plant organs with a herbicide and recording the effects on plant growth or some metabolic activity related to the primary site of herbicide action.

Field screening has been used to test the performance of herbicides for many years. Even if a lot of information about crop varieties' tolerances to herbicides in the laboratory or greenhouse could be generated, it is still necessary to make final evaluations in the field. This technique is very useful because it gives information on the effects of herbicides on yield, which is usually the ultimate parameter in crop production. Field screening also helps to confirm suspicious patterns of varietal tolerance (Truelove, 1977; Truelove & Hensley, 985a).

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Field screening, however, has some shortcomings such as uncontrollable effects of soil factors and environmental conditions, and the long duration of field experiments makes it expensive. This implies that other screening methods could be more practical and even more efficient. Cavero, *et al.* (2001) used field screening to determine the tolerance of paprika pepper to clomazone applied pre-emergence. Similarly, Pauley *et al.* (2000) used field screening to determine the tolerance of sorghum to atrazine, ammonium sulphate and glyphosate.

Greenhouse screening is relatively cheap as compared to the field screening in terms of both the execution and duration of experiments. It is also possible to control the environment and the growth media. This method involves growing plants under controlled environments for a limited period during the vegetative growth stage. The herbicide effects are assessed based on visual injury symptoms and/or plant growth parameters such as fresh and dry mass yields of the top growth and/or roots. The disadvantages of this method include the inconsistency of herbicide performance under controlled conditions and in the field, and that the herbicide effects on the ultimate yield component is not assessed (Hardcastle, 1979; Clay & Davison, 1981; Breeze, 1994).

Laboratory screening methods also exist. Truelove & Hensley (1982) described how these methods are applied. Laboratory methods involve treating crop seeds with a herbicide or germinating seeds in a herbicide solution at an appropriate concentration. The herbicide effect is measured based on seed germination and seedling development. Root and shoot length, germination percentage, fresh and dry mass are recorded. Chlorophyll fluorescence screening is another technique gaining popularity (Ahrens *et al.*, 1981; Truelove & Hensley,

1982). Various other laboratory techniques have also been used. These include cell suspension cultures, sulphate transport in roots, measurements of nitrate reductase activity, and ultrastructural changes in the cells of some plant parts, for example leaves and roots (Kleper, 1975; Ferrari *et al.*, 1981; Reinhardt & Nel, 1986; Baum *et al.*, 1998).

Herbicides used in the present study

Several herbicides are registered for weed control in maize. In South Africa, a total of 42 herbicides and herbicide combinations are registered for maize (Vermeulen *et al.*, 1998). These are dominated by herbicides from two families, amides and triazines. Acetamides (acetanilides) in the form of alachlor and metolachlor, and triazines in the form of atrazine are the dominant groups of herbicides in the United States of America (Ellis, 1992), and the situation is similar in South Africa and other parts of Africa (Akobundu, 1987). However, currently various new herbicides belonging to other herbicide groups are available on the market.

Mechanisms of action, chemical and physical properties of herbicides

Ultrastructural changes that are evoked by herbicides in plants occur due to effects at the biochemical level. The structural changes to plant cells or cell organelles are usually caused by several biochemical alterations originating from one primary site of action; meaning that an inhibition or disturbance in one step of a pathway will trigger the inhibition in other steps of other pathways and the entire complex system will not work accordingly because these are inter-linked (Akobundu, 1987).

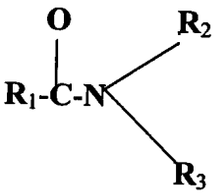
Herbicides are developed to act on one or more important plant processes in order to be

effective. Plant processes that are affected by herbicides include vital life processes such as respiration, photosynthesis, cell division and enlargement (Ashton & Monaco, 1991; Zimdahl, 1993). The effect on these vital plant functions may be directly on the process pathway or indirectly through disruption of the integrity of cell organelles such as chloroplasts, mitochondria, cell membranes, vacuole membranes, and general disturbance of the integration of cell organelles' functions.

The herbicides used in this study were selected based on their usage. Most commonly used herbicides and some promising new herbicides and herbicide mixtures were selected.

Amides

This is a large group of herbicides of which the molecules have the basic chemical structure shown below:



The substitutions on positions R_1, R_2 and R_3 vary greatly, making this a diverse group of chemicals. All herbicides in this group are substituted acid amides. The name of an acid amide herbicide is derived from the R_1 group. Depending on the acid group, the herbicide could be an acetamide, benzamide, propiomide, etc. If a phenyl group replaces one hydrogen of the ammonium group (R_1 or R_2) the resulting herbicide will be an anilide. Chloroacetamides have a monochloronated methyl group (R_1=cl-CH_2-) of the amide structure (Klingman & Ashton, 1982; Akobundu, 1987).

The majority of herbicides in this group are soil-applied and are effective on germinating grasses and some broadleaf weeds. However, some have post-emergence activity on very young susceptible plants. They inhibit the shoot and root growth of susceptible seedlings. Injury symptoms of these herbicides include inhibition of early seedling growth and emergence after germination, stunted roots and shoots, twisted or malformed leaves, and improper unrolling of leaves from the sheath.

Many studies have been carried out to study the mechanism of action of this group of herbicides but this is still not well understood. However, the mechanism of action for these herbicides appears to be the inhibition of seedling growth after germination. They inhibit root and shoot growth of susceptible plants. These herbicides are known to inhibit protein and lipid synthesis (Jaworski, 1975; Kearney & Kaufman, 1975; Zimdahl, 1993). They also inhibit the biosynthesis of isoprenoids and flavonoids. All these effects involve the conjugation of acetyl co-enzyme A and other sulfhydryl-containing biomolecules by amide herbicides.

The mechanism of action of alachlor is the inhibition of protein synthesis. It has been reported to cause disintegration of cell vacuole membranes, double membranes of chloroplasts and membranes of nuclei (Reinhardt & Nel, 1986). Similarly, metolachlor has been reported to cause ultrastructural changes like those caused by alachlor (Kearney & Kaufman, 1975). However, this kind of information still lacks for some herbicides, such as metazachlor, in this group. It is therefore important to study the effects of such herbicides on the cell structure to fully understand their mechanism of action.

In the present study, six herbicides belonging to amide group were used: acetochlor, alachlor, dimethenamid, flufenacet, metazachlor and metolachlor. Chemical and physical properties of these herbicides are listed below (BASF, 1984; Tomlin, 1994; Ahrens, 1994).

Chemical and physical properties of acetochlor

Chemical name: 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl) acetamide

Chemical formula: $C_{14}H_{20}ClNO_2$

Chemical family: chloroacetamide, chloroacetanilide, or acetanilide (acetamide)

Trade name: Guardian:

Molecular weight: 269.77

Physical state: Thick, oily liquid, light amber to violet, aromatic odour.

Melting point: <0°C

pKa: none

Vapour pressure: 3.4×10^{-8} mm Hg at 25°C

Solubility:

Water 223 mg L^{-1} at 25°C

Organic solvents at 25°C:

Acetone (soluble)

Benzene (soluble)

Chloroform (soluble)

Ethanol (soluble)

Ether (soluble)

Toluene (soluble)

Density: 1.136 g ml^{-1} at 20°C

Soil behaviour: Average field half-life is 8-12 weeks

Chemical and physical properties of alachlor

Chemical name: 2-cloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl) acetamide

Chemical formula: $C_{14}H_{20}ClNO_2$

Chemical family: chloroacetamide, chloroacetanilide, or acetanilide (acetamide)

Trade name: Lasso:

Molecular weight: 269.77

Physical state: Cream and odorless

Melting point: 39.5-41.5°C

pKa: none

Vapour pressure: 1.6 x 10^{-5} mm Hg at 25°C

Solubility:

Water 200 mg L^{-1} at 20°C, 240 mg L^{-1} at 25°C

Organic solvents at 25°C:

Acetone (soluble)

Benzene (soluble)

Chloroform (soluble)

Ethanol (soluble)

Density: 1.133 g ml^{-1} at 25/15.6°C

Soil behaviour: Average field half-life is 21 days

Chemical and physical properties of dimethenamid

Chemical name: 2-chloro-N-(2,4-dimeethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)=acetamide

Chemical formula: $C_{12}H_{18}ClNO_2S$

Trade name: Frontier

Chemical family: chloroacetamide, chloroacetanilide, or acetanilide (acetamide)

Molecular weight: 275.8

Physical state: yellowish-brown and odorless

Boiling point: 127°C at 26.7 Pa

pKa: none

Vapour pressure: 36.7 mPa at 25°C

Solubility at 25°C

Water 1174 +/- 12 mg L^{-1}

Organic solvents:

Hectane 28.2 g $100g^{-1}$

Isooctane 22.0 g $100g^{-1}$

Ethanol >50%

Ether >50%

Density: 1.187 g ml^{-1} at 25°C

Soil behaviour: half-life is 2-5 weeks

Chemical and physical properties of metazachlor

Chemical name: 2-cloro-*N'*-(2,6-dimethylphenyl)-*N'*-(1H-pyrazol-1-ylmethyl)-acetamide

Chemical formula: $C_{14}H_{16}ClN_3O$

Trade name: Preecede

Chemical family: chloroacetamide, chloroacetanilide, or acetanilide (acetamide)

Molecular weight: 277.76

Physical state: crystalline, colorless and odorless

Melting point: 85°C

Solubility:

Water 0.1g/100 ml

Good solubility in:

Acetone

Ethyl acetate

Chloroform

Chemical and physical properties of metolachlor

Chemical name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

Chemical formula: $C_{15}H_{22}ClNO_2$

Trade name: Dual

Chemical family: chloroacetamide, chloroacetanilide, or acetanilide (acetamide)

Molecular weight: 283.80

Physical state: white and odorless

Melting point: -40°C

pKa: none

Vapour pressure: 1.3×10^{-5} mm Hg at 20°C

Solubility

Water 488 mg L^{-1} at 20°C

Organic solvents at 25°C:

Acetone (miscible)

Benzene (miscible)

Ethanol (miscible)

Ethylene dichloride (miscible)

Density: 1.117 g ml^{-1} at 20°C

Soil behaviour: half-life is 3-5 months

Triazines

This is a large and very important group of herbicides. They are the most widely used herbicides in food production in the tropics (Akobundu, 1987). The herbicides in this group have been extensively studied (Gast, 1970; Kearney, 1970; Esser e*t al.*, 1975; Bandeen *et al.*, 1982; Le Court De Billot, 1985; Reinhardt, 1993). These herbicides are all well-known inhibitors of photosynthetic electron transport. Atrazine belongs to this group of herbicides and it is principally an inhibitor of photosynthesis, although many other processes are also affected. Triazines inhibit photosynthesis by disrupting electron transport in Photosythem II (Ashton & Crafts, 1981; Ahrens, 1994).

Of particular importance is the herbicide atrazine that dominates broadleaf weed control in maize in South Africa and in many other countries where its use is still allowed. This herbicide is the most important or most widely used in maize in this country, and it is the only one from this group of herbicides that was used in the present study. This herbicide is also sold as a component of several formulated herbicide mixtures with other herbicides such as alachlor, metolachlor or cyanazine, to mention but a few.

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Injury symptoms of triazines include interveinal and/or veinal chlorosis affecting mostly older leaves. Roots and shoots are not affected directly. Chemical and physical properties of atrazine are listed below.

Chemical and physical properties of atrazine

Chemical name: 6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine.

Chemical formula: $C_8H_{14}ClN_5$

Trade name: Atrazine, Flotrazine, etc

Chemical family: Triazine

Molecular weight: 215.69

Physical state: white, crystalline.

Melting point: 175-177

pKa: 1.7 at 21°C

Vapour pressure: 2.9×10^{-7} mm Hg at 25°C

Solubility:

Water 33 mg L^{-1} at 22°C

Organic solvents g $100ml^{-1}$ at 20°C:

Chloroform 5.2

Ethyl acetate 2.8

Methanol 1.8

Density: 0.363 g ml^{-1} at 20°C

Soil behaviour: Average half-life in the field is 60 days

Other herbicides used in this study are herbicide mixtures of acetochlor + atrazine/sulcotrione and flumetsulam/metolachlor. Herbicide mixtures are convenient in certain situations by increasing the range of weeds that can be controlled. For example in a mixture of flumetsulam/metolachlor, flumetsulam mainly controls broadleaf weeds while metolachlor mainly controls grass weeds. A combination of the two solves both broadleaf and grass weed problems. These herbicide mixtures are widely used in South Africa and hence their inclusion in the study. Below are properties of some of the other herbicides used in the present study, which are commonly used in mixtures with other herbicides:

Chemical and physical properties of sulcotrione

Chemical name: 2-[2-chloro-(4-methylsulfonyl)benzoyl]cyclohexane-1,3-dione

Chemical formula: $C_{14}H_{13}ClO_5S$

Trade name: ICI-A0051; SC-0051

Chemical family: Benzoylcyclohexane-1,3-diones or triketones

Molecular weight: 328.77

Description: Light tan solid

Melting point: 139°C

pKa: Not known

Vapour pressure: 4×10^{-8} mm Hg at 25°C

Solubility:

Water 164 mg L^{-1} at 25°C

Soluble in acetone and chlorobenzene

Density: Not known

Soil behaviour: Average half-life in the field is 15-72 days

This herbicide causes plants to be bleached with reduced chlorophyll and carotenoids with elevated phytoene levels. Elevated phytoene levels interfere with plastoquinone biosynthesis by inhibiting the enzyme *p*-hydroxyphenylpyruvate dioxygenase which catalyses the formation of *p*-hydroxyphenylpyruvate, (Ahrens, 1994).

Chemical and physical properties of flumetsulam

Chemical name: *N*-(2,6-diflurophenyl)-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide

Chemical formula: $C_{12}H_9F_2N_5O_2S$

Trade name: DE 498

Chemical family: Triazolopyrimidine

Molecular weight: 325.29

Physical state: Off-white to light tan solid.

Melting point: Unknown

pKa: 4.6

Vapour pressure: 2.8×10^{-15} mm Hg at 25°C

Solubility:

Water 49 mg L^{-1} at 25°C

Organic solvents g $100ml^{-1}$ at 25°C:

Acetone <1.6

Methanol <0.4

Density: 1.77 gm L^{-1}

Soil behaviour: Average half-life in the field is 60 days

This herbicide's mechanism of action is that it inhibits acetolactate synthase (ALS) which is a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine and valine (Ahrens, 1994).

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CHAPTER 2

SENSITIVITY OF MAIZE INBREDS AND HYDRIDS TO SELECTED HERBICIDES

Introduction

Maize (*Zea mays* L.), the most important crop in sub-Saharan Africa, is susceptible to weed interference. If weeds are not properly controlled during the first 6-8 weeks after planting, maize yields will be greatly reduced (Bhau & Singh, 1979; Akobundu, 1987). In the absence of any weed control maize yield loss of 40-60 % may occur in the tropics, while some weeds, such as *Rottboellia cochinchinensis* and *Striga* spp can cause complete crop loss. Weeds should therefore be properly controlled to achieve optimum maize yields.

Herbicides have been used to control weeds, particularly in large-scale farming, and have played a very important role in increasing maize production for the past five decades and will continue to do so for the foreseeable future. Herbicide use is the most practical way of coping with weeds in large-scale maize production (Akobundu, 1987). However, lack of herbicide selectivity that cause crop injury is reported from time to time, and varietal differences have also been noted.

Maize and other crop inbreds and hybrids vary greatly in their response to specific herbicides, and this aspect has been extensively studied elsewhere in the world (Francis & Hamill, 1980; Charlotte *et al.*, 1989; Randall *et al.*, 1995; Wilson, 1999; Grey *et al.*, 2000; O'Sullivan *et al.*, 2001). In South Africa, and other parts of Africa, very little work has been done in this regard. The little research there is has mostly been on maize tolerance to atrazine, which is the most

widely used herbicide in maize production in the country (Nel & Reinhardt, 1984; Le Court De Billot *et al.*, 1986; 1990). Alachlor, a commonly used herbicide in maize production, has been reported to exhibit differential effects on sweet and field maize (Niccum, 1970; Ashley, 1972; Narsaiah & Harvey, 1977; Francis & Hamill, 1980). Metolachlor, another commonly used herbicide in maize production was found to exhibit different effects on maize inbreds and hybrids (Rowe *et al.*, 1990). Differential tolerance of maize inbreds and hybrids to herbicides were also reported by others (Charlotte *et al.*, 1989; Landi *et al.*, 1989; Van Wychen *et al.*, 1999; O'Sullivan, *et al.*, 1999; O'Sullivan *et al.*, 2001). These differential responses of genotypes as well as variable weed control efficacy of herbicides are probably dependent on environmental factors, but could also be due to genetic differences.

Currently, in South Africa and other parts of Africa, very few crop cultivars have been screened for tolerance to registered herbicides. The few that have been screened were screened for tolerance to very few herbicides. Moreover, in most screening trials, parent lines have seldom been included. There exists, therefore, the need to screen crop genotypes for herbicide tolerance in order to recommend appropriate compounds for use on seed and commercial production fields. The objective of a series of experiments was to determine the tolerance of selected maize inbreds and hybrids to important herbicides under both greenhouse and field conditions.

Materials and Methods

Greenhouse experiments

In the first series of trials, comprising seven pot experiments, a total of 60 maize inbreds and 10 hybrids were screened for tolerance to seven herbicides (Table 2.1). A separate single pot

experiment was conducted in the following season of 1999/2000, and the herbicides used appear in Table 2.1. The methodology in all experiments was as described below. The names of genotypes screened are coded as a requirement by the seed suppliers to maintain confidentiality. The series of pot experiments were conducted in a greenhouse at the University of Pretoria phytotron where day/night temperature was maintained at 25-30°C/15-18°C, with a photoperiod of 12-14 hours.

Pots were each filled with 2.5 kg of soil, which was sieved through a 4-mm screen. The soil used was collected from the University of Pretoria experimental farm. The soil consisted of 71.5 % sand, 8.2 % silt, 17.1 % clay, 0.4 % organic matter (%C) and had a pH (H_2O) of 6.5. The pots were lined with plastic bags to avoid herbicide leaching. Pots were arranged in a completely randomised design with four replicates for each treatment.

Five seeds were planted at a depth of 30 mm in each pot. Three days after emergence, seedlings were thinned to three per pot. Watering was done to ensure that moisture was maintained near the field capacity level. To achieve this pots were first weighed immediately after planting and initial watering, and subsequently, once per week in order to avoid under- or over-watering. After emergence, either a complete nutrient solution (Nitsch, 1972) or tap water was applied on alternate days. In order to avoid variation in nutrient supply the same volume of nutrient solution (100 ml) was always applied to each pot, irrespective of water loss, but the volume of tap water applied was dependent on the amount lost.

All herbicides used were registered for pre-emergence application (Vermeulen, *et al.*, 1998), and they were applied one to two days after planting (Table 2.1). Application rates were the

highest recommended for a particular formulated product on the soil used. The rationale being that if an inbred or hybrid is tolerant to a herbicide at this maximum rate it is unlikely that it will be injured by the herbicide in the field. Plant counts were made three days after emergence. Visual injury rating (VIR) was done at seven days after emergence using a 1-10 rating scale; where 1 indicates no effect and 10 indicates complete kill. Shoot dry mass (SDM) was measured three weeks after planting by cutting the shoots at the soil surface and drying them in an oven at 65°C for 48 hours. To determine root dry mass (RDM), roots were thoroughly washed and oven-dried. Samples were then weighed individually.

Field experiments

Two field experiments were conducted at the University of Pretoria experimental farm, at the site where soil was collected for use in the greenhouse experiments. The first trial was done in the 1998/1999 growing season, and the second in the 1999/2000 season. Information regarding the site description, weather conditions and operational dates for both field experiments is provided in Table 2.2.

The seedbed was prepared by tandem discing followed by spike tooth harrowing. Fertiliser application was done before planting at the rate of 350 kg 2:3:2 ha^{-1}. A top dressing of 150 kg LAN ha^{-1} was applied. Maize was planted by hand at a spacing of 910 mm between rows, 300 mm between planting stations and at a depth of 30 mm. The trial with thirteen maize genotypes that had previously been evaluated in the greenhouse was done from September 1998 to May 1999. The selection of genotypes was based on the greenhouse results. The selection was made to include both tolerant and sensitive genotypes. The effects of three of the herbicides used in the greenhouse experiments were assessed in the field. The herbicides

dimethenamid, metazachlor and acetochlor + atrazine/sulcotrione were applied at the same rates used in the greenhouse (Table 2.1). The same herbicides used in the second series of greenhouse screening experiments were used in the (second) field experiment (Table 2.1). The genotypes tested in the field were also the same as those used in the second series of pot experiments.

