

**FACTORS AFFECTING MAIZE (*Zea mays* L.) SENSITIVITY TO
ACETOCHLOR**

by

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DECLARATION

I hereby declare that the dissertation submitted by me for the qualification Magister Scientiae Agriculturae degree at the University of the Free State is my own independent work and has not previously been submitted by me at another University/faculty for a degree either in its entirety or in part.

I furthermore cede copyright of the dissertation in favour of the University of the Free State.

Signed:.....

Date:.....

DEDICATION

To my parents Mr P.E. & Mrs E. Mphundi who opened the doors to my academic achievement.

To my brothers and sisters for their moral support and always being there for me.

Above all, to almighty God for the grace and mercy which He showered upon me.

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CHAPTER 1

INTRODUCTION

Acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)-acetamide) is a pre-emergence herbicide used to control annual grass weeds such as barnyardgrass, crabgrass and fall panicum, certain broadleaf weeds, as well as yellow nutsedge in maize and soyabean. It is used to control weeds in cabbage, citrus, coffee, green peas, maize, onion, orchards, peanuts, potatoes, soyabean, sugarbeet, sugarcane, sunflower and vineyards (Landi *et al.*, 1994, Vasilakoglou *et al.*, 2001; WSSA, 2002; Department: Agriculture, Forestry and Fisheries, 2007; Hiller *et al.*, 2009).

Acetochlor is classified in the chloroacetamide group of herbicides. This group contains other important herbicides such as alachlor, butachlor, dimethachlor, metazachlor, metolachlor, pretilachlor and propachlor (Rao, 2000; WSSA, 2002). These herbicides share the same molecular core of 2-chloroacetanilide and differ only in the type and arrangement of side branch substitution. These structural differences affect the reactivity of functional groups and ultimately their relative sorptivity. For example acetochlor was found to be adsorbed more than alachlor and propachlor (Wang *et al.*, 1999; Liu *et al.*, 2000; Vasilakoglou *et al.*, 2001; Liu *et al.*, 2002).

Acetochlor is compatible with most other pesticides and liquid fertilizers when used at recommended rates. Usually 10-15 mm of rainfall within 7-10 days of application is required to leach acetochlor into the root zone of the weeds. It is applied prior to crop and weed seedling emergence or post crop emergence. Acetochlor should not be used in areas where soils are very permeable, particularly where the groundwater is shallow or applied to coarse soils with less than 3% organic matter where the groundwater is within 9 m of the soil surface in order to avoid groundwater contamination (Rao, 2000; Monsanto, 2002; WSSA, 2002; Hiller *et al.*, 2009).

During 1994 acetochlor was conditionally registered in the United States to the Acetochlor Registration Partnership (ARP), made up of the Monsanto company, the Agricultural Group and ZENECA, for use on maize with the purpose of reducing the use of alachlor and other maize herbicides by one-third. Acetochlor replaced alachlor and other related compounds due to its better biodegradability and relatively small carcinogenic effect (Deryabina *et al.*, 2005). By 1997 acetochlor had replaced alachlor and was rapidly becoming one of the most widely used herbicides in the United States (Gilliom *et al.*, 2007).

In South Africa, the active ingredient acetochlor is found in 25 commercial herbicides and 9 herbicide mixtures, provided by 12 different companies (Department: Agriculture, Forestry and Fisheries, 2007). Some trade names for products containing acetochlor include Acenit, Guardian, Harness, Relay, Sacemid, Surpass, Top-Hand, Trophy and Wenner. Most products are available as emulsifiable concentrates and 11, registered for pre-emergence use contain a safener to prevent crop damage (Directorate: Food safety and quality assurance, 2004; Department: Agriculture, Forestry and Fisheries, 2007).

Soil, plant and climatic factors are known to affect the efficacy and activity of herbicides (Rao, 2000; Vasilakoglou *et al.*, 2001; El-Nahhal, 2002; Schwab *et al.*, 2006). Soil factors that affect herbicide activity include temperature, moisture content, micro-organisms, pH and organic matter (Parochetti, 1973; Hata & Isozaki, 1980; Sahid & Wei, 1993; Franzen & Zollinger, 1997). High soil moisture content can enhance herbicide activity, while soil organic matter reduces activity by adsorbing the herbicide (Putman & Rice, 1979; Reinhardt & Nel, 1990; Rao, 2000; Vasilakoglou, 2001; Steckel *et al.*, 2003). Soil micro-organisms is one of the factors that affect herbicide degradation (Zimdahl & Clark, 1982; Rao, 2000; WSSA, 2002).

Plant factors such as genetic make-up, age, growth rate, morphology, physiology, and biochemistry also play a very critical role on herbicide activity (Rao, 2000; Bayer, 2002; WSSA, 2002). Different crop cultivars tend to be tolerant or sensitive to certain herbicides due to their genetic make up (Breux, 1987; Allemann, 1993; Kanyomeka, 2002; Bernards, *et al.*, 2006; Breux *et al.*, 2009). The herbicide absorption rate by plants is

influenced by their age, growth rate and morphology. Absorption rate tends to be greater in young growing plants as their growth rate is high (Rao, 2000; Bayer, 2002; WSSA, 2002).

Climatic factors such as temperature and water play a critical role in both plant development and herbicide activity. Temperature influences plant growth and development through effects on the rate of physiological and biochemical reactions (Alessi & Power, 1971; Coelho & Dale, 1980). It also affects herbicide activity as losses due to volatility, solubility, and sorption and desorption can result in a reduction in the amount of herbicide available for uptake by plants (Mulder & Nalewaja, 1978; Rao, 2002; Bayer, 2002).

As soil, plant and climate factors affect activity of herbicides in different ways, there is need for a critical study of these factors to fully understand the role they play in acetochlor bioactivity. A series of experiments were conducted to determine the effects of genotype, temperature, soil texture, planting depth and seed size on acetochlor selectivity towards in maize. Understanding the influence of these factors on maize sensitivity to acetochlor will assist in determining suitable management practices to alleviate any problems caused by the herbicide.

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CHAPTER 2

LITERATURE REVIEW

2.1 Chemical and physical properties of acetochlor

Each herbicide has unique chemical and physical properties and these determine how the herbicide acts when applied in a specific area or to specific plants. Herbicides from the same group may have some similarities in their features but at the same time they have specific properties which differentiate them from the other herbicides within the same group (Liu *et al.*, 2000). The chemical and physical properties of acetochlor are listed below (Tomlin, 2000; WSSA, 2002).

Formulation : Emulsifiable Concentrate

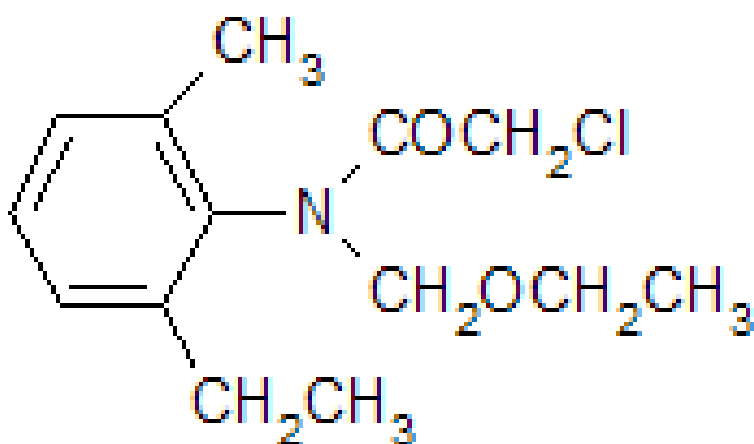
Physical state : Thick, oily liquid, light amber to violet in colour with an aromatic odour

Density : 1.136g ml⁻¹ at 20°C, 1.107g ml⁻¹ at 25°C and 1.1g ml⁻¹ at 30°C

Molecular weight: 269.77

Molecular formula: C₁₄H₂₀ClNO₂

Structural formula:



Melting point : < 0°C

- Boiling point : Unknown
- Vapour pressure : 4.5×10^{-9} kPa (3.4×10^{-8} mm Hg) at 25°C
- Solubility : Water 223 mg L⁻¹ at 25°C
- Organic Solvents at 25°C - Soluble in acetone benzene, carbon tetrachloride, chloroform, ethanol, ether, ethylacetate, and toluene.
- Corrosiveness : Harness and Guardian are slightly corrosive to mild steel
- Leaching index : Has low leaching potential in most soils. Its mobility correlates well with K_d and organic matter content.
- Sorption : Readily adsorbed by soil
- Persistence : Generally provides 8-12 weeks of control, but this may vary depending on soil type and weather condition.
- Compatibility : Compatible with most other pesticides and liquid fertilizers.
- Storage stability : Stable for at least two years under normal warehouse condition
- Acute toxicity : Acute oral LD₅₀ (rat) = 2 148 mg kg⁻¹
- Acute dermal LD₅₀(rabbit) = 4 166 mg kg⁻¹

2.2 Mode of action

Acetochlor is a selective systemic herbicide which is applied to the soil as a pre-emergence treatment (Nemeth-Konda *et al.*, 2002). It can also be used as a post-emergence treatment in order to give extended weed control, being applied after the crop has emerged and before weeds develop beyond the recommended growth stage (Dow AgroSciences, 2002). This herbicide is mainly absorbed by emerging plant shoots, in grass it is absorbed by the coleoptiles and by hypocotyls or epicotyls in broadleaf plants,

although secondary absorption can take place through the seedlings roots (Le Court de Billot & Nel, 1977, Rao, 2000; Peterson *et al.*, 2001; WSSA, 2002). Once plants have gone beyond the seedling stage they will readily absorb acetochlor through the roots, from where it is translocated acropetally to the shoots where it accumulates mainly in the vegetative parts (WSSA, 2002).

The acid amide herbicides (alachlor, metolachlor, propachlor and acetochlor) inhibit the growth of seedlings. Growth inhibitors can be grouped into three sections, *viz.* shoot inhibitors, mitotic poisons, and the shoot and root inhibitors. The amide group of herbicides falls in the latter group, being inhibitors of shoot and root growth (Mosier *et al.*, 1990). Acetochlor is classified in the chloroacetamide subgroup, and this group of herbicides is known to inhibit cell division and enlargement, so inhibiting growth (Ashton & Crafts, 1981).

The chloroacetamides are also reported to inhibit protein synthesis (Ashton & Crafts, 1981), although Matthes *et al.* (1998) reported that these herbicides inhibit the elongation of the very long chain fatty acids (VLCFAs). The precise site of action of acetochlor is not known, but it is currently thought that it inhibits the synthesis of the VLCFAs (WSSA, 2002). The phytotoxic chloroacetanilides have no effect on the formation of long chain fatty acids with up to 18 C-atoms, but the synthesis of fatty acids with 20 – 23 C-atoms are strongly inhibited (Jayesh *et al.*, 2007).

The mechanism of action of the chloroacetamides such as acetochlor has recently been reported to be due to the depletion of the VLCFAs in susceptible plants. These VLCFAs are important constituents of the plasma membrane, and their depletion leads to a loss of cell integrity, eventually leading to death of the plant (Jayesh *et al.*, 2007).

Acetochlor does not inhibit seed germination, but acts on susceptible weed seedlings before they emerge from the soil, causing the majority of susceptible grass and broadleaf weeds to die before they emerge from the soil (WSSA, 2002). The shoots of susceptible monocotyledonous plants treated with acetochlor that do emerge from the soil appear twisted and malformed. The leaves are tightly rolled in the whorl and are unable to unroll normally. In many cases the leaves may emerge from the coleoptiles below the surface of

the soil (Mosier *et al.*, 1990; Rao, 2000; WSSA, 2002). Leaves sometimes do not emerge from the coleoptiles or whorl of the plant resulting in a characteristic “buggy whip appearance” (Mosier *et al.*, 1990; Rao, 2000).

Symptoms of acetochlor toxicity on broadleaf plants include enlarged cotyledons, restricted growth of the leaves and dark green colour (Matthes *et al.*, 1998; Nemeth-Konda *et al.*, 2002). The restricted leaf growth results in slightly cupped or crinkled leaves. Under cold conditions leaf midribs may be shortened, producing a “drawstring” effect at the leaf tip (WSSA, 2002). Roots of susceptible plants treated with acetochlor become shortened, thickened, brittle, and club shaped (Matthes *et al.*, 1998; Nemeth-Konda *et al.*, 2002).

Acetochlor is rapidly metabolized by seedlings of tolerant maize (a grass) and soyabean (a broadleaf) to glutathione (GSH) and homoglutathione (hGSH) conjugates, respectively (WSSA, 2002). Tolerance of species to the chloroacetanilide herbicides appears to be physiological, and depends on rate at which the herbicides are metabolized to GSH and hGSH conjugates (WSSA, 2002; Jayesh *et al.*, 2007), as well as the levels of GSH and glutathione-S-transferases (WSSA, 2002). These glutathione transferases (GSTs) in crops are not only important in detoxifying chloroacetanilide herbicides, but also other major classes of herbicides such as the chloro-s-triazines, sulfoxide derivatives of thiocarbamates, diphenyl ether and several aryloxyphenoxypropionate and sulfonyleurea herbicides (Breaux, 1987; Cummins *et al.*, 1997).

The diverse and abundant GSTs in maize (*Zea mays* L.) have been relatively well characterized at biochemical and molecular level. This GST activity towards herbicides is also present in other cereals such as sorghum (*Sorghum bicolor* L.) and wheat (*Triticum aestivum* L.). The glutathione conjugation mediated by GSTs has a well defined role in the selectivity of chloroacetanilide and chloro-s-triazine herbicides in maize and grass weeds. Sensitive wheat species have also been found to detoxify metolachlor, another chloroacetanilide, by glutathione conjugation (Breaux, 1987; Cummins *et al.*, 1997).

2.3 Factors affecting herbicide activity and selectivity

Herbicide selectivity is a phenomenon where a chemical kills the target plant species in mixed plant populations without harming or only slightly affecting the other plant, while herbicide activity is related to the phytotoxic effect that the herbicide has on plant growth and development. These two concepts are closely related to each other (Rao, 2000). According to Cudney (1996), herbicide selectivity is a dynamic process that involves complex interactions between the plant, the herbicide and the environment. A number of factors, including plant, soil and climatic factors affect both the selectivity and activity of any herbicide. These are discussed in the following sections.

2.3.1 Plant factors

The sensitivity of a given plant species to a specific herbicide can be affected by genetic inheritance, age, growth rate, morphology, physiology and biochemistry. The genetic make-up of a plant determines not only how that plant responds to herbicides but also to its environment (Rao, 2000). Acetochlor is only phytotoxic to emerging seedlings (WSSA, 2002), so the age of plants does not play a role in the sensitivity of plants to this specific herbicide. All of the other factors that influence the sensitivity of the plant to a specific herbicide are affected by the genetic make-up of the plant, so genotype would appear to be the most important factor in determining the tolerance of the plant to a given herbicide

A number of researchers have found differences in sensitivity between varieties and cultivars within the same species of plant to the chloroacetanilide herbicides. Allemann (1993) reported that sunflower cultivars differed in their tolerance to alachlor. Similar results were found by Allemann & Ceronio (2007; 2009). Kanyomeka & Reinhardt (2006) found that maize (*Zea mays* L.) inbred lines and hybrids responded differently to different herbicides, with hybrids being more tolerant to metazachlor than inbred lines. Hirase & Molin (2002) also found that inbred maize was more susceptible to alachlor, probably due to variation in the levels of cysteine synthase between the inbred lines and hybrids. Le Court de Billot & Nel (1977) found that metolachlor was phytotoxic to waxy maize. This difference in maize response to acetochlor has been reported to be occurring in both inbreds and hybrids (Rowe *et al.*, 1990; Rowe & Penner, 1990; Cottingham & Hatzios, 1992).

Bernards *et al.* (2006) found that inbred lines of maize were sensitive to acetochlor, although Landi *et al.*, (1990) found a large variability in tolerance to acetochlor among inbred lines. Tolerance to acetochlor proved to be predominant and hybrids from tolerant lines had a greater tolerance to the herbicide than the corresponding parental lines (Landi *et al.*, 1990). Therefore, it would appear that those cultivars that have at least one tolerant parent line should exhibit tolerance to the herbicide (Bayer, 2002). The inbred lines which are used for the production of hybrid maize seeds respond more sensitively to external environmental effects than the hybrids produced by crossing them (inbred crosses). This greater sensitivity is manifest both in their habits and response to herbicides (Landi *et al.*, 1990).

The age of the plant often determines how well a herbicide works, as younger plants tend to be more sensitive to herbicides than more developed plants. In sunflower it was found that alachlor, applied shortly after sowing did not have phytotoxic effects whereas metolachlor and propachlor applied before transplanting were damaging to lettuce (Scheffer *et al.*, 2002). This effect also depends on the primary site of herbicide absorption as it has been reported that plants beyond seedling stage absorb acetochlor through the roots (WSSA, 2002).

Pre-emergence herbicides such as acetochlor are only active on plants during the germination process and have little effect on older plants. Plants which are growing rapidly are usually more susceptible to herbicides as their biochemical processes such as transpiration, respiration and translocation of herbicides occur rapidly. This facilitates absorption and movement of the herbicide through the plant so enhancing its activity (Rao, 2000; Bayer, 2002).

Plant growth is influenced by the seed size as the seed is regarded as the starting point of plants (Chaudhry & Ullar, 2001). Seed size determines the performance of the seedling that originates from the seed, and ultimately its competitive ability. The larger the seed the more vigorous the seedling when compared to those developing from smaller seeds. This is possibly due to the larger food reserves contained in larger seeds (Smith & Camper, 1974; Singh & Rai, 1988; Bonfil, 1998).

The size of seeds can also play a role in the tolerance of plants to herbicides. Seedlings from smaller seeds of waxy maize have been shown to be more sensitive to metolachlor, possibly due to the length of time that it took them to emerge. It was postulated that the prolonged exposure of the coleoptiles, the main site of chloroacetanilide uptake, of these seedlings to the treated soil resulted in an increase in herbicide absorption and increased phytotoxicity (Le Court de Billot & Nel, 1977). Reduced phytotoxicity in soyabean and kidney bean plants developing from larger seeds has also been observed (Andersen, 1970; Meissner, 1974)

2.3.2 Climatic factors

Climatic factors such as temperature, rainfall, air movement, humidity and radiation influence the processes that affect the herbicide activity (Rao, 2000). Temperature, for example, affects the physical and chemical properties of the herbicide (Koskinen & Harper, 1987), as well as plant processes (Alessi & Power, 1971; Coelho & Dale, 1980). Rainfall on the other hand, is required to leach pre-emergence herbicides such as acetochlor into the soil (Rao, 2000). Excessive rainfall, however, increases acetachlor dissipation and reduces its efficacy (Buhler & Burnside, 1984; Walker, 1987).

2.3.2.1 Temperature

Temperature affects the solubility and vapor pressure of a herbicide, as well as the processes through which herbicides may be lost from the soil (Figure 2.1). High temperatures, for example, can lead to increased herbicide losses through faster chemical and microbial degradation and volatilization (Rao, 2000). Volatilization, however, plays no part in the loss of acetochlor from the soil, and losses due to photodecomposition are negligible (Rao, 2000; Bayer, 2002).

Temperature not only affects the activity of herbicides, but also the degree of control that can be achieved. High temperatures have been found to greatly increase herbicide toxicity, mainly due to an increase in absorption and translocation rate of the herbicide (Kozlowski *et al.*, 1967; Rao, 2000). Crop injury is sometimes increased by extremely high temperatures as the plant is placed under multiple stresses, so making it more susceptible to herbicide injury (Peterson *et al.*, 2001). High temperatures, typical during the hot and dry summer months, can cause some plant species to become dormant. Reduced control of

these species is the result as these dormant plants are unable to absorb the herbicide (Huffman, 2004).

All these outcomes are due to the influence of temperature on the rates of physiological and biochemical reactions that are taking place in the plant (Mulder & Nalewaja, 1978; Cudney, 1996). Temperature plays an important role in the rate of herbicide uptake. High temperatures favor rapid uptake and good weed control generally results when the temperature at the time of herbicide application was high (Mathers, 2006). However, chloroacetanilide herbicides such as acetochlor are not only affected by high temperatures, as dissipation of several herbicides in this group has been shown to be prolonged under reduced temperature conditions (Daniel *et al.*, 2005).

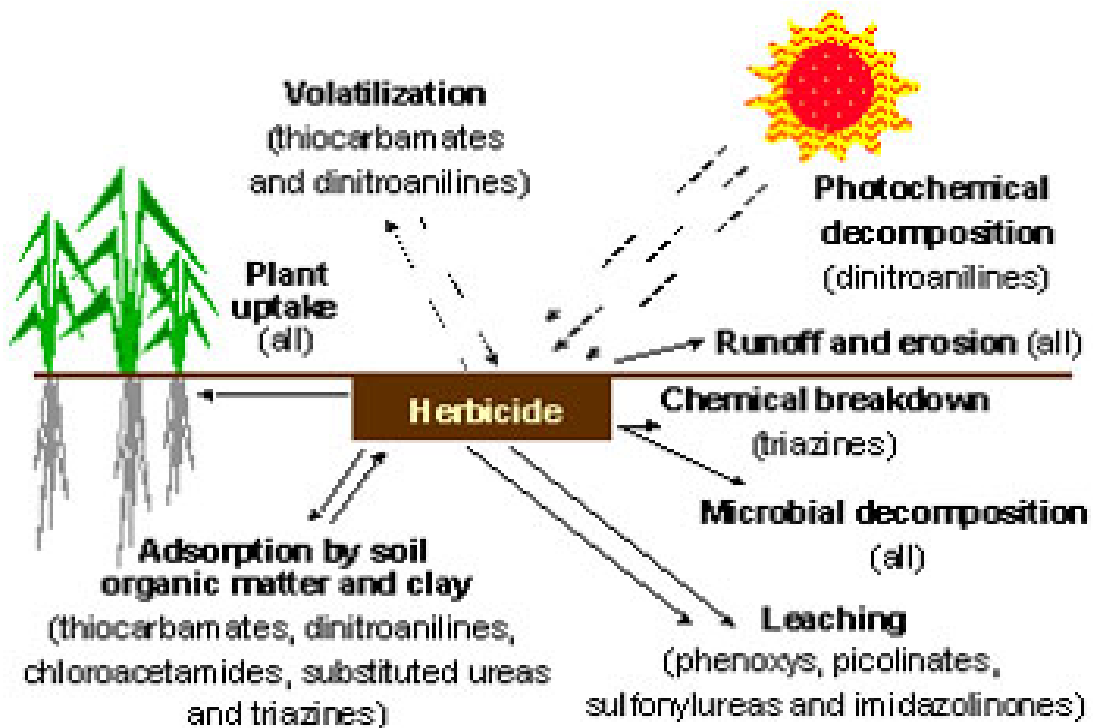


Figure 1.2 Ways in which soil applied herbicides can be lost from the soil (Miller & Westra, 1998).

Temperature also affects the activity of soil applied herbicides through its influence on the rate of seed germination, seedling emergence and growth. Seedlings tend to be more susceptible to soil applied herbicides under cool conditions than under warm temperatures as plant emergence is delayed and metabolism is slowed (Wolfe, 1991; Peterson *et al.*, 2001). The uptake and translocation of most herbicides by both roots and leaves increases with increasing temperature, while low temperatures decrease absorption of water and solutes by roots although species differences occur (Vostral *et al.*, 1970; Lambrev & Goltsev, 1999). Temperature also influences some factors which contribute to herbicide activity, such as, solubility, volatility, sorption and desorption, (Mulder & Nalewaja, 1978; Rao, 2000; Bayer, 2002).

Temperature affects the respiration rate of the plant, being very low at temperatures close to 0°C, and increasing with rising temperature, eventually reaching a maximum at 30°C to 40°C. High temperature may cause injury to plants indirectly by altering the balance between water absorbed by roots and that lost through transpiration (Vostral *et al.*, 1970; Hammerton, 1967; Lambrev & Goltsev, 1999). An increase in temperature from 10 to 30°C has been reported to increase phytotoxicity of triazine in pine (Kozlowski *et al.*, 1967). Similarly Muzik & Mauldin (1964) found that plant sensitivity to 2,4-D was increased at 26°C compared to 10°C and 5°C. On the other hand Le Court de Billot & Nel, (1977) reported that metolochlor phytotoxicity to maize increased with a decrease in temperature. Similar results were reported by Belote & Monaco (1977), Rice & Putman (1980), Viger *et al.* (1991) and Kanyomeka (2002).

2.3.2.2 Rainfall

In order to be effective the herbicide must be present in the zone of the soil profile where the majority of weed seeds germinate. Placement of herbicides within this zone was typically accomplished using mechanical incorporation during the 1970's, but as energy costs increased the majority of farmers began to rely on rainfall or irrigation to move the herbicide into deeper soil layers (Cudney, 1996).

The amount, intensity and frequency of rainfall or irrigation will affect the movement of herbicides to and away from target plants, as well as the ability of the herbicide to go into solution. Under dry conditions, some precipitation is necessary to activate soil applied

herbicides by moving the chemical into the rooting zone, where the herbicide can readily be absorbed and easily translocated throughout the plant (Rao, 2002). Heavy rains immediately after herbicide application can lead to surface runoff, removing some of the applied herbicide, and so decreasing herbicide effectiveness (Koskinen & Harper, 1987).

A 12 mm shower of rain is sufficient to activate most herbicides although this amount varies among soil types and the soil moisture content prior to the rainfall event. A dry soil requires more rain than a wet soil as the initial rainfall must first wet the soil before significant movement of the herbicide occurs. There are relatively small differences among soil applied herbicides in the amount of rain needed to mobilize them within the profile. Hartzler (1997) compared the rainfall requirements of acetochlor, dimethenamid and metolachlor, and found that 6 mm of rain, resulted in an increase in the activity of dimethenamid and acetochlor was increased over that of metolachlor. In order to obtain good weed control at least 10 - 15 mm of continual rainfall or sprinkler irrigation is essential after acetochlor application to leach the product into the soil zone where weed seeds germinate prior to their emergence (Monsanto, 2002).