Plots were kept weed free by means of hand hoeing throughout each growing season in order to eliminate the possibility of reduced yields or any variation in the measured parameters due to weed-crop interaction. A randomised complete block design, with three replicates for each treatment, was used. Plot size was 5 x 4 m. All data were collected from the two centre rows out of a total of four rows per plot. Plant counts were made 2-3 days after emergence started. Visual assessments of crop injury were made at 2, 4, 8 and 14 weeks after emergence. Shoot dry mass was recorded at four weeks after planting, and days to 50 % tasselling and 50 % silking, as well as grain yield were measured.

All data were expressed as percent reduction from the untreated control for each inbred and hybrid. Data were subjected to analysis of variance by means of the SAS programme on the main-frame computer of the university of Pretoria (SAS user's Guide Stat., 1989). Treatment means were compared using Turkey's Least Significant Difference (LSD) test at the 5 % level of significance.

TABLE 2.1 Herbicides used in the maize tolerance screening study in the greenhouse

Product	Active ingredient (a.i)	a.i.content ($g\ L^{-1}$)	Dosage** ($L\ ha^{-1}$)
Experiment Series I 1998/1999: (seven experiments; 70 genotypes)			
Bateleur	Flumetsulam/metolachlor	20/630	1.3
Dual S GOLD	Metolachlor	915	0.9
Frontier	Dimethenamid	900	0.75
Flotrazine	Atrazine	500	3.25
Lasso	Alachlor	480	4.0
Preecede	Metazachlor	400	1.5
*Galleon	Atrazine/sulcotrione	300/125	0.8
*Wenner	Acetochlor	700	1.0
Experiment Series II	*1999/2000: (one experiment; 10 genotypes)*		
Basagran	Bendioxide	480	5.0
Tiara	Flufenacet	600	400 $g\ ha^{-1}$
Gardomil	Atrazine/metolachlor/ terbuthalazine	262.5/175/ 262.5	2.3
Guardian S	Acetochlor	840	1.3

* Applied in combination

** Equivalent applied to 170 cm^2 (soil surface area of each pot), dosage is for the formulated product.

Common names: acetochlor,2-chloro-*N*-(ethoxymethyl)-*N*-(-ethyl-6-methylphenyl)acetamide, alachlor, 2-chloro-*N*-(2,6-diethyl phenyl)-*N*-(methoxymethyl)acetamide; acetochlor,2-chloro-*N*-(ethoxmethyl)-*N*-(2-ethl-6-methyl phenyl)acetamide; atrazine, 6-chloro-N-ethyl-N^1-(1-methylethyl)-1,3,5-triazine-2,4-diamine; dimethenamid, 2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)acetamide; flumetsulam, *N*-(2,6-difluorophenyl)-5-methyl[1,2,4]trazolo[1,5-á]pyrimidine-2-sulfonamide; metazachlor, 2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2'-6'-xylidide; metolachlor, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide; and sulcotrione, 2[2-chloro-4(methylsulfonyl)benzoyl]-1,3-cyclohexanedione; (Tomlin, 1994).

TABLE 2. 2 Site description and dates of operations for the two field experiments

Item	Description	
	Experiment I	Experiment II
Previous crop	Maize	Maize
Herbicide used last season	Metolachlor	Metazachlor & dimethenamid
Soil pH (H_2O)	6.5	6.8
Organic matter content (%C)	0.4 %	0.3 %
Clay content	17.7 %	16.8 %
Sand content	72.0 %	72.0 %
Silt content	7.0 %	7.8 %
Planting date	28/10/98	1/12/99
Herbicide application date	29/10/98	2/12/99
Crop harvesting date	18/3/99	26/4/00
Min. temp. 24hr	15.1°C	12.9 °C
Max. temp. 24hr	27.3 °C	29.6 °C
Total rainfall (growing season)	552.2 mm	894.0 mm
Irrigation	200 mm	Nil
Disease and pest control	Nil	Nil

Results and Discussion

For the Series I pot experiments, the SDM and RDM data for four of the seven experiments (3 x 10 inbreds and 1 x 10 hybrids) are given in Table 2.3 and 2.4 respectively. None of the herbicides significantly reduced the rate of emergence of seedlings in the greenhouse (data not presented). VIR data are presented in Table 1A in the appendix. For the Series II experiments, the VIR and SDM data are presented in Tables 2.10 and 2.11, respectively. Results for the first field experiment, comprising crop injury rating, shoot dry mass, days to 50 % tasselling and silking and grain yield, are presented in Tables 2.5, 2.6, 2.7, 2.8 & 2.9, respectively. Rate of emergence results for the first field experiment are presented in Table 2B. The second field experiment's results for the rate of emergence, days to 50 % tasselling, days to 50 % silking and grain yield are presented in Tables 3B, 2.12, 2.13 & 2.14, respectively. There were no visual injury symptoms observed, and therefore, no data on VIR are presented.

Inbred responses in the 1998/1999 growing season

Maize emergence was not significantly reduced by any of the herbicides in the greenhouse (data not shown), while significant differences occurred in the field experiment (Table 2B). Both in the greenhouse and in the field (Tables 2.5 & 1B), visual injury symptoms ranged from no effect to very severe effects. For example, P11 (CL) showed severe injury due to metazachlor application at the recommended rate. However, no injury symptoms were observed when the same herbicide, was applied to P2 (SX1) at the recommended rate.

TABLE 2.3 Growth inhibition (% reduction from controls) caused by herbicides on maize shoot dry mass in the greenhouse (ANOVA in Table 1A, 2A, 3A &4A) (Experiment Series I)

Genotype	Herbicide							
	FL/-MET	MET	DIM	ATR	AC+ATR/-SU	ALA	METZ	Mean
Batch I: Inbreds								
P1	0	8	9	0	6	3	59	12
P2	10	0	5	3	7	0	0	4
P3	21	27	5	4	24	24	0	15
P4	11	4	11	11	9	13	11	10
P5	10	15	19	11	11	12	13	13
P6	25	0	6	7	13	17	28	14
P7	11	6	9	3	8	10	7	8
P8	11	12	6	8	7	1	9	8
P9	13	0	2	28	5	0	14	9
P10	29	6	8	0	25	3	60	19
Mean	14	8	8	8	12	8	20	
$LSD_{T(0.05)}$ Genotype x Herbicide = 13				SE_{means} = 5				
Batch II:Inbreds								
P11	18	0	0	2	7	1	13	6
P12	5	5	0	0	0	2	1	2
P13	13	16	4	12	20	17	0	12
P14	0	0	15	10	0	8	13	7
P15	12	7	5	11	22	5	21	12
P16	14	2	1	10	11	0	16	8
P17	8	4	0	1	6	0	15	5
P18	20	8	18	4	18	13	10	13
P19	20	0	19	0	24	5	23	13
P20	13	5	4	4	21	4	17	10
Mean	12	5	7	5	13	6	13	
$LSD_{T(0.05)}$ Genotype x Herbicide = 13				SE_{means} = 4				

Batch III. Inbreds								
P21	0	0	0	0	3	0	0	0
P22	0	0	0	0	4	0	0	1
P23	0	2	0	0	0	0	0	0
P24	23	12	18	9	12	25	17	17
P25	4	6	1	0	0	0	0	2
P26	0	0	0	0	0	1	12	2
P27	11	0	6	14	7	9	25	10
P28	13	0	11	16	7	5	3	8
P29	0	0	0	1	0	0	0	0
P30	10	12	0	1	15	3	12	8
Mean	5	3	2	3	5	4	7	
$LSD_{T(0.05)}$ Genotype x Herbicide = 14				SE_{means} = 5				
Batch IV. Hybrids								
CV1	0	0	0	0	0	0	0	0
CV2	11	18	14	0	8	0	12	9
CV3	0	1	8	30	0	20	0	8
CV4	0	1	0	5	17	4	24	7
CV5	21	0	0	7	9	0	26	9
CV6	2	0	0	0	0	0	7	1
CV7	0	4	3	18	0	0	0	4
CV8	5	0	1	8	0	0	0	2
CV9	33	0	7	0	0	0	6	7
CV10	20	26	22	17	14	7	24	19
Mean	9	5	6	8	5	3	10	
$LSD_{T(0.05)}$ Genotype x Herbicide = 21				SE_{means} = 8				

Turkey's LSD is for comparing means within maize genotypes and herbicides. A zero implies no reduction or an increase above the untreated control.

Key: FL=flumetsulam, MET=metolachlor, DIM=dimethenamid, ATR=atrazine, AC=acetochlor, SU=sulcotrione, ALA= alachlor, METZ=metazachlor.

TABLE 2.4 Growth inhibition (% reduction from untreated controls) caused by herbicides on maize root dry mass in the greenhouse (ANOVA in Table 5A, 6A, 7A & 8A) (Exp. Series I)

	Herbicide							
Genotype	FL/MET	MET	DIM	ATR	AC+ATR/SU	ALA	METZ	Mean
BatchI:Inbreds								
P1	27	11	18	33	49	17	1	22
P2	0	16	0	20	6	15	0	8
P3	5	0	0	0	0	26	0	4
P4	11	6	0	0	0	14	0	4
P5	0	0	0	27	21	14	1	9
P6	20	23	21	8	0	0	11	12
P7	2	2	6	1	20	10	12	8
P8	39	32	23	10	19	31	16	24
P9	12	14	0	0	0	0	0	4
P10	4	28	0	17	46	0	43	20
Mean	12	13	7	12	16	13	8	
$LSD_{T(0.05)}$ Genotype x Herbicide = 9				SE_{means} = 7				
Batch II: In-breds								
P11	17	4	0	13	14	0	13	9
P12	8	8	0	0	0	0	10	4
P13	30	28	24	22	28	29	25	27
P14	0	0	0	4	4	0	8	2
P15	0	0	0	0	6	0	36	6
P16	23	0	0	1	0	0	0	3
P17	0	11	1	19	1	24	13	10
P18	0	6	2	0	0	0	0	1
P19	23	12	25	29	12	7	15	18
P20	18	4	35	28	25	22	19	22
Mean	12	7	9	12	9	8	14	
$LSD_{T(0.05)}$ Genotype x Herbicide = 10				SE_{means} = 7				

Batch III. In-breds								
P21	37	0	23	10	0	8	2	11
P22	0	0	0	0	0	0	0	0
P23	34	5	31	0	0	18	39	18
P24	14	9	25	4	28	0	8	13
P25	0	0	0	0	0	0	0	0
P26	29	0	0	0	12	0	12	8
P27	0	0	0	0	0	0	0	0
P28	14	16	26	36	37	16	2	21
P29	20	16	35	18	24	14	19	21
P30	30	1	0	8	0	29	29	14
Mean	18	5	14	8	10	8	11	
$LSD_{T(0.05)}$ Genotype x Herbicide = 15			SE_{means} = 14					
Batch IV. Hy-brids								
CV1	0	0	0	8	0	7	12	4
CV2	17	26	24	7	18	22	16	19
CV3	8	15	18	8	23	19	32	18
CV4	8	21	9	2	33	17	21	16
CV5	0	19	0	16	11	0	28	11
CV6	13	15	2	2	0	0	0	5
CV7	0	0	0	10	9	0	10	4
CV8	18	4	27	20	0	27	2	14
CV9	34	33	21	0	19	25	25	22
CV10	3	6	0	0	0	0	0	1
Mean	10	14	10	7	11	12	15	
$LSD_{T(0.05)}$ Genotype x Herbicide = 12			SE_{means} = 8					

Turkey's LSD is for comparing means within maize genotypes and herbicides. A zero implies no reduction or an increase above the untreated control.

Key: FL=flumetsulam, MET=metolachlor, DIM=dimethenamid, ATR=atrazine, AC=acetochlor, SU=sulcotrione, ALA= alachlor, METZ=metazachlor.

Similarly, other herbicides caused injury symptoms and some did not. Injury symptoms varied from one herbicide to another, both in the greenhouse and in the field. Most of the severe injury symptoms were observed on inbreds exposed to metazachlor. Absence of visual injury symptoms did not, however, indicate that the inbred was tolerant because some lines showed no visual injury symptoms although their SDM and/or RDM was significantly reduced. For example, P9 showed no visual injury symptoms (Table 1B) due to atrazine treatment but SDM was significantly reduced (Table 2.3). In the field experiment it was observed that some cultivars have the ability to recover from herbicide injury (Table 2.5). For example, P2, P7 and P32 were slightly injured by metazachlor for up to four weeks, but the injury symptoms were outgrown by eight weeks after planting. However, injury symptoms persisted up to maturity in some of the genotypes.

In the greenhouse, the interaction effect inbreds x herbicide was significant (Tables 2.3 & 2.4). With respect to SDM reduction, the inbreds P2, P7, P21, P25, and P29 were the most tolerant to all herbicides tested, while the most sensitive were P3, P10 and P24. Metolachlor, dimethenamid and atrazine influenced the SDM of the inbreds the least. Metazachlor was the most injurious herbicide, causing SDM reduction for more inbreds than any other herbicide. Similarly, field experiment results showed that the interaction of maize genotypes and herbicides was significant. Metazachlor was again the most injurious herbicide (Table 2.6).

Averaged across herbicides, percentage RDM reduction in the greenhouse ranged from marked reductions for P13, P29 and P28 to no effect on P25, P22, P14, P18 and P27 (Table 2.4).

TABLE 2.5 Ratings for visual injury symptoms caused by herbicides on maize in the field (Experiment Series I)

Genotype	Herbicide											
	Metazachlor				Acetochlor + atrazine/ sulcotrione				Dimethenamid			
	Weeks after treatment (WAT)											
	2	4	8	14	2	4	8	14	2	4	8	14
CV7	1	1	1	1	1	1	1	1	1	1	1	1
CV5	1	1	1	1	1	1	1	1	1	1	1	1
P11	6	6	7	6	1	1	1	1	1	1	1	1
P12	1	1	1	1	1	1	1	1	1	1	1	1
P56	4	3	1	1	1	1	1	1	3	1	1	1
P2	3	2	1	1	1	1	1	1	2	1	1	1
P1	3	5	5	5	1	1	1	1	2	1	1	1
P31	1	1	1	1	1	1	1	1	3	1	3	1
P37	5	6	4	4	1	1	1	1	1	1	1	1
P44	4	4	3	4	1	1	1	1	1	1	1	1
P7	5	4	2	1	1	1	1	1	1	1	1	1
P32	2	2	2	1	1	1	1	1	1	1	1	1
P38	4	5	3	3	1	1	1	1	1	1	1	1

Injury rating scale: 1 - 10; indicating: 1 = no effect, 2-3 = slight effect, 4-5 = medium effect, 6-7 severe effect, 8-9 = very severe effect, 10 = plants dead.

TABLE 2.6 Growth inhibition (%) caused by herbicides on maize shoot dry mass in the field (ANOVA in Table 10A) (Experiment Series I)

Genotype	Herbicide			
	Metazachlor	Acetochlor + atrazine/sulcotrione	Dimethenamid	Mean
CV7	14	17	3	11
CV5	58	12	4	24
P11	36	0	8	7
P12	10	0	9	6
P56	62	10	32	35
P2	35	16	10	20
P1	71	15	9	31
P31	10	3	0	5
P37	10	28	21	20
P44	55	29	37	40
P7	58	0	36	31
P32	55	39	12	35
P38	50	33	37	40
Mean	40	16	17	

$LSD_{T(P=0.05)}$ Herbicide x Genotype = 22
SE = 6

A zero implies no reduction or an increase above the untreated control.

No injury symptoms were observed on the above-ground plant parts of the tolerant genotypes, though some roots of these genotypes were substantially injured at the recommended rate, and even more severely at double the rate. Roots were visibly less where metazachlor was applied than at the untreated control. Susceptible genotypes exhibited metazachlor injury symptoms both on the roots and shoots. Visually, roots were fewer, thinner and had less root hairs than the untreated control. From metazachlor data it was observed that no effect on SDM does not necessarily mean that roots are not affected or that the genotype is tolerant to a specific herbicide. For example, the SDM of P1 shoot dry mass was significantly reduced by metazachlor and yet its RDM was not affected (Table 2.3 & 2.4).

Presented in Table 2.7 are data on the effect of three herbicides on number of days to 50 % tasselling. The first order interaction of maize genotypes and herbicides was not significant. Main effects of maize genotypes and herbicides were, however, significant. Metazachlor significantly delayed the tasselling of most of the genotypes as compared to the other two herbicides. The most delayed, by at least four days relative to the controls, were CV7, P11 and P37. Number of days to tasselling for P31, P2 P1 and P44 genotypes were not affected by metazachlor. The other two herbicides, dimethenamid and acetochlor + atrazine/sulcotrione, did not significantly affect the number of days to tasselling for most of the genotypes.

Data for the effect of herbicides on the number of days to 50 % silking are presented in Table 2.8. The maize genotype x herbicide interaction was not significant. However, both the main effects of maize genotype and herbicide were significant.

TABLE 2.7 Effect of three herbicides on maize days to 50 % tasselling (relative to control) in the field (ANOVA in Table 11A) (Experiment Series I)

Genotype	Herbicide			
	Metazachlor	Acetochlor + atrazine/sulcotrione	Dimethenamid	Mean
CV7	4.6	-0.5	1.0	1.7
CV5	2.6	1.0	1.0	1.6
P11	4.0	0.5	0.5	1.7
P12	2.0	0	0	0.7
P56	2.7	0.5	3.2	2.2
P2	-0.5	0	0.5	0
P1	1.0	0.5	2.4	1.3
P31	0	-0.5	1.0	0.2
P37	4.4	2.9	0.5	2.6
P44	-1.0	-2.9	0	-1.3
P7	2.6	0.5	0	1.0
P32	3.6	5.0	2.2	3.6
P38	2.0	0	2.0	1.4
Mean	2.2	0.5	1.1	

$LSD_{T(P=0.05)}$ Herbicide x Genotype = ns, Genotype = 1.7, Herbicide = 1.2
SE = 1.4

Key: (-) tasselled earlier than the control;
(0) tasselled at the same time with the control;
( ) tasselled after the control.

TABLE 2.8 Effect of three herbicides on maize days to 50 % silking (relative to control) in the field (ANOVA in Table 12A) (Experiment Series I)

Genotype	Herbicide			
	Metazachlor	Acetochlor + atrazine/sulcotrione	Dimethenamid	Mean
CV7	3.0	-2.0	-1.5	-0.2
CV5	4.0	1.5	3.0	2.9
P11	0.4	-1.8	-0.4	-0.3
P12	3.0	1.0	0	1.3
P56	4.6	0.5	4.1	3.1
P2	0.4	0.9	1.4	0.9
P1	4.1	0.9	0.9	2.0
P31	1.8	-0.9	0	0.3
P37	3.7	3.7	1.8	3.1
P44	-1.8	-1.8	-4.4	2.6
P7	3.9	-1.5	0.5	1.0
P32	-0.8	0	-0.4	0.4
P38	1.5	2.0	1.5	1.6
Mean	2.1	0.2	0.6	

$LSD_{T(P=0.05)}$ Herbicide x Genotype = ns, Genotype = 1.9, Herbicide = 1.4
SE = 1.5

Key: (-) tasselled earlier than the control;
(0) tasselled at the same time with the control;
( ) tasselled after the control.

TABLE 2.9 Effect of three herbicides (% reduction from the untreated control) on maize grain yield in the field (ANOVA in Table 13A) (Experiment Series I)

Genotype	Herbicide			
	Metazachlor	Acetochlor + atrazine/sulcotrione	Dimethenamid	Mean
CV7	12	1	7	7
CV5	27	16	14	19
P11	34	1	7	14
P12	12	6	6	8
P56	31	15	31	26
P2	0	17	3	7
P1	31	6	2	13
P31	6	10	0	5
P37	30	20	2	17
P44	35	8	5	16
P7	2	16	15	11
P32	0	7	22	10
P38	37	22	28	29
Mean	20	10	11	

$LSD_{T(P=0.05)}$ Herbicide x Genotype = 13
SE = 6

A zero implies no reduction or an increase above the untreated control.

Metazachlor application significantly increased the number of days to 50 % silking for most of the maize genotypes, significantly more than the other two herbicides. The greatest effect was on CV5, P56 and P1, while the least effect was on P2 and P11.

The effect of herbicides on maize parent lines' grain yield varied significantly (Table 2.9). Metazachlor depressed grain yield for more inbreds than did the other herbicides. The range of percent yield reduction caused by metazachlor was significantly higher (0 - 36.6 %) than that caused by the other two herbicides. The greatest significant yield reduction caused by metazachlor was observed for P11, P1, P44 and P38.

Hybrid responses in the 1998/1999 growing season

In the greenhouse, hybrids showed significant reductions in SDM and RDM in response to different herbicides (Table 2.3 & 2.4). Shoot dry mass for hybrids CV1, CV2, CV6, CV7 and CV8 was not significantly reduced by any of the herbicides (Table 2.3). The hybrid CV10 was the most significantly sensitive towards most herbicides. Metazachlor caused significant reductions in the SDM of more hybrids than any other herbicide.