Leaching is also affected by soil texture, as the coarser the texture of the soil the greater the chance for the herbicide to leach (Rao, 2000). Research carried out by Jordan & Harvey (1980) showed that injury to peas from preemergence applications of acetanilide herbicides was highly dependent on rainfall. Phytotoxicity occurred when rain fell immediately after herbicide application. Moisture from rainfall, thawing cycles and snow may prevent a herbicide from entering the soil in the concentrations necessary to achieve the desired degree of control. Moreover, excessive rainfall may lead to serious herbicide damage to vegetation outside the target area as each soil applied herbicide has a specific requirement of water for it to perform effectively (Huffman, 2004). This concurs with Hartzler (1997) who reported that rapid leaching of herbicides can cause two problems; 1) movement of herbicides out of weed seedlings germination zone, and 2) movement of herbicides into the ground water. As the majority of weed seedlings germinate in the upper layer of the soil, herbicide movement out of this zone can result not only in ineffective weed control, but also in crop damage (Wilson *et al.*, 1990).

Several factors such as water solubility of the herbicide, soil structure and texture, as well as the persistence of herbicide adsorption to soil particles influence leaching of a herbicide. If the herbicide is strongly adsorbed to soil particles, it is less likely to leach, regardless of its solubility, unless the soil particles themselves move with the water flow. Herbicides which are low in solubility are removed from the soil water and become associated with soil colloids and organic matter. While certain herbicides may have low solubility, under certain conditions (such as sandy soils or clay soils with large cracks) they may tend to leach in free-flowing water rather than adsorb to soil particles (Rao, 2000; Hembree, 2004).

Acetochlor has a fairly low leaching potential in most soils, as it is readily adsorbed by the soil (WSSA, 2001). The water solubility of the product is 242 mg L^{-1} , this is similar to that of alachlor which has a solubility of 230 mg L^{-1} . The solubility of propachlor and metolachlor does not vary much, but metazachlor is reported to have the greatest water solubility among all. It has a water solubility of 1200 mg L^{-1} (Ross & Lembi, 1985).

2.3.3 Soil factors

A number of soil factors such as texture, soil pH, soil organic matter, soil moisture, soil microbes and soil temperature affect the herbicide activity in the soil, from all these factors soil organic matter and clay content are the most important soil factors that indirectly influence all the processes affecting herbicide activity (Reinhardt & Nel 1984; Vasilakoglou *et al.*, 2000; Liu *et al.*, 2005). The greater the organic matter and clay content the greater the adsorption of the herbicide to the soil particles resulting in decreased bioactivity (Day *et al.*, 1968; Koskinen & Harper, 1987).

2.3.3.1 Soil texture

Soil texture has a large effect on the activity of all soil applied herbicides. Those herbicides that have residual effect in the soil are more effective in soils with low clay content due to their higher availability to plants. In soils with a high clay percentage, the herbicide molecules adsorb to the clay particles and are not available for uptake by plants. Due to the effect of soil texture on herbicide activity, clay content is used when arriving at the recommended rate of herbicides (Ballard & Santelman, 1973; Blumhorst *et al.*, 1990). Soil texture also affects herbicide loss through leaching and runoff. Lighter soils were

reported to increase losses due to leaching (Rao, 2000), while heavy soils have an influence on run-off. The heavier the soil, the faster the pore spaces get filled and run-off commences (Menalled & Dyer 2005; Hartzler, 1997).

There are three major groups of clay *viz.* swelling type (montmorillonite) and non-swelling types (kaolinite and illite). Herbicides are adsorbed more strongly on the swelling type of clay than on non swelling types, and so tend to be unavailable in the soil for weed control. This results in poor weed control unless the herbicide application rate is increased (Rao, 2000; Monsanto, 2002; Bayer, 2002). Adsorption of herbicides to soil particles occurs through several mechanisms depending on both herbicide and soil characteristics (Rao, 2000).

Different chloroacetanilide herbicides are adsorbed at different rates in the soil depending on the soil and the herbicide structure. In humic acid soils acetochlor was adsorbed more than propachlor and alachlor, this was also found on clay soils (Liu *et al.*, 2000). They concluded that different moieties attached to 2-chloro-acetanilide core and their unique arrangement may influence the binding mechanisms and so the sorptivity of these herbicides.

2.3.3.2 Soil pH

The soil pH affects detoxification of herbicides by affecting the ionic or molecular character of the chemical, the ionic character and the cation exchange capacity (CEC) of the soil colloids, as well as the activity of soil microorganisms (Rao, 2000). Non-ionic herbicides such as the chloroacetanalides do not react with water and do not carry any electrical charge, but they are still affected by soil pH as they are polar in nature.

Differences in the pH of the soil affect its ability to adsorb and retain herbicide molecules, thereby affecting leaching of the herbicide through the soil profile. Different herbicides respond differently to changes in soil pH. For example, adsorption of atrazine increased as the pH is reduced, resulting in reduced bioactivity as less herbicide is available for uptake by plants (Haris & Warren, 1964).

Soil pH had little effect on the activity of acetochlor, (Reinhardt & Nel, 1990), although it has been shown to affect the speed of degradation. Liu *et al.* (2005) reported that the greatest degradation of acetochlor took place under strong alkaline conditions (pH.12) and was lower under acidic conditions (pH < 5). This means that the period of residual activity of acetochlor would be reduced in alkaline soils and enhanced in acidic soils. However, soil pH also affects microbial degradation of the herbicide as it influences the microbial life in the soil. Microbe numbers tend to increase in soils with a neutral pH, resulting in a faster loss of activity in these soils due to greater microbial activity (Rao, 2000). Anything that results in faster growth or activity of microbes in the soil would result in a reduction in activity and persistence of acetochlor as the product is degraded mainly through the activity of microbes, with minor losses due to non-microbial degradation (WSSA, 2002).

2.3.3.3 Soil temperature

The effect of soil temperature on herbicides relates to the rate of chemical degradation through hydrolysis as well as the population and activity of soil microbes (Rao, 2000; Hembree, 2004). Degradation of alachlor, metolachlor and propachlor was found to increase with an increase in temperature to 30°C (Zimdahl & Clark, 1982). Similarly, herbicide degradation was enhanced in non sterile soils compared with sterile soils as soil temperature increased from 15 to 30°C (Jefrey *et al.*, 2003).

Chloroacetamide injury to maize seedlings has been shown to increase through a decrease in soil temperature and an increase in soil moisture. Soil temperature also affects herbicide persistence in the soil. The lower the soil temperature the lower the microbial activity hence the longer the time the herbicide will stay active in the soil (Kulshrestha & Singh, 1992; Gestel *et al.*, 2007).

Lower soil temperatures play an important role in herbicide activity through delaying germination and seedling growth. This results in a delay in plant emergence, and increases the time needed for plants to reach the one leaf stage. Delayed emergence prolongs the exposure of the coleoptiles, the main site of chloroacetanalide herbicide uptake by grasses,

to the herbicide in the soil, so leading to enhanced sensitivity (Boldt & Barrett, 1989, WSSA, 2002).

2.3.3.4 Soil microorganisms

Soil-applied herbicides can be lost through microbial degradation whereby the herbicide is broken down by microorganisms present in the soil. This occurs when microorganisms such as fungi and bacteria use the herbicide molecule as a food source. Conditions that favour microbial growth will result in faster degradation of the herbicide, leading to reduced persistence. Factors favouring microbial growth include warm temperatures, favorable pH levels, adequate soil moisture, oxygen and fertile soils (Rao, 2000).

Zimdahl & Clark (1982) found that degradation of chloroacetanilide herbicides is affected by soil temperature and soil moisture. They also found that degradation rate of both alachlor and propachlor was greater at high temperature and high soil water content. Beestman & Deming (1974) and Vasilakoglou & Eleftherohorinos (2003) reported that microbial degradation is the most important factor affecting the activity and dissipation of the chloroacetanilide herbicides in soil.

Adsorbed herbicides are more slowly degraded because they are less available to some microorganisms (Hembree, 2004; Menalled & Dyer, 2005; Daniel *et al.*, 2005). Degradation of metolachlor took place twice as fast in a clay loam than in a sandy loam soil due to the increase in microbial activity in the clay soil. Under aerobic conditions, degradation increases with increase in soil water content and drops at saturation levels. This would also apply to acetochlor as it belongs to the same group of herbicides as metolachlor (Zimdahl & Clark, 1982). Degradation of acetochlor by soil microbes under aerobic conditions produces the following major metabolites: *N*-ethoxymethyl-2'-ethoxy-6'-methyl-oxanalic acid; [*N*-ethoxymethyl-*N*-(2'-ethoxy-6'-methyl)-2-amino-2-oxoethyl]sulphinylacetic acid; and *N*-ethoxymethyl-(2'-ethoxy-6'-methyl)-2-sulphoacetamide (Rao, 2000; WSSA, 2002).

2.3.3.5 Soil moisture

Herbicide adsorption and phytotoxicity is very dependent on soil moisture, which is important for herbicide movement, particularly the herbicide is moving through mass flow (Rao, 2000). The amount of moisture in the soil affects the amount of the herbicide particles that can be adsorbed by the soil, as these molecules tend to compete with water molecules for absorption sites on mineral colloids. The space available for herbicides to go into solution also decreases as soils dry out, so affecting activity as less free herbicide is present in dry soils. Under dry conditions, plants are therefore less likely to absorb toxic concentrations of herbicide (Rao, 2000; Carolyn, 2007). When soil moisture is replenished, herbicide will desorb from the colloids and re-enter the soil solution.

Herbicides that are readily translocated in the xylem and active in the leaves (photosynthesis and pigment inhibitors) may control established weeds, or injure the crop, shortly after rainfall events due to the release of herbicide into the soil solution from where they can be absorbed by plants (Carolyn, 2007). This concurs with what Green & Obien (1969) suggested, in that herbicide phytotoxicity would increase with increasing soil water content.

Herbicides that are effective on emerging seedlings, such as acetochlor, do not rely on translocation to leaves for their effect. They are, however, still dependent on soil moisture for their activity, as without moisture seeds will not germinate (Wagenvoort, 1981). Rowe & Penner (1990) found that soil moisture content has an effect on chloroacetanilide herbicides activity. They indicated that there was a linear response between crop injury by alachlor and metolachlor and moisture. The injury was found to increase with an increase in soil moisture. Allemann (1993) found that alachlor phytotoxicity to sunflower was increased with a decrease in soil moisture.

2.3.3.6 Soil organic matter

Soil organic matter is one of the most important soil properties which affect herbicide activity (Weber & Peter, 1982; Liu *et al.*, 2002; Rao, 2000). In South Africa, however, this may not be the case as most soils have an organic matter content of <1% (Reinhardt & Nel, 1984; Bayer, 2002). Organic matter has been reported to have a greater adsorption

capacity (Weber & Peter, 1982; Reinhardt & Nel, 1990; Vasilakoglou *et al.*, 2000). Generally, however, the application rate of soil-applied herbicides needs to increase as the organic matter content of the soil increases due to the enhanced adsorption of herbicides by the organic matter.

The organic matter content of the soil will, therefore, also play an important role in determining the mobility of a herbicide in the soil. Soils low in organic matter content that have a high sand fraction have the greatest potential for herbicide leaching (Rao, 2000). The mobility of acetochlor in soils has been shown to be well correlated with the organic matter content of the soils (WSSA, 2001).

Adsorption of the acetanilide herbicides by soils occurred on both organic and clay surfaces and herbicidal activity was affected by the amount of herbicide adsorbed (Weber & Peter, 1982). A number of researchers concluded that soil organic matter was the soil constituent that contributed most to the adsorption of acetanilide herbicides (Weber & Peter, 1982; Reinhardt & Nel, 1990). Weber & Peter (1982) found that acetochlor was adsorbed more than metolachlor and alachlor in the calcium organic matter complex. The activity of acetochlor was reduced with increasing organic matter content and this could be attributed mainly to adsorption differences caused by the increase of organic matter content.

Although the organic matter content of most South African soils is <1%, Reinhardt & Nel (1984; 1989) showed that the organic matter content of the soil is the best predictor of alachlor activity. These results were confirmed by Allemann (1993) who found that an increase in soil organic matter content of 0.12% C was sufficient to negate the bioactivity of four times the recommended application rate of alachlor on sunflower.

Research conducted on the bioactivity of acetochlor in South African soils verified the importance of soil organic matter as a predictor of chloroacetanilide activity, despite the low organic matter content of the soil (Reinhardt & Nel, 1990). Research has determined that the K_d values for acetochlor increased rapidly, from 0.4 to 2.7, with an increase in soil

organic matter content from 0.7% to 3.4%. This correlates well to an increase in the adsorption, and a reduction in mobility, of acetochlor as the organic matter content of the soil increases.

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CHAPTER 3

CULTIVAR SCREENING FOR ACETOCHLOR SENSITIVITY

3.1 Introduction

The recommended application rates for a herbicide are determined in such a way as to be safe for the crops on which they are used, and usually depend on the soil type on which they are to be applied. It does, however, occasionally happen that crop damage occurs in the field when a tried and tested pre-emergence herbicide is applied at recommended application rates that have proven to be safe in the past. The sensitivity of a plant to a given herbicide depends on the amount and rate at which it absorbs the herbicide, as well as its ability to detoxify the specific herbicide (Ashton & Crafts, 1981). Any factor, therefore, that affects the amount of herbicide absorbed would be likely to affect the plants susceptibility to that herbicide (Le Court de Billot & Nel, 1977). Factors such as the type of herbicide, application rate, climatic conditions following application, as well as a number of soil factors can affect the activity of soil-applied herbicides (Rao, 2000).

Acetochlor, (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)-acetamide), is a pre-emergence herbicide used to control annual grass weeds such as barnyardgrass, crabgrass and fall panicum, and certain broadleaf weeds, as well as yellow nutsedge in maize (Landi *et al.*, 1990; WSSA, 2002). This herbicide has been in use since its registration by the Acetochlor Registration Partnership in America during 1994. By 1997 this herbicide had become one of the most widely used herbicides in maize in the USA due to its better biodegradability and relatively small carcinogenic effect (Deryabina *et al.*, 2005; Gilliom *et al.*, 2007).

In South Africa acetochlor formulations containing a safener are registered for pre-emergence use on maize at rates ranging from 490 g ai ha⁻¹ on sand soils (<10% clay) to 1 890 g ai ha⁻¹ on soils with a clay content of between 41 and 55% (Directorate: Food Safety and Quality Assurance, 2004). Despite there being no cultivar restrictions on the use of this herbicide, some apparent herbicide-induced injury on some maize has been

observed in the field, indicating that there might be cultivar differences in sensitivity to the herbicide.

It is a well-known fact that different genotypes do not always react to a herbicide in the same way (Hodgeson *et al.*, 1964). This differential tolerance among cultivars has been reported for various herbicides and in a number of different crops. A number of researchers have reported cultivar response differences in a number of crops to herbicides within the acetamide group of herbicides (Eastin, 1971; Voges & Nel, 1974; Narsaiah & Harvey, 1977; Rowe *et al.*, 1990; Bernards *et al.*, 2006; Allemann & Ceronio, 2007; 2009). Bernards *et al.* (2006) reported that the greatest factor in acetamide injury to crops may be sensitivity of inbred lines or cultivars to the herbicide.

Maize has been reported to exhibit differential response among genotypes for a number of herbicides, such as atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine], trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine], EPTC (*S*-ethyl dipropylcarbamothioate), and imazaquin [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-3-quinolinecarboxylic acid] (Anderson, 1964; Eastin, 1971; Voges & Nel, 1974; Sagara & Foy, 1982; Penner *et al.*, 1988; Roggenbuck & Penner, 1987). This differential response to the acetamide herbicides in maize has also been demonstrated amongst both inbred lines and cultivars to alachlor [2-chloro-2,6-diethyl-*N*-(methoxymethyl)acetanilide] and metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] (Voges & Nel, 1974; Rowe *et al.*, 1990; Rowe & Penner, 1990; Cottingham & Hatzios, 1992).

Breaux *et al.* (2002) rated maize as being highly tolerant to acetochlor, although it appears as though they only worked with a single genotype. Bernards *et al.* (2006), on the other hand, demonstrated differential tolerance to acetochlor in three inbred maize lines. No published information on the tolerance of South African maize cultivars to acetochlor could be found. It was, therefore, decided to screen a number of maize cultivars for their tolerance to this herbicide to see if differential response could be identified as a causal factor for the field observations.

3.2 Material and Methods

The trial was conducted in a temperature controlled glasshouse on the Bloemfontein campus of the University of the Free State. The temperature in the glasshouse was set to a 28°/18°C day/night temperature regime. The trial was conducted under natural daylight conditions, with a daylength of approximately 13 hours. Twenty one commercially available maize cultivars (Table 3.1) used in the central areas of South Africa were screened for their tolerance to acetochlor in a randomized complete block design with four replicates.

Table 3.1 Maize cultivars used in the acetochlor tolerance evaluation

SEED MARKETING COMPANY		
MONSANTO	PANNAR	AGRICOL
CRN 3505	PAN 6Q521R	NK ARMA
DKC 78 - 15B	PAN 6432B	SC 701
DKC 77 - 61B	PFX 428B	BRASCO (4P)
DKC 80 - 30R	PAN 3P – 432B	Q5.7608 (4A/R)
DKC 73 - 76R	PAN 6616	PATHERA (4F)
DKC 62 - 74R	PAN 4P – 516R	QS 7608 (4/F)
	PAN 6D - 256	GROENMIELIE (7P)
		IMPAK 52-11

Polyethylene pots, 150 mm in diameter and 120 mm high, were lined with plastic bags to prevent leaching and herbicide contamination from the sides of the pot. Each pot was filled with 1.5 kg of sandy loam soil ($pH_{KCl} = 4.9$ and 15% Clay). Eight maize seeds were planted at a depth of 25 mm in each pot.

Acetochlor (WENNER* 700 S EC[®]) was applied at five rates, *viz.* 0 (control), 0.74, 1.47, 2.94, and 5.88 kg ai ha⁻¹, being 0, 0.5, 1, 2, and 4 times the recommended application rate for the soil being used. The herbicide was applied to the soil surface the day after planting using the laboratory spraying apparatus described by Allemann & Ceronio (2007). The

system was calibrated for a delivery rate of 200 L ha⁻¹ at a pressure of 2.6 bars. Control pots were sprayed with reverse osmosis (RO) water.

The soil water content at field capacity (drained upper limit) was determined gravimetrically to be 22% (m/m). Prior to herbicide treatment all pots were watered with RO water to within 130 mm of the volume of water required to wet the dry soil to field capacity. Following herbicide application the remaining 130 mm of water (approximating a rain shower of 6 mm) was applied evenly over the surface of each pot in order to leach the herbicide into the soil and bring the soil to field capacity.

Pots were weighed daily and RO water added when necessary to bring the water content of the pot back to 70% of the water available at field capacity. If necessary plants were thinned out two weeks after planting so that three plants remained in each pot. A modified EWRC rating scale (Table 3.2) was used to rate phytotoxic symptoms on the seedlings 21 days after planting.

Table 3.2 Modified EWRC scale to rate herbicide phytotoxicity

Category	Herbicide Damage to Crop
1	No damage
2	No symptoms on lower leaves, Plants stunted
3	Slight symptoms
4	Malformed leaves and chlorosis
5	Severe, whipping of the leaves

Plants were harvested 30 days after treatment, and plant height as well as above ground fresh mass determined. Plants were then dried to constant mass in an oven at 70°C and dry

mass determined. Data were analysed using the SAS Ver. 9.1 for Windows statistical package (SAS Institute, 2003). The data were expressed as a percentage of the control treatments prior to statistical analysis in order to negate inherent growth differences between cultivars. Significant results were analysed using Tukey's Least Significant Difference test, described by Steel & Torrie (1980), at the 5% level of significance to determine statistically significant differences between treatment means, even though the ANOVA may have indicated a higher level of significance.

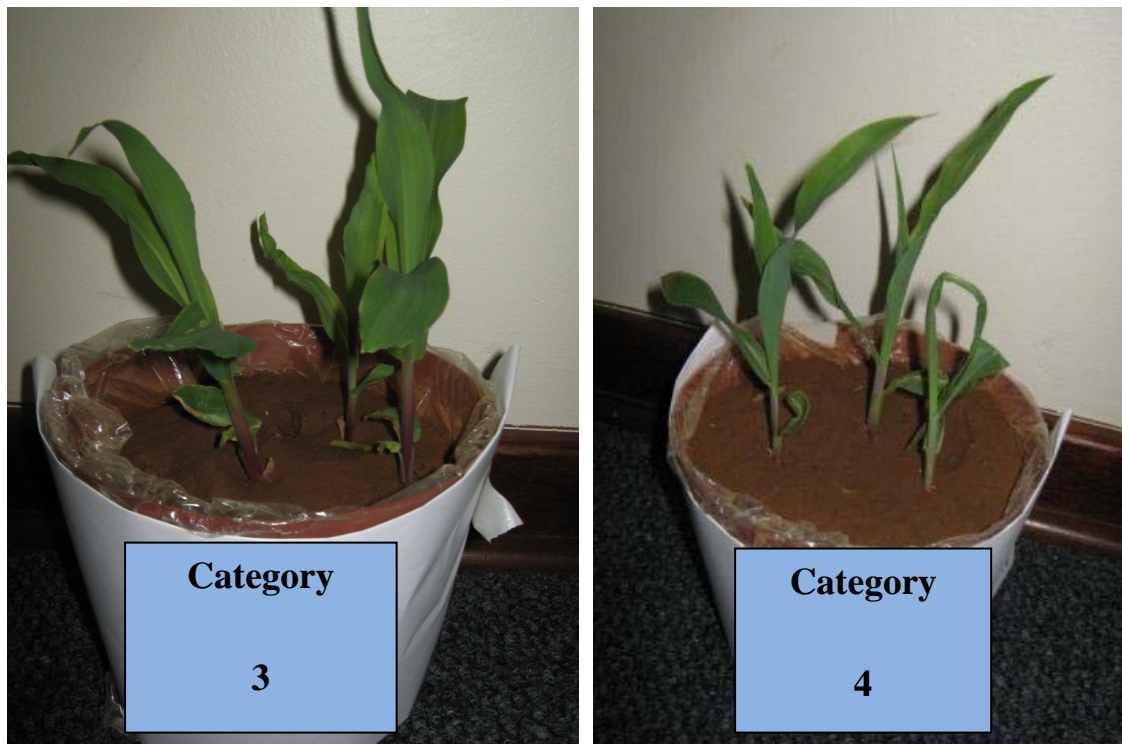
3.3 Results and discussion

Symptoms

Characteristic symptoms of acetochlor phytotoxicity were noted, particularly at the higher application rates of 2.94 and 5.88 kg ai ha⁻¹. These plants appeared shorter and had thicker leaves than those at lower application rates. The leaves did not appear to be able to unroll normally and were tightly rolled in the whorl, giving the appearance of shortened internodes. Seedlings also appeared twisted and malformed, these symptoms being most severe at the highest rate of application. Cupping and twisting of the coleoptile and first leaves were also noted. Seedlings of some cultivars also exhibited a "whip" effect, with the leaf tips appearing to be fused into the tip of the coleoptile, with the leaves emerging along the side of the coleoptile (Figure 3.1). Chlorosis was also noted in seedlings at higher application rates.

These symptoms are consistent with those noted on susceptible monocotyledons by other authors (WSSA, 2002). Hickey & Krueger (1974a;b) also noted leaf distortion and the "whip" effect in both sorghum and maize treated with alachlor, another acetamide herbicide.

At an early stage it was already apparent that the cultivars differed with respect to their sensitivity to this herbicide. Cultivars such as DKC 77-61B, SC 701 and DKC 73-76R showed good symptom development, even at low application rates, and also exhibited poor germination at higher application rates. The latter concurs with the symptomology noted for monocotyledonous species that are susceptible to the acetamide herbicides (Hickey & Krueger, 1974a;b; Ashton & Crafts, 1981; Fuerst, 1987; WSSA, 2002).



A

B

Figure 3.1 Symptoms of acetochlor damage to a sensitive cultivar where the leaf stuck to the coleoptiles (A) and malformed (B)

It was also observed that the coleoptiles of treated plants of susceptible cultivars opened either below, or just above ground level, something that did not occur in the control treatments, which opened several centimeters above the ground. This showed that the plants were being affected by acetochlor following the start of germination, but before emergence. This observation is supported by Hickey & Krueger (1974a), Deal & Hess (1980) and WSSA (2002), who state that the acetamide herbicides are absorbed primarily by the emerging coleoptile in grasses, with secondary absorption taking place via the roots of the seedlings. In this case absorption would take place via both routes due to the limited soil volume available in the pot. This would probably exacerbate the sensitivity of cultivars in pot trials over that being found in the field.

Acetochlor injury was evaluated visually and rated on a scale of 1 – 5 according to the symptoms exhibited by the plants in a pot (Table 3.2). A series of photographs were taken showing plants exhibiting the levels of damage for each of the levels shown in Table 3.2, and these were then used as a standard against which all the plants were evaluated (Figure 3.2). This evaluation proved that the cultivars differed in their reaction to the herbicide, and showed that DKC 73-76R (average value = 4) was far more sensitive than PAN 6Q521R with an average value of 1.5. This data is not presented.

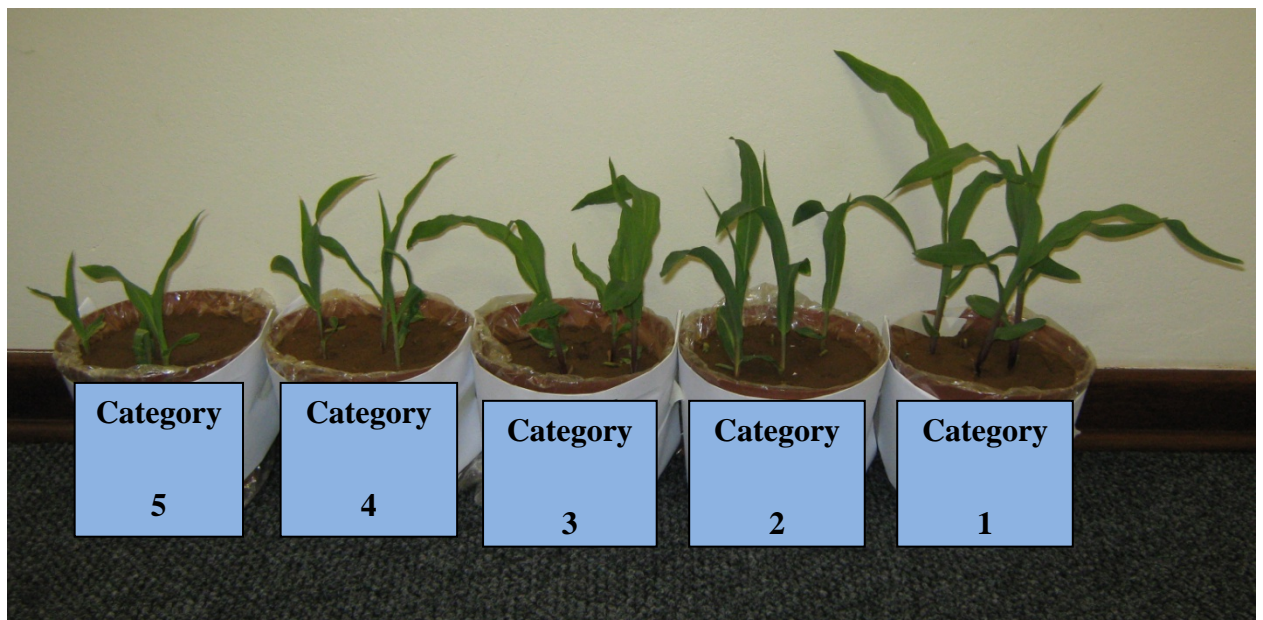


Figure 3.2 Rating scale photograph

Plant height, fresh mass and dry mass of the seedlings all showed good reactions to acetochlor application rate, and as such all appeared to be good indicators of acetochlor activity in maize. However, only plant height and dry mass will be discussed as many other factors, such as differences in time between cutting and weighing, could influence the fresh mass.

Days to emergence

The number of days to the emergence of the first seedling, and days to emergence of all seedlings in the pot were analyzed. In both cases cultivar and acetochlor application rate had a highly significant ($P < 0.0001$) effect (Data not presented).

In all cases the time taken for the seedlings to emerge increased with increasing rate of acetochlor application, with seedlings at both the double and four time recommended rate of application taking significantly longer to emerge than those at the 0, 0.5 and recommended rates of application. No significant differences in the time to emergence were noted between the latter three treatments. However, when looking at the average time that it took all seedlings to emerge in the various treatments a significant decline was noted from the control treatment to the recommended application rate, although this difference was only 0.28 days.

These results are to be expected due to the inhibitory effect of the herbicide on cell division in the shoot tips (Ross & Lembi, 1985). This can be expected to lead to growth inhibition, which would in turn lead to a reduction in the rate of shoot elongation, and so increase the time taken for the seedling to emerge from the soil. An increase in the herbicide concentration could, therefore, be expected to increase the time to emergence due to a more inhibitory effect. This was confirmed by the data obtained during this trial.

Plant height

Both cultivar and acetochlor application rate had a highly significant ($P < 0.01$) effect on this parameter, while the interaction effect was not significant. Despite the fact that effects were significant at the 1% level of significance, treatment means were only analysed at the 5% level of significance. Herbicide application rate had a far greater effect ($P < 0.0001$) than that of cultivar ($P = 0.0037$) on seedling height. The insignificant Cultivar x Acetochlor application rate interaction indicated that all the cultivars reacted to the herbicide in the same way (Table 3.3).

From Table 3.3 it can be seen that the cultivars can be divided into three groups on the basis of their reaction to the acetochlor, with PAN 6Q521R exhibiting the most tolerance

to the herbicide, while PAN 6616, DKC 62-74R and DKC 73-76R are significantly more sensitive. The tolerance of the other 17 cultivars is intermediate to these four cultivars.

It can be seen that the height of seedlings of the three sensitive cultivars is reduced by an average of more than 20% (taken over all application rates) when compared to their respective control treatments. The seedling height reduction of PAN 6Q521R, on the other hand was only reduced by an average of 7.8% over all rates of acetochlor application.

The three sensitive cultivars showed a reduction in plant height of 15.24%, 15.86% and 19.9% for PAN 6616, DKC 62-74R and DKC 73-76R respectively at an acetochlor application rate of 0.5 times the recommended rate, while the corresponding height reduction in PAN 6Q521R was only 3.06%. The differences between these cultivars became more marked as the application rate increased (Table 3.4).

Table 3.3 Effect of cultivar and acetochlor application rate on the height of maize seedlings

Cultivar (C)	Acetochlor application rate (kg ai ha ⁻¹) (A)					Mean
	0.00	0.74	1.47	2.94	5.88	
PAN 6Q521R	100.00	96.94	98.18	80.01	85.88	92.20 ^a
PAN 3P-432B	100.00	89.64	89.75	87.40	77.17	88.79 ^{ab}
PATHERA	100.00	85.03	94.56	81.82	77.53	87.79 ^{ab}
QS 7608	100.00	97.57	80.84	78.95	78.15	86.99 ^{ab}
IMPAK52-11	100.00	95.01	79.02	87.42	72.71	86.93 ^{ab}
GROENMIELIE	100.00	94.50	89.21	80.73	67.81	86.45 ^{ab}
BRASCO	100.00	87.62	93.74	82.49	68.27	86.42 ^{ab}
DKC 80-30R	100.00	87.06	88.43	83.01	69.44	85.59 ^{ab}
PAN 6D-256	100.00	88.42	89.03	78.66	71.74	85.57 ^{ab}
CRN 3505	100.00	87.37	83.96	77.95	75.01	84.86 ^{ab}
PFX 428B	100.00	87.82	88.62	69.52	77.01	84.60 ^{ab}
NK ARMA	100.00	87.86	84.73	78.50	70.67	84.35 ^{ab}
PAN 4P-516R	100.00	83.40	94.42	76.48	66.70	84.20 ^{ab}
DKC 78-15B	100.00	85.93	83.76	73.94	76.57	84.04 ^{ab}
SC 701	100.00	90.92	81.88	78.92	65.02	83.35 ^{ab}
PAN 6432B	100.00	89.55	70.70	76.20	68.19	80.93 ^{ab}
DKC 77-61B	100.00	78.70	79.49	78.39	64.38	80.19 ^{ab}
Q5.7608 (4A/R)	100.00	84.94	81.86	63.60	68.82	79.85 ^{ab}
PAN 6616	100.00	84.76	81.07	69.42	58.39	78.73 ^b
DKC 62-74R	100.00	84.14	73.07	69.89	65.10	78.44 ^b
DKC 73-76R	100.00	80.10	71.67	74.64	58.48	76.98 ^b
Mean	100.00 ^a	87.97 ^b	84.67 ^b	77.50 ^c	70.62 ^d	
LSD _{T(0.05)}	C = 13.27		A = 4.93		C x A = ns	

ns = not significant

Means followed by the same letter in either rows or columns do not differ from each other at the 5% level of significance

Table 3.4 Percentage height reduction caused by increasing acetochlor application rates in a tolerant and three more sensitive maize cultivars.

Cultivar (C)	Acetochlor application rate (kg ai ha ⁻¹)					Mean
	(A)					
	0.00	0.74	1.47	2.94	5.88	
PAN 6Q521R	0.00	3.06	1.82	19.99	12.12	7.80
PAN 6616	0.00	15.24	18.93	30.58	41.61	21.27
DKC 62-74R	0.00	15.86	26.93	30.11	34.90	21.56
DKC 73-76R	0.00	19.90	28.33	25.36	41.52	23.02

From Table 3.4 it can be seen that plant height in the most sensitive cultivar is already reduced by 28.3% at the recommended application rate, while the more tolerant cultivar only shows a 1.8% height reduction at this rate of herbicide application. The difference between the most tolerant and the most sensitive of the cultivars tested are shown in Figure 3.3.

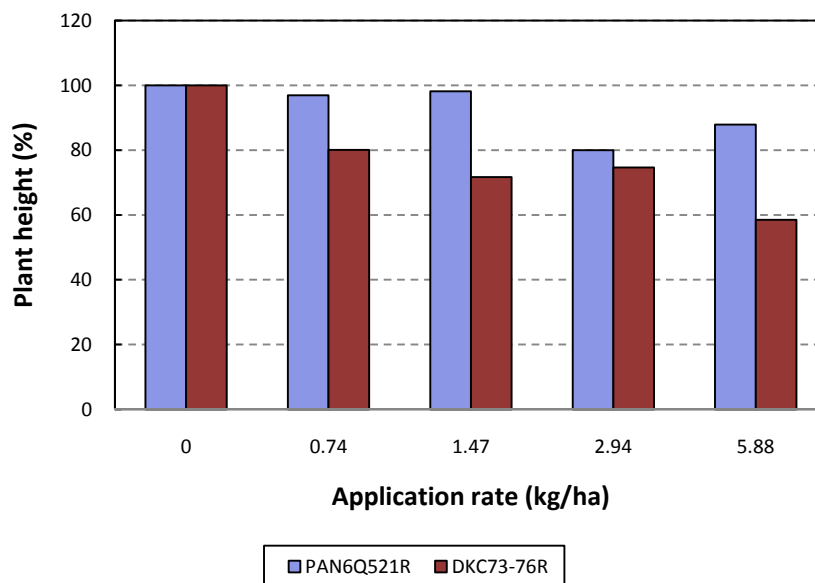


Figure 3.3 Effect of increasing acetochlor rate on height of a tolerant (PAN 6Q521R) and a sensitive (DKC 73-76R) maize cultivar.

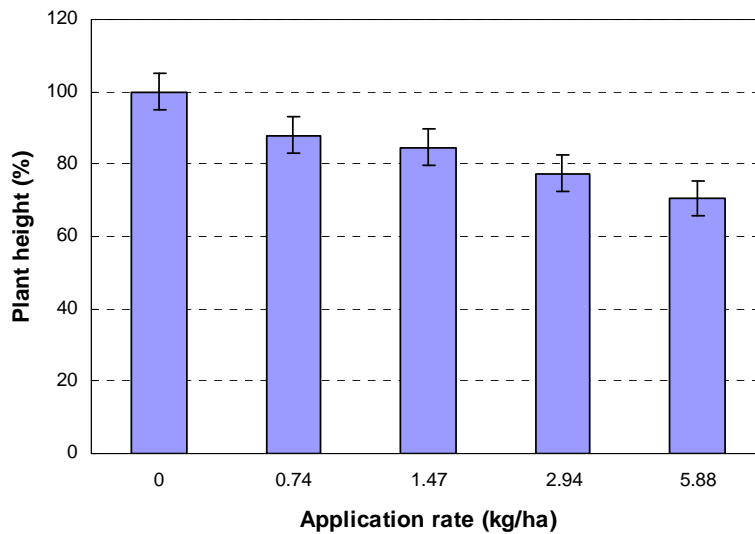


Figure 3.4 Effect of increasing acetochlor application rate on the height of maize seedlings (Bars indicate the $LSD_{T(0.05)}$ value).

From Figure 3.4 it can be seen that an acetochlor application of $0.74 \text{ kg ai ha}^{-1}$ (half the recommended application rate) already resulted in a significant reduction in the height of seedlings when analysed over all maize cultivars tested. Plant height did not suffer a further significant reduction when the rate was increased to $1.47 \text{ kg ai ha}^{-1}$, but it did decrease significantly when the rate was increased to $2.94 \text{ kg ai ha}^{-1}$, and $5.88 \text{ kg ai ha}^{-1}$.

Dry mass

Seedling dry mass was also highly significantly ($P < 0.01$) affected by both cultivar and acetochlor application rate. Once again the effect of herbicide application rate was far greater ($P < 0.0001$) than that of cultivar ($P = 0.0092$). The insignificant Cultivar x Acetochlor application rate interaction indicated that all the cultivars reacted to acetochlor application in the same way (Table 3.5).

From Table 3.5 it can be seen that the cultivars can be divided into three groups on the basis of their reaction to acetochlor application, in the same way as with plant height.

Once again PAN 6Q521R exhibited the most tolerance to the herbicide, actually showing an increase in dry mass when analysed over all rates of acetochlor application, while 10 cultivars showed a significant reduction in dry mass over that shown by PAN 6Q521R. The reaction of the other 10 cultivars did not differ significantly from that of either PAN 6Q521R or the 10 more sensitive cultivars.

The dry mass of the sensitive cultivars was reduced by an average of between 19 and 28% when taken over all application rates of the herbicide. Seedlings of PAN 6Q521R in actual fact increased in mass up to the recommended application rate of $1.47 \text{ kg ai ha}^{-1}$. This phenomenon of an increase in mass at sub-lethal rates of pesticide application is known as hormesis, and has been reported for a number of herbicides (Wiedeman & Appleby, 1972; Allemann, 1993; Cedergreen, 2008). This reaction was also noted when acetochlor was applied at half the recommended application rate ($0.74 \text{ kg ai ha}^{-1}$) in the cultivars PAN 6D-256, BRASCO, DKC 62-74R and PAN 6616.

Differences between the height and dry mass reductions in cultivars with increasing acetochlor applications could be explained by the fact that seedlings treated with the herbicide were shorter and thicker than the control treatments (Figures 3.3 and 3.4).

This concurs with the findings of Allemann & Reinhardt (1995) regarding the effect of alachlor, a related herbicide, on sunflower. The degree of thickening occurring in the seedlings could affect the dry mass of seedlings, so affecting the order of reaction to the herbicide as measured by dry mass. This should be investigated in more detail.

Table 3.5 Effect of cultivar and acetochlor application rate on the dry mass of maize seedlings (% of control)

Cultivar (C)	Acetochlor application rate (kg ai ha ⁻¹) (A)					Mean
	0.00	0.74	1.47	2.94	5.88	
PAN 6Q521R	100.00	140.49	118.09	79.67	97.31	107.11 ^a
QS 7608	100.00	93.02	98.21	83.22	71.49	89.19 ^{ab}
PAN 6D-256	100.00	113.62	92.32	86.59	52.14	88.93 ^{ab}
BRASCO	100.00	104.44	96.75	81.45	54.19	87.37 ^{ab}
DKC 62-74R	100.00	115.29	92.27	72.66	55.62	87.17 ^{ab}
IMPAK 52-11 23	100.00	88.75	93.74	83.85	65.42	86.35 ^{ab}
PAN 6432B	100.00	93.77	81.02	78.16	73.67	85.33 ^{ab}
CRN 3505	100.00	90.28	95.25	76.63	62.53	84.94 ^{ab}
PAN 6616	100.00	105.29	80.56	87.15	46.84	83.97 ^{ab}
PAN 4P-516r	100.00	90.28	84.90	75.56	65.51	83.25 ^{ab}
PFX 428B	100.00	87.31	80.74	66.79	79.10	82.79 ^{ab}
PATHERA	100.00	86.15	63.14	79.80	76.49	81.12 ^b
Q5.7608	100.00	82.07	71.52	76.42	75.05	81.01 ^b
DKC 80-30R	100.00	72.03	88.94	74.50	67.95	80.68 ^b
DKC 78-15B	100.00	82.23	76.90	82.29	60.85	80.45 ^b
NK ARMA	100.00	95.13	75.06	71.02	57.95	79.83 ^b
GROENMIELIE	100.00	79.15	87.20	78.20	49.02	78.71 ^b
PAN 2P-432B	100.00	87.66	66.26	63.61	71.56	77.82 ^b
DKC 73-76R	100.00	89.17	73.92	75.78	46.33	77.04 ^b
DKC 77-61B	100.00	65.49	74.46	66.33	61.41	73.54 ^b
SC 701	100.00	93.06	61.64	59.63	50.30	72.93 ^b
Mean	100.00 ^a	93.08 ^{ab}	83.47 ^{bc}	76.16 ^c	63.84 ^d	
LSD _{T(0.05)}	C = 25.91		A = 9.63		C x A = ns	

ns = not significant

Means followed by the same letter in either rows or columns do not differ from each other at the 5% level of significance

Figure 3.5 shows the dry mass reaction of the two cultivars identified as tolerant and sensitive to the herbicide on the basis of their height reduction with increasing acetochlor application rates. From this figure the hormesis reaction of PAN 6Q521R can be seen, as well as the reduction in mass with increasing acetochlor application rates exhibited by the sensitive cultivar (DKC 73-76R).

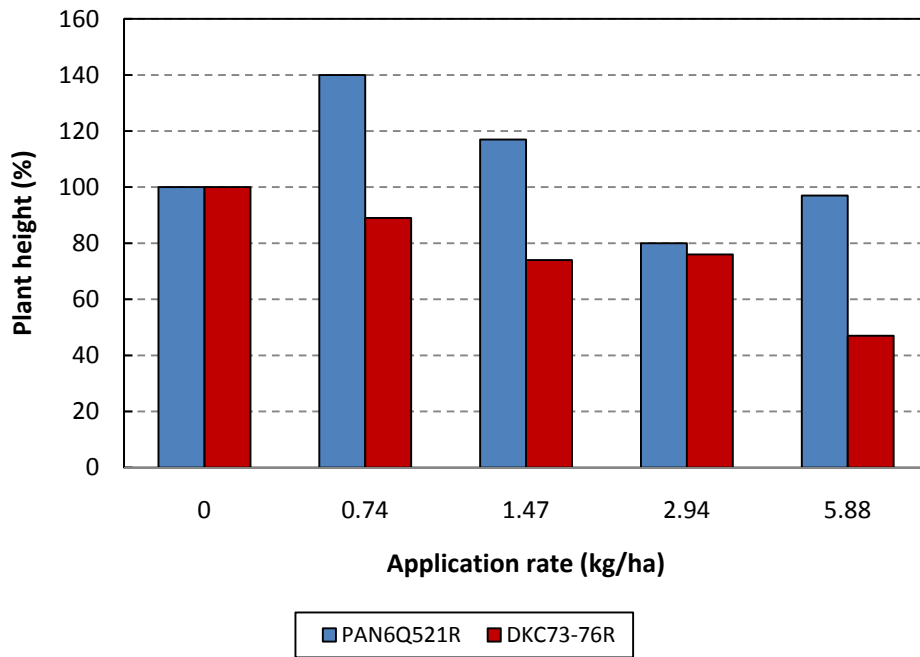


Figure 3.5 Effect of increasing acetochlor application rate on the dry mass of seedlings from a tolerant and a sensitive maize cultivar.

The reaction of the seedlings to increasing acetochlor application rates followed a similar trend to that noted in plant height, with the dry mass decreasing as acetochlor applications increased (Figure 3.6). The dry mass obtained at 0.74 kg ai ha⁻¹ did not differ significantly from the mass at either the 0 or 1.47 kg ai ha⁻¹ application rates. The dry mass of seedlings at the latter rate exhibited a significant reduction over the mass of the control treatment, while not differing significantly from that at double the recommended application rate. However, a significant decrease in seedling dry mass occurred between the two highest application rates. The statistically significant reduction in dry mass between the control and 1.74 kg ha⁻¹ application rates is noteworthy, as this means that acetochlor is having a

significant inhibitory effect on the maize seedlings even at the recommended rate of application.

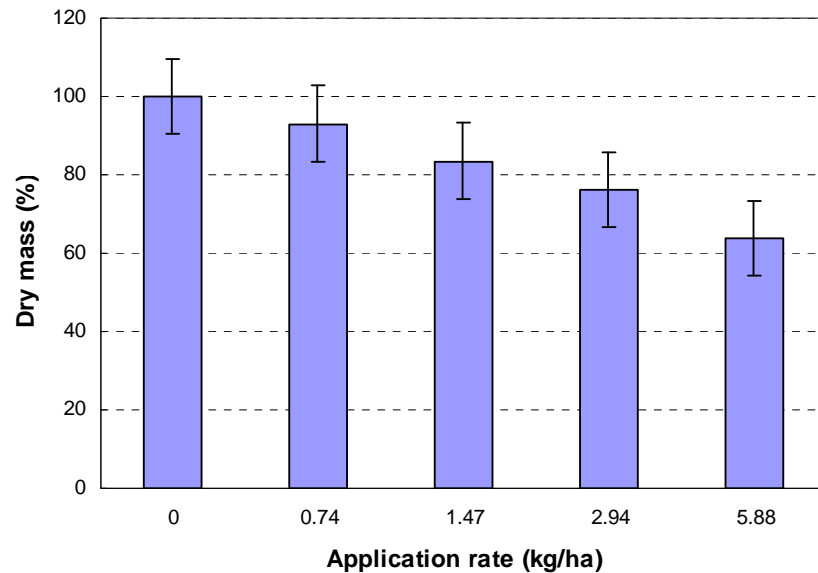


Figure 3.6 Effect of increasing acetochlor rate on the mean dry mass of maize cultivars ($LSD_A=9.63$)

Cultivars were grouped into sensitivity classes using the data from Tables 3.3 and 3.5. In order to be classified as tolerant the height and dry mass reductions should have been <10%, while cultivars were considered sensitive if the percentage height and dry mass reductions exceeded 20% over all acetochlor application rates. All other cultivars were considered to be intermediate in their reaction (Table 3.6).

This type of grouping, showing only a single cultivar in each of the tolerant and sensitive groups, while the rest are classified as intermediate, appears to be in agreement with the findings of Rowe *et al.* (1990), who found that a normal distribution regarding sensitivity of both inbred lines and hybrids to metolachlor existed, with most being moderately tolerant, but some very sensitive and others very tolerant.

Table 3.6 Categories of tested maize cultivars based on their reaction to acetochlor

Tolerant	Intermediate	Sensitive
PAN 6Q521R	PAN 3P-432B*	DKC 73-76R
	PATHERA*	
	QS 7608	
	IMPAK 52-11	
	GROENMIELIE*	
	BRASCO	
	DKC 80-30R*	
	PAN 6D-256	
	CRN 3505	
	PFX 428b	
	NK ARMA*	
	PAN 4P-516R	
	DKC 78-15B*	
	SC 701*	
	PAN 6432B	
	DKC 77-61B*	
	Q5.7608 ^a	
	PAN 6616 ^a	
	DKC 62-74R ^a	

* - Sensitive on dry mass reaction only

^a - Sensitive on height reaction only

The different categories of these maize cultivars may be proof that tolerance to acetochlor in maize is genetically controlled. This is in agreement with the findings of Bernards *et al.* (2006) that the variable response of hybrids to acetochlor was probably due to genetic inheritance. These results may be a true reflection of what may happen under field conditions, a result that was noted by Kanyomeka (2002) on his research on sensitivity of inbreds and hybrids to selected herbicides.

The advantage of pot trials, however, is that they represent the worst case scenario in terms of simulating conditions promoting herbicide damage. This means that if crop damage does not occur under these conditions, it is unlikely to occur under “normal” field conditions, so translating into a low risk of herbicide damage.

3.4 Conclusion

This study found that both plant height and dry mass were good indicators of acetochlor activity to maize seedlings, as both parameters decreased with increasing acetochlor application rate. It may also be concluded that maize cultivars differ with respect to their sensitivity to acetochlor, with PAN 6Q521R being tolerant and DKC 73-76R being sensitive. These findings must, however, be confirmed in the field.

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CHAPTER 4

EFFECT OF PLANTING DEPTH ON ACETOCHLOR ACTIVITY

4.1 Introduction

Planting depth is an important area of maize production management, as it influences plant growth and development. It also affects crop emergence, with deeper planted seeds taking longer to emerge. Not only does planting deeper delay crop emergence, but also reduces emergence percentage. Research has indicated that time to 80% emergence of maize increased by one day for every 50 mm increase in planting depth (Alessi & Power, 1971).

Herbicide selectivity is affected by planting depth. The deeper the crop seed is planted below the upper zone where the herbicide is active, the less the injury (Gaillardon *et al.*, 1988; Rao, 2000; Bayer, 2002). However, an increase in planting depth was also reported to increase herbicide phytotoxicity (Prendeville *et al.*, 1967). This happens as the time to emergence increased with an increase in planting depth, and this has an influence on the amount and rate at which the crop absorbs the herbicide (Ashton & Crafts, 1981).

The effect of planting depth on herbicide phytotoxicity depends on herbicide placement and site of uptake (Prendeville *et al.*, 1967). The site of uptake of chloroacetanilide herbicides has been shown to be important with respect to their activity, although it differs among species. Most broad leaf (Dicotyledonous) plants absorb chloroacetanilides through the hypocotyls and secondarily through the roots (Allemann, 1993; WSSA, 2002), while most monocotyledonous plants, such as maize, absorb these herbicides through the shoot zone or coleoptiles (Narsiah & Harvey, 1977; Pillai *et al.*, 1979; Jordan & Harvey, 1980; WSSA, 2002).

A number of researchers have reported that planting depth has an effect on herbicide phytotoxicity in crops. Pacanoski *et al.* (2007) found that atrazine and a cynazine / atrazine / alachlor mixture caused more serious injury to maize planted at 70 mm than that planted at 40 mm. Butylate injury to maize also increased as planting depth increased, due to prolonged shoot exposure to the herbicide (Wright & Rieck, 1974). Similar results were

found in sugar beet by Wilson *et al.* (1990), where several herbicides injured seedlings as the planting depth increased from 25 to 46 mm. It has been found that metolachlor, a chloroacetanilide herbicide had a greater inhibitory effect on maize as planting depth decreased from 55 mm to 22 mm (Gaillardon *et al.*, 1988). In this experiment, however, the depth of herbicide incorporation was increased from 4 to 16 mm, resulting in increased exposure of the coleoptiles to the herbicide.