All herbicides significantly reduced the RDM of specific hybrids (Table 2.4). The most tolerant hybrids to all herbicides were CV1, CV7 and CV10, while CV2 and CV9 were the most sensitive, with their RDM significantly reduced by six herbicides. None of the herbicides caused obvious injury symptoms on any of the hybrids (Table 1B). Generally, hybrids were more tolerant to the herbicides than inbreds. The trend of hybrid response to herbicides in the field was similar to those obtained from the greenhouse. There were varying responses and metazachlor had the greatest adverse effect on the hybrids.

Crop responses in the second series of experiments in the 1999/2000 growing season

The results for the second series of greenhouse and field experiments showed a similar trend of herbicide effects on maize genotypes to those observed in the first series. In the greenhouse, visual injury symptoms caused by the herbicides ranged from zero to severe effects (Table 2.10). None of the herbicides caused any visual injury on some genotypes such as CPH, CPE, CPC and CPB. However, some genotypes were affected by certain herbicides. For example, visual injury symptoms were observed on CPD where acetochlor was applied. Flufenacet caused medium to severe injury symptoms on cultivars P7, CPF, CPD and CPG. Among the herbicides, flufenacet caused the most visible injury to plants, while bendioxide and a mixture of atrazine/metolachlor/terbuthylazine did not cause any visual injury symptoms. In the field, none of the herbicides caused any visual injury symptoms.

Results for SDM reductions in the greenhouse, were significantly different among genotypes (Tables 2.11). Maize genotypes varied in their tolerance to herbicides. The SDM of some genotypes such as CPA, CPB, CPC and P12, were not significantly reduced by any of the herbicides, whilst that of other genotypes were, significantly reduced by some herbicides. For example, the SDM of genotypes CPB was significantly reduced by acetochlor and flufenacet. Herbicide effects on maize SDM varied from herbicide to herbicide with flufenacet generally causing the greatest SDM reductions. Bendioxide and a mixture of atrazine/metolachlor/ terbuthylazine did not cause any significant SDM reductions in any of the genotypes.

In the field experiment the effect of herbicides on days to 50 % tasselling were significant (Table 2.12). These effects varied among genotypes. For genotype CPB the parameter days-to-50% tasselling were significantly reduced compared to other genotypes when calculated

across herbicides. In the case of genotype CPC the number of days to 50% tasselling, calculated across herbicides, was significantly increased compared to most other genotypes (Table 2.12). In spite of the aforementioned effects on growth and development of maize, no significant yield reductions were caused by any of the herbicides (Table 2.14).

TABLE 2.10 Ratings for visual injury symptoms caused by herbicides on maize in the greenhouse (Experiment Series II)

	Herbicide				
Genotype	Acetochlor	Bendioxide	Flufenacet	Atrazine/metolachlor/terbuthylazine	Mean
CPA	2	1	1	1	1
CPB	1	1	1	1	1
CPC	1	1	1	1	1
CPD	3	1	7	1	4
CPE	1	1	1	1	1
CPF	1	1	5	1	2
CPG	1	1	7	1	2
CPH	1	1	1	1	1
P12	1	1	1	1	1
P7	1	1	4	1	2
Mean	1	1	3	1	

Injury rating scale: 1 - 10; indicating: 1 = no effect, 2-3 = slight effect, 4-5 = medium effect, 6-7 severe effect, 8-9 = very severe effect, 10 = plants dead.

TABLE 2.11 Growth inhibition (%) caused by herbicides on maize shoot dry mass in the greenhouse (ANOVA in Table 14A) (Experiment Series II)

	Herbicide				
Genotype	Acetochlor	Bendioxide	Flufenacet	Atrazine/metolachlor/terbuthylazine	Mean
CPA	8	2	0	0	2
CPB	0	0	9	0	2
CPC	12	0	15	0	6
CPD	20	0	35	0	14
CPE	15	0	0	0	4
CPF	16	0	28	0	11
CPG	0	5	46	0	13
CPH	20	2	17	5	11
P12	13	9	0	7	7
P7	0	0	26	0	6
Mean	10	2	13	1	

$LSD_{T(P=0.05)}$ Herbicide x Genotype = 20
SE = 10

A zero implies no reduction or an increase above the untreated control.

TABLE 2.12 Effect of herbicides on maize days to 50 % tasselling in the field (ANOVA in Table 16A) (Experiment Series II)

	Herbicide				
Genotype	Acetochlor	Bendioxide	Flufenacet	Atrazine/metolachlor/terbuthylazine	Mean
CPA	0	1	0	7	2
CPB	-3	-4	-3	-1	-3
CPC	5	3	3	5	4
CPD	1	0	1	1	0
CPE	3	-2	0	4	1
CPF	-2	0	-3	2	0
CPG	4	0	-1	3	1
CPH	0	0	-3	-4	0
P12	0	0	1	0	0
P7	0	0	-1	0	0
Mean	0	0	0	2	

$LSD_{T(P=0.05)}$ Herbicide x Genotype = ns Genotype = 2.5 Herbicide = 1.3
SE = 2

Key: (-) tasselled earlier than the control;
(0) tasselled at the same time with the control;
( ) tasselled after the control.

TABLE 2.13 Effect of herbicides on maize days to 50 % silking in the field (ANOVA in Table 17A) (Experiment Series II)

	Herbicide				
Genotype	Acetochlor	Bendioxide	Flufenacet	Atrazine/metolachlor/terbuthylazine	Mean
CPA	0	2	2	0	1
CPB	-7	-4	-6	-6	-6
CPC	6	3	4	3	4
CPD	4	3	5	8	5
CPE	4	0	0	5	2
CPF	4	0	3	6	3
CPG	2	-2	-2	1	0
CPH	3	0	3	6	3
P12	-1	0	4	6	2
P7	-7	-1	-1	-1	2
Mean	1	0	3	3	

$LSD_{T(P=0.05)}$ Herbicide x Genotype = ns Genotype = 2.5 Herbicide = 1.5
SE = 2

Key: (-) silked earlier than the control;
(0) silked at the same time with the control;
( ) silked after the control.

TABLE 2.14 Effect of herbicides (% reduction from the untreated control) on maize grain yield in the field (ANOVA in Table 18A) (Experiment Series II)

	Herbicide				
Genotype	Acetochlor	Bendioxide	Flufenacet	Atrazine/metolachlor/terbuthylazine	Mean
CPA	1	2	2	3	2
CPB	2	0	4	0	1
CPC	0	1	0	4	1
CPD	4	5	3	0	3
CPE	4	2	0	3	2
CPF	0	2	5	0	1
CPG	3	0	0	0	0
CPH	2	0	1	0	0
P12	4	9	2	11	6
P7	0	0	0	0	0
Mean	2	2	2	2	

$LSD_{T(P=0.05)}$ = NS
SE = 6

A zero implies no reduction or an increase above the untreated control.

Results, for all the experiments both in the greenhouse and in the field, show that there are distinctive responses among maize inbreds and hybrids to herbicides. Differential herbicide tolerance among crop genotypes has been reported previously (Le Court De Billot & Nel, 1985a; Le Court De Billot, 1985; Currie *et al.*, 1995; Green & Ulrich, 1993; Hinz *et al.*, 1997; Doohan *et al.*, 1998). This suggests that genetic composition of crop genotypes, in addition to certain environmental factors, play a major role in determining the responses of maize genotypes to herbicides. Tolerant genotypes probably metabolise herbicides faster than susceptible genotypes.

Injury symptoms, resulting from some herbicides, were observed in some maize genotypes. However, in this study it was found that some genotypes, which showed phytotoxic symptoms at an early stage, did not suffer significant growth or yield reduction later on. This implies that some maize genotypes have the ability to recover after the initial herbicide injury. The ability to recover varies among genotypes and it occurs in varying degrees. This finding confirms previously reported differential recovery rates of maize genotypes to initial herbicide injury (Narsaiah & Harvey, 1977; Hagood *et al.*, 1980; Le Court De Billot, 1985). Therefore, the presence of injury symptoms at an early stage of crop development may not necessarily be a good predictor of crop genotype tolerance to herbicides because there is a possibility of recovery.

Root dry mass apparently did not satisfactorily correspond well with SDM reductions. Some genotypes, which had no shoot dry mass reductions, had significant root dry mass reductions. Visually, root development was impaired, for both tolerant and susceptible genotypes, due to metazachlor application but the shoots were not injured for the tolerant genotype.

Metazachlor and other herbicides in the chloroacetamide family are known to affect roots more than shoots in susceptible species. The tolerant genotypes' root system possibly recovers quicker than the susceptible genotypes. This implies that the absence of aboveground effects may not necessarily indicate tolerance because the roots of a particular genotype may be seriously affected by the herbicide. This in turn might affect the grain yield, especially if root injury persists for a long time during crop development. Root dry mass measurements should routinely be made for herbicides known to affect root development, e.g. the acetanilides (Kearney & Kaufman, 1975; Ashton & Crafts, 1981; Klingman & Ashton, 1982).

Among the herbicides registered for maize, metazachlor was more injurious to inbreds and hybrids than any other herbicide. Perhaps, many maize genotypes do not inactivate this herbicide fast enough to avoid accumulation of the herbicide at the site of action. Thus it was not surprising that the registration of the product on maize was withdrawn at about the time this finding was made.

Hybrids were found to be more tolerant to herbicides than inbred lines. This has also been reported by other researchers (Le Court De Billot, 1985; Landi, *et al.*, 1989; Green & Ulrich, 1993). Inbred lines are generally weaker genotypes than hybrids. This is due to inbreeding (inbreds) and heterosis (hybrids).

Number of days to flowering (tasselling and silking) was significantly affected by some herbicides for certain maize genotypes. Days to flowering is a very important factor in seed production. If this is altered for one parent of a cultivar by certain factors, for example herbicide application, pollination may be affected because the two parents will not flower at

the same time. For pollination to be successful, the parents' (of a specific cultivar) flowering should be synchronised and protected from any factor that may disturb the synchronisation.

It should, however be noted that, although there are statistically significant differences among maize genotypes in their days to flowering, due to some herbicide applications, it is possible for pollination not to be affected in practice. This is because, the period that a female line silk remain receptive and male flower produce pollen may be long enough for pollination not to be affected by these changes in days to flowering. Therefore, it is important to know the pollen shedding period and the period silk remain receptive in order to safely determine whether the changes in days to flowering, due to herbicide applications, will affect pollination in practice.

Generally, there seems to be good correspondence of results from the greenhouse and those from the field; meaning that it may be possible to determine maize tolerance to specific herbicides at an early stage of crop development. This issue is pursued further in the next chapter

The results showed that there is the need to classify maize genotypes into tolerant and susceptible classes with respect to their responses to specific herbicides in order to make practical use of such information. Although many parameters are measured to determine crop tolerance to herbicides, only one parameter could be used to categorise genotypes. This is because of the difficulties that arise from lack of total consistence in responses to herbicide application as measured by different parameters. The most appropriate parameter would be crop yield, since this is the ultimate measure of genotype performance. However, screening

may be done in greenhouses where it is more cost effective, and in which case other parameters, may be considered. It is therefore suggested that the general herbicide recommendations be based on shoot dry mass results. This parameter is more reliable and more commonly used in greenhouse screening. It is further suggested that maize genotypes be classified according to broad tolerance categories (Table 2.15).

TABLE 2.15 Crop tolerance categories

Shoot dry mass reduction (%)	Tolerance category
0 - 25	Tolerant
26 - 35	Moderately tolerant
36 - 100	Susceptible

The categories in Table 2.15 imply the following: (i) tolerant category of 0 – 25 %, if SDM is reduced up to 25% the plants will probably recover fully from the initial herbicide injury by the time it reaches maturity: the crop yield is therefore not expected to be significantly reduced: (ii) moderately tolerant category of 26 – 35 %; if SDM is reduced by 26 to 35 % there will often be as light reduction in yield: in other words, plants should recover from the initial injury but probably not completely; (iii) susceptible category of 36 – 100 %; any SDM reduction above 35 % will ultimately in all probability reduce the crop yield significantly: plants affected this way are unlikely to recover substantially from the initial herbicide injury.

Based on the proposed general crop tolerance categories, selected maize genotypes are classified according to their tolerance to specific herbicides in Table 2.16. The classification reflects the differential tolerance that exists among maize genotypes to specific herbicides.

Some genotypes are more tolerant that others to all herbicides tested. For example cultivars P7, P8, P17, P30 and P26 were tolerant to all herbicides while P6, P10 and P27 were susceptible to some herbicides. Similarly, herbicides varied in their effects on maize. Metazachlor was the most injurious. It is conceivable that the proposed system of categorising maize genotypes into tolerance classes could be applicable for all herbicides. A similar type of categorisation has been possible for soybean genotypes with metribuzin (Hardcastle, 1979; Gosset *et al.*, 1982).

This study has shown that maize inbreds and hybrids are variable in their response to herbicides. Generally, inbreds were more sensitive to herbicides than hybrids. Metazachlor was found to have the lowest selectivity in maize, and also elicited the most variable responses in maize. In any cultivar development programme, routine screening of all material is advisable in order to avoid crop injury after commercialisation. A general crop tolerance classification based on shoot dry mass reductions in greenhouse screening is recommended to make it easy for companies/farmers to select herbicides for use in the field.

TABLE 2.16 Classification of genotypes according to the extent of SDM reduction in response to different herbicides. Herbicide recommendations for selected maize genotypes

Genotype	Herbicide						
	FL/-MET	MET	DIM	ATR	AC+ATR/-SU	ALA	METZ
Batch I: Inbreds							
P1	T	T	T	T	T	T	S
P2	T	T	T	T	T	T	T
P3	T	M	T	T	T	T	T
P4	T	T	T	T	T	T	T
P5	T	T	T	T	T	T	T
P6	M	T	T	T	T	T	M
P7	T	T	T	T	T	T	T
P8	T	T	T	T	T	T	T
P9	T	T	T	M	T	T	T
P10	M	T	T	T	M	T	S
Batch II:Inbreds							
P11	T	T	T	T	T	T	T
P12	T	T	T	T	T	T	T
P13	T	T	T	T	T	T	T
P14	T	T	T	T	T	T	T
P15	T	T	T	T	T	T	T
P16	T	T	T	T	T	T	T
P17	T	T	T	T	T	T	T
P18	T	T	T	T	T	T	T
P19	T	T	T	T	T	T	T
P20	T	T	T	T	T	T	T
Batch III. Inbreds							
P21	T	T	T	T	T	T	T
P22	T	T	T	T	T	T	T
P23	T	T	T	T	T	T	T
P24	T	T	T	T	T	M	T
P25	T	T	T	T	T	T	T
P26	T	T	T	T	T	T	T
P27	T	T	T	T	T	T	M
P28	T	T	T	T	T	T	T
P29	T	T	T	T	T	T	T
P30	T	T	T	T	T	T	T

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Batch IV. Hybrids							
CV1	T	T	T	T	T	T	T
CV2	T	T	T	T	T	T	T
CV3	T	T	T	M	T	T	T
CV4	T	T	T	T	T	T	T
CV5	T	T	T	T	T	T	M
CV6	T	T	T	T	T	T	T
CV7	T	T	T	T	T	T	T
CV8	T	T	T	T	T	T	T
CV9	M	T	T	T	T	T	T
CV10	T	M	T	T	T	T	T

Key: FL=flumetsulam, MET=metolachlor, DIM=dimethenamid, ATR=atrazine, AC=acetochlor, SU=sulcotrione, ALA= alachlor, METZ=metazachlor

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CHAPTER 3

CORRELATION BETWEEN MAIZE TOLERANCE TO HERBICIDES DETERMINED IN THE GREENHOUSE AND IN THE FIELD

Introduction

Prediction of crop performance in the field based on laboratory or greenhouse studies is difficult, and is regarded by many as being tenuous, although many biological disciplines attempt to do it due to its importance. If it is found to be both practical and reliable this concept will be advantageous because it would allow for quick screening of crop cultivars and herbicides under controlled conditions. Furthermore, laboratory and greenhouse conditions can be repeated and they can also be varied at will (Pike, 1994; Suett, 1994). Although scientists endeavour to effectively predict field performance, such that the reality is not different from the greenhouse results, there are always difficulties in the extrapolation of such results (Garrod, 1989; Krahmer & Russell , 1994). The problem is that field conditions cannot always be exactly duplicated in the greenhouse, and even if they can, it is probable that some unique combination of conditions in the field is crucial to the results of any trial. Type of herbicide, genetic composition and environmental factors are amongst several factors contributing to these differences between experimental environments (Klingman & Ashton, 1982; Akobundu, 1987).

Differential maize tolerance to herbicides exists and cases of maize injury due to herbicides have been reported from time to time in South Africa (Le Court De Billot, 1985; Le Court De Billot *et al.*, 1986; Le Court De Billot *et al.*, 1990; Reinhardt, 1993; Kanyomeka & Reinhardt,

2000). Ideally all maize genotypes should be screened in terms of their tolerance to all registered herbicides so that only appropriate herbicides are recommended for use in specific maize genotypes. The practical problems associated with this ideal situation for maize have been alluded to earlier, and if all crop species are considered, the scope of the work involved is staggering and simply beyond the research capacity of most institutions.

Because the maize genotypes that require screening are so many and varied that field screening may not cope with the situation due to the time it takes and the costs involved. There is need to develop techniques for rapid, yet reliable, maize screening in terms of herbicide tolerance. Such alternative techniques could be laboratory and greenhouse screening that are far cheaper, less time consuming and makes it possible for more genotypes to be screened at the same time. These methods have been used previously for other crops (Barrentine *et al.*, 1976; Hardcastle, 1979; Zhaohu *et al.*, 1999). Due to the possible variability in the performance of maize genotypes under field, greenhouse and laboratory conditions, it is important to ascertain the reliability of such methods to enable field performance to be predicted with some confidence. This may be possible through correlation of laboratory and greenhouse results with those obtained from the field, particularly the grain yield, which is usually the ultimate parameter for productivity. The objective of this exercise was to determine the correlation of maize genotypes' tolerance to herbicides between greenhouse and field conditions through use of data reported in the previous Chapter.

Materials and Methods

Data reported in Chapter 2, for both greenhouse and field experiments were used to determine the correlation between greenhouse and field results. Data for the two sets (series) of

greenhouse and field experiments were used. In each case simple linear correlation coefficients were calculated for relationships between pairs of growth parameters from the greenhouse and the field. Herbicides involved are listed in Table 3.1.

TABLE 3.1 Herbicides evaluated under both greenhouse and field conditions

Experiment	Herbicide	Trade name	Application rate
Expt. Series I (1998/99)	Metazachlor	Preecede	1.5 L ha^{-1}
	Acetochlor+atrazine/sulcotrione	Wenner/galleon	1.0/0.8 L ha^{-1}
	Dimethenamid	Frontier	0.75 L ha^{-1}
Expt. Series II (1999/2000)	Flufenacet	Tiara	400 g ha^{-1}
	Acetochlor	Guardian	1.3 L ha^{-1}
	Atrazine/metolachlor/terbuthylazine	Gardomil	2.3 L ha^{-1}

Results and Discussion

Relationships between maize grain yield and major parameters used to assess the tolerance of maize genotypes to herbicides are presented in Tables 3.2, 3.3, 3.4 and 3.5. Simple correlation coefficients (r) were compared to judge the relative strengths of these relationships. In these comparisons, the coefficient of determination (r^2) is relevant also. This quantity shows the percentage of variation attributable to the relationship between parameters. Coefficient of determination required to obtain acceptable and good correlation varies with the objective and type of the research. Van Ark (1995) states that a generally accepted r^2 minimum is in the region of 0.49 (r=+/- 0.7). In the present study it is proposed that an acceptable minimum r-value is in the region of 0.50.

According to findings reported in the previous chapter, maize tolerance to herbicides varied according to genotypes and specific herbicides. The variations of maize tolerance to the herbicides are attributed to genetic variations. Tolerant genotypes probably metabolise herbicides faster than susceptible ones (Akobundu, 1987). Injury symptoms caused by some herbicides in the greenhouse corresponded well with those observed in the field. More than 80 % of the herbicide visual injury in the greenhouse and in the field corresponded well. Other researchers (Barrentine *et al.*, 1976; Hardcastle, 1979; Zhaohu *et al.*, 1999) have reported similar correspondence of injury symptoms in the greenhouse and the field. The visual injury symptoms observed at the vegetative stage in the field persisted up to crop maturity in some cases. This persistent injury is likely to significantly reduce maize yield.

TABLE 3.2 Simple linear correlation coefficients (r) and r^2 values to describe the relationships between various parameters, used to assess maize tolerance to herbicides, and grain yield (Experiment Series I: 1998/99 growing season)

Parameter	Herbicide					
	Metazachlor		Acetochlor+atrazine/ sulcotrione		Dimethenamid	
	r	r^2	r	r^2	r	r^2
SDM(GH)	0.80**	0.64	0.78**	0.61	0.73**	0.53
VI(GH)	0.92**	0.85	0.19ns	0.04	0.63*	0.40
VI(Field)	0.91**	0.83	0.55*	0.30	0.59*	0.35
DT(Field)	0.65*	0.42	0.13ns	0.02	0.89**	0.79
DS(Field)	0.65*	0.42	0.66*	0.44	0.70**	0.62

*Significant at 5 % probability level.
**Significant at 1 % probability level.
SDM=shoot dry mass reduction VI=visual injury rating, DT=days to 50 % tasselling, DS=days to 50 % silking, GH=greenhouse.