Research conducted by Le Court De Billot & Nel (1977) found that the greater the planting depth of maize, the longer the plant took to emerge. As the time to emergence was prolonged, exposure of the shoots to metolachlor, the main site of chloroacetamide uptake in the soil, also increased. Ayeni *et al.* (1997) found similar results with mexican sunflower. Young emerging shoots absorbed higher concentrations of the herbicide as they were pushing through the deeper soil profile. There is a possibility that the energy reserves in the seeds emerging from greater soil depths were depleted more than in the seeds emerging from shallow depths. This may result in an increase in susceptibility in plants that have been planted at a greater depth (Ayeni *et al.*, 1997).

Acetochlor, a chloroacetamide herbicide, is used as a pre-emergence herbicide in maize. It is applied to the soil surface or incorporated into the top layer of the soil. In order for it to move into the soil after being applied on the surface, it requires between 10 and 15 mm of rainfall or irrigation (Monsanto, 2002). The more water applied the further it leaches into the soil.

Several approaches are used by farmers to determine planting depth of maize, one of which is planting deep enough so that the seed is placed in moist soil (Van Zeggelaar, 2004). In South Africa farmers tend to plant seeds deeper due to the lack of soil moisture in upper soil layers. This may have an effect on herbicide phytotoxicity as shoots may be weak due to depletion of food reserves as they have to grow through a deeper soil layer and so become more susceptible to the herbicide. An investigation was therefore conducted in order to establish if planting depth affected maize sensitivity to acetochlor.

4.2 Materials and Methods

The trial was conducted in a temperature controlled glasshouse on the Bloemfontein campus of the University of the Free State. The glasshouse was set to a 28°/18°C day/night temperature regime, and the trial conducted under natural daylight conditions, with a day length of approximately 13 hours. Two maize cultivars, one tolerant (PAN6Q521R) and one sensitive (DKC 73-76R) to acetochlor, were selected according to the results of the cultivar screening trial reported in Chapter 3.

Polyethylene pots, 150 mm in diameter and 120 mm high, were lined with plastic bags to prevent leaching and herbicide contamination from the sides of the pot. Each pot was filled with 1.5 kg of sandy loam soil ($\text{pH}_{\text{KCl}} = 4.9$ and 15% Clay). Six maize seeds were planted in each pot, and planting depths of 15 mm, 25 mm, 30 mm and 45 mm were used. A randomized complete block experimental design was used, with each treatment being replicated four times.

Acetochlor (WENNER* 700 S EC[®]) was applied at five rates, 0 (control), 0.74, 1.47, 2.94, and 5.88 kg ai ha⁻¹, being 0, 0.5, 1, 2, and 4 times the recommended application rate for the soil being used. The herbicide was applied to the soil surface the day after planting as described in Chapter 3. Control pots were sprayed with reverse osmosis (RO) water.

The soil water content at field capacity was determined gravimetrically to be 22% (m/m). Prior to herbicide treatment all pots were watered with RO water to within 130 mm of the volume of water required to wet the dry soil to field capacity. Following herbicide application the remaining 130 mm of water (approximating a rain shower of 6 mm) was applied evenly over the surface of each pot in order to leach the herbicide into the soil and bring the soil to field capacity.

Pots were weighed daily and RO water added when necessary to bring the water content of the pot back to 70% of the water available at field capacity. If necessary, plants were thinned to three plants per pot, two weeks after planting. A modified EWRC rating scale (Table 3.2) was used to rate phytotoxic symptoms on the seedlings 21 days after planting.

Plants were harvested 30 days after treatment, and plant height, as well as above ground fresh mass determined. Plants were then dried to constant mass in an oven at 70°C and dry mass determined. Data were analysed using the SAS Ver. 9.1 for Windows statistical package (SAS Institute, 2003). The data were expressed as a percentage of the control treatments prior to statistical analysis in order to negate inherent growth differences between cultivars. Significant results were analysed using Tukey's Least Significant Difference test, described by Steel & Torrie (1980), at the 5% level of significance to determine statistically significant differences between treatment means, even though the ANOVA may have indicated a higher level of significance.

4.3 Results and discussion

Emergence patterns

The first seedlings started to emerge six days after planting (DAP). Time to emergence was affected by planting depth, with seedlings from the 15 and 25 mm planting depth emerging first, and those from the 45 mm depth only starting to emerge three days later (Figure 4.1). These results are in agreement with research results on both Mexican sunflower and maize, where it was found that the emergence of seedlings was delayed as planting depth increased (Alessi & Power, 1971; Ayeni *et al.*, 1997).

The time taken to emergence was similar for both cultivars used although it was found that the percentage of seedlings emerged from a given depth differed between cultivars. A larger percentage of PAN6Q521R seedlings emerged faster than those of DKC 73-76R. This reaction was true for all planting depth used (Figure 4.1).

Ultimately, it was found that fewer seedlings of the acetochlor sensitive cultivar (DKC73-76R), as a percentage of seeds planted, emerged than those of the acetochlor tolerant cultivar (PAN6Q521R). This effect was exacerbated by planting depth, with the percentage reduction in emergence at the 15 to 30 mm planting depth being far lower than that noted at 45 mm. The emergence percentage of PAN6Q521R at the 45 mm depth was reduced by approximately 10% than the average at the shallower depths, while for DKC 73-76R this reduction was similar at 9%.

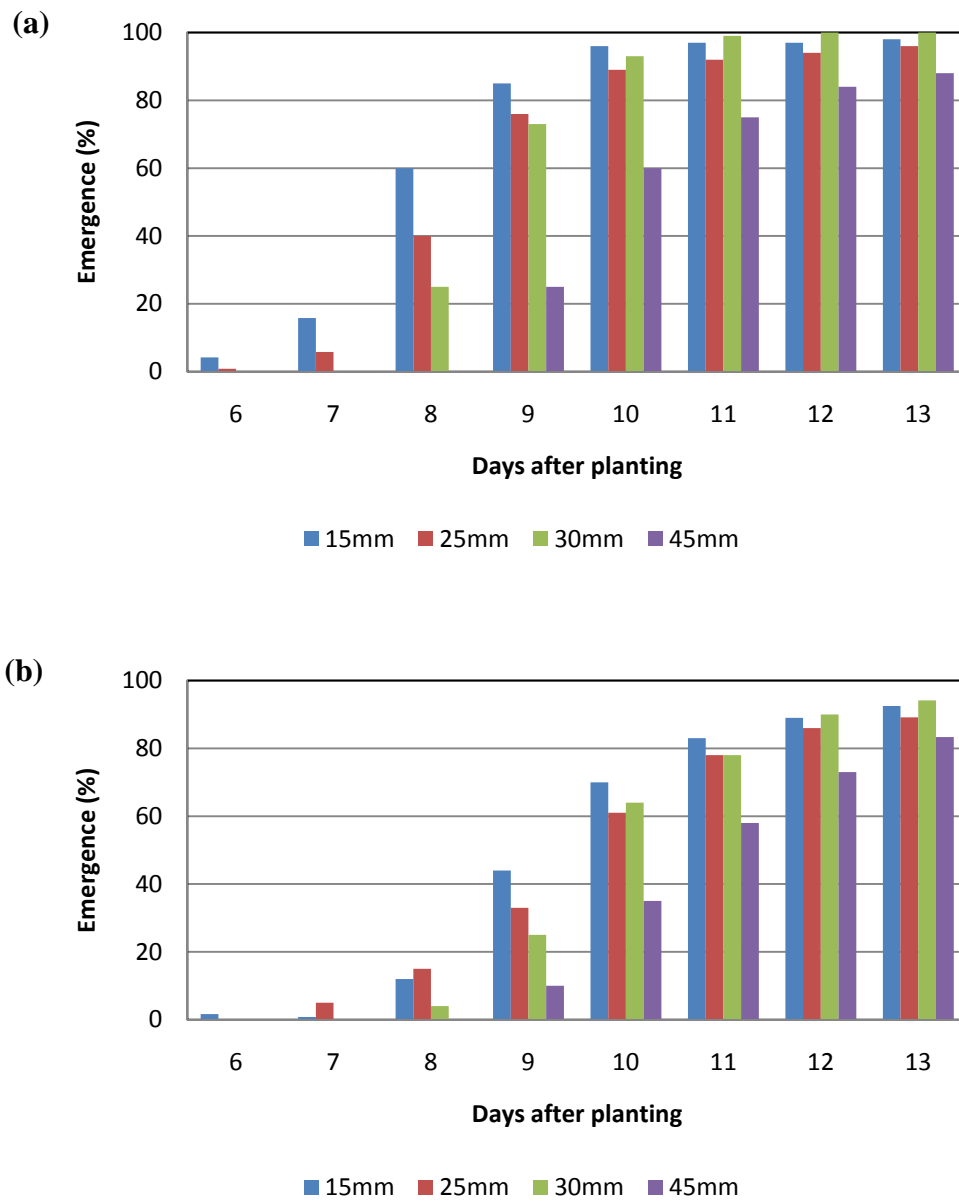


Figure 4.1 Seedling emergence pattern from different planting depth for (a) PAN6Q521R (b) DKC 73-76R.

After analyzing the emergence data statistically, none of the interactions was found to be significant at the 5% significance level. This indicated that both the cultivars reacted the same way to increasing planting depth, although DKC 73-76R did appear to be slower to emerge and gave a lower emergence percentage than PAN6Q521R. As this was over

herbicide application rate, it would appear to confirm that the former cultivar is more sensitive to the herbicide than the latter.

Acetochlor application rate appeared to affect the emergence of the seedlings. At higher application rates (2.94 and 5.88kg ai ha⁻¹) the emergence percentage was significantly reduced when compared to that of the control treatment (Figure 4.2).

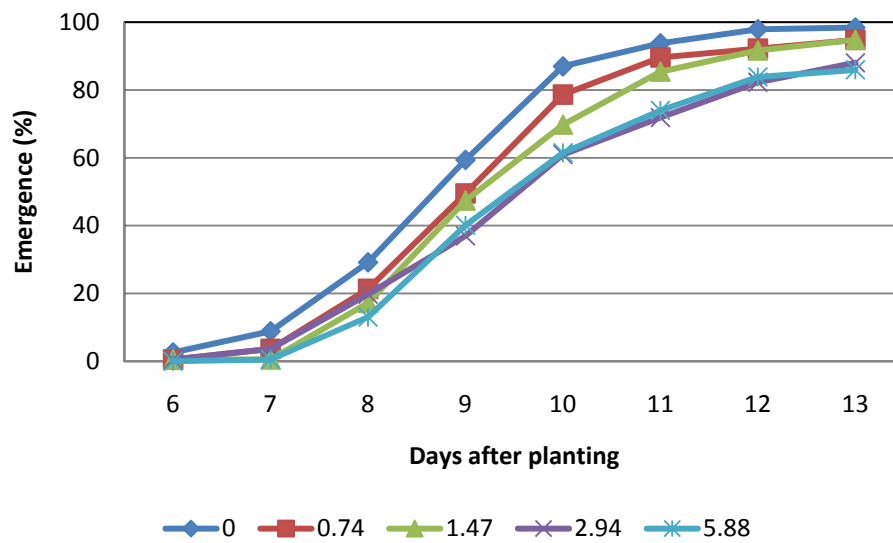


Figure 4.2 Seedling emergence pattern from different acetochlor application rate over cultivar and planting depth.

Plant height

Plant height showed highly significant differences between cultivars ($P < 0.0002$), acetochlor application rate ($P < 0.0001$) and planting depth ($P < 0.0001$). No interactions were significant. The insignificant cultivar x acetochlor application rate and cultivar x planting depth interactions indicated that both maize cultivars reacted in the same way to acetochlor application rate and planting depth.

The results of this trial confirmed the findings of the cultivar sensitivity trial (Chapter 3) that PAN 6Q521R was less sensitive to acetochlor than DKC 73-76R (Table 4.1). The height was found to be affected differently at different planting depths and application rates though the interaction between application rate and planting depth was not significant at the 5% significance level. A significant decrease in plant height occurred at 0.74 kg ai ha⁻¹, although seedling height did not vary significantly from that at 1.47 and 2.94 kg ai ha⁻¹. Plant height at 1.47, 2.94 and 5.88 kg ai ha⁻¹ rates did not vary significantly.

Table 4.1 Effect of acetochlor on plant height expressed as percentage of the control

Cultivar (C)	Acetochlor application rate (kg ai ha ⁻¹) (A)					Mean
	0.00	0.74	1.47	2.94	5.88	
PAN 6Q521R	100.00	82.99	78.89	73.60	73.30	81.76 ^a
DKC 73-76R	100.00	71.24	71.20	71.63	65.58	75.93 ^b
Mean	100.00 ^a	77.12 ^b	75.04 ^{bc}	72.62 ^{bc}	69.44 ^c	
LSD _{T(0.05)}	C = 2.98		A = 6.59		C x A = ns	

ns = not significant

Means followed by the same letter in either rows or columns do not differ from each other at the 5% level of significance.

Plant height decreased as planting depth increased from 15 to 45 mm, although no significant differences in height were noted between plants from 15, 25 and 30 mm planting depth. However, plant height was significantly reduced when seed was planted at 45 mm (Figure 4.3). Although not significant, slight differences between the cultivars were noted, although this decreased with increasing planting depth, from 9.5% at 15 mm to 2.7% at 45 mm (Figure 4.4). This would appear to indicate that the tolerant cultivar becomes more sensitive to the herbicide as planting depth increases. However, this would need to be verified in further experiments.

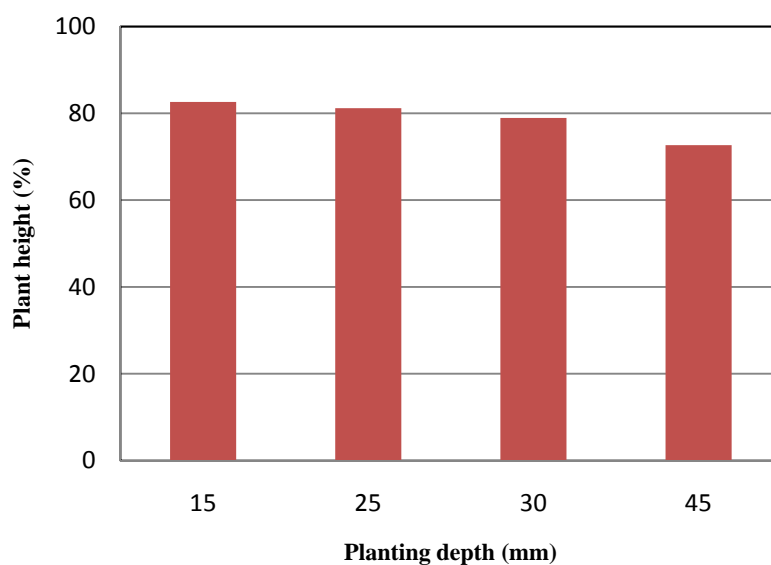


Figure 4.3 Effect of increasing planting depth on mean height of maize seedlings treated with acetochlor ($LSD_{(T0.05)} = 5.54$).

The effect of acetochlor application rate and planting depth is illustrated in Table 4.2. Here it can be seen that the effect of increasing acetochlor concentration was exacerbated by increasing planting depth. Although this interaction was not significant, this could be due to the greatest planting depth of 45 mm not being deep enough to ensure sufficient herbicide absorption. These results are similar to the findings of Ayeni *et al.* (1997) who reported that plant height of mexican sunflower was reduced more as planting depth and herbicide application rate is increased. Similarly, Pacanoski *et al.* (2007) reported that height of maize plants treated with soil applied herbicides was greatly reduced as planting depth increased from 40 to 70 mm.

Coleoptiles of seedlings from the deeper planting depths (30 mm & 45 mm) opened either just below, or at the soil surface, while those from 15 mm opened some centimeters above the ground. According to Le Court De Billot & Nel (1977) this is an indication of acetanilide phytotoxicity.

Table 4.2. Effect of acetochlor application rate and planting depth on the height of maize seedlings (% of control)

Acetochlor application rate (kg ai ha ⁻¹) (A)	Planting depth (mm) (P)				Mean
	15	25	30	45	
0.00	100	100	100	100	100.00 ^a
0.74	83.33	80.22	78.23	67.68	77.12 ^b
1.48	82.09	78.25	70.93	68.91	75.04 ^{bc}
2.98	74.95	71.99	76.24	67.30	72.62 ^{bc}
5.88	73.77	75.41	69.19	59.39	69.44 ^c
Mean	82.63 ^a	81.17 ^a	78.92 ^a	72.65 ^b	
LSD _{T(0.05)}	A = 6.59		P = 5.54	A x P = ns	

ns = not significant

Means followed by the same letter in either rows or columns do not differ from each other at the 5% level of significance.

Using the modified EWRC scale (Table 3.2), plant injury increased as planting depth increased, being more severe when the highest acetochlor rate (5.88 ai kg ha⁻¹) was applied at the greatest planting depth (45 mm). Most of the plants growing from shallow depths and at lower application rate were grouped into rate 2 (data not presented). This is in agreement with Wright & Rieck (1974) who found that butylate injury to maize was increased as planting depth and application rate were increased concurrently. Growing shoots of seedlings emerging from the greater depths took longer to emerge from the soil. Therefore, they were more exposed to the herbicide than those from shallow depth. This led to an increase in herbicide absorption and plant damage, as the growing shoots are the main site of chloroacetanilides uptake (Le Court De Billot & Nel, 1977; Narsaiah & Harvey, 1977; WSSA, 2002).

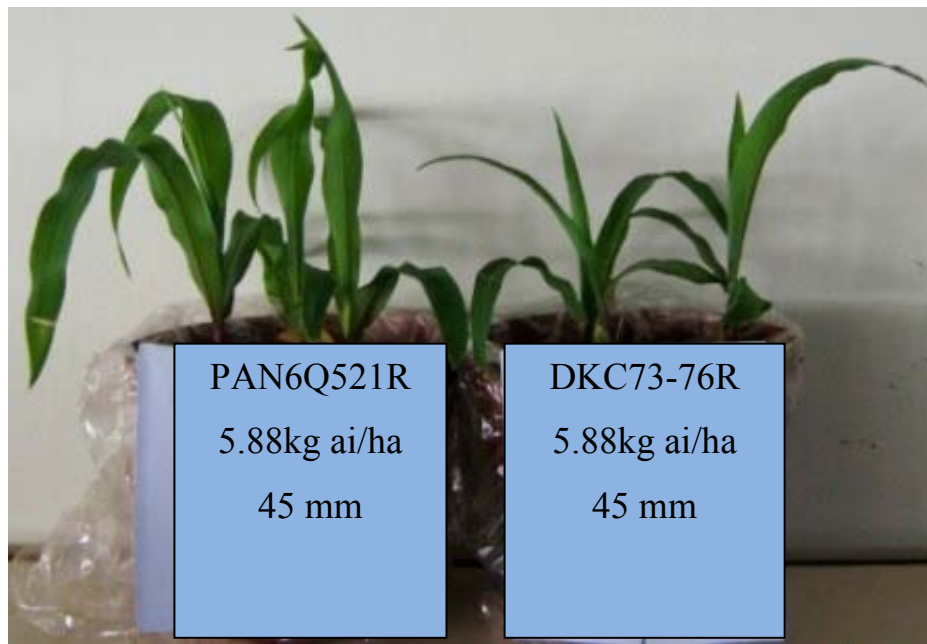


Figure 4.4 Cultivar differences at the highest rate of acetochlor application and the deepest planting depth.

Dry mass

The dry mass of both cultivars (PAN6Q521R and DKC 73-76R) was influenced by both planting depth and acetochlor application rate. They both reacted to these (planting depth and application rates) in the same way. No interactions were significant at 5% significance level, while the effects of cultivar, application rate and planting depth were highly significant. The tolerance of PAN6Q521R and sensitivity of DKC 73-76R to acetochlor were again confirmed.

Plant dry mass decreased as both the planting depth and acetochlor application rate increased, although the interaction (planting depth x application rate) was not significant (Table 4.3). Dry mass of seedlings from 15, 25 and 30 mm planting depths did not vary significantly from each other, while that at 45 mm planting depth was significantly lower than at 15 and 25 mm depth. At 30 mm the reduction in dry mass did not differ significantly from that at either the shallower or deeper planting depths (Table 4.3).

Table 4.3 Effect of acetochlor and planting depth on dry mass (% of control)

Acetochlor application rate (kg ai ha ⁻¹) (A)	Planting depth (mm) (P)				Mean
	15	25	30	45	
0.00	100.00	100.00	100.00	100.00	100.00 ^a
0.74	100.57	88.38	75.83	66.22	82.75 ^b
1.48	76.06	84.77	66.56	63.96	72.83 ^{bc}
2.98	82.01	62.57	59.26	48.99	63.21 ^{cd}
5.88	58.02	60.21	62.53	43.87	56.16 ^d
Mean	83.33 ^a	79.18 ^a	72.84 ^{ab}	64.61 ^b	
LSD _{T(0.05)}	A = 12.52		P = 10.54		A x P = ns

ns = not significant

Means followed by the same letter in either rows or columns do not differ from each other at the 5% level of significance.

Very little difference in dry mass was observed between cultivars at 15 mm depth, where there was less than a 6% difference between DKC 73-76R and PAN6Q521R. As the depth was increased, the difference kept on increasing until it reached a point where it again declined. This occurred at greatest planting depth (45 mm). At this point all the cultivars were equally affected. These results were in accord with findings by Wilson *et al.* (1990) in that planting deeper than 25 mm reduces the risk of phytotoxic effect with preplant herbicides. However, Gaillardon *et al.* (1988) found that the deeper sowing depth tended to position the coleoptiles deeper in the soil so decreasing metolachlor injury to maize. The latter results may be due to different placement of the herbicide as metolachlor incorporation was increased from 4 to 16 mm while the sowing depth was decreased from 55 to 22 mm. This meant that not only does the planting depth influence herbicide phytotoxicity but that the herbicide placement together with planting depth has an influence.

Although the interaction effect between planting depth and acetochlor application rate was not significant at 5% significance level, it did appear as though this interaction may have affected seedling dry mass. This would appear to indicate that the data might become significant if a greater planting depth were included. As both the planting depth and acetochlor rate were increased, the dry mass decreased. At deepest planting depth the percentage reduction in dry mass increased from approximately 24% at the lowest rate of acetochlor application ($0.74 \text{ kg ai ha}^{-1}$) to 56% at the highest rate. This compares with 0% and 42% reduction in the shallow planting depth. This observation concurs with findings by Pacanoski *et al.* (2007) that the most serious injury to maize by atrazine or the cynazine / atrazine / alachlor mixture occurred at 70 mm rather than 40 mm, probably due to leaching and accumulation of the herbicides deeper in the soil. In addition to this, deeper planted seeds took more time to emerge, so leaving the coleoptiles exposed to the herbicide for a longer period and leading to an increase in herbicide absorption.

4.4 Conclusion

The results of this experiment indicate that planting depth does affect the sensitivity of maize to acetochlor. Increasing planting depth to 45 mm increased the sensitivity to the herbicide irrespective of the cultivar used. Given the tendency of farmers to use planting depths of up to 100 mm, this trial should be repeated to study the effect of these greater planting depths.

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CHAPTER 5

EFFECT OF SEED SIZE ON ACETOCHLOR ACTIVITY IN MAIZE

5.1 Introduction

Seed size is one of the most important characters of seed quality of a crop plant. Seed is regarded as the starting point for a new generation of plants, which is influenced by the health and vigor of seed (Chaudhry & Ullah, 2001). The size of seeds partly determines the seedling performance as well as its competitive ability, as seedlings which emerge from larger seeds, grow more vigorously and develop better root systems than those from smaller seeds. This is due to larger seeds containing more food reserves than the smaller seeds (Smith & Camper, 1974; Singh & Rai, 1988; Bonfil, 1998).

Emergence percentage has been reported to be influenced by seed size as well (Singh & Rai, 1988; Chaudhry & Ullah, 2001). Le Court De Billot & Nel (1977) found that emergence of waxy maize seedlings from small seeds was slower than that from large seeds when treated with metolachlor. This resulted in prolonged exposure of the coleoptiles, the main site of chloroacetamide uptake (WSSA, 2002), to the herbicide, leading to increased herbicide absorption which resulted in increased herbicide injury.

Seedlings emerging from small seeds tend to be more susceptible to herbicides since most of the energy reserves are depleted as they are pushing through the soil (Ayeni *et al.*, 1997, Bayer, 2002). The bigger the seed, the more surface it has for water absorption. Larger seedlings produced from larger seeds tend to be more tolerant to herbicides, as they have a smaller absorbing surface relative to the total seedling volume, than the seedlings produced by small seeds (Andersen, 1970; Scott & Phillips, 1971).

Research has shown that seed size plays an important role in the tolerance of crops to herbicide. Atrazine phytotoxicity in soyabean plants developing from large seeds was reduced as there were more reserves in the cotyledon. The seedlings were surviving on food reserves until atrazine levels reached non-lethal levels, unlike those from the smaller seeds where the reserves were depleted before atrazine levels became non-lethal resulting

in plant damage (Anderson, 1970). Inbred maize lines which have smaller seeds, were found to be more susceptible to alachlor and EPTC than maize hybrids (Voges & Nel, 1974). Similar results were reported by Cargill & Santelmann (1970) when they found that peanut plants growing from small seeds were more susceptible to several herbicides than those from larger seeds. As no information on the effect of seed size on sensitivity of maize to acetochlor could be found, a trial was conducted to determine if seed size plays a role in maize sensitivity to acetochlor.