TABLE 3.3 Simple linear correlation coefficients (r) and r^2 values to describe the relationships between various parameters, used to assess maize tolerance to herbicides, and grain yield (Experiment Series II: 1999/2000 growing season)

Parameter	Herbicide					
	Acetochlor		Atrazine/metolachlor/ Terbuthylazine		Flufenacet	
	r	r^2	r	r^2	r	r^2
SDM(GH)	0.60*	0.36	0.65*	0.42	-0.25ns	0.06
VI(GH)	0.77**	0.59	0.62*	0.38	-0.31ns	0.10
VI(Field)	0.80**	0.64	0.70**	0.49	0.65*	0.42
DT(Field)	0.71**	0.50	0.76**	0.58	0.69*	0.48
DS(Field)	0.73**	0.53	0.69*	0.48	0.64*	0.41

*Significant at 5 % probability level.
**Significant at 1 % probability level.
SDM=shoot dry mass reduction, VI=visual injury rating, DT=days to 50 % tasselling, DS=days to 50 % silking, GH=greenhouse.

TABLE 3.4 Simple correlation coefficients (r) among parameters used to assess herbicide damage to maize (Experiment Series I)

Parameters	Correlation coefficients						
	V1	V2	V3	V4	V5	V6	V7
V1	-	034*	0.45**	0.33*	0.62**	0.34*	0.41*
V2	0.34*	-	0.36*	0.55**	0.28NS	-0.02NS	0.30NS
V3	0.45**	0.36*	-	0.59**	0.51**	0.26NS	0.28NS
V4	0.33*	0.55**	0.59**	-	0.50**	0.15NS	0.35*
V5	0.62**	0.28NS	0.51**	0.50**	-	0.34*	0.24NS
V6	0.34*	-0.02NS	0.26NS	0.15NS	0.34*	-	0.50**
V7	0.41*	0.30NS	0.28NS	0.35*	0.24NS	0.50**	-

*Significant at 5 % probability level; **Significant at 1 % probability level.
V1=grain yield reduction; V2=shoot dry mass reduction (greenhouse); V3=shoot dry mass reduction (field); V4=visual injury rating (greenhouse); V5=Visual injury rating (field); V6=Days to 50 % tasselling; V7=Days to 50 % silking.

TABLE 3.5 Simple correlation coefficients (r) among parameters used to assess herbicide damage to maize (Experiment Series II)

Parameters	Correlation coefficients					
	V1	V2	V3	V4	V5	V6
V1	-	0.38*	0.34*	0.65**	0.46**	0.56**
V2	0.38*	-	0.60**	0.25NS	0.28NS	0.16NS
V3	0.34*	0.60**	-	0.27NS	0.24NS	0.16NS
V4	0.65**	0.25NS	0.27NS	-	0.35*	0.28NS
V5	0.46**	0.27NS	0.24NS	0.35*	-	0.60**
V6	0.56**	0.16NS	0.16NS	0.28NS	0.60**	-

*Significant at 5 % probability level; **Significant at 1 % probability level.
V1=grain yield reduction; V2=shoot dry mass reduction (greenhouse); V3=visual injury rating (greenhouse); V4=Visual injury rating (field); V5=Days to 50 % tasselling; V6=Days to 50 % silking.

The relationships between grain yield reduction and other tolerance parameters from the greenhouse or field were generally significant (Table 3.2 & 3.3). Interesting to note is the positive correlation between SDM reduction and visual injury rating (VIR) in the greenhouse with grain yield in the field. This relationship was positive and significant for at least two of the three herbicides in each experiment. In the second series of experiments (Table 3.3) there was no relationship between SDM reduction and visual injury rating with grain yield for flufenacet, while VIR was not positively correleted with the yield reduction for the herbicide combination of acetochlor and atrazine/sulcotrione in the first series (Table 3.2).

Comparisons of the relationships among all parameters indicate that they were herbicide-dependent in both sets of experiments. In the first series of experiments (Table 3.2), parameters measured in the greenhouse showed significant correlation with yield reduction for metazachlor and dimethenamid, while visual injury rating and days to 50 % silking showed no relationship with grain yield for the herbicide mixture of acetochlor and atrazine/sulcotrione. Similarly, in the second set of experiments (Table 3.3) SDM reduction

and VIR showed no relationship with grain yield for flufenacet, although other parameters were significantly correlated with yield in the case of other herbicides.

Results in Tables 3.4 and 3.5 indicate that there is generally positive and significant correlation among parameters used to measure herbicide tolerance in maize. As mentioned earlier, important to note are the positive relationships between SDM reduction and VIR in the greenhouse with grain yield reduction in the field.

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CHAPTER 4

INFLUENCE OF TEMPERATURE ON MAIZE TOLERANCE TO ALACHLOR, METAZACHLOR AND METOLACHLOR

Introduction

Alachlor, metazachlor and metolachlor are chloroacetamide herbicides that were developed primarily for selective control of mostly germinating grass and some broadleaf weeds in maize and some other crops (Tomlin, 1994). Metazachlor was, however, deregistered for use in maize in South Africa during 1999, at about the time of concluding this experiment, as a result of inadequate selectivity. Although these herbicides are considered safe for use in maize, some maize genotypes have been reported to be sensitive to them (Ashley, 1972; Francis & Hamill, 1980; Rowe, *et al.*, 1990). Similarly, in the maize screening experiments reported in Chapter 2, some maize genotypes showed outstanding tolerance to these herbicides while some were very sensitive (Kanyomeka & Reinhardt, 2000). Differential tolerance of crop cultivars, including maize, to herbicides has been documented (Malan *et al.*, 1984; O'Sullivan *et al.*, 1998; Van Wychen, *et al.*, 1999; Wilson, 1999; O'Sullivan *et al.*, 2001).

Crop tolerance to herbicides is influenced by genetic composition, type of herbicide and environmental factors (Klingman & Ashton, 1982; Akobundu, 1987). These latter factors in particular may increase the activity or uptake of a herbicide and reduce the normal crop tolerance, thereby causing crop injury. Environmental factors that can affect the efficacy of herbicides are relative humidity, temperature, light intensity, rainfall and soil conditions. The efficacy of chloroacetamides is particularly influenced by temperature and soil moisture content. Temperature is an important environmental factor that influences plant growth and development by influencing respiration, transpiration, nutrient and water uptake, and other

biochemical processes (Didwell, 1974; Eastin, *et al.*, 1982).

Temperature changes have been reported to affect herbicide selectivity. Tolerance of maize and peas to alachlor decreased with increasing soil moisture and decreasing soil temperatures (Putnam & Rice, 1979). Reinhardt & Nel (1982) reported that alachlor is more active at lower temperature regimes of 20/10°C and at higher ones of 30/20°C (day/night) than at 25/15°C. Skipper *et al.* (1977) showed that alachlor and metolachlor are more active at 22/13°C than at high temperature regimes of 27/18°C and 32/24°C. Metolachlor activity was reported to be greater at high temperature of 29°C than at low temperature of 18°C (Gerber *et al.*, 1974). Van Biljon (1991) reported that metolachlor was most injurious to maize at temperature regimes of 30/20°C day/night. Gauvrit & Gaillardon (1991) found that 2,4-D selectivity towards maize is drastically reduced under cold conditions. Low temperature increases spray retention per unit dry matter and retards 2,4-D degradation. Similarly, McMullan (1994) reported that compared to diclofop-methyl, fenoxafop-p-ethyl mixtures cause more injury to barley at lower temperatures. This shows that effect of temperature on crop tolerance varies from herbicide to herbicide.

Under normal temperatures maize is able to metabolise the absorbed alachlor, metazachlor and metolachlor. Cool and hot environments probably cause changes in the plant physiology that could contribute to crop injury due to reduced rates of inactivation of these herbicides. Generally, most crop injury from chloroacetamide herbicides occurs on sandy soils with low organic matter, i.e., on soil with low adsorptive capacity for the compounds. Deep planting, cool wet conditions and soil crusting may also increase the potential for crop injury. These factors prolong the time the emerging maize seedling is in contact with the soil-herbicide solution, and the plant is likely to ultimately take up more herbicide than expected. In addition, under those conditions, the plant may not be able to detoxify the absorbed herbicide effectively (Didwell, 1974; Akobundu, 1987).

Many reports of maize injury from these herbicides have been linked to temperature changes, other than those mentioned above. Cool temperatures during or soon after herbicide application are more associated with this kind of injury. It is therefore important to determine how temperature affects the tolerance of genotypes. The objective of this study was to determine the influence of temperature on the tolerance of maize genotypes to alachlor, metazachlor and metolachlor (Ashton & Crafts, 1981; Akobundu, 1987; Butzen, 2000).

Materials and Methods

Three separate experiments were conducted in growth chambers to determine the influence of temperature on the tolerance of maize genotypes to alachlor, metazachlor and metolachlor. The application rates for the formulated products Lasso, Preecede, Dual S GOLD were 4.0, 1.5 and 0.9 L ha^{-1}, respectively. Herbicides were applied immediately after planting, since they are registered as pre-emergence herbicides in maize.

Maize plants were grown in pots containing 2.5 kg of soil for five weeks. Four maize inbreds were used in the experiments involving metolachlor and alachlor, while two inbreds and two hybrids were used in the metazachlor experiment. These genotypes included tolerant and susceptible types, which were selected based on results from earlier greenhouse and field experiments reported on in Chapter 2.

The experiments were conducted in growth chambers at the University of Pretoria phytotron. The night/day temperature regimes were 10/18°C, 15/25°C, 20/35°C and 10/30°C. The first experiment involving metazachlor did not include the 10/30°C temperature regime. The photoperiod was maintained at 12 hours for the day regime. The experimental design in each case was completely randomized. The control treatment at each temperature regime was a zero herbicide treatment.

Pots were filled with 2.5 kg of soil, which was sieved through a 4 mm screen prior to filling.

The soil consisted of 75.5 % sand, 8.2 % silt, 17.1 % clay with 0.4 % organic C, and had a pH (H_2O) of 6.5. The soil used was collected from the University of Pretoria experimental farm. The pots were fitted with plastic bags before filling them with soil in order to avoid herbicide leaching.

Five seeds of each genotype were planted in each pot at a depth of 30 mm. Three days after emergence, seedlings were thinned to three per pot. Watering was done to ensure that moisture was maintained at field capacity level. Pots were first weighed soon after planting and after the initial watering, and subsequently once per week to avoid under- or over-watering. After emergence, either a complete nutrient solution (Nitsch, 1972) or tap water was applied on alternate days. The same volume of nutrient solution (100 ml) was always applied to each pot, but the volume of tap water was dependent on the amount of water lost.

Three days after plant emergence, the rate of emergence was determined by taking plant counts. Visual injury was assessed according to a 1-10 rating scale, where 1 indicates no effect and 10 indicates complete kill. Shoot dry mass was measured five weeks after planting by cutting the plants at soil surface level and oven-drying them at 65°C for 48 hours. To determine the root dry mass, roots were thoroughly washed and dried as the shoots were. Data for shoot and root dry mass were expressed as percentage reduction from the untreated control for respective genotypes.

Data were subjected to analysis of variance. Treatment means were compared using Turkey's Least Significant Difference (LSD) test at the 5 % level of significance.

Results and Discussion

Data on the influence of temperature on the tolerance of maize genotypes to alachlor, metazachlor and metolachlor are presented in Tables 4.1, 4.2, 4.3, 4.4, 4.5, and 4.6. There

was no effect of herbicides on the rate of emergence (data not shown).

The study demonstrated that temperature has an influence on maize tolerance to alachlor, metazachlor and metolachlor. For both susceptible and tolerant maize genotypes, shoot dry mass was significantly reduced from the untreated control by metazachlor at 10/18°C (night/day). However, at 15/25°C and 20/35°C temperature regimes, only SDM for susceptible genotypes, cultivars CV5 and P1, were significantly reduced (Table 4.1). Similarly, all genotypes treated with metazachlor showed visual injury symptoms ranging from severe to very severe at the low temperature regime of 10/18°C. At higher temperature regimes of 15/25°C and 20/35°C severe effects of metazachlor were only observed on susceptible genotypes (Table 4.2).

Table 4.1 Percentage shoot dry mass reduction, from the untreated control, of maize genotypes treated with metazachlor (ANOVA in Table 19A)

Genotype	Temperature regimes (night/day)			
	10/18°C	15/25°C	20/35°C	Mean
CV5 (susceptible)	35.9	22.6	31.4	30.0
CV1 (tolerant)	22.9	0	14.1	12.3
P1 (susceptible)	46.0	25.9	40.1	37.3
P2 (tolerant)	33.5	0	13.1	15.5
Mean	34.6	12.1	24.6	

$LSD_{T(0.05)}$ Genotype x Temperature regimes = 16.3 SE = 7.2

Table 4.2 Visual injury rating of maize genotypes treated with metazachlor

Genotype	Temperature regimes (night/day)			
	10/18°C	15/25°C	20/35°C	Mean
CV5 (susceptible)	7	2	5	4
CV1 (tolerant)	4	1	2	2
P1 (susceptible)	6	5	6	6
P2 (tolerant)	7	2	3	3
Mean	8	2	4	

Scale used: 1-10, indicating: 1=no effect, 2-3=slight effect, 4-5=medium effect, 6-7=severe effect, 8-9=very severe effect, 10=plants dead.

The tolerance of maize to alachlor was reduced at the low temperature regime of 10/18° C. At this temperature regime the SDM, for both susceptible and tolerant genotypes, was significantly reduced from the untreated control (Table 4.3). The tolerance of maize to alachlor was not affected significantly at both the 15/25°C and 20/35°C temperature regimes. Even at relatively low and high temperatures of 10°C (night) and 30°C (day) the tolerance of maize to alachlor was not affected. Visual injury symptoms caused by alachlor were only observed at the low temperature regime of 10/18°C for both tolerant and susceptible genotypes (Table 4.4).

Table 4.3 Percentage shoot dry mass reduction, from the untreated control, of maize genotypes treated with alachlor (ANOVA in Table 20A)

Genotype	Temperature regimes (night/day)				
	10/18°C	15/25°C	20/35°C	10/30°C	Mean
P2 (tolerant)	30	0	10	6	8
P3 (susceptible)	38	12	15	11	19
Mean	34	6	13	8	

LSD $_{(T=0.05)}$: Genotypes = 11, Temperature regimes = 20
SE =7.2

Table 4.4 Visual injury rating (VIR) of alachlor effect on maize

Genotype	Temperature regimes (night/day)				Mean
	10/18°C	15/25°C	20/35°C	10/30°C	
P2 (tolerant)	3	1	1	1	2
P3 (susceptible)	3	1	1	1	2
Mean	3	1	1	1	

Scale used: 1-10, indicating: 1=no effect, 2-3=slight effect, 4-5=medium effect, 6-7=severe effect, 8-9=very severe effect, 10=plants dead.

Similarly, the tolerance of maize genotypes to metolachlor was only affected at low temperature conditions of 10/18°C (Table 4.5). The SDM, for both susceptible and tolerant genotypes, was significantly reduced from the untreated control at the low temperature regime. Only at this regime was visual injury caused by metolachlor observed for both tolerant and susceptible genotypes (Table 4.6).

Table 4.5 Percentage shoot dry mass reduction, from the untreated control, of maize genotypes treated with metolachlor (ANOVA in Table 21A)

Genotype	Temperature regimes (night/day)				Mean
	10/18°C	15/25°C	20/35°C	10/30°C	
P2 (tolerant)	22	0	0	7	7.2
P31 (susceptible)	24	19	13	13	17.2
Mean	23	9.5	6.5	10	

LSD $_{(T=0.05)}$ Genotype x Temperature regimes = 14 SE = 6

Table 4.6 Visual injury rating for metolachlor effect on maize

Genotype	Temperature regimes (night/day)				Mean
	10/18°C	15/25°C	20/35°C	10/30°C	
P2 (tolerant)	3	1	1	1	2
P31 (susceptible)	3	1	1	1	2
Mean	3	1	1	1	

Scale used: 1-10, indicating: 1=no effect, 2-3=slight effect, 4-5=medium effect, 6-7=severe effect, 8-9=very severe effect, 10=plants dead.

The observed influence of temperature on the efficacy of herbicides is in accordance with the findings of Putnam & Rice (1979) who reported that snap bean tolerance to alachlor was reduced at low temperatures and low rainfall. Similarly, McWillian (1967), and Hodgins & Van Huystee (1976) found low maize tolerance to 2,4-D at low temperatures of less than 18°C. Generally, low temperatures reduced the tolerance of plants to herbicides, thereby reducing the selectivity of a herbicide (Thompson *et al.*, 1970; Ritter & Coble, 1981; Blair *et*

al., 1983; Pillmoor, 1985; Polge & Barrett, 1997; Harrison & Peterson, 1999). Cool temperatures during germination and emergence could reduce the rates of these plant processes, prolonging the time the emerging shoot remains in contact with the soil-herbicide solution in the rooting zone, thus providing a greater opportunity for more uptake. It has been reported that plants of the Gramineae family, such as maize, absorb the chloroacetamides mainly through the coleoptile and mesocotile parts of the emerging shoot. This would explain why low temperatures and wet conditions promote injury to maize where these herbicides are used (Gerber, *et al.*, 1974; Narsaiah & Harvey, 1977; Klingman & Ashton, 1982; Akobundu, 1987). If rainfall during the germination and emergence stages of development is low enough so as not to cause herbicide leaching beyond the zone where crop seeds were placed, yet not so low as to prevent absorption of the herbicide, the amount available for uptake may be maximized.

There is also a possibility of reduced plant metabolism at low temperatures. Even tolerant plants may not adequately metabolise the herbicide under cool conditions due to reduced plant metabolic activities. Herbicide translocation could also be decreased (Didwell, 1974; Putnam & Rice, 1979), and hence, the absorbed herbicide may not be translocated to a site where it can be detoxified, and as such the crop could be damaged.

The magnitude of sensitivity of maize genotypes to the chloroacetamide herbicides was dependent on the type of herbicide and also genotype. Susceptible genotypes were injured much more than tolerant genotypes at low temperatures. This is possibly due to the differences in their genetic constitution. Metazachlor showed higher injury both on SDM and visual injury symptoms than either alachlor or metolachlor. Perhaps this is caused by differences in their modes of action. Metazachlor is probably less metabolised by maize than the other two herbicides.

At moderate temperatures (15/25°C), results were similar to those obtained in the greenhouse maize screening experiments reported earlier. Only susceptible genotypes were injured by all three herbicides. At higher temperatures (20/35°C), the tolerance of maize genotypes to the herbicides was similar to that shown at intermediate temperatures (15/25°C). There was, however, at this regime apparent injury of tolerant genotypes exposed to metazachlor. The SDM reduction was higher, while visual injury was observed on all genotypes.

Plant metabolic processes are probably not seriously affected at both intermediate and high temperature regimes, and therefore, tolerant genotypes were able to detoxify the herbicides. This is in agreement with the findings of Mulder & Nalewaja (1978) who reported that atrazine and alachlor toxicity to oats increased with decreasing temperatures. Nel & Reinhardt (1984) reported that both low and high temperatures influence the toxicity of atrazine to maize.

Results from this study show that temperature affect maize tolerance to alachlor, metazachlor and metolachlor. Low temperatures reduced the tolerance of maize to these herbicides, while higher temperatures did not affect tolerance as much. It is, therefore, very important to screen maize genotypes for tolerance to these type of herbicides under specific environmental conditions, particularly those that could be expected early in the growing season, e.g., high soil moisture and low temperatures. Genotypes that are tolerant to commonly used herbicides under varying temperatures could be recommended for areas where temperatures fluctuate a lot.

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CHAPTER 5

INHERITANCE OF METAZACHLOR TOLERANCE IN MAIZE

Introduction

Differential tolerance of maize genotypes to several herbicides has been reported (Francis & Hamill, 1980; Ogg & Drake, 1982; Sagara & Foy, 1982; Le Court De Billot, 1985; Charlotte *et al.*, 1989; Eberlein & Miller, 1989; Le Court De Billot *et al.*, 1990; Reinhardt, 1993; Green & Ulrich, 1993, Van Wychen, *et al.*, 1999; O'Sullivan *et al.*, 2001). This is an important factor for all herbicides, for most if not all crops, including maize. Own observations in the previous experiments also confirmed the differential tolerance of maize to herbicides (Kanyomeka & Reinhardt, 2000). Maize genotype responses to specific herbicides varied from 0 to 60 % shoot dry mass reduction from the untreated control; and low temperatures affected tolerance negatively.