5.2 Materials and Methods

The trial was conducted under natural daylight conditions, with a day length of approximately 13 hours in a temperature controlled glasshouse on the Bloemfontein campus of the University of the Free State. The temperature in the glasshouse was set to a 28°/18°C day/night temperature regime. During this trial the available seed sizes of two cultivars, one tolerant (PAN6Q521R) and one sensitive (DKC 73-76R) to acetochlor selected from the cultivar screening trial (Chapter 3), were planted in a randomized block design with four replicates.

Polyethylene pots, 150 mm in diameter and 120 mm high, were lined with plastic bags to prevent leaching and herbicide contamination from the sides of the pot. Each pot was filled with 1.5 kg of sandy loam soil ($\text{pH}_{\text{KCl}} = 4.9$ and 15% Clay). In this experiment different seed shapes (flat & round) and sizes for each cultivar (PAN 6Q521R & DKC 73 - 76R) were planted. The seeds were weighed in order to determine their sizes (Table 5.1 & Figure 5.1a & b). Most of the flat shaped seeds were found to have a lower 100 seed mass irrespective of the cultivar, possibly due to lower reserves (Singh & Rai, 1988). Five seeds were planted in each pot at a depth of 25 mm.

Acetochlor (WENNER[®] 700 S EC[®]) was applied at five rates, *viz.* 0 (control), 0.74, 1.47, 2.94, and 5.88 kg ai ha⁻¹, being 0, 0.5, 1, 2, and 4 times the recommended application rate for the soil being used. The herbicide was applied to the soil surface the day after planting as described in Chapter 3. Control pots were sprayed with reverse osmosis (RO) water.

The soil water content at field capacity was determined gravimetrically to be 22% (m/m). Prior to herbicide treatment all pots were watered with RO water to within 130 mm of the volume of water required to wet the dry soil to field capacity. Following herbicide application the remaining 130 mm of water (approximating a rain shower of 6 mm) was applied evenly over the surface of each pot in order to leach the herbicide into the soil and bring the soil to field capacity.

Table 5.1 Different seed sizes used in the experiment

Cultivar	Seed size	100 - seed weight (g)	Description
PAN 6Q521R	MR	32.67	Round in shape
	MRS	29.42	Round in shape but smaller than MR
	MF	27.36	Flat in shape
	MFS	24.61	Flat in shape but smaller than MF
	SFS	21.49	Flat in shape and smallest of all the above
DKC 73 - 76R	R23	39.50	Round in shape
	PFL1	38.25	Flat in shape & large in size
	R21	34.14	Round in shape
	R22	34.03	Round in shape
	F12	31.17	Flat in shape
	F11	30.07	Flat in shape & small in size

Pots were weighed daily and RO water added when necessary to bring the water content of the pot back to 70% of the water available at field capacity. If necessary plants were thinned out two weeks after planting so that three plants remained in each pot.

Plants were harvested 30 days after treatment, and plant height as well as above ground fresh mass determined. Plants were then dried to constant mass in an oven at 70°C and dry

mass determined. Data were analyzed using the SAS Ver. 9.1 for Windows statistical package (SAS Institute, 2003). The data were expressed as a percentage of the control treatments prior to statistical analysis in order to negate inherent growth differences between cultivars.

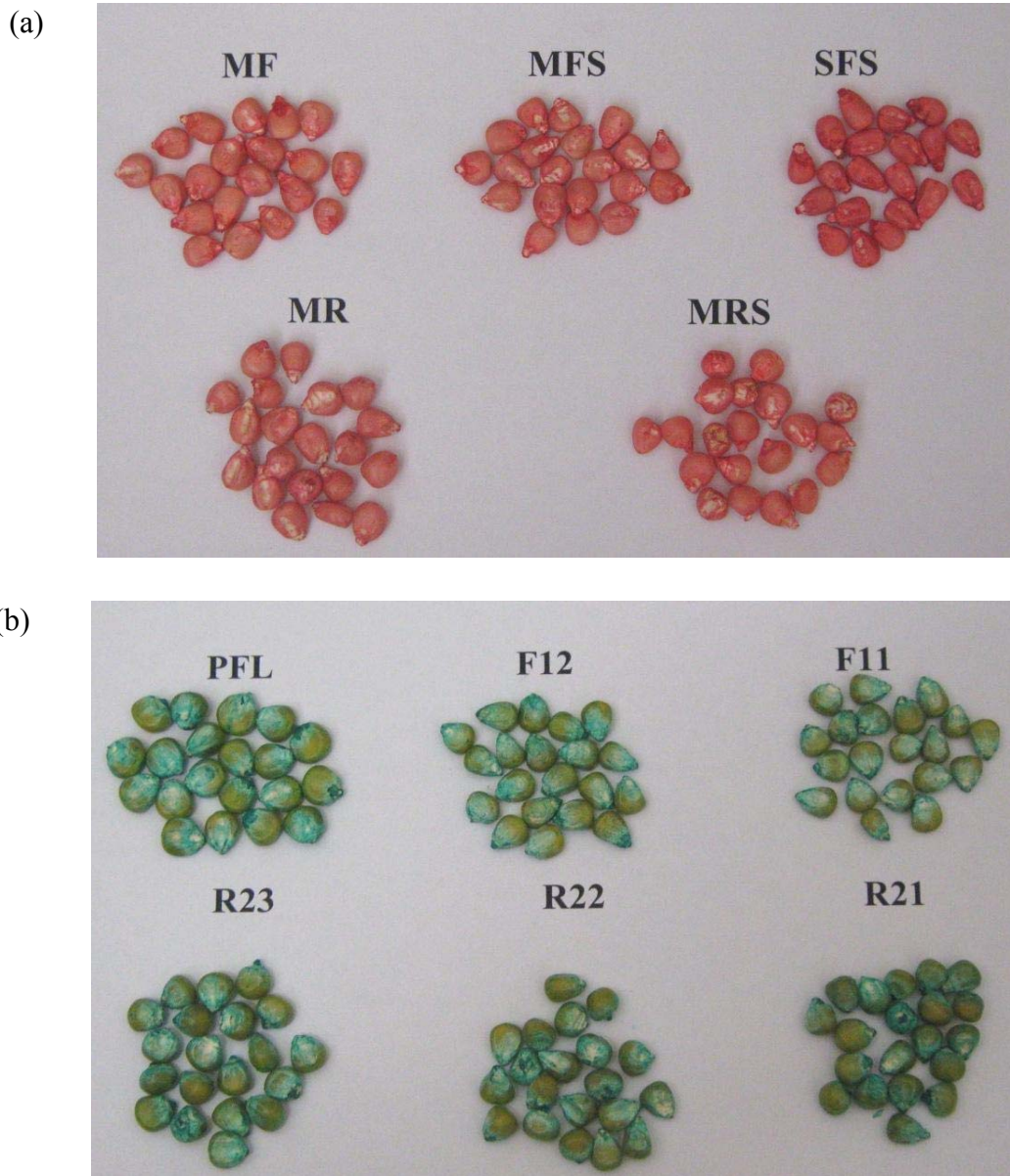


Figure 5.1 Different seed sizes used in the trial (a) PAN 6Q521R and (b) DKC 73 – 76R.

Data from each cultivar was analyzed separately in order to see if seed size had an effect on cultivar susceptibility to acetochlor. Thereafter data were pooled in order to determine

if a distinct pattern of plant reaction to the herbicide due to seed size could be determined. Significant results were analyzed using Tukey's Least Significant Difference test, described by Steel & Torrie (1980), at the 5% level of significance to determine statistically significant differences between treatment means, even though the ANOVA may have indicated a higher level of significance.

5.3 Results

Emergence patterns

Time to emergence appeared to be affected by both seed size and acetochlor rate. Seedlings developing from all five different seed sizes of PAN 6Q521R, started to emerge six days after planting, whereas only seedlings from three seed sizes of DKC 73 - 76R (PFL1, R23 & R22) started to emerge at this time. Emergence rate from larger seeds appeared to be higher than that from smaller seeds. This observation was similar for both cultivars used, although PAN 6Q521R seeds had significantly greater emergence rate than those of DKC 73-76R (Figure 5.2a & b). This finding is similar to those of Alam & Locascio (1965) and Khan *et al.* (2005), who reported an increase in emergence and germination percentage in large size seeds of broccoli, beans and maize respectively.

The effect of acetochlor on seedling emergence was more pronounced as application rate was increased, and was true for all seed sizes irrespective of the cultivar. Emergence percentage per day was reduced. However, this was more obvious in smaller seeds than larger ones (data not presented). This might be due to larger reserves which resulted in fast and vigorous sprouting of seedlings (Alam & Locasio, 1965; Khan *et al.*, 2005; Na Chiangmai *et al.*, 2006). In PAN6Q521R, very small differences between acetochlor rates were observed, but pronounced effects were noticed for DKC 73-76R where all acetochlor rates differed significantly from the control (Figure 5.3).

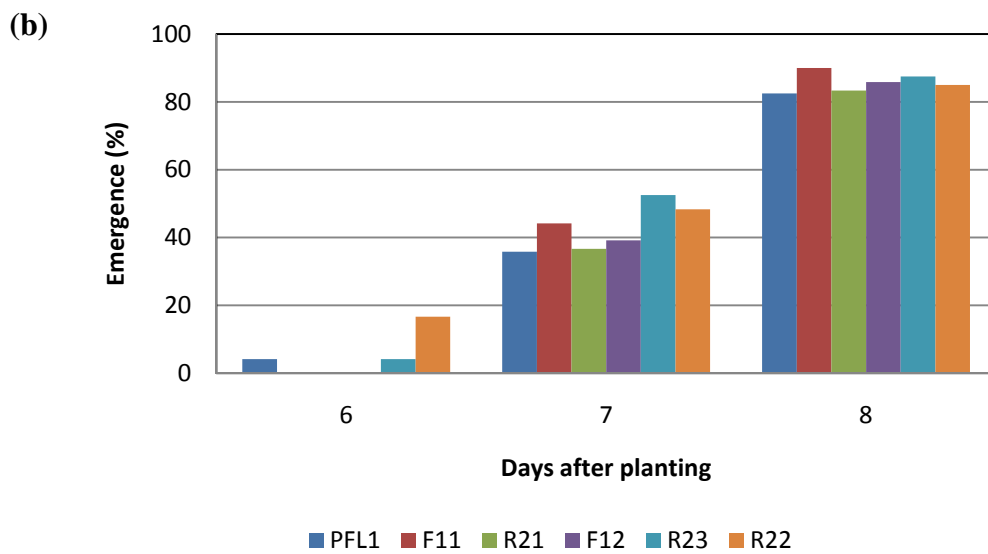
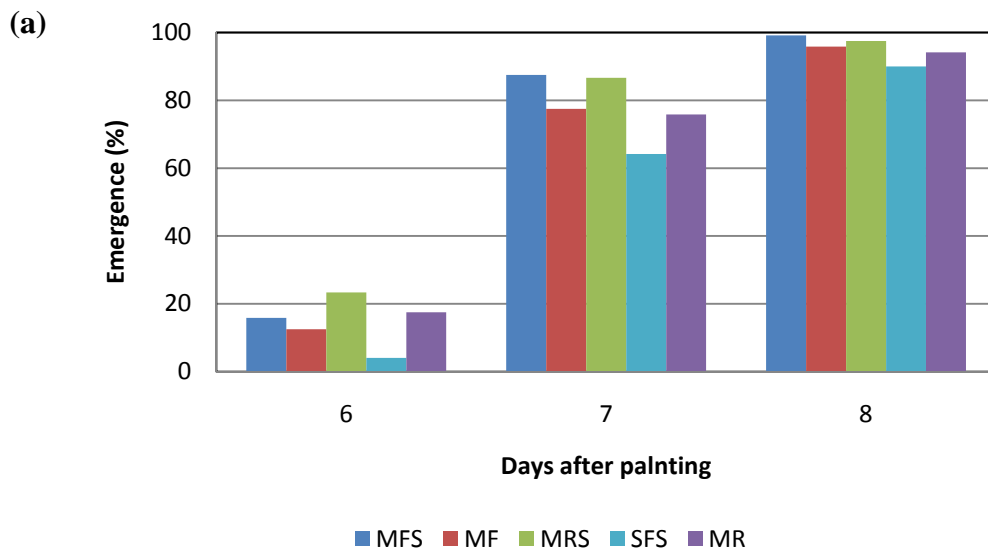


Figure 5.2 Seedling emergence pattern from different seed sizes for (a) PAN6Q521R (b) DKC 73-76R.

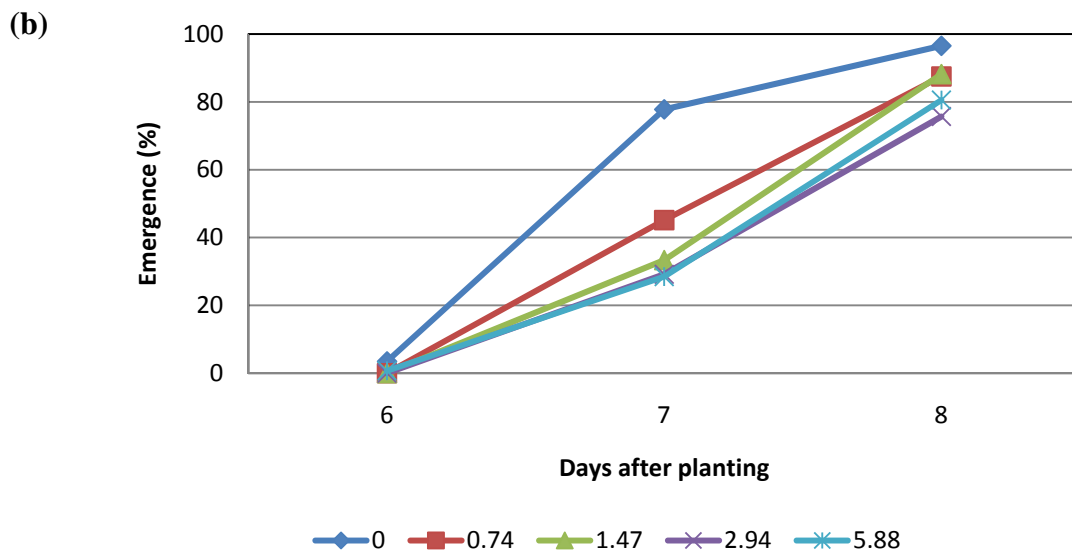
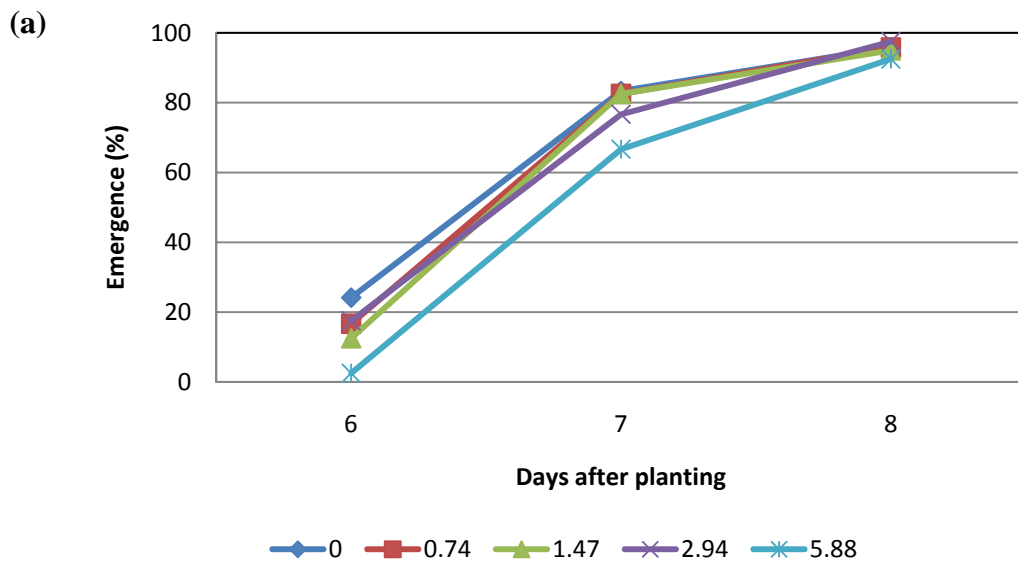


Figure 5.3 Seedling emergence pattern at different acetochlor application rates over seed size (a) PAN 6Q521R and (b) DKC 73 - 76R

Over all seed sizes for both cultivars

When data for seedling height over all seed sizes in both cultivars was analyzed it was found that acetochlor application rate ($P < 0.0001$), seed size ($P = 0.0241$) and the interaction between application rate and seed size ($P = 0.0481$) were significant at the 5% significance level.

Table 5.2 Effect of acetochlor on the seedling height of various seed sizes of DKC 73 - 76R and PAN6Q521R

Seed size (S)	Acetochlor application rate (kg ai ha ⁻¹)					Mean
	0.00	0.74	1.47	2.94	5.88	
PFL1	100.00	99.89	85.08	83.42	92.06	92.09 ^a
F11	100.00	90.51	83.04	76.12	85.61	87.06 ^{ab}
R21	100.00	93.63	88.24	72.38	67.41	84.33 ^{ab}
F12	100.00	71.75	84.98	95.85	66.78	83.87 ^{ab}
R23	100.00	92.80	88.47	74.80	76.79	86.58 ^{ab}
R22	100.00	91.57	102.5	76.65	59.42	86.03 ^{ab}
MFS	100.00	90.21	88.65	63.81	65.74	81.68 ^{ab}
MF	100.00	101.37	91.81	82.55	77.71	90.69 ^a
MRS	100.00	87.48	77.64	74.87	78.93	83.78 ^{ab}
SFS	100.00	70.31	78.03	72.35	66.84	77.51 ^b
MR	100.0	86.56	83.97	78.12	70.07	83.74 ^{ab}
Mean	100.00 ^a	88.74 ^b	86.58 ^b	77.36 ^c	73.40 ^c	
LSD _{T(0.05)}	S = 12.7		A = 7.23		S x A = 30.81	

From Table 5.2 it can be seen that there was no clear picture regarding the effect of seed size as the seedling height from most seed sizes did not differ significantly from each other at different acetochlor rates. However, the seedlings developing from the largest seed (PFL1) were taller than from the smaller seed (SFS). This could, however, have been affected by the difference in tolerance between the cultivars.

Seedling dry mass was affected by acetochlor application rate ($P < 0.0001$), and seed size ($P < 0.0001$), while the interaction (acetochlor application rate x seed size) was not significant at the 5% level.

Although large seeds of each cultivar outperformed the smaller seeds of that cultivar (Figure 5.4), the picture regarding the effect of seed size is still not clear. It can be seen that seedlings from MRS (29.42 g 100⁻¹ seeds) were heavier than those from F11 (30.07 g 100⁻¹ seeds) and R22 (34.03 g 100⁻¹ seeds), even though these seeds were heavier. It appeared therefore as though cultivar differences were playing a role as MRS was from the tolerant PAN6Q521R and the other two (F11 & F12) were both from DKC 73–76R which is sensitive to the herbicide.

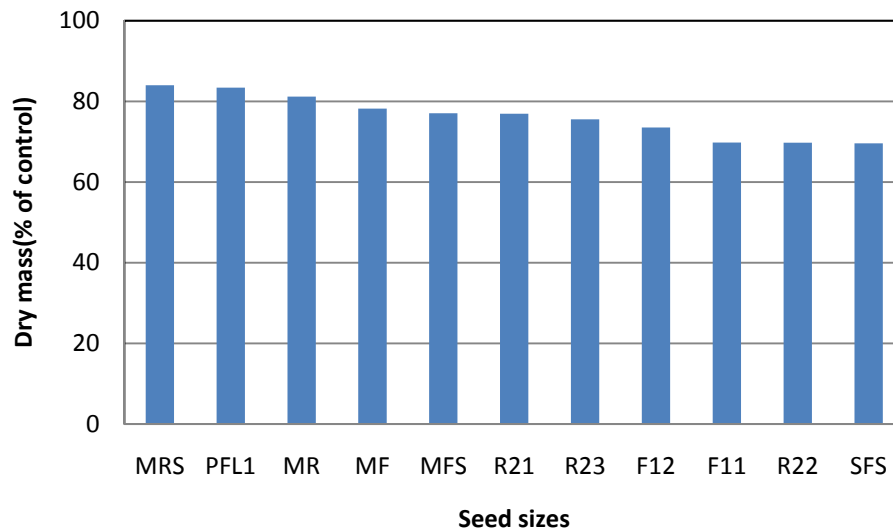


Figure 5.4 Effect of seed size on seedling dry mass of PAN6Q521R and DKC 73-76R treated with acetochlor ($LSD_{T(0.05)} = 12.10$).

It was as a result of this finding decided to separate the two cultivars and redo the statistical analysis in order to see if seed size played a role in acetochlor activity without the added cultivar effect.

Tolerant Cultivar PAN6Q521R

(a) All seed sizes

When data was analyzed over all seed sizes irrespective of their shape, seedling height was significantly affected by seed size ($P = 0.0246$) and acetochlor application rate ($P < 0.0001$). The interaction seed size x acetochlor application rate was not significant at 5% significance level, indicating that all seed sizes responded in the same way at all acetochlor rates.

Acetochlor application rate was found to have an effect on seedling height. There was a significant drop from the control to $0.74 \text{ kg ai ha}^{-1}$ (0.5x) although height at this application rate did not vary significantly from that at $1.47 \text{ kg ai ha}^{-1}$ (1x). It did, however, differ significantly from that at the 2.98 and $5.88 \text{ kg ai ha}^{-1}$ application (Data not shown).

The picture regarding seed size was still not clear as there was one of the larger seed sizes (MR) that did not differ significantly from either the smaller seeds or the larger seeds (Figure 5.5).

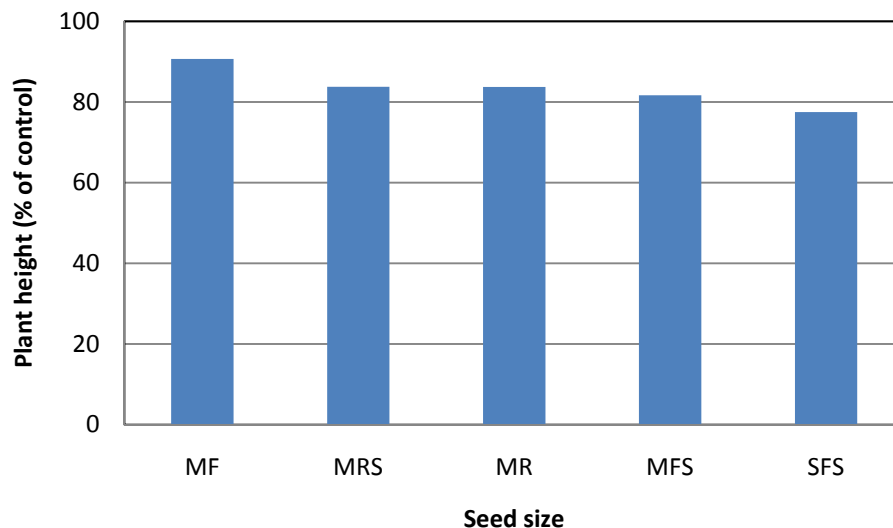


Figure 5.5 Effect of seed size on plant height over acetochlor application of PAN6Q521R ($LSD_{T(0.05)} = 10.94$).

Dry mass was affected by acetochlor application rate ($P < 0.001$) and seed size ($P = 0.0123$). The interaction was not significant at 5% significant level. This indicated that seedling dry mass from all seed sizes were affected in the same way. Dry mass was significantly reduced by acetochlor at $0.74 \text{ kg ai ha}^{-1}$, which mass did not significantly differ from that at $1.47 \text{ kg ai ha}^{-1}$, which in turn did not differ significantly from that at $2.99 \text{ kg ai ha}^{-1}$ (Data not shown).

A picture on the effect of seed size on maize sensitivity started to emerge at this point as seedling mass from the two heavier seeds (MRS & MR) performed significantly better than that from the lightest seed (SFS). However, the larger seeds in this case were both round in shape. A factor analysis could be carried out in order to determine if the seed shape was playing a role. However, this analysis was not possible for this cultivar as there were only two round versus three flat shaped seeds.

Round versus Flat seed

When the round seeds were compared, no differences in seedling height or dry mass were found between the two seed sizes (MR & MRS). However, when the seedlings from flat seeds were compared with each other significant differences due to seed size were found in both seedling height ($P = 0.0068$) and dry mass ($P = 0.0453$).

Seedling height from the heaviest flat seeds (MF $27.36 \text{ g } 100^{-1}$ seeds) was significantly greater than that of seedlings from the lightest seed (SFS $21.49 \text{ g } 100^{-1}$ seeds). Seedlings from the intermediate seed size (MFS $24.61 \text{ g } 100^{-1}$ seeds) did not differ significantly from those from either heavier or lighter seeds (Figure 5.6).

It appeared, therefore, as though seed size could play a role in the activity of acetochlor in PAN 6Q521R. Seedling dry mass from all seed size was reduced significantly at $0.74 \text{ kg ai ha}^{-1}$ (0.5x). Although the F-test indicated that there were significant differences between the dry mass of seedlings from various seed sizes, when the data was analyzed using the Tukey test no difference emerged. This shows that the data was tending towards

significance. However, looking at the data it showed the same trend as that seen for seedling height.