Herbicide tolerance in maize and other crops has been reported to be under genetic control (Edwards *et al.*, 1976; Faulkner, 1982; Sauza Machado, 1982; Le Court De Billot *et al.*, 1990). The genes responsible for tolerance can either be dominant or recessive. The nature of inheritance for herbicides has also been reported to be associated with both single and multiple genes (Grogan *et al.*, 1963; Comstock & Andersen, 1968). Polygenic inheritance and the existence of additive gene effects have been reported (Geadelman & Andersen, 1977; Le Court De Billot, *et al.*, 1986; Landi *et al.*, 1989; Landi *et al.*, 1999).

It is clear that the inheritance of crop tolerance to herbicides involves a single gene or many genes. Information on the nature of inheritance of some herbicides is known or at least has been investigated. Examples are atrazine (Grogon *et al.*, 1963; Le Court De Billot, 1990), trifluralin (Landi *et al.*, 1999), Hoe 23408 (Greadelman & Andersen, 1977) and chlorsulfuron (Landi *et al.*, 1989). For many other herbicides, such as metazachlor, the nature of inheritance

is not known despite the importance of this information.

The nature of herbicide inheritance in crops, in addition to genetic variability, is crucial for any successful breeding program. This information could enable plant breeders to select for this trait and use it in cultivar development to increase the tolerance of new material to a specific herbicide. Though this kind of information has not been abundantly exploited, for various reasons, there is potential to breed for herbicide tolerance (Faulkner, 1982; Sauza Machado, 1982).

In previous experiments (Chapter 2) it was observed that maize responses to herbicides varied most with metazachlor. Some genotypes were almost completely killed by metazachlor while others were not affected at all. This indicates that there is a possibility of improving maize tolerance to metazachlor through breeding. This could be done by crossing tolerant with tolerant genotypes, tolerant with susceptible or susceptible with susceptible genotypes, depending on the nature of inheritance of metazachlor tolerance.

Information on the pattern of inheritance of metazachlor tolerance in maize is non-existent. Although the initial plan of this study did not consider investigating the nature of metazachlor tolerance in maize, it is important to try to establish this from the data generated from the maize screening experiments. The objective of this study was to investigate the nature of inheritance of metazachlor tolerance in maize.

Materials and Methods

Various methods to determine the nature of inheritance of herbicide tolerance in crops are available. The most common one is the Diallel crossing system that involves making all possible crosses among a group of genotypes. This offers an opportunity to generate patterns of inheritance information from all possible combinations of parents used (Griffing, 1956; Landi *et al.*, 1989; Sughrove & Hallauer, 1997). In other words, this method allows one to

study and compare the performance of the parent lines in a hybrid combination. With this method, general and specific combining abilities can be determined. According to Griffing (1956) general combining ability is used to designate the average performance of a line in hybrid combination, and specific combining ability is used to designate those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved.

Diallel crossing techniques vary depending upon whether or not the parental inbreds or reciprocal inbreds or the reciprocal F1's, or both are included. This results in four possible experimental approaches; (a) parents, one set of F1's and reciprocal F1's are included; (b) parents and one set of F1's are included but reciprocal F1's not; (c) one set of F1's and reciprocals are included but not parents, and (d) one set of F1's is included. Each one of these methods necessitates a different form of analysis (Griffing, 1956). The appropriateness of diallel crossing methods depends on the experimental material and the objectives of the experiment. The diallel crossing systems are useful and frequently used in plant breeding research to obtain genetic information. One would obtain genetic effects for a fixed set of parent lines or estimates general and specific combining ability variance components and heritability for population from randomly chosen parental lines (Zhang & Kang, 1997).

To obtain genetic information on herbicide tolerance in maize, diallel crossing systems are the appropriate methods to use. Even our study would have done well to employ this method, in which case a certain number of tolerant and susceptible parent lines could have been selected and crossed in all possible combinations. Subsequently the performance of parent lines and their hybrids would have been evaluated as regards their tolerance to metazachlor. However, since the original plan was not to generate any genetic information, no diallel was constructed. Nevertheless, an attempt is retrospectively being made here to obtain genetic information from the data.

Using data from the greenhouse screening reported earlier (Chapter 2, Table 2.3), eight hybrids with varying tolerances to metazachlor, and their respective parents, were used in this study. These hybrids included one single cross, three three-way crosses and four double crosses (Table 5.1).

Data interpretation was done based on the statistically analysed results from Table 2.3. To determine the gene effects on metazachlor tolerance, it was assumed that three genes for tolerance exist (A, B, C). This was based on the fact that shoot dry mass reductions ranged from 0 % to 60 %, the three genes are assumed to share this equally. Meaning that each one contributes 20 % (A=20 %, B=20 %, C=20 %). Then a table was constructed (Table 5.3) to elaborate all possible combinations and the respective possible performance of their hybrids (*Eisenberg, 2000, Personal communication)

Table 5.1 Hybrids and their respective parents used to determine the genetic inheritance of metazachlor tolerance in maize

Hybrid	Parent lines	Type of cross
CV1	[P45(f) X P17(m)](F) X P1(M)	Three-way cross
CV2	[P22(f) X P25(m)](F) X P5(M)	Three-way cross
CV3	[P46(f) XP21(m)](F) X [P24(f) X P14(m)](M)	Double cross
CV5	[P41(f) X P10(m)](F) X [P5(f) X P9(m)](M)	Double cross
CV7	[P26(f) X P4(m)](F) X [P5(f) X P21(m)](M)	Double cross
CV8	P10 (F) X P5 (M)	Single cross
CV9	[P31(f) x P2(m)](F) X P8 (M)	Three-way cross
CV10	[P45(f) X P17(m)](F) X [P14(f) X P18(m)](M)	Double cross

Key: f=female for parent, m=male for parent, F=female for a hybrid, M=male for a hybrid

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Results and Discussion

Data reflecting the tolerance of genotypes to metazachlor, and their respective tolerance levels, are presented in Table 5.2. The data presented are percentage shoot dry mass reductions from the untreated controls obtained from greenhouse experiments that were reported in Chapter 2, Table 2.3, except genotypes P1, P44, P45 and P46 which were included in series of experiments whose results are not presented. Projected hybrid tolerances are shown in Table 5.3.

Shoot dry mass reductions ranged from 0 to 60 % (see Chapter 2), with the highest reductions, therefore the greatest sensitivity, recorded for parent lines (Table 5.2). A combination of hybrid parents that are all tolerant to metazachlor produced tolerant hybrids such as CV9. Hybrid parents that were all susceptible produced susceptible hybrids, for example CV5. Crosses of some tolerant and susceptible genotypes produced hybrids with varying tolerances to metazachlor. For example, CV1 hybrid is highly tolerant despite being a progeny of only one tolerant and two susceptible lines. One of the parents was very susceptible with a SDM reduction of 59%. In other words, although 66.7 % of parents of CV1 were susceptible to metazachlor application, they still produced a tolerant hybrid. Similarly, a single cross hybrid (CV8) was very tolerant to metazachlor, despite being a product of both highly susceptible parents.

The results suggest that metazachlor tolerance in maize is genetically controlled because in most cases when susceptible parent lines were crossed, they produced a susceptible hybrid and when tolerant lines were crossed they produced a tolerant hybrid (Table 5.2). This could mean that there are three types of genes for tolerance (A, B, C) (*Eisenberg, 2000, Personal communication), thus, if all genotypes with recessive genes are crossed they will produce a hybrid with recessive genes for tolerance and therefore the hybrid will be susceptible, and the opposite is true.

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Table 5.2: Shoot dry mass reduction of the hybrids and their respective parents used in the study (Data from Chapter 2, Table 2.3)

Hybrids and parents	Tolerance levels
CV1	T
P45	T
P17	S
P1	S
CV2	T
P22	T
P25	T
P5	S
CV3	T
P46	T
P21	T
P24	S
P14	S
CV5	S
P41	S
P10	S
P5	S
P9	S
CV7	T
P26	T
P4	T
P5	S
P21	T

CV8	T
P10	S
P5	S
CV9	T
P31	T
P2	T
P8	T
CV10	S
P45	T
P17	S
P44	T
P18	T

Key: P=parent' **CV**=hybrid, T=tolerant, S=susceptible

Table 5.3 Crosses of all possible gene combinations and their projected tolerances (% SDM reduction from the untreated control) to metazachlor (*Eisenberg, 2000, Personal communication)

Gene type	ABC	ABc	AbC	Abc	aBC	ABc	abC	abc
ABC	0	0	0	0	0	0	0	0
Abc	0	20	0	20	0	20	20	20
AbC	0	20	20	20	0	0	20	20
Abc	0	20	20	40	0	20	20	40
ABC	0	20	0	0	20	20	20	20
ABc	0	20	0	20	20	40	20	40
AbC	0	0	20	20	20	40	40	40
Abc	0	20	20	40	20	40	40	60

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Based on the latter assumption that there are three genes for tolerance to metazachlor in maize, a table (Table 5.3) was constructed to show all possible combinations of genes and their expected responses to metazachlor.

In Table 5.3, it is assumed that each gene contributes 20 % to the reaction of a genotype to metazachlor application. This means that each dominant gene (A,B,C) increases tolerance of a genotype by 20 % and each recessive gene (a,b,c) reduces tolerance by 20 %. For example if parent lines with only dominant genes are crossed, the resulting hybrid will be dominant and therefore will be tolerant to metazachlor (ABC x ABC = AABBCC). The opposite scenario is where genotypes with recessive genes are crossed and will produce a hybrid with recessive genes, which will be susceptible to metazachlor (a,b,c x a,b,c = aabbcc). Several other combinations are also possible with varying tolerances to metazachlor.

Results of this investigation correspond with that of other researchers, that it is possible to improve herbicide tolerance through breeding. Faulkner (1982) reported that crop tolerance to herbicides is genetically controlled and that it is possible therefore to improve this tolerance through breeding. Similarly, Le Court De Billot (1985) observed that atrazine tolerance could be improved through breeding. Cytoplasmic inheritance of atrazine in maize was reported by Souza Machado (1982) who suggested that tolerance could be improved by breeding. In practical terms, it is possible to improve the metazachlor tolerance in maize by crossing parent lines with dominant genes for tolerance. However, it is very important to identify parent lines with dominant genes prior to making crosses. Once these lines are identified it would be possible to work towards improving metazachlor tolerance in maize by crossing appropriate genotypes. It should, however, be noted that some researchers have not observed appreciable improvement in crop tolerance through breeding (*DeFelice, 2000, Personal communication).

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CHAPTER 6

ULTRASTRUCTURAL CHANGES CAUSED BY METAZACHLOR ON MAIZE SEEDLING LEAVES AND ROOT CELLS

Introduction

Metazachlor is a chloroacetamide herbicide registered for control of weeds in rapeseed, potatoes, broccoli, canola, cabbage, dry beans, groundnuts, hops, soybean, sugarcane and tobacco. It is mainly used for the control of annual grass weeds and some broadleaf weeds (BASF, 1984; Ekler & Stephenson, 1989; Tomlin, 1994; Vermeulen, 1998). Metazachlor was also registered for use in maize in South Africa for some years. It is selective in maize when applied with a safener (Ekler & Stephenson, 1989). Lack of stable selectivity for maize led to the withdrawal of this herbicide from the South African market in 1999.

Differential tolerance of maize genotypes to metazachlor exists (Kanyomeka & Reinhardt, 2000). These differences could be due to varietal differences in herbicide uptake, translocation and metabolism. Responses exhibited by susceptible plants to this family of herbicides are inhibition of root and shoot growth resulting from impaired cell division and elongation. Nucleic acids and protein synthesis is inhibited in susceptible plants (Klingman & Ashton, 1982; Akobundu, 1987; Zimdahl, 1993). However, not all this has been confirmed for all herbicides, including metazachlor, in this group of compounds. The effect of these herbicides on cell organelles is not well described and also their effect on fatty acid biosynthesis and thus on membranes has not been determined.

Many studies have been done on metolachlor and alachlor, the two most widely used herbicides of the chloroacetamide group, to understand the mechanism of action. Luanne & Hess (1979) reported that alachlor and metolachlor inhibited growth at concentrations of 1 x 10^{-7} M alachlor and 5 x 10^{-8} M metolachlor, and 5 x 10^{-7} M alachlor and 1 x 10^{-6} M

metolachlor for peas and oats, respectively. The growth inhibition was due to the herbicide inhibition of both cell division and cell enlargement. Reinhardt & Nel (1986) reported that alachlor-treated sorghum plants showed disintegration of cell vacuole membranes, double membranes of chloroplasts and membranes of nuclei. Mellis *et al.* (1982) found no evidence that the loss of cotton and maize root cell membrane integrity is due to the inhibition of total lipid, phospholipid or PC synthesis by either alachlor or metolachlor.

Similar work has been done on other plant species and herbicides to describe the effects of herbicides on the ultrastructure of root and shoot cells. Ferreira *et al.* (1999) reported that sulcotrione/atrazine, atrazine/terbuthylazine + 2,4-D and bendioxide caused ultrastructural changes in the leaves of *Commelina bengalensis* L. These changes included change of chloroplast shape, disappearance of starch from stroma and ultimately disintegration of the chloroplast envelope. Stoynova *et al.* (1997) found that chlorsulfuron caused ultrastructural changes in the mitochondria, nucleoi and chloroplasts of root and leaf cells in pea plants.

The effects of metazachlor on the ultrastructure of susceptible plants have not been determined. This study was aimed at describing ultrastructural changes in root and leaf cells of maize seedlings damaged by metazachlor.

Materials and Methods

Plant material

Maize plants treated pre-emergence with metazachlor were grown in a greenhouse for a period of two weeks after treatment. Greenhouse day/night temperatures were maintained at 25-30°C/15-18°C, with a photo-period of 12-14 hours. Five maize seeds were planted in pots containing 2.5 kg of soil. This soil had 72 % clay content, with a pH (H_2O) of 6.5. Watering was done to ensure that there was always adequate moisture for plant growth. After emergence, a complete nutrient solution (Nitsch, 1972) and water were applied on alternate days. The plants were thinned to three per pot after emergence.

Metazachlor (product Preecede) was applied immediately after planting at the rate of 1.5 kg ha^{-1}. Two maize genotypes were used; one tolerant and one susceptible genotype, based on the earlier screening experiments. Each genotype was treated with the herbicide, and for each there was an untreated control.

Two weeks after emergence, plants were taken to the electron microscopy laboratory where fresh leaf tissue segments were cut for determination of metazachlor effect on the leaf cells.

Root plant material was prepared by placing maize seeds in petri dishes that were lined with Whatman No.3 filter paper. Four maize genotypes were used - two tolerant and two susceptible. Germination was done in an incubator at 27°C for five days. Metazachlor (10 ml of a concetration of 1.5 x 10^{-4}M) was applied to each petri dish of each genotype. The other treatment of each genotype was an untreated control. Five days after planting, root tips were removed with a clean, sharp incision.

Electron microscopy

The cut plant material was prepared for light microscopy (LM) and transmission electron microscopy (TEM) using methods described by Coetzee & Van der Merwe (1996).

Plant tissue segments were fixed in 2.5 % glutaraldehyde in 0.075 M phosphate buffer at pH 7.4 for two hours. They were then rinsed three times in 0.075 M phosphate buffer for five minutes each. After that, specimens were fixed in 0.25 % aqueous osmium tetroxide for two hours and then rinsed three times in distilled water. The samples were dehydrated in a graded series of ethanol where 100 % ETOH was mixed with epoxy resin at 33 % resin and 66 % resin both standing for one hour each, and finally 100 % resin standing over-night. The specimens were then embedded in moulds and polymerised at 65°C for 36 hours. Ultrathin sections were cut with a diamond knife and picked up on grids. They were then contrasted in 4 % aqueous uranyl acetate for 10 minutes and then rinsed in water. The sections were

contrasted in Reynolds' lead citrate for two minutes and rinsed in water. Monitor sections of 0.5µm were cut, stained in Toluidine blue and mounted in immersion oil. Sections were examined with a Nikon LM and a Philips EM 301 transmission electron microscope before representative photographs were taken.

Specimens for the scanning electron microscope (SEM) were prepared according to the technique used by Ecklin (1992). Plant tissue segments were mounted with carbon dag on carbon stubs and plunge frozen in liquid nitrogen. The material was fractured level with the carbon dag surface and freeze-dried overnight. Stubs were coated with chromium in an ion beam coater and viewed with a JEOL 6000F SEM.

Results and Discussion

The effect of metazachlor on the morphology of maize seedlings is shown in Figure 6.1. The ultrastructural effect of metazachlor on the root and leaf cell organelles is shown in electron microscope micrographs in Figures 6.2 – 6.8.

Plant growth

The effect of metazachlor on the rate of emergence and seedling development was similar to that observed earlier in the screening experiment (Chapter 2). There was no effect of metazachlor on the rate of emergence for both susceptible (CL) and tolerant (SX1) genotypes. The tolerant genotype's shoot growth was not affected at the recommended herbicide rate while at double this rate it was greatly reduced (Fig. 6.1A). However, the susceptible genotype's shoot growth was greatly reduced at both application rates (Fig. 6.1B). Root growth was affected in similar fashion as for the shoots. Roots grew normally in the tolerant plants at the recommended rate but at double the normal rate the growth was greatly reduced (Fig. 6.1C). In the susceptible genotype root growth was reduced at both herbicide application rates (Fig. 6.1D).

Ultrastructural changes

Light microscopy

In the leaf sections of tolerant plants there were no differences in the structures between the treated at the normal application rate and the control (Fig. 6.2A & C). However, at double the rate (Fig. 6.2E) there were more chloroplasts, which were disorganised as compared with the control. In the susceptible plant leaf sections, differences were observed at both application rates (Fig. 6.2D & F) and the control (Fig. 6.2B). Leaf sections from the treated plants showed increased thickness of leaves resulting from enlarged epidermal cells. Clusters of large and empty parenchyma cells were observed (Fig, 6.3D & E). In addition, more and disorganised chloroplasts were not arranged evenly against the cell walls as in the control samples. This is also evident when comparing the bundle sheath chloroplasts (Fig. 6.4).

Transmission electron microscopy

A comparison of bundle sheath chloroplasts from treated plants and those from the control plants (Fig. 6.4B) show that membranes were dilated in the former plants at both recommended and double the recommended rates for the susceptible genotype (Fig. 6.4D & F). This was only observed at double the rate in the tolerant plants (Fig. 6.4E) and not at the recommended rate (Fig. 6.4C) or the control (Fig. 6.4A). In addition, a lot of empty spaces were observed in the treated plants. The grana orientation in the mesophyll chloroplasts was affected in the treated susceptible plants at both application rates (Fig. 6.5D & E) as compared to the control (Fig. 6.5B), while this was only observed at double the recommended rate (Fig. 6.5E) in the tolerant plants, and not at the recommended rate (Fig. 6.5C) or the control (Fig. 6.5A). The affected grana are elongated.

Other observations from Figure 6.6 are that mitochondria were not adversely affected by metazachlor treatment (Fig. 6.6 B&D). In the injured leaves the epidermal cells were fused along the cuticles (Fig. 6.6C). Abnormal cell wall growth was observed in treated susceptible

plants (Fig. 6.6 E). Many crystals or dark bodies were observed in cell walls of treated susceptible plants (Fig. 6.6F). These appear to be protein crystals. The vacuole membranes are disintegrated or absent in some cases or only visible as vesicles.

Sections of the root samples showed that there were larger and empty vacuoles in the treated susceptible plants, and their nuclei contained disorganized with disorganized chromatids. Nuclear and plasma membranes appeared to have disintegrated (Fig. 6.7). However, in the treated tolerant plants these changes were not observed (Fig. 6.8).

Metazachlor belongs to a group of herbicides, chloroacetamide, that are known to affect the root and shoot growth of susceptible plants and not the germination (Akobundu, 1987; Ashton & Monaco, 1991). This perhaps is the reason why the rate of emergence of both the susceptible and the tolerant genotypes was not affected.

The observed effect of metazachlor on plants is similar to what was found in the earlier screening experiments. This particular investigation confirms the existence of differential tolerance of maize genotypes to specific herbicides (Charlotte *et al.*, 1989; Green & Ulrich, 1993; Landi *et al.*, 1999). There was a consistent pattern of metazachlor effect on plant growth and on the ultrastructure of the seedlings. In other words, a genotype that was injured by metazachlor in the earlier experiments was also injured in this experiment, and similarly the leaves and root cells' ultrastructures were affected. Tolerant genotypes were not affected at all.