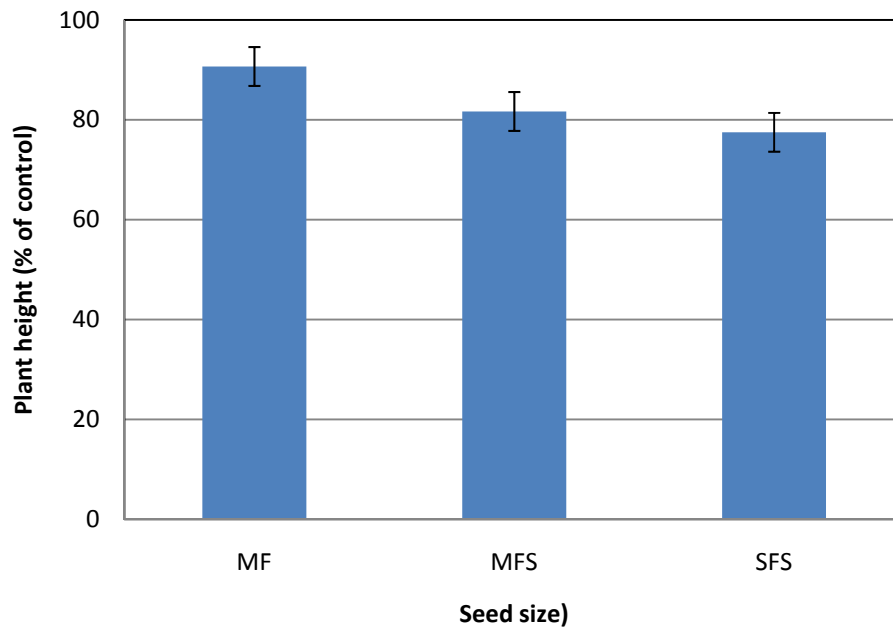


Figure 5.6 Effect of seed size on seedling height in PAN6Q521R ($LSD_{T(0.05)} = 9.76$).

Sensitive Cultivar DKC 73 -76R

(a) All seed sizes

Data for seedling height from all seed sizes showed a significant effect due to acetochlor application rate ($P < 0.0001$), while seed size and the interaction were not significant at the 5% level. There was a significant decrease in height at the $0.74 \text{ kg ai ha}^{-1}$ application rate, although it did not vary significantly from that at the $1.47 \text{ kg ai ha}^{-1}$ rate (data not shown).

When the data for dry mass was analyzed it was found that results for acetochlor application rate ($P < 0.0001$) and seed size ($P = 0.0008$) were significant, whereas their interaction was not significant at the 5% significance level.

The picture regarding the effect of seed size on acetochlor activity in this cultivar was also not 100% clear as it is again being troubled by the different seed shapes. This was demonstrated by the fact that dry mass of seedlings from R22 ($34.04 \text{ g } 100^{-1}$ seeds) a fairly

large seed was lower than those from F11 (30.07 g 100⁻¹ seeds) the smallest of all seed tested (Figure 5.7). Again the analysis was split in order to determine the effect of seed size within various seed shapes.

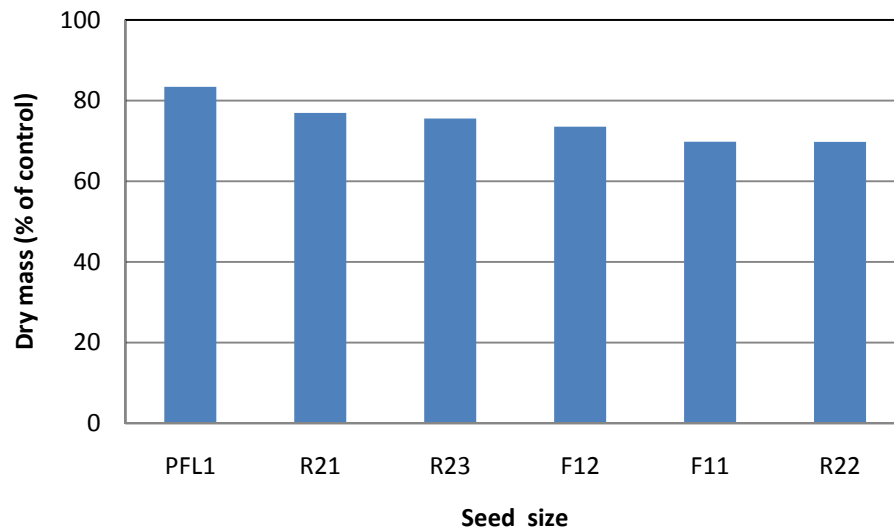


Figure 5.7 Effect of seed size of DKC 73 – 76R on seedling dry mass over acetochlor rates (% of control) ($LSD_{T(0.05)} = 9.79$).

Round versus Flat seed

Seedling height from round seeds was found to be influenced significantly only by acetochlor application rate ($P < 0.0001$). The seed size and the application rate x seed size interaction were not significant at 5% significance level. This indicated that seedlings from all round seeds were affected in the same way. The seedling height at the control treatment was significantly greater than that at the rest of the application rates (data not shown). At 0.74 kg ai ha⁻¹ there was already reduction in height though not significantly different from the recommended rate of 1.47 kg ai ha⁻¹. This did not differ significantly from that from 2.98 kg ai ha⁻¹. Seedling height at 5.88 kg ai ha⁻¹ was significantly different from all the other acetochlor rates. These results concur with those from Chapter 3, where an increase in acetochlor application rate resulted in a decrease in seedling height.

The dry mass of seedlings from the round seeds was only significantly affected by acetochlor application rate ($P < 0.0001$) at the 5% level of significance, while seed size and interaction between seed size and acetochlor application rate were not significant.

As acetochlor rate was increased the dry mass was reduced (data not shown). The control varied significantly with the rest of the acetochlor rates, although dry mass from the 0.74, 1.47 and 2.98 kg ai ha⁻¹ treatments did not differ significantly from each other.

When the data for seedling height from the flat seeds were analyzed, it was found that the seed size did not have a significant effect while acetochlor application rate ($P = 0.0037$), and the interaction between application rate and seed size was significant ($P = 0.0122$). This indicated that seedlings growing from different sized seeds responded differently at different acetochlor rates.

The plants growing from PFL1 (largest flat seeds) were taller than those growing from F11 and F12 at almost all acetochlor rates (Table 5.3), whereas seedlings from F12 at highest acetochlor rate (5.88 kg ai ha⁻¹) were significantly smaller than those from the control, 0.74, 1.47 and 2.94 kg ai ha⁻¹. On the other hand they did not vary significantly with seedlings from PFL1 and F11 when similar application rate of acetochlor (5.88 kg ai ha⁻¹) was applied (Table 5.3). This may be due to fewer reserves in the cotyledon, as a result the seedling vigour was reduced therefore making them more sensitive (Alam & Locascio, 1965; Anderson, 1970).

Dry mass of seedlings originating from flat seeds was affected by both acetochlor application rate ($P < 0.0001$) and seed size ($P < 0.0002$). The interaction between these two factors was not significant at the 5% level of significance.

As the acetochlor application rate was increased a concomitant decrease in dry mass occurred (data not shown). This reduction was significant at 0.74 kg ai ha⁻¹ and it was again reduced significantly at the recommended rate (1.47kg ai ha⁻¹), although this mass did not differ significantly from that from 2.98 kg ai ha⁻¹ (2x).

Table 5.3 Effect of acetochlor on the seedling height of flat seeds of DKC 73 - 76R (% of control)

Seed size (SZ)	Acetochlor application rate (kg ai ha ⁻¹)					Mean
	(A)					
	0.00	0.74	1.47	2.94	5.88	
PFL	100.00	99.89	85.08	83.42	92.06	92.09 ^a
F11	100.00	90.51	83.04	76.12	85.61	87.06 ^a
F12	100.00	71.75	84.98	95.85	66.78	83.87 ^a
Mean	100.00 ^a	87.39 ^{ab}	85.13 ^b	84.37 ^b	81.48 ^b	
LSD _{T(0.05)}	SZ=8.99		A=13.62		SZ x A = 29.26	

Means followed by the same letter in either rows or columns do not differ from each other at the 5% level of significance.

5.4 Discussion

Acetochlor caused stunting in seedlings developing from small seeds. This effect was greater in PAN6Q521R, where seedlings from SFS were shorter than those from MRS. This could be attributed to fewer reserves in the seed as indicated by the lower seed weight, as seed weight was reported to have a linear relationship with seed size and reserves (Paul & Ramaswamy, 1979). Le Court De Billot & Nel (1977) showed that seed size had an effect on the sensitivity of waxy maize to metolachlor. Data analysis for seedling height from all seed sizes of DKC 73-76R showed that the effect of seed size was not significant. This was an indication that seedlings from all seed sizes were influenced in the same way irrespective of the shape and the size of the seed. This could be due to seeds weighing in the same ranges (30g's), or possibly due to the sensitivity of the cultivar to the herbicide hiding subtle differences in sensitivity.

In both cultivars it was observed that seedlings developing from heavier flat seeds were more tolerant to acetochlor than those from lighter seeds, possibly due to fewer reserves in the cotyledon of the latter seedlings. As a result seedling vigour was possibly reduced,

making them more sensitive (Alam & Locascio, 1965; Anderson, 1970). In DKC 73-76R it was found that seedlings from PFL1 (largest seed) tended to be taller than those from F11 and F12 (although not significant) whereas in PAN6Q521R seedlings from MF were more tolerant compared to those from MFS and SFS. These findings are in agreement with Cargill & Santelmann (1971) who found that plants from small seeds were more susceptible to injury from herbicides than those from larger seeds.

It appeared that as herbicide application rate increased, seed shape became more critical. Seedlings from the round seeds of PAN6Q521R were more tolerant to the acetochlor than those from flat seeds (Figure 5.8a), probably due to more reserves and smaller surface area to volume ratio which may result in a reduction in the amount of the herbicide being absorbed by the seed. It may as well be due to larger seedlings produced by larger seeds, having a smaller absorbing surface relative to the total seedling volume, than that of seedlings produced by small seeds as it was found by Scott & Phillips (1971).

It did not appear as though seed shape played a role in the sensitivity of seedlings of DKC 73 -76R to acetochlor (Figure 5.8b). As can be seen from Figure 5.8b, no pattern emerged regarding the effect of seed shape on seedling dry mass.

5.5 Conclusion

No clear picture regarding the effect of seed size on acetochlor tolerance could be found in maize. However, seedlings from flat seeds in both cultivars followed the trend that heavier seeds were more tolerant to the herbicide. As the results seem to be confusing, further research should be done to elucidate these differences.

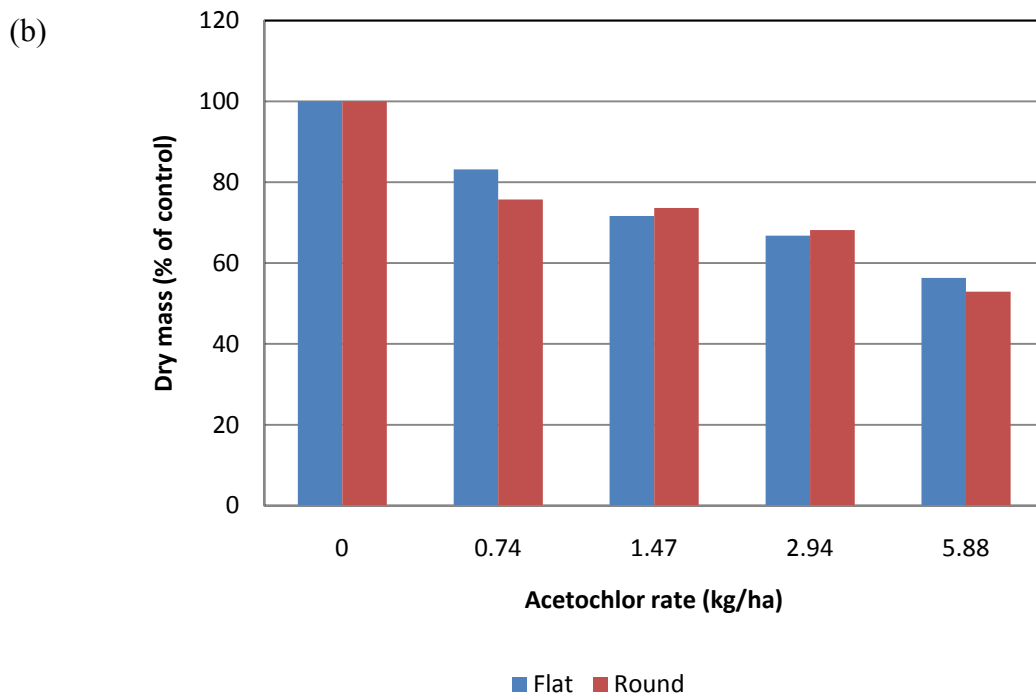
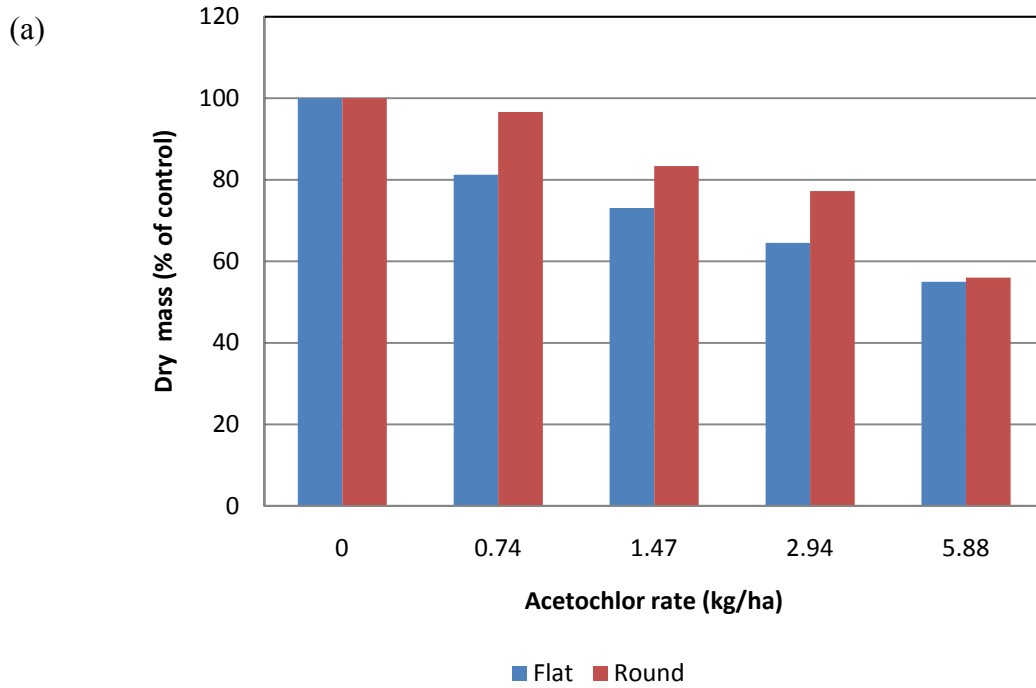


Figure 5.8 Effect of increasing acetochlor rate on the dry mass of seedlings from flat and round seeds (a) PAN 6Q521R (b) DKC 73 -76R (% of control).

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CHAPTER 6

INFLUENCE OF SOIL TYPE ON ACETOCHLOR ACTIVITY IN MAIZE

6.1 Introduction

A number of soil properties, such as pH, organic matter and texture, are reported to affect activity of soil applied herbicides (Blumhorst *et al.*, 1990; Franzen & Zollinger, 1997; Vasilakoglou *et al.*, 2000; Liu *et al.*, 2002; Ye, 2003). Among these soil properties, organic matter content and clay content have been found to be the most important properties affecting herbicide activity (Weber & Peter, 1982; Reinhardt & Nel, 1984; Rao, 2000; Liu *et al.*, 2002).

Due to its effect on herbicide activity soil texture (clay content) also influences herbicide application rates, and the recommended application rates of soil applied herbicides are based on the clay content of the soil (Ballard & Santelman, 1973; Blumhorst *et al.*, 1990). The different amounts of clay minerals found in soils contribute to variations in herbicide phytotoxicity that can occur (Scott & Weber, 1966; Reinhardt & Nel, 1990; Allemann, 1993; Rao, 2000). The greater the clay content of the soil, the greater the herbicide application rate (Bayer, 2002; Monsanto, 2002).

Several investigations have been conducted on the influence of different soil properties on the activity of chloroacetanilide herbicides. Alachlor and metolachlor were found to adsorb more to clay and organic matter (Reinhart & Nel, 1984; Blumhorst *et al.*, 1990). Acetochlor was found to adsorb more to organic matter and this reduced its activity (Weber & Peter *et al.*, 1982; Hiller *et al.*, 2009). Peter & Weber (1985) reported that soil organic matter influenced adsorption of acetochlor, alachlor and metolachlor, and that organic matter together with clay content are regarded as the most important soil properties that influence adsorption of chloroacetanilides.

In South Africa the organic matter content of most soils is lower than 1%. Due to this, clay content is considered more important when determining recommended application rate of

herbicides (Reinhardt & Nel, 1984; 1989; Bayer, 2002). Clay soils tend to decrease herbicide activity, unlike sandy soils, since they adsorb the herbicide molecules more than sandy soils do, hence the herbicide become less available in the soil solution and so to plants (Scott & Weber, 1967; Allemann, 1993).

The objective of this study was to determine the effect of soil texture on the activity of acetochlor on maize. In order to do this, two trials were conducted. The first trial utilized a single application rate of acetochlor irrespective of clay content, while the second trial used application rates based on the clay content of the soil.

6.2 Materials and Methods

Two maize cultivars, one tolerant (PAN6Q521R) and one sensitive (DKC 73-76R) to acetochlor selected from the cultivar screening trial, were tested for their reaction to acetochlor on different soils, in an air conditioned glasshouse on the Bloemfontein campus of the University of Free State under natural day length conditions. The temperature in the glasshouse was controlled at 28/18°C (day/night). Three different types of soil were collected and soil texture determined (Table 6.1).

Table 6.1 Properties of the soils used in the experiment

Soil type	Clay %	Silt %	Sand %			Source
			Coarse	Medium	Fine	
Sand	8	5.6	0.36	4.6	82.36	Kenilworth
Sandy loam	12	8	1.88	6.62	72.22	Bainsvlei
Clay loam	38	22	1.16	1.62	34.38	Glen

The trial was laid out in a randomized complete design with four replicates. Polyethylene pots, 150 mm in diameter and 120 mm high, were lined with plastic bags to prevent

leaching and herbicide contamination from the sides of the pot. Each pot was filled with 1.5 kg of each soil type. Five seeds were planted in each pot at a depth of 25 mm.

The soil water content at field capacity for each soil type was determined gravimetrically to be 18%, 22% and 32% (m/m) for sand, sandy loam and clay loam respectively. This was done by filling the pots with a known mass of air dry soil then wetting them until free drainage takes place. After 24 hours the soil was weighed again, the difference between the two masses was the mass of water required to bring the soil to field capacity.

Two trials were conducted in order to determine the effect of soil texture on maize reaction to acetochlor. In trial 1 acetochlor was applied at the following five rates, 0 (control), 0.74, 1.47, 2.94, and 5.88 kg ai ha⁻¹, being 0, 0.5, 1, 2, and 4 times the application rate recommended for use on the sandy loam soil. Herbicide was also applied at 0, 0.5, 1, 2, and 4 times the recommended application rate in the second trial, but these rates were based on the rate recommended for each soil type, i.e. 1.19 kg ai ha⁻¹ on sand, 1.47 kg ai ha⁻¹ on the sandy loam and 1.89 kg ai ha⁻¹ on clay loam. In both trials acetochlor was applied the day after planting. The herbicide was applied to the soil surface using a laboratory spraying apparatus already described in cultivar screening trial (Chapter 3).

Reverse osmosis (RO) water was applied to the control pots. All pots were watered to within 130 ml of the amount of water required to fill them to field capacity using RO water, prior to herbicide application. Following herbicide application, the remaining 130 ml of water (approximating a shower of 6 mm of rain) was applied to each pot in order to leach the herbicide into the soil. Pots were weighed daily and RO water added when necessary to bring the water content of the pot back to 70% of the water available at field capacity. If necessary plants were thinned out two weeks after planting so that three plants remained in each pot.

Plants were harvested 30 days after treatment, and seedling height as well as above ground fresh mass determined. Plants were then dried to constant mass in an oven at 70°C and dry mass determined. Data were analyzed using the SAS Ver. 9.1 for Windows statistical package (SAS Institute, 2003). The data were expressed as a percentage of the control treatments prior to statistical analysis in order to negate inherent growth differences

between cultivars. Significant results were analyzed using Tukey's Least Significant Difference test, described by Steel & Torrie (1980), at the 5% level of significance to determine statistically significant differences between treatment means, even though the ANOVA may have indicated a higher level of significance.

6.3 Results and discussion

Emergence

The first seedlings started to emerge six days after planting (DAP) irrespective of a trial and soil type. However the emergence percentage varied. In both trials the emergence rate was high in the clay loam soil (Figure 6.1). This may possibly be due to high water retention and ability of the soil to absorb heat which is one of the important factors affecting emergence. This finding is in agreement with that of Jalota *et al.* (2009).

When the standard acetochlor application rate based on recommended rate for the sandy loam soil was used (Trial 1), emergence percentage was significantly lower in sand and sandy loam soil than in the clay loam. This was particularly so at six, seven and eight DAP (Figure 6.1a). After acetochlor was applied based on clay content of each soil (Trial 2), emergence was significantly influenced by soil ($P < 0.0001$), cultivar ($P < 0.0001$), and acetochlor application rate ($P < 0.0001$). Emergence rate increased in sand and sandy loam soils, whereas in clay loam it was reduced. For example at seven DAP, emergence rate in clay loam was reduced by 20% in comparison to that observed in trial 1 (Figure 6.1a & b).

This is an indication that acetochlor should be applied based on the clay content of each soil so that a similar effect should occur in different soils. Time to emergence was similar in both cultivars although PAN6Q521R appeared to have a higher emergence rate than DKC 73-76R in all different soils used in this experiment.

Time to emergence was significantly affected by acetochlor rate, with an increase in acetochlor rate resulting in a decrease in emergence rate in both trials (Figure 6.2 a & b). The effect of the various application rates was more marked in the second trial. In this trial at seven and eight DAP, the emergence percentage from two and four times the recommended rate was significantly lower than that of the control and the recommended rate.

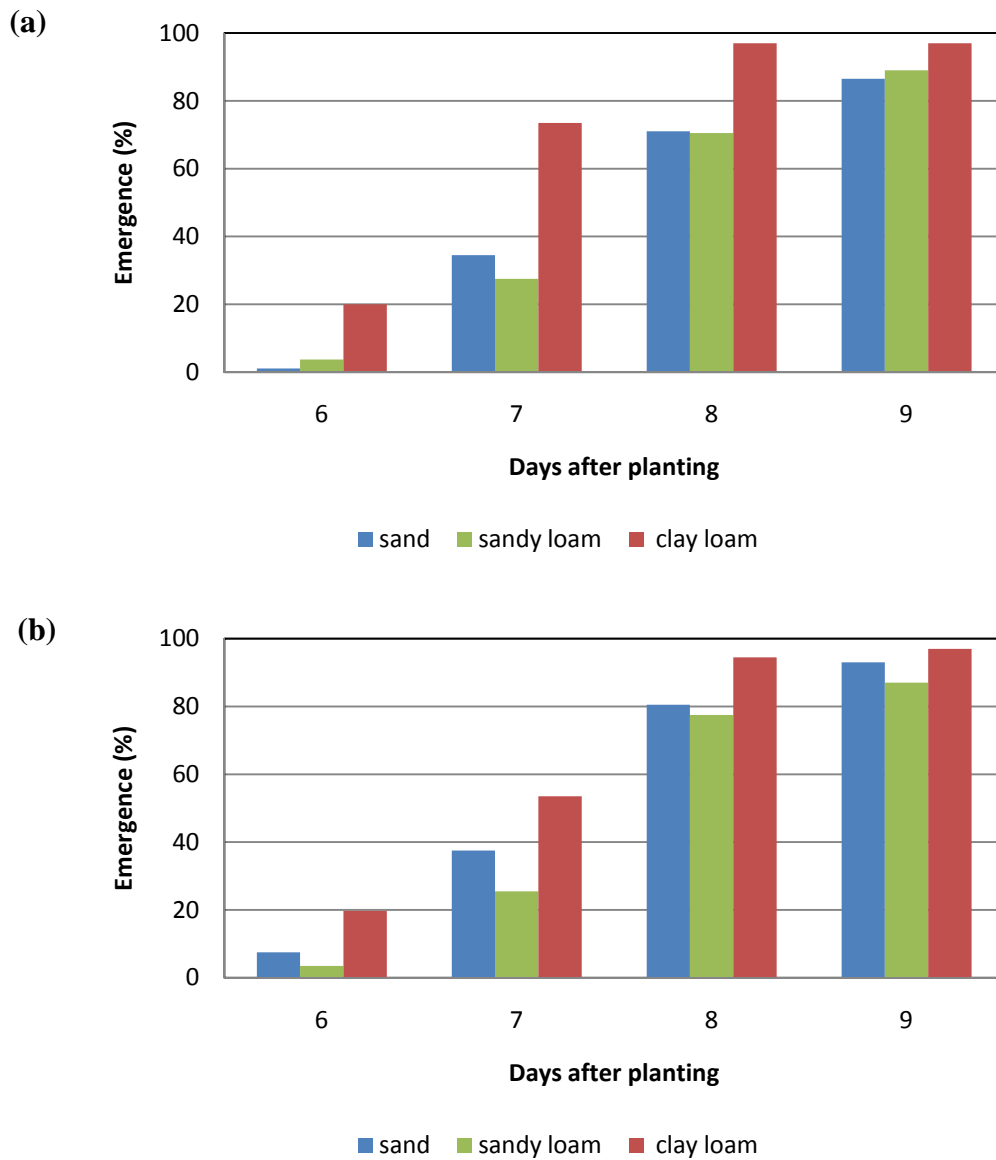


Figure 6.1 Seedling emergence pattern from different soils (a) at standard application rate as recommended for sandy loam (b) acetochlor rate based on the soil type.

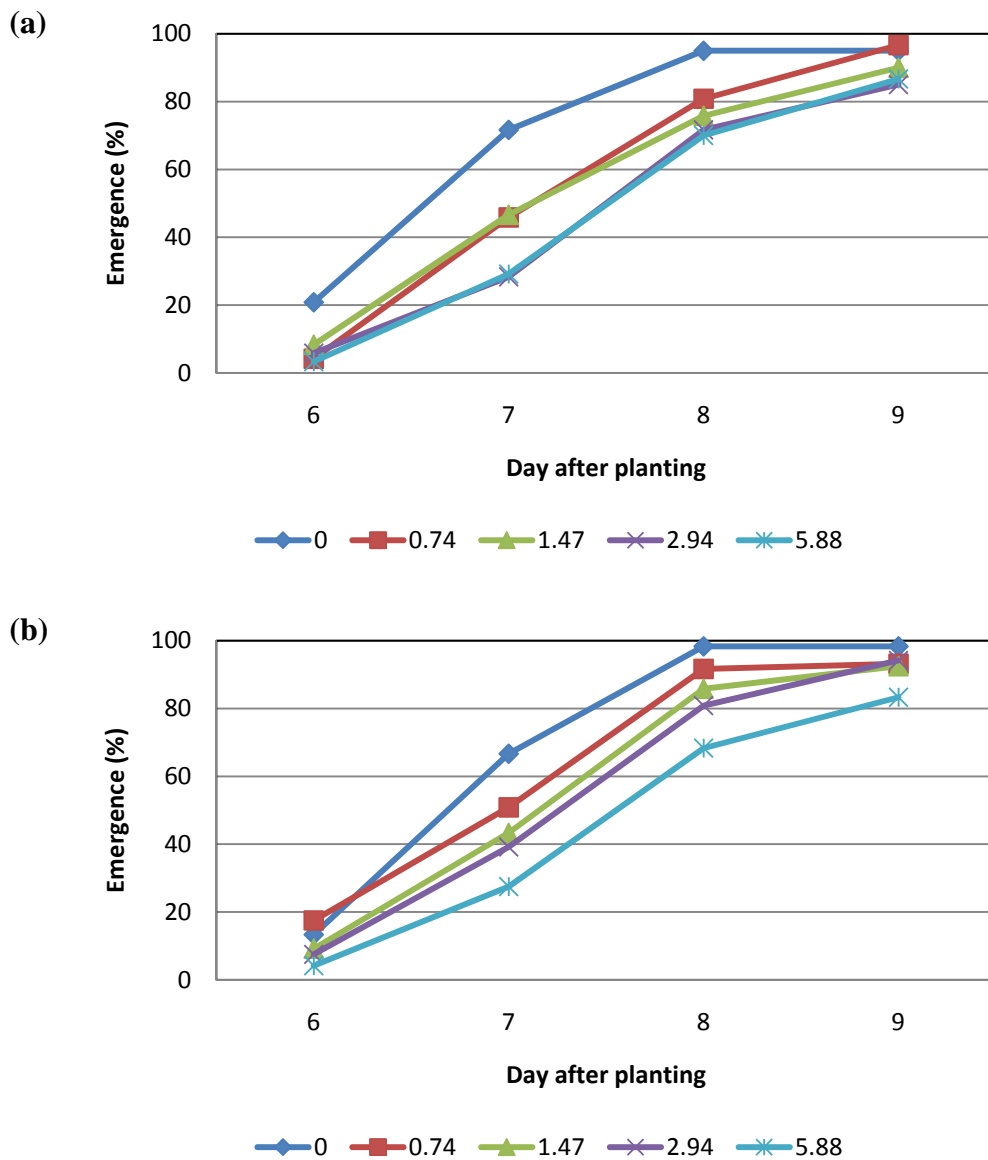


Figure 6.2 Seedling emergence pattern from different acetochlor application rates over cultivar and soil type. (a) acetochlor application rate recommended for sandy loam, (b) acetochlor application rate based on the soil type

Seedling height

Trial 1

Plant height was significantly affected by acetochlor application rate ($P < 0.0001$), soil type ($P < 0.0001$) and the interaction between application rate and soil type ($P < 0.0001$).

None of the other factors or interactions had a significant effect at the 5% level of significance. The response of maize seedlings to acetochlor on the different soils is shown in Figure 6.3.

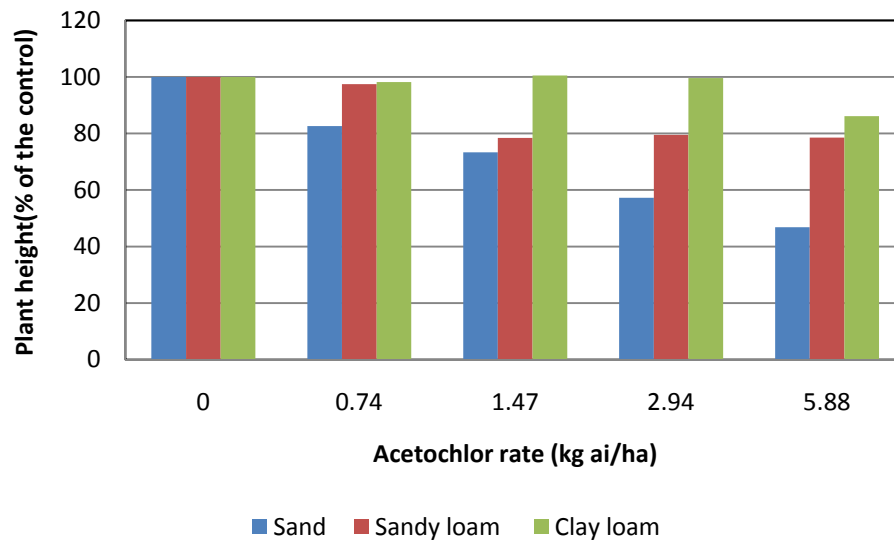


Figure 6.3 Effect of different soils on seedling height at different acetochlor rates in trial 1
($LSD_{T(0.05)} = 23.07$)

Seedlings in the sandy soil appeared to be more sensitive to acetochlor, as their height was reduced significantly with an increase in acetochlor rate. The significant reduction was observed first at the recommended acetochlor rate, although seedling height at this rate did not vary significantly with that at 0.74 kg ai ha⁻¹ and 2.94 kg ai ha⁻¹. At 0.74 kg ai ha⁻¹ (0.5 times the recommended rate) seedling height in the sandy loam and clay loam soils was reduced by 1.8% while in the sand it was reduced by 17% although this difference was not significant. Seedling height on sandy soil was significantly reduced at highest acetochlor rate (5.88 kg ai ha⁻¹) compared to that from both sandy loam and clay loam soils, while seedling heights in the clay loam and sandy loam soils did not differ significantly from each other at any of the acetochlor rates.

Bioactivity was found to decrease in clay loam soils and to decrease moderately in sandy loam soils (Figure 6.4), whereas in sand it was significantly increased. It was observed that the higher the clay content of the soil the less the reduction in seedling height at all acetochlor rates. Similar results were reported by Vasilakoglou *et al.* (2001) who found that growth of oats was reduced by increasing acetanilide herbicide (alachlor, acetochlor & metolachlor) concentration in both sand and silty clay loam soils. Bioactivity was lower in the clay loam, probably due to a decrease in the availability of acetochlor, as it has been reported that acetochlor is readily adsorbed to clay particles (WSSA, 2002; Ye, 2003). This finding concurs with that of Scott & Weber (1967) who found that the phytotoxicity of several herbicides was reduced as the amount of clay in the soil increased. Similarly, several authors have reported that both herbicidal activity and phytotoxicity are influenced by soil properties such as clay content (Hata & Isozaki, 1980; Allemann, 1993; Reinhart & Nel, 1989; Rao 2000; Bayer, 2002; Ye, 2003)

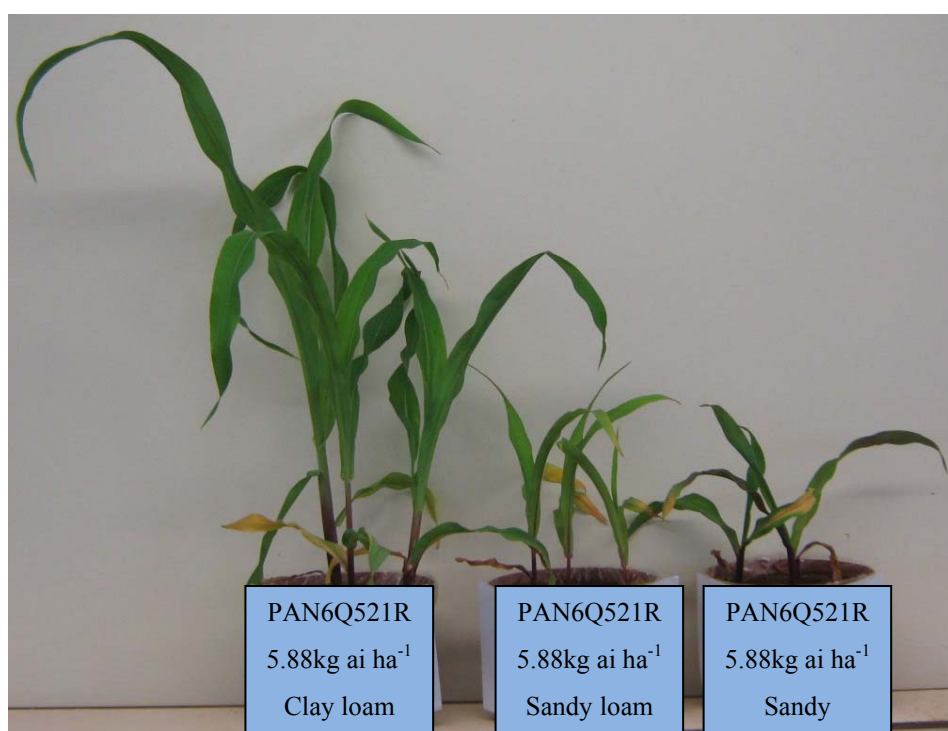


Figure 6.4 Influence of soil type on acetochlor activity at the highest application rate (4 times the recommended rate)

Trial 2

When acetochlor was applied based on the clay content of the soil as per label instructions, it was found that herbicide application rate, soil type and the application rate x soil type had a highly significant ($P < 0.0001$) effect on seedling height. No other factor had significant effects at the 5% level of significance. It was observed that although height was less reduced in the clay loam soil at all acetochlor rates (Figure 6.5), it did not differ significantly from that in sand and sandy loam at half the recommended rate for each soil. As acetochlor application rate was increased from the recommended rate to four times the recommended rate, seedling height in the sand soil tended to be significantly less than in both the sandy loam and clay loam soils. Height of seedlings from the sandy loam did not differ significantly from that of seedlings on the clay loam, at any rate of acetochlor application.

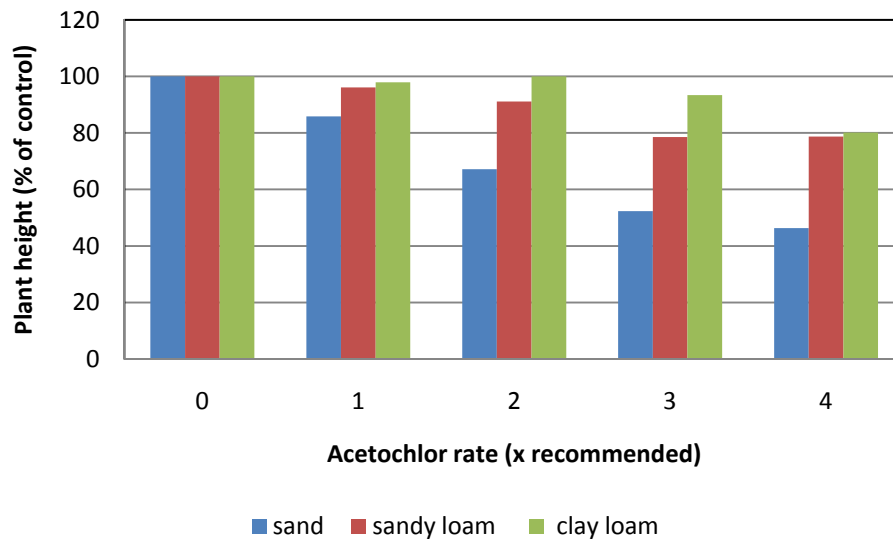


Figure 6.5 Effect of different soils on seedling height at different acetochlor rates
($LSD_{T(0.05)} = 20.42$)

General discussion

In trial 1 the herbicide application rate was based on the clay content of the sandy loam soil. This resulted in lower activity on the clay loam soil, probably due to greater adsorption of herbicide due to the increased clay content in this soil resulting in a lower

availability for plant uptake. In trial 2 acetochlor was applied based on the clay content of the soil as per label instructions. The higher application rate on the clay loam soil, resulted in more herbicide being available for uptake by the plants, and led to greater phytotoxicity occurring than in trial 1. These results are similar to those reported by Hiller *et al.* (2009), in that as the concentration of acetochlor increases the percentage of acetochlor being adsorbed by the soils decreases, resulting in an increase in bioactivity. According to Hiller *et al.* (2009) this may be due to the difficulty of acetochlor molecules to access active sorption sites as its concentration is increased.

In trial 2, it can be seen that the bioactivity of acetochlor in sand soil was reduced, particularly at half the recommended rate, while in clay loam it was increased when compared to the results of trial 1. This illustrates that the clay content of the soil plays an important role in determining the activity of acetochlor and so it should be considered when coming up with herbicide recommendation rates.

Dry mass

Trial 1

When acetochlor was applied to all soils based on the recommended rate for the sandy loam soil, it was found that herbicide application rate, soil type and application rate x soil type had highly significant ($P < 0.0001$) effects. The rest of the factors and interactions were not significant at the 5% significance level. The significant interaction indicated that the soil type influenced acetochlor activity differently. At all acetochlor rates plant dry mass on the clay loam was greater than that of the sand soil, (Table 6.2) although this difference was only significant at the two highest application rates. These results are similar to those observed for seedling height.

No significant differences occurred between the sand, sandy loam soil and clay loam soil at any application rate.

Bioactivity appeared to be greater in sand soil, moderate in sandy loam and lowest in the clay loam soil. At 0.74 kg ai ha⁻¹ and 1.47 kg ai ha⁻¹, dry mass from all the three soils did not vary significantly. While at 2.94 kg ai ha⁻¹ and 5.88 kg ai ha⁻¹, dry mass from sand and

sandy loam did not vary significantly from each other, but that from sand varied significantly from clay loam at these rates.

Table 6.2 Effect of acetochlor rate and soil type on plant dry mass (% of control)

Soil type (S)	Acetochlor application rate (kg ai ha ⁻¹)					Mean
	(A)					
	0.00	0.74	1.47	2.94	5.88	
Clay loam	100.00	101.44	96.74	87.89	76.92	92.98 ^a
Sandy loam*	100.00	93.41	78.90	72.42	68.11	82.57 ^b
Sand	100.00	96.15	79.24	57.00	48.38	76.15 ^c
Mean	100.00 ^a	97.00 ^a	84.96 ^b	72.44 ^c	64.47 ^c	
LSD _{T(0.05)}	S = 5.65		A = 8.52		S x A = 25.44	

*Acetochlor recommended for this soil used on all soils

Means followed by the same letter in either rows or columns do not differ from each other at the 5% level of significance.

Trial 2

The data collected on the dry mass when acetochlor was applied based on the clay content of each soil showed highly significant differences between cultivar ($P < 0.0001$), acetochlor application rate ($P < 0.0001$) and soil type ($P = 0.0014$). None of the other factors or interactions were significant. This demonstrates that both cultivars reacted in the same way to the acetochlor application rate on all three soils, although it was noted that dry mass of DKC 73-76R seedlings was reduced by a greater percentage than those of PAN6Q521R over all rates of acetochlor application (Figure 6.6). This confirms that DKC 73-76R is more sensitive to acetochlor than PAN6Q521R, as indicated by the significant cultivar difference.

The dry mass was reduced incrementally as the application rate was increased. Although it was not significantly reduced at half the recommended rate, a significant reduction occurred at the recommended rate of application, and at each further increase in application rate (Figure 6.7).

Seedling dry mass on the clay loam was significantly greater than that on sand, but it did not differ significantly from that on the sandy loam. The dry mass of seedlings in the sandy loam did not vary significantly from that of seedlings in the sand (data not presented). In this trial, bioactivity was greater in the sand soil, although not significantly different from that in the sandy loam soil.

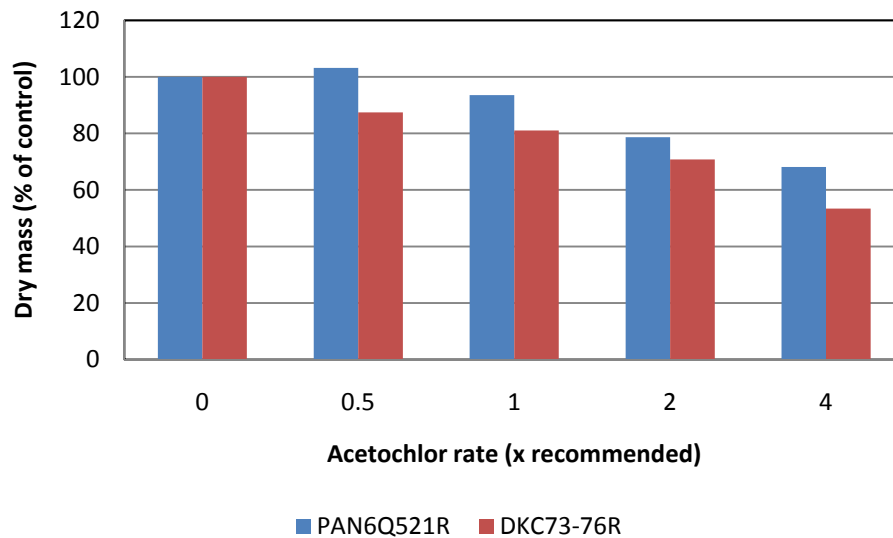


Figure 6.6 Cultivar differences at different application rate when acetochlor was applied based on clay content of each soil

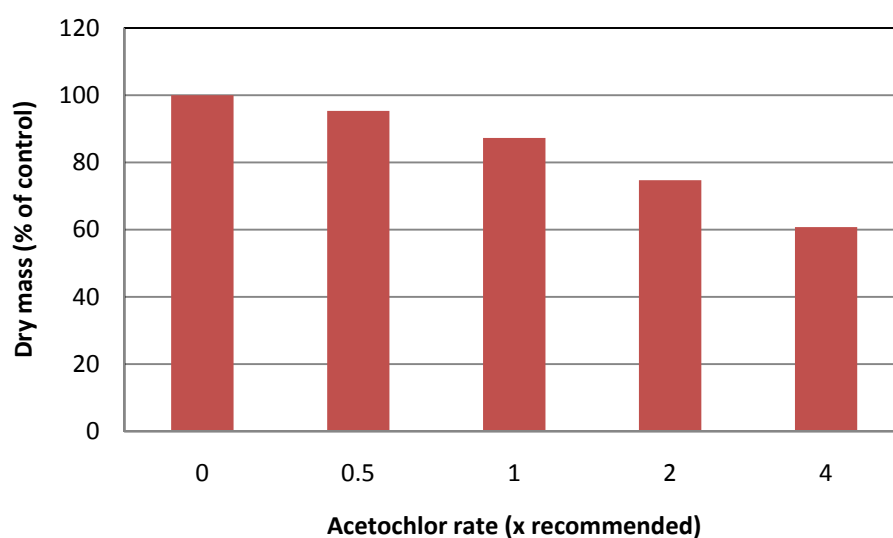


Figure 6.7 Effect of acetochlor application rate on dry mass of maize seedlings over different soils ($LSD_{T(0.05)}=11.25$).

General discussion

Mean dry mass of maize seedlings in the clay loam soil was reduced by approximately 3% when the herbicide was applied at the rate determined by the soil clay content, compared to that from trial 1 when a single recommended rate was used on all soils. On the other hand maize dry mass in the sand soil was increased by about 2% when herbicide was applied at rate determined by soil clay content. This was probably due to less herbicide being available for uptake by plants as compared to when it was applied based on the clay content of the sandy loam soil.

Studies on the phytotoxicity of metolachlor, butalachlor and alachlor, herbicides in the same family as acetochlor, showed that clay content influenced herbicidal activity through adsorption, so making less herbicide available to plants on soils with a high clay content (Reinhardt & Nel, 1984; Vasilakoglou *et al.*, 2001; Liu *et al.*, 2008). The lower the clay content the more herbicide is available for uptake by plants resulting in increased bioactivity, so causing more damage (Day *et al.*, 1968; Rao, 2000). These results show

that acetochlor application rates should be differentiated on the basis of soil type, with the rate being based on the clay content of the soil.

Although organic matter has a greater adsorption capacity than clay (Weber & Peter, 1982; Reinhardt & Nel, 1990; Vasilakoglou *et al.*, 2000), clay content is generally used when arriving at the recommended application rates of herbicides. This is particularly true in South Africa as the organic matter content of most soils is very low at <1% (Reinhardt & Nel, 1984; Bayer, 2002), so making the use of clay percentage a better predictor of herbicide activity and application rate.

6.4 Conclusion

Soil clay content had a definite effect on acetochlor activity in maize seedlings, showing that the acetochlor application rates should be adjusted based on the clay content of the soil. Despite the recommendation for acetochlor application rates being changed based on the clay content of the soil, the bioactivity of this herbicide on sand still appeared to be significantly higher than that obtained on sandy loam and clay loam soils. This could result in herbicide damage occurring on light soils under certain circumstances. Further research in this regard should be carried out.

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CHAPTER 7

EFFECT OF TEMPERATURE ON ACETOCHLOR ACTIVITY

7.1 Introduction

Temperature influences plant growth and development through its effect on the rate of physiological and biochemical reactions (Alessi & Power, 1971; Coelho & Dale, 1980). Karim *et al.*, (2000) and Medany *et al.* (2007) reported that when temperature exceeds the optimum for biological processes, crops often respond negatively with a reduction in net growth. This was observed in maize where an increase in temperature from the optimum (25-30°C) to 35°C resulted in a reduction in seedling growth. Wolfe (1991) found that the relative growth rate of maize was decreased by 10% when temperature was reduced from 28/18°C to 18/13°C. An increase in temperature has been reported to accelerate seed germination and growth (Pearson, 1975; Milford & Riley, 1980).

Some factors which contribute to herbicide activity, such as solubility, volatility, and sorption and desorption of the herbicide are also affected by temperature (Bowen, 1967; Mulder & Nalewaja, 1978; Rao, 2000; Bayer, 2002). An increase in temperature within the range of 10-30°C has been reported to enhance the phytotoxicity of s-triazine herbicides to pine seedlings (Kozlowski *et al.*, 1967). These results were similar to those of Muzik & Mauldin (1964) who found that plant sensitivity to 2,4-D was greater at 26°C than at 10°C and 5°C. This increase in phytotoxicity may be attributed to an increase in absorption and translocation of the herbicides (Vostrál *et al.*, 1970; Lambrev & Goltsev, 1999). In a review done by Hammerton (1967), it was stated that high temperatures generally increases the susceptibility of plants to herbicides. However, Xie *et al.* (1996) found that Fenoxaprop-ethyl phytotoxicity was reduced by high temperatures (30/20°C).

The activity of some herbicides from the chloroacetanilide group is also affected by temperature. Research by Le Court de Billot & Nel (1977) found that metolachlor toxicity in maize increased with a decrease in temperature, a result that was confirmed by Viger *et al.* (1991). Similar results have been reported by Belote & Monaco (1977), Rice & Putman

(1980) and Kanyomeka (2002). Allemann (1993) reported that alachlor phytotoxicity to sunflower was increased with an increase in temperature.

The influence of temperature on herbicide phytotoxicity is contradictory and seems to depend on the herbicide and the crop being used. As no reference on the effect of temperature to acetochlor activity on maize was found in the literature, it was decided to determine the effect of temperature on acetochlor activity in maize.

7.2 Materials and Methods

The trial was carried out in modified Conviron controlled environment cabinets on the Bloemfontein campus of the University of Free State. Two maize cultivars were used, one tolerant (PAN6Q521R) and one sensitive (DKC 73-76R) to acetochlor as shown in the cultivar screening trial (Chapter 3), were used.

Polyethelene pots, 150 mm in diameter and 120 mm high were lined with polyethelene bags in order to prevent leaching and contamination from the sides of the pot, and filled with 1.5 kg of sandy loam soil ($\text{pH}_{\text{KCl}} = 4.9$ and 15% Clay). Five maize seeds were planted at a depth of 25 mm in each pot.

Acetochlor was applied at five rates, viz. 0 (control), 0.74, 1.47, 2.94, and 5.88 kg ai ha⁻¹, being 0, 0.5, 1, 2, and 4 times the recommended application rate for the soil being used. The herbicide was applied to the soil surface the day after planting as described in Chapter 3. The control pots were sprayed with RO water.

The soil water content at field capacity was determined gravimetrically to be 22% (m/m). Prior to herbicide treatment all pots were watered with RO water to within 130 mm of the volume of water required to wet the dry soil to field capacity. Following herbicide application the remaining 130 mm of water (approximating a rain shower of 6 mm) was applied evenly over the surface of each pot in order to leach the herbicide into the soil and bring the soil to field capacity.

The experiment was carried out in three controlled environment cabinets, each set at a different temperature regime with a 12 hour photoperiod. The temperature regimes used in this trial were selected by referring to work done by Ehlers (1981). The first temperature regime [15/8°C (day/night)] was selected to be below the optimum temperature for maize germination and seedling growth, the second temperature regime of 25/18°C (day/night) was within the optimal temperature range, while the third temperature regime [35/28°C (day/night)] was higher than the optimal temperature range. The trial was laid out in a completely randomized block design in each growth chamber, with each treatment replicated six times.