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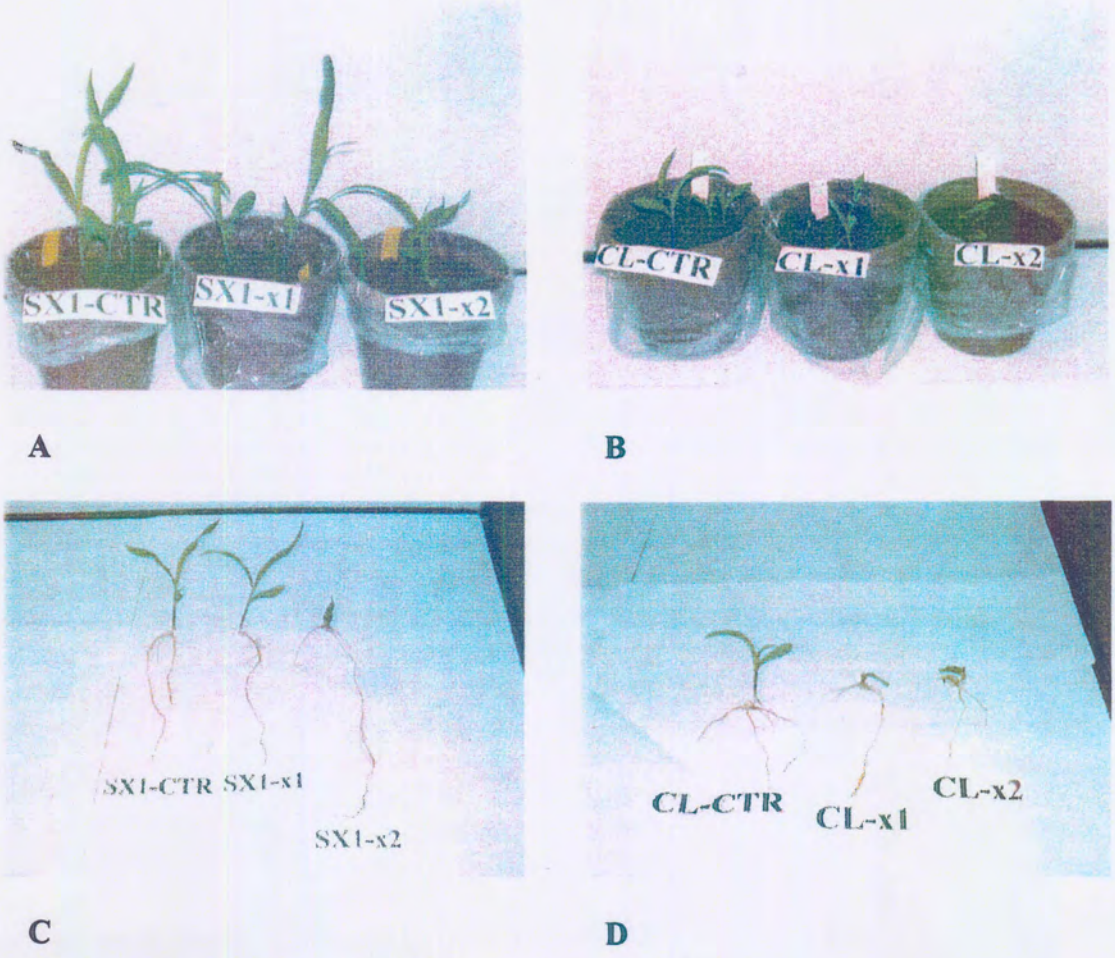

Figure 6.1 **Maize seedlings treated with metazachlor;**
A = tolerant genotype at normal dose (x1) and double dose (x2)
B = susceptible genotype at normal dose (x1) and double dose (x2)
C = tolerant genotype at normal dose (x1) and double dose (x2) showing shoot and roots
D = susceptible genotype at normal dose (x1) and double dose (x2) showing shoot and roots

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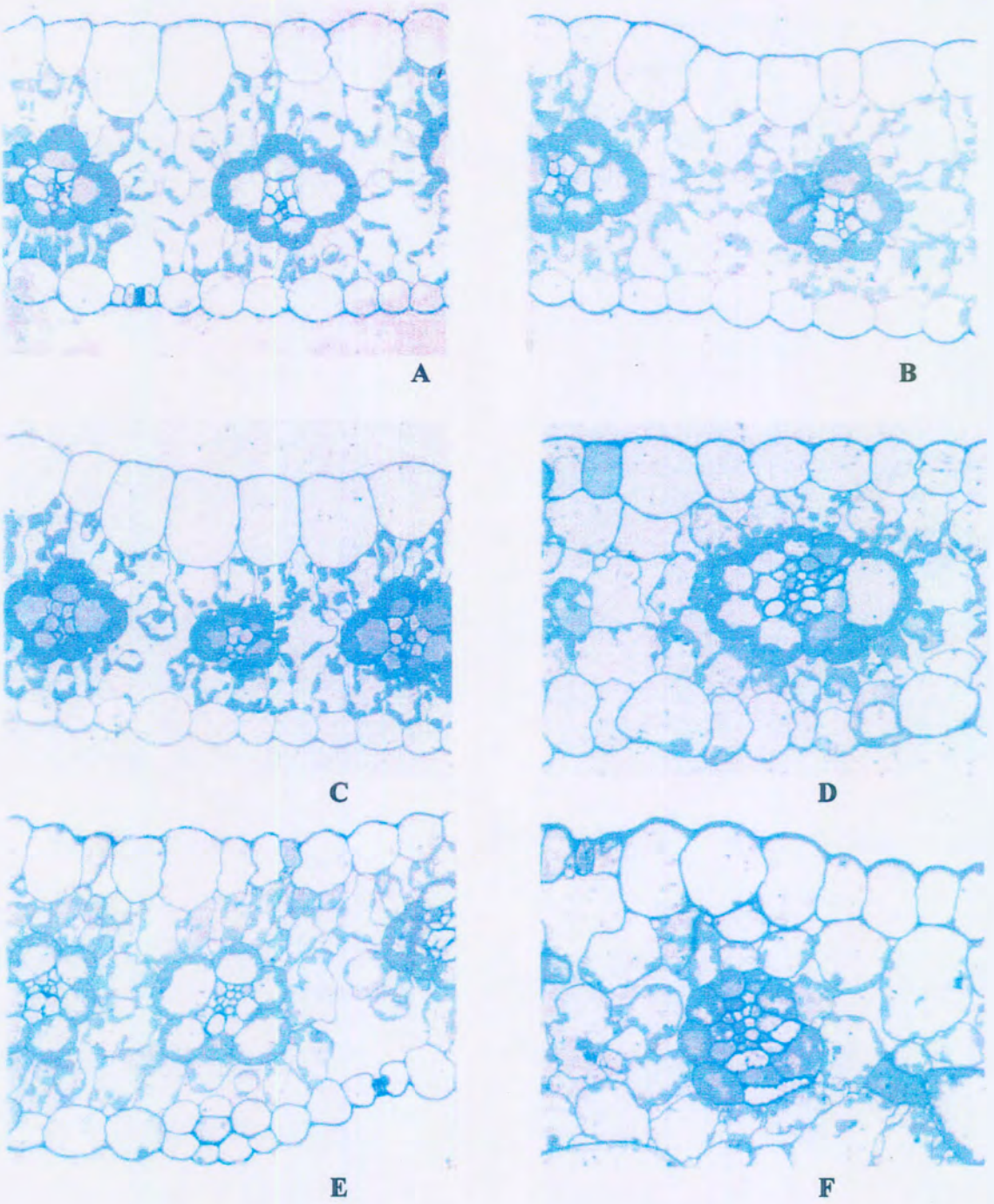

Figure 6.2. Micrographs of cross sections through the leaves: A = tolerant genotype untreated control; B = susceptible genotype untreated control; C = tolerant genotype treated at normal dose; D = susceptible genotype treated at normal dose; E = tolerant genotype treated at double dose; F = susceptible genotype treated at double dose.

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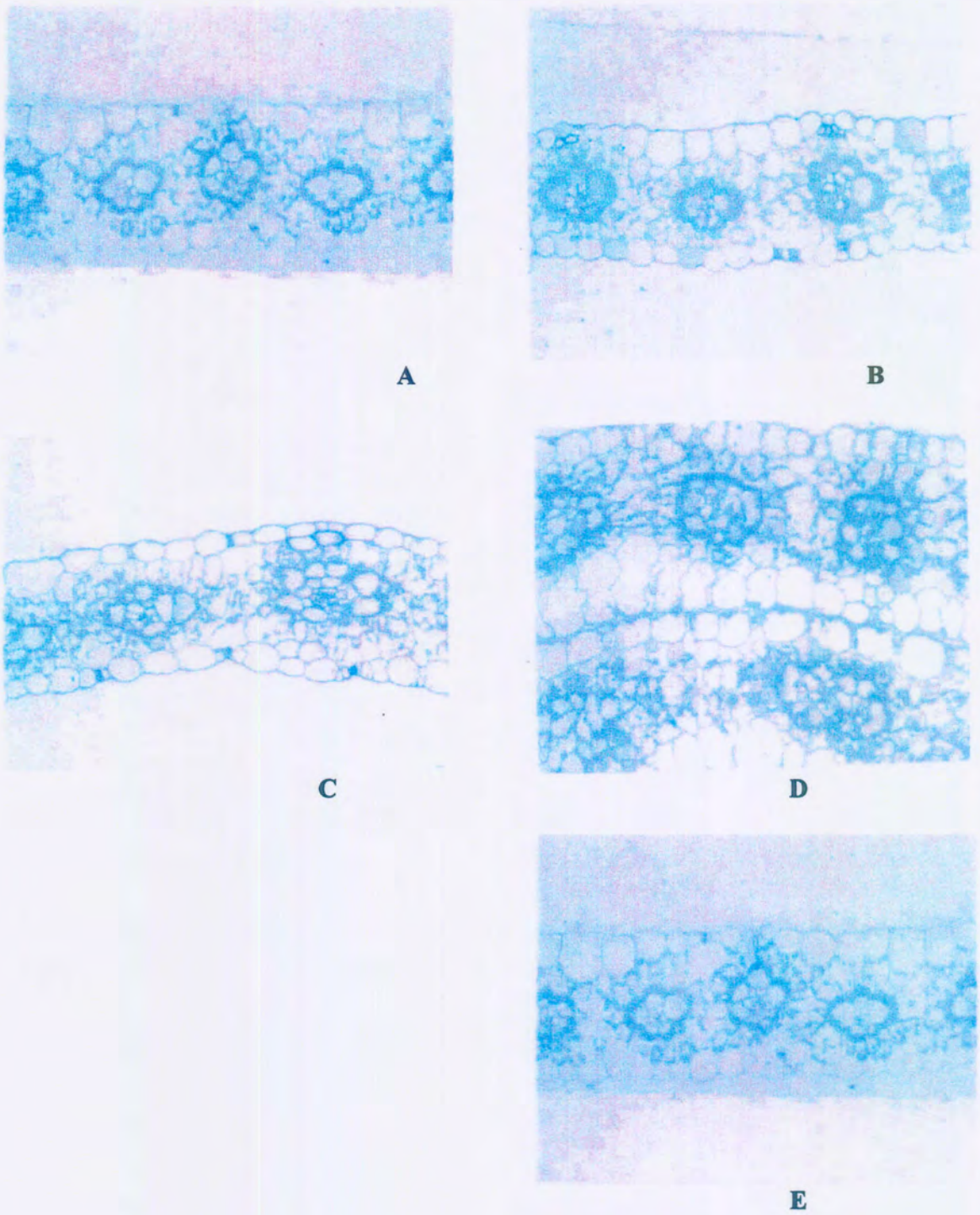

Figure 6.3. **Micrographs of cross sections through the leaves showing enlarged parenchyma cells: A = tolerant genotype untreated control; B = susceptible genotype untreated control; C = tolerant genotype treated at double dose; D & E = susceptible genotype treated at double dose.**

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Bundle sheath chloroplasts:

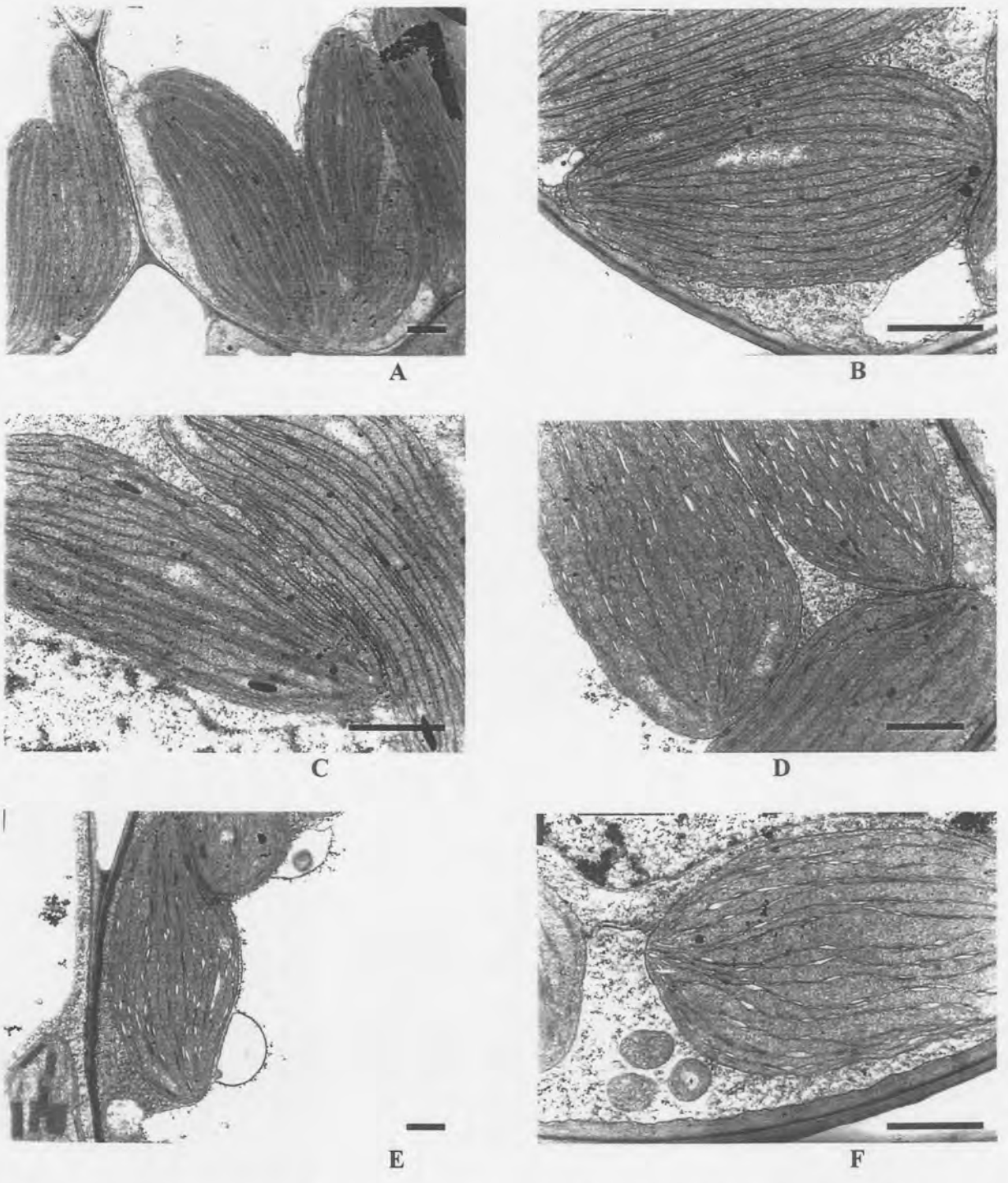

Figure 6.4. Micrographs showing bundle sheath chloroplasts. : A = tolerant genotype untreated control; B = susceptible genotype untreated control; C = tolerant genotype treated at normal dose; D = susceptible genotype treated at normal dose; E = tolerant genotype treated at double dose; F = susceptible genotype treated at double dose. Scale bars = 1μm.

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Mesophyl chloroplasts:

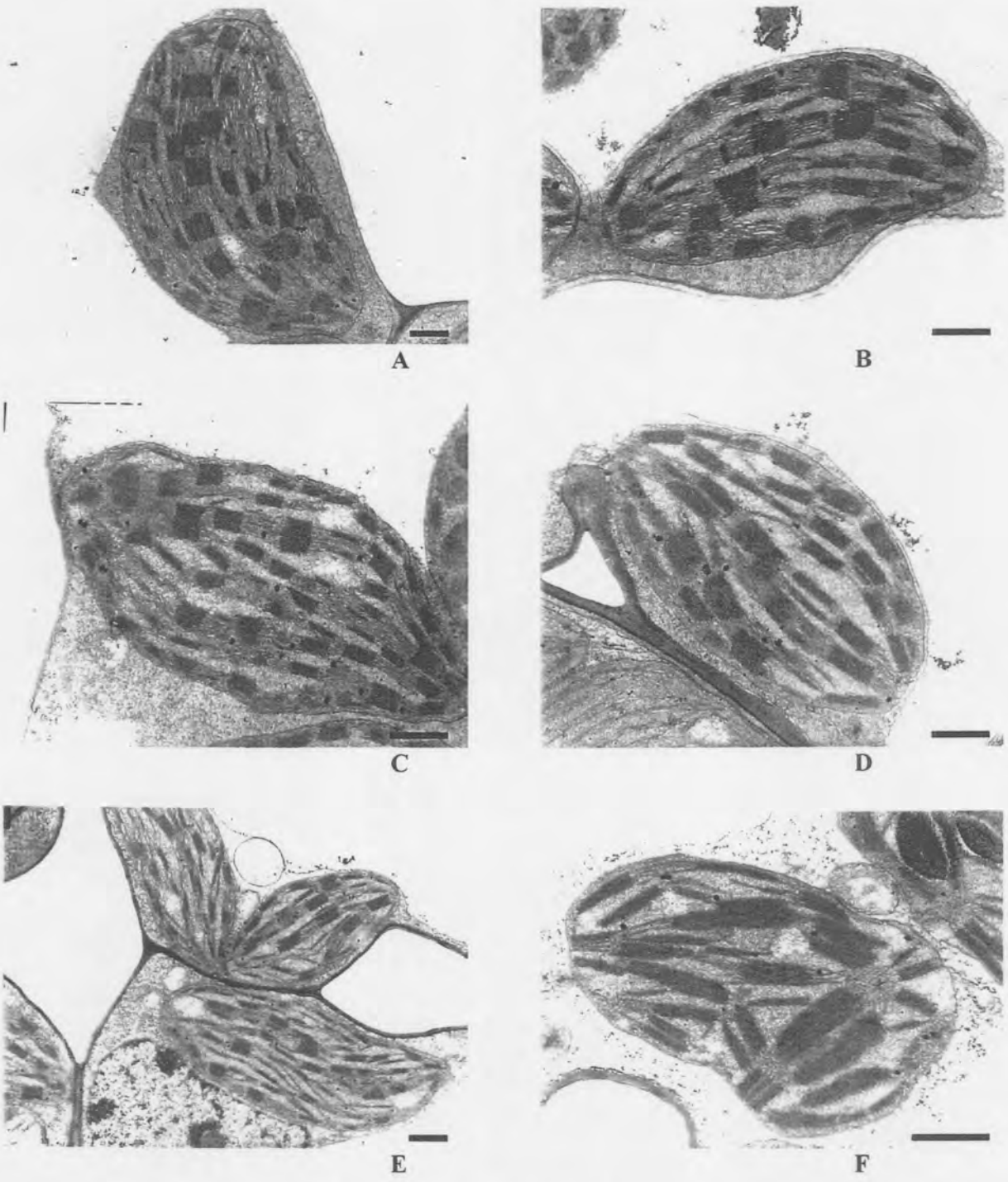

Figure 6.5. Micrographs showing mesophyl chloroplasts: A = tolerant genotype untreated control; B = susceptible genotype untreated control; C = tolerant genotype treated at normal dose; D = susceptible genotype treated at normal dose; E = tolerant genotype treated at double dose; F = susceptible genotype treated at double dose. Scale bars = 1μm.

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Extra:

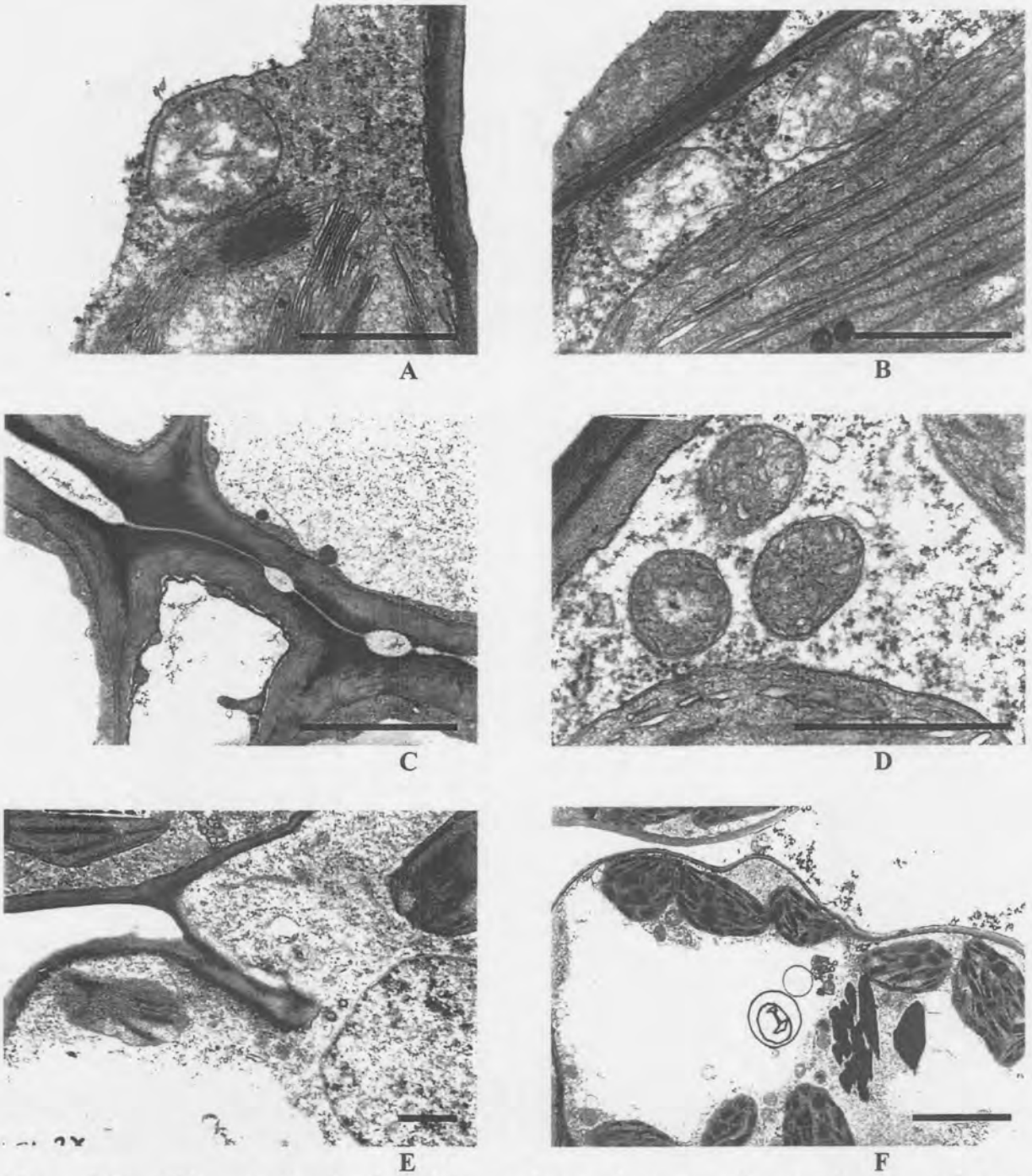

Figure 6.6. Micrographs showing different changes in some cell organelles: A = tolerant genotype treated at double dose; B = susceptible genotype untreated control; C = susceptible genotype treated at double dose; D = susceptible genotype treated at normal dose; E = susceptible genotype treated at double dose; F = susceptible genotype treated at double dose.
Scale bars: Figs. A, B, D & E = 1µm. Figs. C & F = 5µm.

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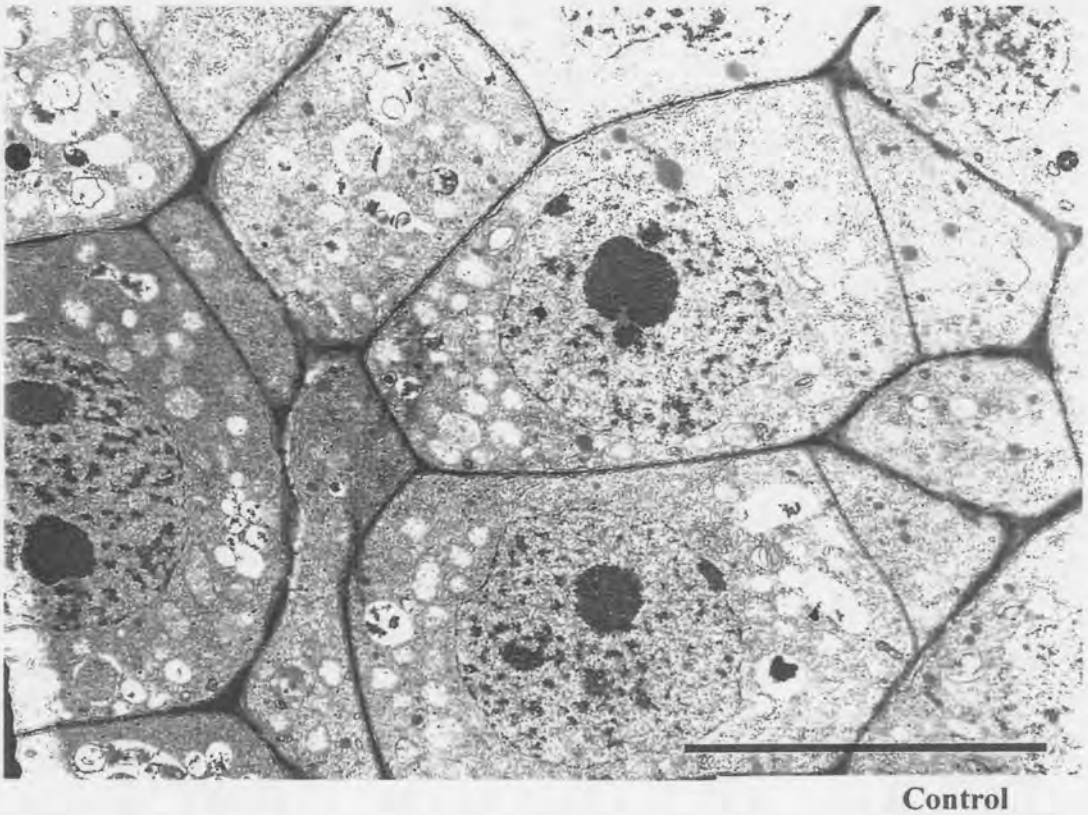
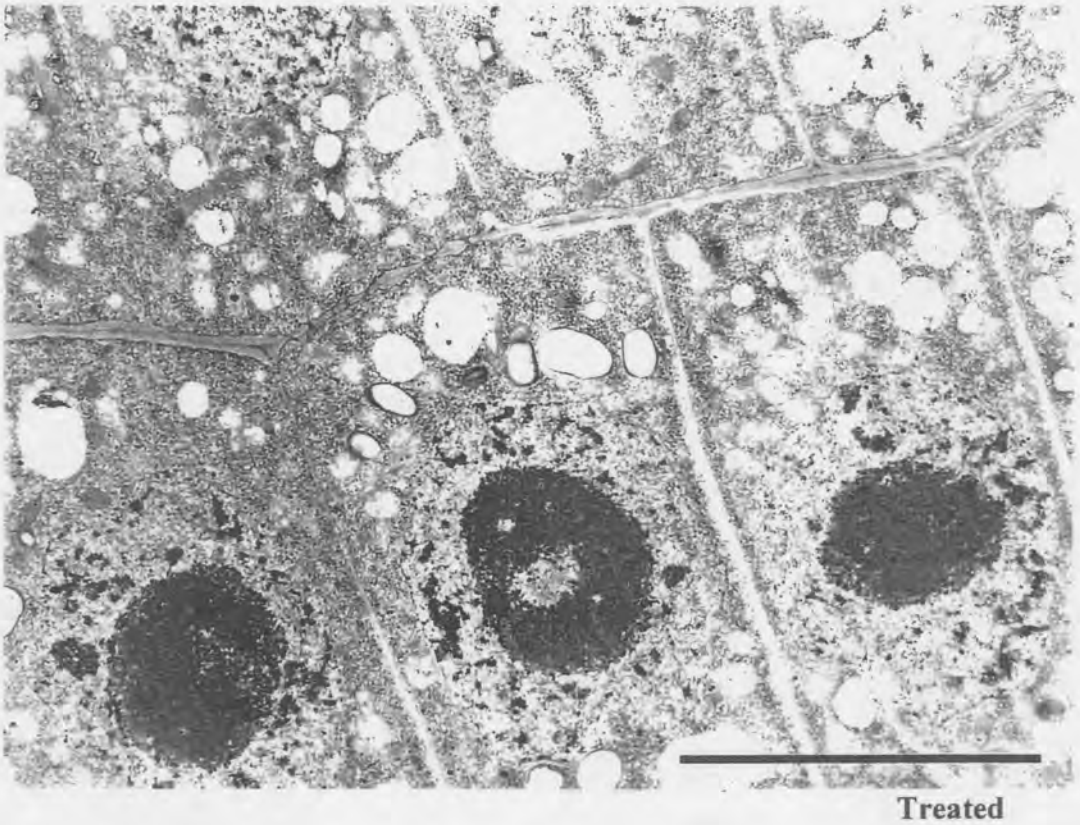


Control



Treated

Figure 6.7 **Micrographs showing root cell structure of a susceptible genotype (SXI) Scale bar = 10μm**

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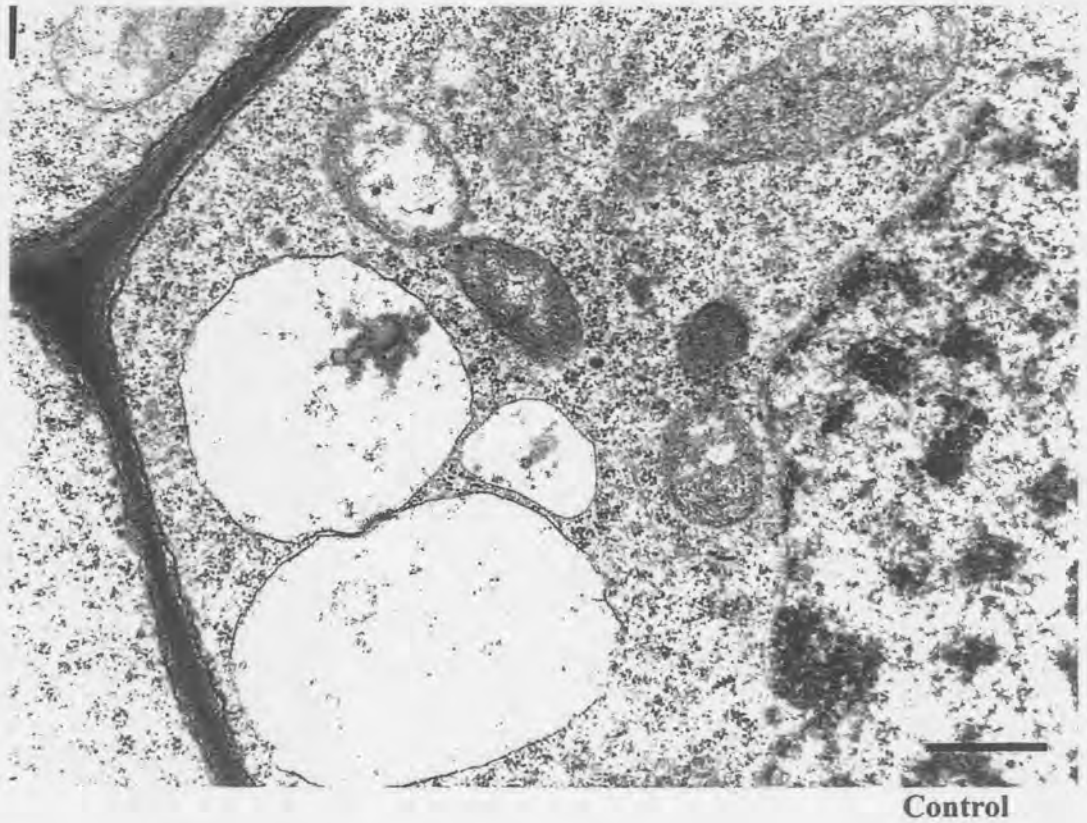
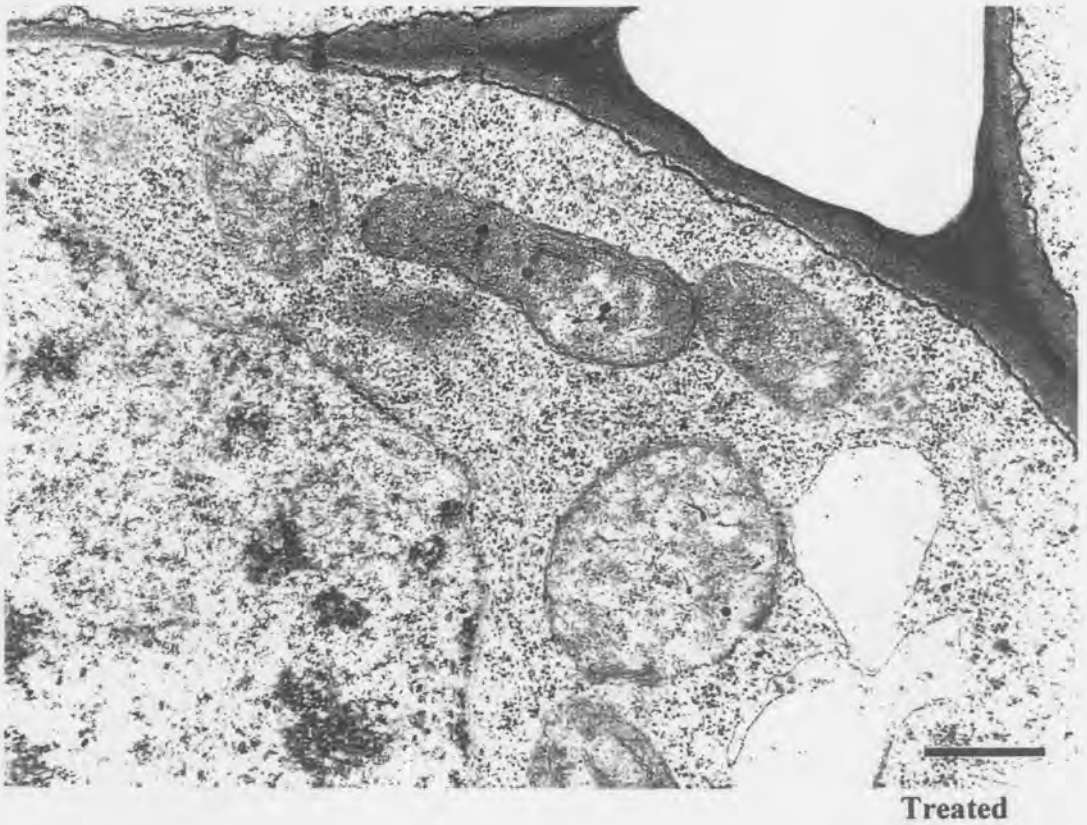


Control



Treated

Figure 6.8. **Micrographs showing root cell structure of a tolerant genotype (SXI) Scale bar = 1μm**

The effects of metazachlor on the ultrastructure of roots and leaves of maize seedlings are similar to those observed where some herbicides in the same family, and other groups, were applied on susceptible plants. Reinhardt & Nel (1986) reported that alachlor caused disintegration of vacuole membranes, double membranes of chloroplasts and membranes of nuclei in sorghum. Similarly, Mellis *et al.* (1982) reported that metolaclor and alachlor affected the membrane permeability. Malan *et al.* (1985) reported that atrazine affected ultrastructure of maize leaves. Mesophyll chloroplasts became more spherical, grana were swollen and chloroplasts membranes were disintegrated. Duke (1985) also reported chloroplast senescence when thylakoids of grana are swollen and extended. Stoynova, *et al.* (1997) reported that mitochondria, nucleoli and chloroplasts showed ultrastructural disturbances due to chlorsulfuron. They suggested that cell growth is inhibited by accumulation of toxic intermediates. The effect of metazachlor on cell organelle membranes could have an effect on lipid and protein synthesis (Zimdahl, 1993; Chao *et al.*, 1994).

From the results, it was realised that through this kind of experiment it is fairly difficult to clearly study the ultrastructural changes caused by herbicides. The common practice in this kind of study is to treat plants with a herbicide and study the effect soon after application. In other words the process of cell death can easily be observed and described. In this case the plants were not close to death. Instead they were struggling to remain alive, and hence, were attempting to develop normally.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

This study was undertaken after reports over many years of various cases of maize damage caused by inadequate herbicide selectivity in farmers' fields. Differential cultivar tolerance was observed in these fields. Variable tolerance of crop cultivars to a particular herbicide is well known (Dayan *et al*, 1997; Kassim *et al*., 1997; Sprague *et al*., 1999; O'Sullivan *et al*., 2001). Differential crop cultivar tolerance to herbicides is therefore a very important factor to consider for all herbicides. All these differences are associated with the type of herbicide, genetic composition of the cultivars, and the environmental conditions.

In South Africa, no crop herbicide screening has routinely been done on a large scale. This probably, at least partly, explain the many misunderstandings that often occur when crop injury is reported. Sometimes the injury is suspected to be the result of other production inputs, such as either over- or under-supply of fertilizers. It was therefore important to verify or confirm the existence of differential tolerance to herbicides in South African maize genotypes, in order to provide information for use in seed breeding and marketing strategies of seed and agrochemical companies, respectively. Furthermore, it was considered important to better understand the effects of temperature on crop tolerance to herbicides that tend to cause injury to maize under "extreme" temperature conditions, especially low temperature. Also, the effect of metazachlor on the ultrastructural level in maize plants was investigated, because nothing has been reported in this regard, and hopefully such effects could provide some insight into the probable mode of action of the herbicide.

This study demonstrated the existence of differential maize genotype tolerance to herbicides. This confirms previous reports that maize cultivars differ in their response to herbicide applications (Le Court De Billot, 1985; Green & Ulrich, 1993; Hinz *et al*., 1997; Doohan *et*

al., 1998). Variable tolerance within a particular crop species is probably explained by tolerant genotypes metabolizing herbicides faster than susceptible types. Visual injury symptoms were observed for some herbicides. Symptoms persisted until maturity in some cases, while they disappeared early in other cases. This indicates the possibility of recovery from initial injury for some of the herbicide/genotype combinations. Narsaiah & Harvey (1977) and Hagood *et al.*, (1980) reported differential recovery rates of maize from initial injury. In the present study, root and shoot dry mass reductions from the untreated control did not always correspond well. In some cases the herbicide negatively affected above-ground plant parts but not the roots, and *vice versa*. Inhibition of root growth, even without obvious injury above-ground, could affect the yield if the effect persists for a long time. Herbicide effects on maize root system should therefore routinely be measured, especially for herbicide/crop combinations that seem to be prone to this kind of effect, e.g., chloroacetamides and maize.

Among the herbicides included in the study, metazachlor was generally more injurious to maize than any other herbicide. One explanation might be that maize plants do not easily inactivate this herbicide as effectively as other chloroacetamides. Among the genotypes tested, inbreds were generally more sensitive to the herbicides than hybrids. Landi *et al.* (1989) and Green & Ulrich (1993) reported that inbreds were more damaged by herbicides than hybrids. Due to inbreeding, inbreds are generally weaker than hybrids. Present findings suggest that it is necessary that routine screening of maize genotypes be done in order to avoid crop damage in the field, both in seed production and commercial farming situations.

The existence of differential maize cultivar tolerance to important herbicides, and many crop damage reports in recent years necessitate the initiation of an effective maize screening programme to avoid further crop damages. The material requiring screening is so much that the slow and expensive field screening method may not cope with the situation. Alternative methods that are cheap and quick, yet yield reliable data, are recommended. Screening in the

greenhouse was evaluated to ascertain the consistence of herbicide effects under different growing conditions, greenhouse and field. Krahmer & Russel (1994) describe the problems of glasshouse to field transfer of pesticides performance. Despite some drawbacks, it remains important to consider screening crops at greenhouse level because of the high costs associated with fieldwork. The latter approach is also more time-consuming than work in the greenhouse. Greenhouse screening has been used by many researchers to predict pesticide performance in the field (Goff & Miller, 1990; Grime, 1994; Bonnet & Bosschert,1994). In the present study, relationships between herbicidal effects in the greenhouse and effects on grain yield in the field were strong in most cases. This shows that greenhouse effects could be good predictors of herbicide effects on yield in the field. Therefore it is suggested that maize screening for tolerance to herbicides could be reliably done in the greenhouse, provided that herbicide rates and environmental conditions are carefully selected. Research in a controlled environment offers the opportunity to investigate worst-case scenarios in terms of herbicide/crop interaction. Screening in the greenhouse would save valuable time and money in programmes aimed at assessing crop tolerance to herbicides. Results have shown that correspondence of data from the greenhouse and that from the field are herbicide dependent (Table 3.2 & 3.3). This is probably due to differences in herbicide modes of action. It should also be noted that the influence of soil and environmental factors on plant responses to herbicides vary from compound to compound. Ideally, this would necessitate determining the correspondence of greenhouse and field data for individual herbicides.

The effect of temperature on maize genotype tolerance to selected chloroacetamide herbicides was found to be significant. Generally, low temperatures reduced the tolerance of maize to these herbicides, to such an extent in the case of metazachlor that even genotypes that were highly tolerant at normal maize growing temperature regimes were susceptible at lower regimes. Similarly, McWilliam (1967) and Hodgins & Van Huystee (1976) reported low maize tolerance to 2,4-D at low temperatures of less than 18°C. Cool temperatures tend to reduce the plant metabolic processes, thereby impeding the process of detoxifying herbicides

to safe metabolites. Rate of plant metabolism may be slowed down when conditions are cool, and when wet conditions coincide with this, as is often the case early in the summer growing season, plants may not adequately inactivate such high amount of herbicide. Among the herbicides tested, maize tolerance to metazachlor was more dependent on temperature than the tolerance towards metolachlor or alachlor. The results suggest that in cases where tolerance to certain herbicides are known to be temperature-dependent, maize genotypes should preferably be screened under varying temperatures in an controlled environment. The requirement of specialized research facilities, which this approach necessitates, could be circumvented by using excessive herbicide rates (>1-X amounts) to simulate the role of environmental factors which promote the accumulation of phytotoxic amounts of herbicide at the site of action in the plant system.

Results for maize tolerance to metazachlor suggest that genotype response is genetically controlled. Generally, if tolerant parent lines are crossed, they could produce a tolerant hybrid. Herbicide tolerance has been found to be under genetic control in maize and other crops (Edwards *et al.*, 1976; Faulkner, 1982; Le Court De Billot *et al.* 1990). It appears that there are three genes coding for metazachlor tolerance. These genes are either dominant or recessive. If two genotypes with dominant genes are crossed, a tolerant hybrid will be produced, but a cross of genotypes with recessive genes will produce a susceptible hybrid. It was therefore concluded that metazachlor tolerance in maize could be improved through breeding. Proper identification of genotypes with appropriate genes should be done prior to making crosses.

Metazachlor caused ultrastructural changes in both root and leaf cells of maize seedlings of susceptible genotypes. In tolerant genotypes, these changes only occurred at double the recommended rate. Leaves increased in thickness because of the enlargement of the epidermal cells. Clusters of large and empty parenchyma cells were present in susceptible plants, and the chloroplast content was disoganised. Membranes of chloroplasts were dilated and generally

disintegrated. The grana were also disoriented. In the roots, similar effects on cell and organelle membranes were observed. Root cells of susceptible plants had many vacuoles with disintegrated membranes. Nucleoli and their membranes were also disintegrated. These findings are similar to reported effects for certain other chloroacetamides on susceptible plant species (Mellis *et al.*, 1982; Reinhardt & Nel, 1986).

Research benefits to small scale farmers

Herbicide use in small-scale farming is not very common as compared to large-scale farming. This is largely because herbicides are expensive and require special skills to apply; and special equipment for application is needed. Small-scale farmers generally lack knowledge about the importance of herbicides and therefore do not easily adopt this technology. In addition, small-scale farmers apparently do not have a lack of labour for hand-weeding (Shetty *et al.*, 1977; Akobundu, 1987). However, there is a place for use of herbicides in small-scale farming. It is increasingly becoming economical to use herbicides in small-scale farming because labour is becoming scarce and expensive. In addition, the increasing practice of minimum tillage, due to its various benefits, necessitates the use of herbicides.

To simplify the use of herbicides by small-scale farmers some areas of herbicide research need attention. These could include, *inter alia*, simplification of equipment, e.g., granular formulations and low volume sprayers; development of cheap herbicides and/or associated technology; reduced herbicide dosages; herbicides with high selectivity to specific crops, and herbicide-resistant crops.

The study revealed the need for careful selection of herbicides with high selectivity for specific maize genotypes. This would reduce the risk of crop damage for commercial and small-scale farmers, as well as for seed companies. Increased confidence in the safety of herbicides is likely to promote the use of these valuable tools in small-scale crop production systems. Because the risk of crop injury is reduced, subsistence farmers may adopt herbicide

use. This could in turn enable them to increase their farming outputs because they would divert some of their labour from weeding to other farm operations. This could result in increased food production for the farmer and ultimately his income may increase. In addition, the farmer's children may be able to attend school because there may be enough money to pay school fees and also that there is no dire need for labour to weed the fields. Ultimately, there could be increased national food production that would reduce hunger and poverty, not to mention the ramifications of these afflictions. The general welfare and well-being of many communities would ultimately be improved.

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TOLERANCE OF MAIZE GENOTYPES TO SELECTED HERBICIDES

by

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SUMMARY

Maize genotypes' relative tolerance to selected registered herbicides was studied. Maize inbreds and hybrids were grown in the greenhouse and in the field to establish the existence and extent of differential tolerance to the herbicides. The correspondence of maize plant responses to herbicides in the greenhouse and in the field was assessed. This was done to ascertain the correspondence of herbicide effects in the greenhouse and in the field, in order to determine whether relatively rapid maize screening could be done in the greenhouse, which is both faster and cheaper than the field screening method. The effect of temperature on maize tolerance to certain chloroacetamide herbicides was investigated in growth chambers. The effect of metazachlor on the ultrastructural changes in maize seedlings was studied to understand the effects of this herbicide on plant cell organelles. The possibility of improving metazachlor tolerance in maize by means of directed breeding programmes was also studied.

Maize inbreds and hybrids demonstrated significant differences in their responses to herbicides, both in the greenhouse and in the field. Inbreds were generally more sensitive to herbicides than hybrids. Shoot dry mass reductions from the untreated controls ranged from 0

to 60%. Visual injury symptoms ranged from no injury to severe responses. Among the herbicides used, metazachlor damaged more maize genotypes than any other herbicide. It was also observed that some herbicide applications caused crop injury at an initial stage of crop development but these plants later recovered. This indicates that initial injury by some herbicide/genotype combinations may not necessarily affect crop yield. The study has clearly established that there are varying responses of maize genotypes to herbicides, and therefore, there is need to screen maize for herbicide tolerance so that only appropriate ones are recommended for use in seed and commercial maize production. To ensure that maize screening results have practical applicability, a system of classifying herbicides was established. This system divides herbicide tolerance into three classes, namely: tolerant, moderately tolerant and susceptible. The classification is based on shoot dry mass, a parameter which is relatively easy to measure and comparatively more positively correlated with other growth parameters, such as yield, than other parameters used to assess herbicide effects on maize.

Correspondence between greenhouse effects and those observed in the field were generally good. Shoot dry mass and visual injury rating in the greenhouse could be good predictors of the grain yield responses to herbicides in the field. Correspondence of herbicide effects under varying growing conditions and development stages varied from herbicide to herbicide. This is probably a result of their differences in modes of action. This study ascertained that greenhouse screening could be used to save valuable time and money in cultivar development programmes for tolerance to herbicides, although field screening should be conducted to confirm some of the greenhouse results.

The experiment on the effect of temperature on the tolerance of maize to herbicides confirmed some of the previously reported work that low temperatures reduce maize tolerance to chloroacetamides. It was found that tolerance to alachlor, metazachlor and metolachlor were reduced by low temperatures. Even genotypes that showed good tolerance

at normal temperature were significantly injured at low temperatures. This effect was much more pronounced in the case of metazachlor. These results suggest that maize screening should ideally be done under varying environmental conditions to ensure that appropriate genotypes are recommended for production in areas where specific environmental factors are expected to reduce the crop's ability to inactivate a particular herbicide.

The potential for improving metazachlor tolerance in maize through breeding was investigated. Results suggest that metazachlor tolerance in maize is genetically controlled. There is potential for improving metazachlor tolerance by crossing parent lines with dominant genes for metazachlor tolerance. However, the lines with such genes should be clearly identified prior to making crosses.

Metazachlor caused ultrastructural changes in root and shoot cells of susceptible plants. Root cell vacuoles were abnormally large, empty and more in numbers than in the cells from untreated plant seedlings. The cell nucleus were scattered with disorganised chromatids. The nuclear membranes and plasma membranes were also disorganised in treated plants. In the leaf cells of treated plants the membranes of nuclei, chloroplasts and vacuoles appeared dilated and organelle content was disorganized.

The investigation confirmed differential tolerance of maize genotypes to some important herbicides. Routine screening of genotypes to selected herbicides should reduce the risk of crop injury, and hence, limit the occasional losses incurred by seed producers and commercial farmers, and even agrochemical companies as a result of settling claims that emanate from crop damage. Better knowledge about the safety of cultivar/herbicide combinations will promote confidence in herbicide usage, and possibly will also promote the practice amongst small-scale farmers who for various reasons are loathe to adopt chemical weed control.

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APPENDIX A

Table 1A Analysis of variance of percentage shoot dry mass of maize in the greenhouse-Batch I

	Percent damage in shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	1072.5	16.2	0.0001
Herbicide	9	5650.8	85.2	0.0001
Genotype x Herbicide	81	559.1	8.4	0.0001
Error	200	66.3		
Total	299			
R^2		0.89		

Table 2A Analysis of variance of percentage shoot dry of maize genotypes in the greenhouse-Batch II

	Percent damage in shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	400.2	6.0	0.0001
Herbicide	9	3196.3	48.2	0.0001
Genotype x Herbicide	81	304.8	4.6	0.0001
Error	200	66.2		
Total	299			
R^2		0.81		

Table 3A Analysis of variance of percentage shoot dry mass of maize genotypes in the greenhouse-Batch III

	Percent damage in shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	2594.9	15.2	0.0001
Herbicide	9	18067.5	105.9	0.0001
Genotype x Herbicide	81	398.7	2.3	0.0001
Error	200	170.6		
Total	299			
R^2		0.86		

Table 4A Analysis of variance of percentage shoot dry mass of maize genotypes in the greenhouse-Batch IV

	Percent damage in shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	4077.4	52.6	0.0001
Herbicide	9	5356.8	69.2	0.0001
Genotype x Herbicide	81	455.6	5.9	0.0001
Error	200	77.4		
Total	299			
R^2		0.89		

Table 5A Analysis of variance of percentage root dry mass of maize genotypes in the greenhouse-Batch I

	Percent damage in root dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	2506.3	19.0	0.0001
Herbicide	9	9954.4	75.5	0.0001
Genotype x Herbicide	81	845.5	6.4	0.0001
Error	200	131.9		
Total	299			
R^2		0.87		

Table 6A Analysis of variance of percentage root dry mass of maize genotypes in the greenhouse-Batch II

	Percent damage in root dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	4554.9	28.8	0.0001
Herbicide	9	3394.8	21.5	0.0001
Genotype x Herbicide	81	639.0	4.0	0.0001
Error	200	158.0		
Total	299			
R^2		0.80		

Table 7A Analysis of variance of percentage root dry mass of maize genotypes in the greenhouse-Batch II

	Percent damage in root dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	2662.8	12.8	0.0001
Herbicide	9	10092.1	48.4	0.0001
Genotype x Herbicide	81	493.1	2.4	0.0001
Error	200	208.4		
Total	299			
R^2		0.79		

Table 8A Analysis of variance of percentage root dry mass of maize genotypes in the greenhouse-Batch IV

	Percent damage in root dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	21257.4	37.3	0.0001
Herbicide	9	5881.0	10.3	0.0001
Genotype x Herbicide	81	1217.8	2.1	0.0001
Error	200	569.8		
Total	299			
R^2		0.75		

Table 9A Analysis of variance of rate of emergence of maize genotypes treated with metazachlor, dimethenamid and acetochlor + atrazine/sulcotrione in the field

	Rate of emergence			
Source	DF	MS	F value	PR>F
Genotype	12	85.4	1.0	0.4302
Herbicide	2	383.5	4.6	0.0126
Block	2	49.5	0.6	0.5528
Genotype x Herbicide	24	57.7	0.7	0.8393
Error	76	82.8		
Total	116			
R^2		0.64		

Table 10A Analysis of variance of percentage shoot dry mass of maize genotypes caused by metazachlor, dimethenamid and acetochlor + atrazine/sulcotrione in the field

	Percent damage in shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	12	1899.4	5.3	0.0001
Herbicide	2	7626.9	21.1	0.0001
Block	2	965.1	2.7	0.0755
Genotype x Herbicide	24	816.6	2.3	0.0039
Error	76	361.1		
Total	116			
R^2		0.69		

Table 11A Analysis of variance of days to tasselling of maize treated with metazachlor, dimethenamid and acetochlor + atrazine/sulcotrione in the field

	Days to tasselling			
Source	DF	MS	F value	PR>F
Genotype	12	13.8	2.4	0.0107
Herbicide	2	26.6	4.6	0.0124
Block	2	10.0	1.8	0.1800
Genotype x Herbicide	24	4.9	0.9	0.6513
Error	76	5.7		
Total	116			
R^2		0.64		

Table 12A Analysis of variance of days to silking of maize genotypes treated with metazachlor, dimethenamid and acetochlor + atrazine/sulcotrione in the field

	Days to silking			
Source	DF	MS	F value	PR>F
Genotype	12	24.5	3.5	0.0004
Herbicide	2	41.0	5.9	0.0043
Block	2	6.5	0.9	0.3977
Genotype x Herbicide	24	5.3	0.8	0.7830
Error	76	7.2		
Total	116			
R^2		0.62		

Table 13A Analysis of variance of grain yield of maize genotypes treated with metazachlor, dimethenamid and acetochlor + atrazine/sulcotrione in the field

	Grain yield			
Source	DF	MS	F value	PR>F
Genotype	12	503.2	4.0	0.0001
Herbicide	2	1633.7	13.0	0.0001
Block	2	559.9	4.4	0.0149
Genotype x Herbicide	24	283.2	2.2	0.0041
Error	76	125.9		
Total	116			
R^2		0.65		

TABLE 14A Analysis of variance of growth inhibition (%) caused by herbicides on maize shoot dry mass in the greenhouse

	Shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	9	1684.4	5.1	0.0001
Herbicide	7	1511.4	7.7	0.0001
Genotype x Herbicide	63	510.2	1.6	0.0140
Error	160	327.5		
Total	239			
R^2		0.65		

TABLE 15A Analysis of variance of the effect of herbicides (% reduction from the untreated control) on rate of emergence of maize in the field

	Rate of emergence			
Source	DF	MS	F value	PR>F
Genotype	10	668.4	3.1	0.0020
Herbicide	3	7.1	0.03	0.999
Block	2	1845.6	8.6	0.0004
Genotype x Herbicide	30	31.6	0.2	0.1000
Error	86	214.6		
Total	131			
R^2		0.80		

TABLE 16A Analysis of variance of the effect of three herbicides on maize days to 50 % tasselling in the field

	Days to 50% tasselling			
Source	DF	MS	F value	PR>F
Genotype	10	47.2	6.4	0.0001
Herbicide	3	53.0	7.2	0.0002
Block	2	4.9	0.7	0.5168
Genotype x Herbicide	30	6.4	0.9	0.6521
Error	86	7.3		
Total	131			
R^2		0.69		

TABLE 17A Analysis of variance of the effect of three herbicides on maize days to 50 % silking in the field

	Days to 50% silking			
Source	DF	MS	F value	PR>F
Genotype	10	120.3	14.6	0.0001
Herbicide	3	35.2	4.2	0.0075
Block	2	45.2	5.5	0.0058
Genotype x Herbicide	30	12.6	1.5	0.0682
Error	86	8.2		
Total	131			
R^2	0.71			

TABLE 18A Analysis of variance of the effect of herbicides (% reduction from the untreated control) on maize grain yield in the field

	Grain yield			
Source	DF	MS	F value	PR>F
Genotype	10	206516.0	21.7	0.0001
Herbicide	3	83505.7	8.8	0.0001
Block	2	17496.2	1.8	0.1656
Genotype x Herbicide	30	34036.0	3.6	0.0001
Error	86	9529.3		
Total	131			
R^2		0.80		

Table 19.A Analysis of variance of the effect of metazachlor on maize shoot dry mass exposed to different temperatures

	Shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	3	1345.25	8.55	0.0005
Temperature	2	1243.44	7.91	0.0023
Genotype xTemp	6	205.84	1.31	0.0212
Error	24	157.26		
Total	35			
R^2		0.75		

Table 20A Analysis of variance of the effect of alachlor on maize shoot dry mass exposed to different temperatures

	Shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	1	170.20	1.07	0.0315
Temperature	3	1787.57	11.26	0.0003
Genotype x Temp	3	112.19	0.71	0.5619
Error	16	158.77		
Total	23			
R^2		0.70		

Table 21.A Analysis of variance of effect of metolachlor on maize shoot dry mass exposed to different temperatures

	Shoot dry mass			
Source	DF	MS	F value	PR>F
Genotype	1	26.56	0.25	0.6234
Temperature	3	1011.35	9.55	0.0008
Genotype x Temp	3	710.82	6.71	0.0038
Error	16	105.93		
Total	23			
R^2		0.75		

APPENDIX B

Table 1B Visual herbicide injury symptoms on maize inbreds and hybrids (Experiment I)

	Herbicide							
Genotype	FL/-MET	MET	DIM	ATR	AC+ATR/-SU	ALA	METZ	Mean
Batch I: Inbreds								
P1	1	1	1	1	1	1	8	2
P2	1	1	1	1	1	1	1	1
P3	4	1	1	1	1	1	1	1
P4	1	1	1	1	1	1	4	1
P5	4	1	1	1	1	1	1	1
P6	1	1	1	1	1	1	1	1
P7	1	1	1	1	2	4	4	2
P8	1	1	1	1	1	1	1	1
P9	7	1	1	1	1	1	1	2
P10	1	1	1	1	1	3	8	2
Mean	2	1	1	1	1	2	3	
Batch II:Inbreds								
P11	1	1	1	1	1	1	1	1
P12	1	1	1	1	1	1	1	1
P13	1	1	1	1	1	1	1	1
P14	1	1	1	1	1	1	1	1
P15	1	1	1	1	4	1	4	2
P16	1	1	1	1	1	1	2	1
P17	1	1	1	1	1	1	3	1
P18	1	1	1	1	1	1	1	1
P19	1	1	1	1	1	1	3	1
P20	1	1	1	1	1	1	1	1
Mean	1	1	1	1	1	1	2	

Batch III. Inbreds								
P21	1	1	1	1	1	1	1	1
P22	1	1	1	1	1	1	1	1
P23	1	1	1	1	1	1	3	1
P24	1	1	1	1	1	1	1	1
P25	1	1	1	1	1	1	1	1
P26	1	1	1	1	1	1	1	1
P27	1	1	1	1	1	1	2	1
P28	1	1	1	1	1	1	1	1
P29	1	1	1	1	1	1	1	1
P30	1	1	1	1	1	1	1	1
Mean	1	1	1	1	1	1	1	
Batch IV. Hybrids								
CV1	1	1	1	1	1	1	1	1
CV2	1	1	1	1	1	1	1	1
CV3	1	1	1	1	1	1	1	1
CV4	1	1	1	1	1	1	1	1
CV5	1	1	1	1	1	1	1	1
CV6	1	1	1	1	1	1	1	1
CV7	1	1	1	1	1	1	1	1
CV8	1	1	1	1	1	1	1	1
CV9	1	1	1	1	1	1	1	1
CV10	1	1	1	1	1	1	1	1
Mean	1	1	1	1	1	1	1	

Rating scale used: 1-10; indicating: 1=no effect, 2-3=slight effect, 4-5=medium effect, 6-7=severe effect, 8-9=very severe effect, 10=plants dead.

Table 2B. Growth inhibition (% reduction from the control) of metazachlor, dimethenamid and acetochlor + atrazine/sulcotrione on the rate of maize emergence (ANOVA is in Table 9A) (Experiment I)

Genotype	Herbicide			
	metazachlor	Acetochlor + Atrazine/sulcotrione	Dimethenamid	Mean
CV7	8.2	4.9	4.4	5.8
CV5	4.4	0.5	1.1	2.0
P11	15.7	0	0	5.2
P12	3.4	0.6	4.5	2.8
P56	4.9	1.6	6.6	4.4
P2	0	0	0	0
P1	14.4	1.7	2.2	6.1
P31	0	0	1.8	0.6
P37	6.2	14.7	3.4	8.1
P44	12.4	7.8	2.6	7.6
P7	14.2	2.2	1.6	6.0
P32	5.3	4.7	0	3.3
P38	12.2	0	7.8	6.7
Mean	7.8	2.4	2.8	

$LSD_{T(P=0.05)}$ Herbicide x Maize = ns, Maize = ns, Herbicide = 4.6
SE = 1.5

A zero implies no reduction or an increase above the untreated control

TABLE 3B Effect of herbicides (% reduction from the untreated control) on rate of emergence of maize in the field (ANOVA in Table 15A) (Experiment II)

Genotype	Herbicide				Mean
	Acetochlor	Bendioxide	Flufenacet	Atrazine/metolachlor/terbuthylazine	
CPA	0	0	0	0	0
CPB	2	0	8	3	3
CPC	2	1	6	3	3
CPD	3	2	1	2	2
CPE	2	4	3	1	3
CPF	14	3	13	11	10
CPG	0	0	0	0	0
CPH	11	2	10	8	8
P12	1	0	2	0	0
P7	5	3	0	3	3
Mean	4	2	4	3	

$LSD_{T(P=0.05)}$ Herbicide x Genotype = 9
SE = 8
A zero implies no reduction or an increase above the untreated control