Pots were weighed daily and RO water added when necessary to bring the water content of the pot back to 70% of the water available at field capacity. Two weeks after planting, plants were thinned out so that three plants remained in each pot. Two replicates were used to adjust the water application for growth of the maize plants. This was done by harvesting the plants at 14 and 21 days after planting, weighing them, and then adding the average mass to the pot mass when weighing pots in order to adjust the soil water content.

Plants were harvested 30 days after treatment and plant height as well as above ground fresh mass determined. Plants were then dried to constant mass in an oven at 70 °C and dry mass determined. Data were analysed using the SAS Ver.9.1 for windows statistical package (SAS Institute, 2003). The data were expressed as a percentage of the control treatment prior to statistical analysis in order to negate inherent growth differences between cultivars. Significant results were analysed using Tukey's Least Significant Difference test, described by Steel & Torrie (1980), at the 5% level of significance to determine statistically significant differences between treatment means, even though the ANOVA may have indicated a higher level of significance.

7.3 Results and discussion

Emergence pattern

Time to emergence was significantly affected by temperature, acetochlor application rate and the interaction of temperature x acetochlor application rate at 5% significance level. This indicated that germination was affected differently by acetochlor rate at the different

temperature regimes. The first seedlings to emerge were from the supra-optimal temperature regime (35/28°C). These seedlings started to emerge three days after planting. Those from the optimal temperature regime (25/18°C) started to emerge after five days, while those from the sub-optimal temperature regime (15/8°C) took eleven days to emerge (Figure 7.1). These results are consistent with findings by Alessi & Power (1971) and Boldt & Barret (1989) in that an increase in temperature resulted in a reduction in number of days to emergence.

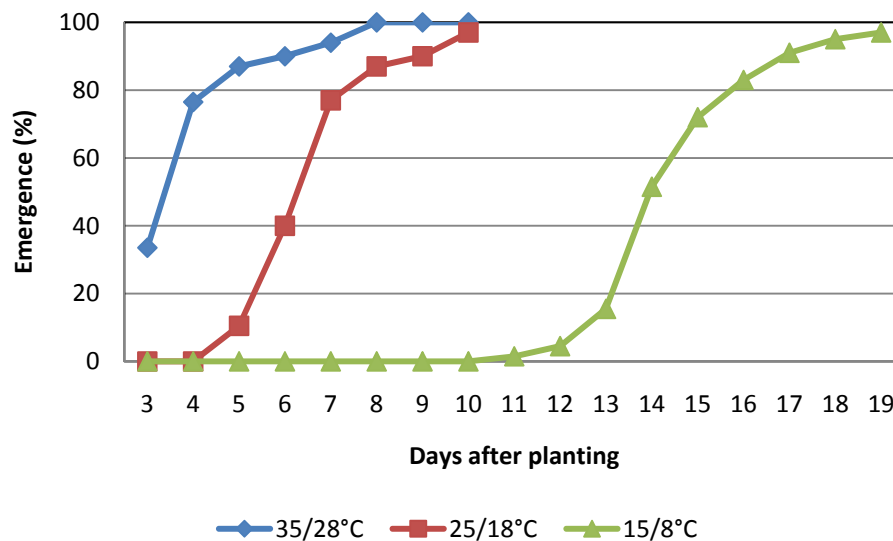


Figure 7.1 Seedling emergence pattern at the different temperature regimes

Not only was time to first emergence affected but also the rate at which emergence occurred. Emergence rate was greater at the supra-optimal temperature regime, moderate at optimal temperatures and slow at the sub-optimal regime. Growth rate was also affected by changes in temperature, being faster at the higher temperatures. This concurs with the results found by Wolfe (1991), that maize growth rate decreased by 10% when the temperature was reduced from 28/18°C to 18/13°C. Seedlings developing in the supra optimal temperature regime were taller and thinner than those from the other temperature

regimes due to their rapid growth, while seedlings at the lowest temperature regime (15/8°C) were thicker and shorter due to a slower growth rate (Figure 7.2). These results concur with those obtained in both maize and pearl millet by Ashraf & Hafeez (2004).

Seedling height

When data were analyzed as percentage of the control, both acetochlor application rate and temperature regime were found to have a statistically significant effect on seedling height. Acetochlor application rate had a far greater effect ($P < 0.0001$) than that of temperature regime ($P = 0.0012$) on seedling height. The interaction between temperature regime and cultivar was also significant, as well as that of temperature and acetochlor application rate. This indicated that the cultivars reacted differently in the different temperature regimes. Whereas the interaction between temperature regime and acetochlor indicated that the activity of acetochlor at different rates was affected by the temperature.

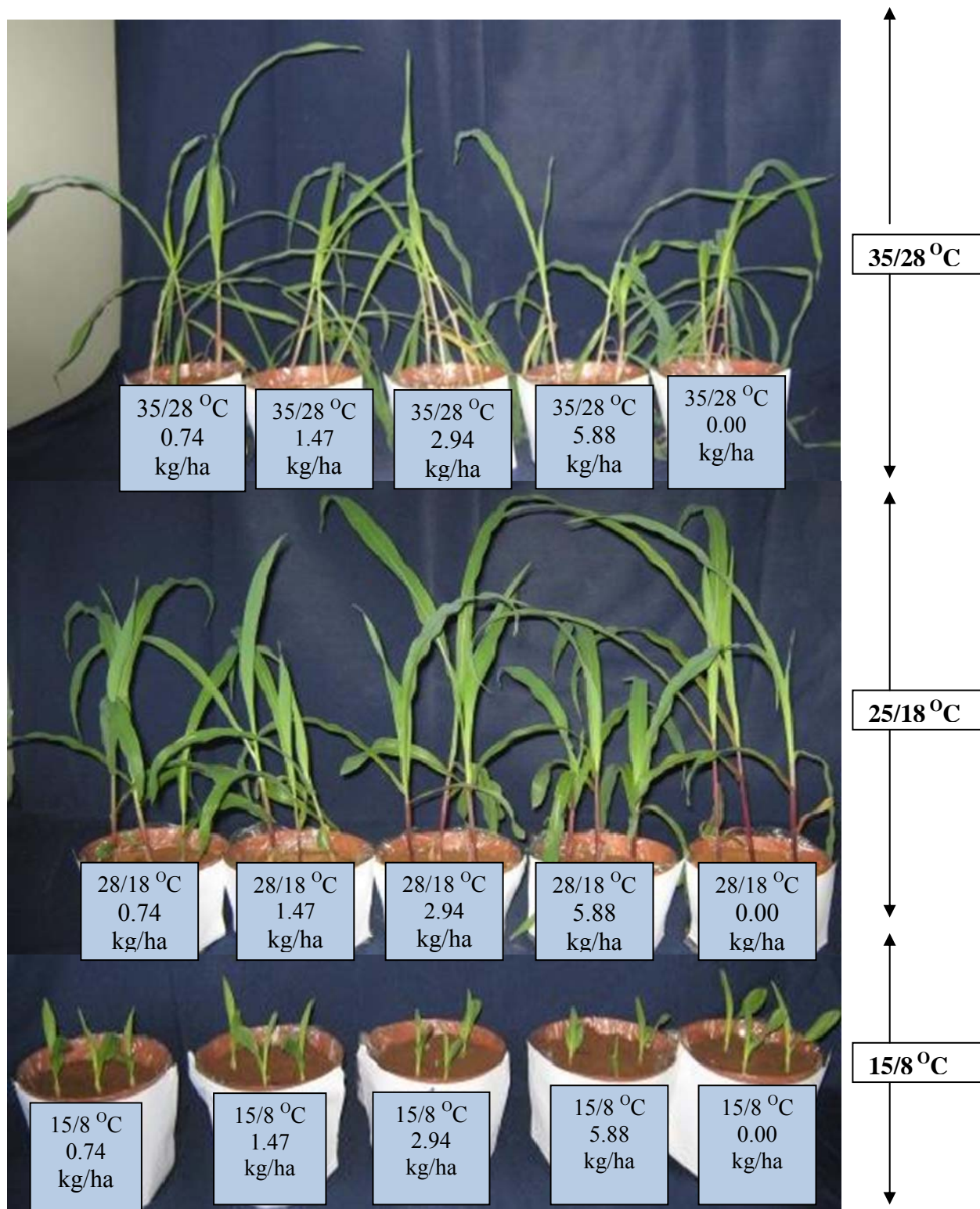


Figure 7.2 Variations in plant appearance in PAN6Q521R at the different temperature regimes.

The greatest acetochlor activity, as shown by reduction in seedling height, in both cultivars was noticed in the lowest temperature regime. Seedling height of PAN6Q521R was reduced by about 3% while that of DKC 73-76R was reduced by 13% as compared with height from supra-optima temperature regime (Figure 7.3), possibly due to the delayed emergence of seedlings at this temperature regime (15/8°C). This resulted in the coleoptiles, the main site of chloroacetanilide herbicides uptake in maize, being exposed to the herbicide for a longer period of time, so absorbing more herbicide which resulted in greater bioactivity. These results are in agreement with those found by other researchers using chloroacetanilide herbicides (Le Court de Billot & Nel, 1977; Boldt & Barret, 1989).

Seedling height at all acetochlor application rates under supra-optimal temperature conditions (35/28°C) was not as affected as that from optimal (25/15°C) and sub optimal (15/8°C) temperature regimes, although the only significant difference occurred at the highest acetochlor rate (5.88 kg ai ha⁻¹) (Table 7.1). This was possibly due to an increase in growth rate as well as the rate of herbicide metabolism, as Rice & Putman (1980) and Viger *et al.* (1991) reported with snap bean and maize respectively. This concurs with findings by Rice (1977) that alachlor toxicity increased with an increase in temperature until reaching a point where further increase in temperature did not lead to alachlor toxicity.

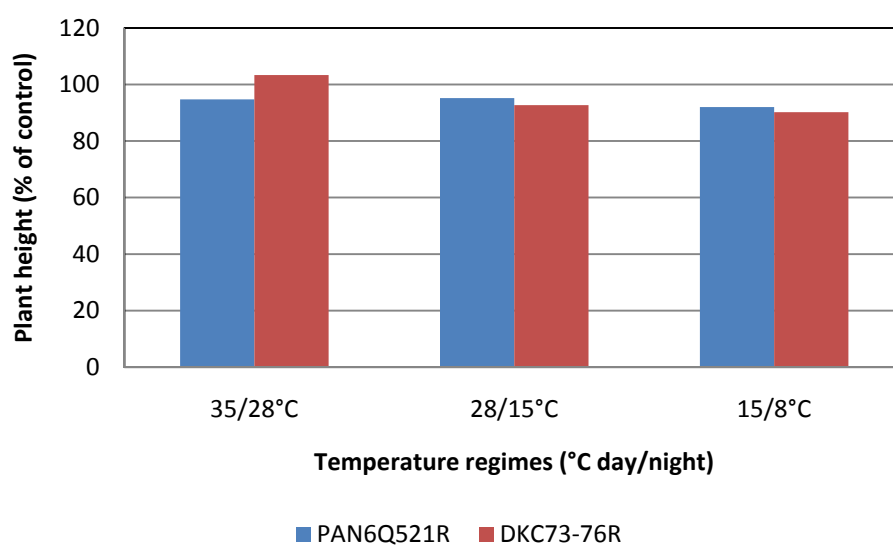


Figure 7.3 Effect of temperature on the seedling height of two maize cultivars over acetochlor application (% of control).

Table 7.1 Effect of temperature on acetochlor phytotoxicity to maize

Temperature regime (°C)	Acetochlor application rate (kg ai ha ⁻¹)					Mean
	(A)					
	0.00	0.74	1.47	2.94	5.88	
35/28°C	100.00	102.19	96.57	99.87	96.50	99.03 ^a
25/18°C	100.00	90.88	95.83	91.20	91.90	93.96 ^b
15/8°C	100.00	100.97	93.44	88.78	72.42	91.12 ^b
Mean	100.00 ^a	98.01 ^a	95.28 ^a	93.28 ^{ab}	86.94 ^b	
LSD _{T(0.05)}	Temp = 5.00		A = 7.55		Temp x A = 3.45	

Means followed by the same letter in either rows or columns do not differ from each other at the 5% level of significance.

At highest acetochlor application rate (5.88 kg ai ha⁻¹), seedling height from optimal temperature regime (25/15°C) did not vary significantly from that of both sub-optimal temperature regime (15/8°C) and supra-optimal temperature regime (35/28°C), while that of supra-optimal temperature regime varied significantly from that of sub-optimal regime.

Seedling height was, however, not a very good predictor of acetochlor activity in this trial. This is indicated by the low R² value of 0.55.

Dry mass

The dry mass of seedlings was significantly affected by cultivar (P = 0.0291), acetochlor application rate (P < 0.0001) and temperature regime (P < 0.0001). Temperature x Cultivar, and Temperature x Acetochlor application rate interactions also had a significant effect, while no other interactions were significant at 5% level. The seedling dry mass appeared to be a far better predictor of acetochlor activity than seedling height in this trial, as shown by the R² value of 0.81. The significant temperature x acetochlor application rate interaction indicated that both temperature regimes and acetochlor application rate played a role in determination of maize tolerance to acetochlor. The interaction between the temperature regimes and cultivar indicated that maize cultivars were affected differently at the different temperature regimes (Figure 7.4).

From Figure 7.4 it can be seen that seedlings from both cultivars had lower dry mass at the optimum temperature regime (25/18°C) compared with that at the other temperature regimes. The difference in seedling dry mass between these temperature regimes was not significant in PAN6Q521R, but in DKC 73–76R dry mass at optimum temperature regime was significantly reduced compared with that at sub-optimal temperature regime (Figure 7.4). Those grown under supra-optimal temperature conditions (35/28°C) had a greater percentage difference from the control than those at the sub-optimal temperature regime (15/8°C). This may be due to heat injury to seedlings at the high temperature, and the limitation in some metabolic processes, so resulting in a reduction in shoot dry mass (Karim *et al.*, 2000; Ashraf & Hafeez, 2004).

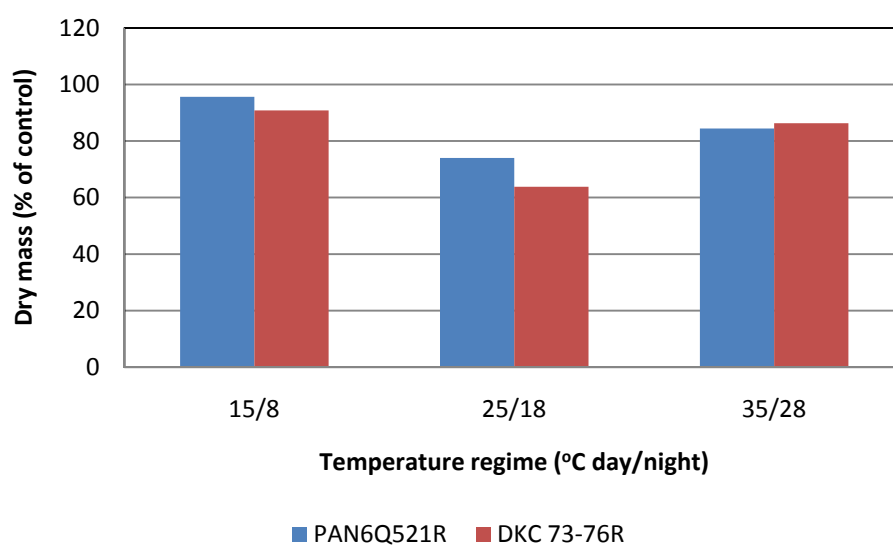


Figure 7.4 Effect of cultivar and temperature on seedling dry mass ($LSD_{T(0.05)} = 27.12$).

An increase in uptake of the herbicide could also have caused this, as the plants at the optimal (25/18°C) and supra-optimal (35/28°C) temperature regimes were growing fast and passed the seedling stage (2-4 leaves) rapidly. Plants are considered to be beyond the seedling stage when they have 2 to 4 leaves (Anon, 2000). It may be possible that seedlings at the higher temperature were absorbing the herbicide through their roots during the latter stages of the trial, as it has been reported that plants beyond the seedling stage may absorb acetochlor through their roots (WSSA, 2002). As the trial was conducted in pots from which the herbicide could not leach, all of the herbicide would remain in the pot for the full duration of the trial, and be leached deeper into the soil, ultimately ending in the root zone. This is an aspect that will require further study.

Although the mass of both cultivars was greatly affected at the optimal temperature regime (25/18°C), PAN6Q521R was less affected than DKC 73-76R. The dry mass of PAN6Q521R seedlings showed significant differences between the temperature regimes. At optimal temperature regime (25/18°C) the dry mass was reduced by 21%, whereas at supra-optimal (35/28°C) dry mass was only reduced by 11%. Seedling dry mass from DKC 73-76R at optimal temperature regime (25/18°C) was reduced by about 27%,

whereas at supra-optimal temperature regimes (35/28°C) it was reduced by only 5%. These results substantiate the ones from Chapter 3 where DKC 73-76R was found to be more sensitive to acetochlor than PAN6Q521R. This is in agreement with the finding by Boldt & Barret (1989) that the greatest potential for chloroacetanilide injury occurs when susceptible maize hybrids are planted at temperatures below 30°C.

Seedling dry mass was reduced with an increase in acetochlor application rate irrespective of temperature regime, although the seedlings from lowest temperature regime (15/8°C) appeared to be less sensitive, followed by highest temperature regime (35/28°C). Those from the middle temperature regime (25/18°C) were the most sensitive. The seedling dry mass at this temperature regime (25/18°C) was significantly lower than that at the sub-optimal (15/8°C) and supra-optimal (35/28°C) temperature regimes. Seedlings dry mass at the latter two temperature regimes did not differ significantly from each other, or from the control. At the recommended application rate (1.47 kg ai ha⁻¹), dry mass at the optimal temperature regime (25/18°C) differed significantly from that at the sub-optimal temperature regime (15/8°C), although it did not differ significantly from that at the supra-optimal temperature regime (35/28°C). Similar results were observed at 2.94 kg ai ha⁻¹, while at highest application rate (5.88 kg ai ha⁻¹), the dry mass at all temperature regimes did not vary significantly from each other (Figure 7.5).

These findings show that acetochlor activity is greater at the optimal growing temperature for maize, followed by that at supra-optimal temperatures, while the least activity can be expected at sub-optimal growth temperatures. These results concurs with what Allemann (1993) found in an alachlor sensitive sunflower cultivar. This was probably due to an increase in growth rate and transpiration which might result in an increase in herbicide absorption and so increased phytotoxicity. These results are also in agreement with findings by Putman & Rice (1979) who reported that alachlor uptake by germinating snap beans was greater under higher temperature which resulted in alachlor injury.

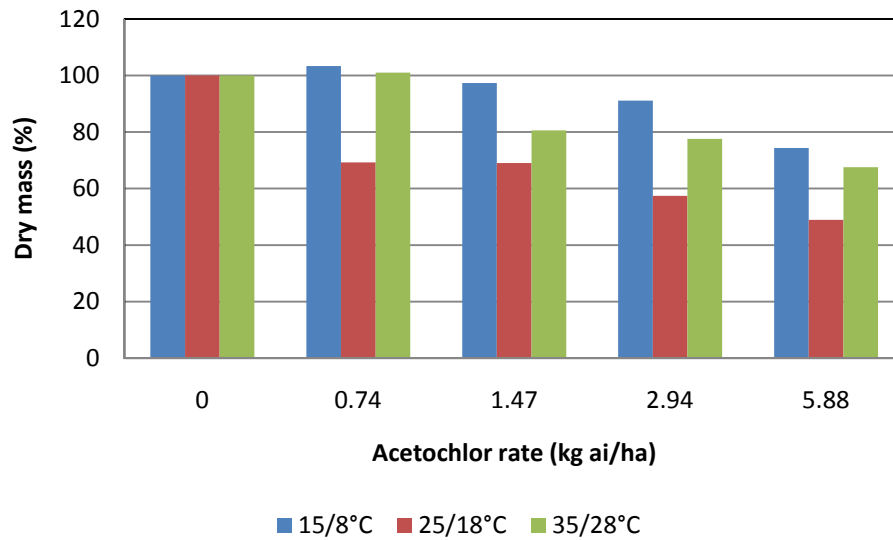


Figure 7.5 Effect of acetochlor rate and temperature on seedling dry mass ($LSD_{T(0.05)} = 27.12$)

7.4 Conclusion

Seedling dry mass appeared to be a better predictor of acetochlor activity than seedling height in this experiment. Maximum bioactivity occurred at the optimum temperature regime (25/18°C), followed by that at the supra-optimal temperature regime (35/28°C). At the optimal temperature regime (25/18°C) significant phytotoxic effects were obtained at the 0.74 kg ai ha⁻¹(0.5x) acetochlor application rate and at 1.47 kg ai ha⁻¹(1x) acetochlor application rate at the supra-optimal temperature regime (35/28°C). This could have major implications in the field as plant damage could occur at rates lower than recommended.

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CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

The screening of twenty one maize cultivars used in central areas of South Africa, showed that differences in tolerance to acetochlor occur in these cultivars. These findings are in agreement with those of Bernardts *et al.* (2006) that differential tolerance to acetochlor occurs in maize. The different maize cultivars could be classified into tolerant, intermediate and sensitive based on their reaction to the herbicide, with most cultivars being classified in the intermediate category. This is in agreement with findings by Rowe *et al.* (1990) who tested a large number of maize hybrids and inbred lines to metolachlor, and found that most were moderately tolerant to the herbicide, but that some were very sensitive and others very tolerant. The results of this trial would therefore appear to indicate that tolerance to acetochlor in maize is genetically controlled, and that resistance could be obtained through breeding. This finding is in agreement with that of Bernardts *et al.* (2006) that the variable response of hybrids to acetochlor was probably due to genetic inheritance. This differential tolerance is of importance in that some cultivars, such as DKC 73-76R, showed signs of acetochlor injury even at the recommended rate of application.

The findings of this experiment should be confirmed in the field as the root growth of plants in a pot trial is restricted to a relatively small volume of soil. Drainage was also prevented in this case, so retaining the herbicide within this restricted soil volume. The entire root system of the seedlings was, therefore, constantly exposed to the herbicide throughout the trial period. More herbicide was available for absorption by the roots of the plants at any given time than would be the case in the field where roots would be able to develop in an area away from a high concentration of herbicide. This has an important implication for absorption of acetochlor as, although it is absorbed primarily through the coleoptile of the emerging maize seedling, secondary absorption does take place through the roots (WSSA, 2002).

Planting depth was found to affect the sensitivity of maize seedlings to acetochlor. Phytotoxicity was found to increase as the planting depth was increased from 15 mm to 45 mm, although significant reductions in seedling height and mass only occurred at the deepest planting depth. This finding concurs with that of other researchers on a number of pre-emergence herbicides from the chloroacetanilide group (Wilson *et al.*, 1990; Ayeni, 1997; Pacanoski *et al.*, 2007). Acetochlor toxicity increased as the application rate was increased, and the effect appeared to be exacerbated by increased planting depth. This was possibly due to the seedlings taking longer to emerge, and so absorbing more herbicide as the coleoptile grew through the herbicide treated soil (Ashton & Crafts, 1981; Ayeni *et al.*, 1997). This finding has important ramifications as South African farmers are tending to plant maize at depths of up to 100 mm in order to be able to plant in moist soil. From these results it would therefore appear as though these plants would be more sensitive to acetochlor than those planted at 25 mm, a danger that would be exacerbated by the use of an acetochlor sensitive cultivar such as DKC 73-76R. The use of a tolerant cultivar like PAN6Q521R would appear to be best in this situation. However, further research in this regard is required as the maximum planting depth in this trial was only 40 mm.

The effect of seed size on the sensitivity of maize to acetochlor was not as cut and dried as the effect of some other acetanilide herbicides on broadleaf species. Generally, it was found that seedlings from larger flat seeds were more resistant to acetochlor than those from smaller seeds. This finding was similar to those obtained with soyabean and kidney bean (Meissner, 1974). The effect of seed size was found to be affected by the tolerance of the cultivar to acetochlor, as well as the shape of the seed, with seedlings from round seed appearing more resistant than those from flat seeds. As a result different results were obtained for each cultivar and each seed shape being used. This is an aspect that will require a great deal more research in order to elucidate the differences in acetochlor sensitivity caused by seed size. Differences could possibly be attributed to differences in the amount of reserves available in the seed affecting growth and vigour of seedlings (Singh & Rai, 1988), with the more vigorous seedlings being more tolerant to the effect of the herbicide.

The effect of soil type was investigated in two trials. In the first, an average application rate of acetochlor based on the recommended rate for a sandy loam soil was applied to a sandy soil, a sandy loam soil and a clay loam soil, while in the second trial acetochlor was applied based on the clay percentage for each soil type. The results from the first trial showed that maize was severely injured on the sandy soil by a very low application rate of acetochlor (0.5 x recommended rate), while no significant phytotoxicity was noted on the clay loam soil even at the highest rate of acetochlor application (4 x recommended rate). These results confirmed the importance of clay content in affecting herbicide activity as determined by a number of researchers (Scott & Weber, 1967; Weber & Peter, 1982; Reinhardt & Nel, 1989).

Using the application rates recommended for each soil resulted in far less phytotoxicity on the sandy soil, and a greater phytotoxicity on the clay loam. Regardless of the soil type, phytotoxicity increased as application rate increased, probably since more herbicides were available in the solution (Vasilakoglou *et al.*, 2000). This confirmed the importance of basing the acetochlor application rate on the clay content of the soil for good weed control as recommended by the manufacturers (Monsanto, 2002; Dow AgroSciences, 2002). However, from these results it appears as though the recommended rate of acetochlor application on sandy soils might still be too high as a significant reduction in maize growth, indicated by seedling height and dry mass, occurred at the recommended application rate when the data were analysed over the two cultivars used. This finding would, however, have to be tested in the field as pot trials represent the worst-case scenario in terms of simulating conditions that promote herbicide damage, as the herbicide is restricted to the relatively small volume of soil occupied by the plant roots (Allemann & Ceronio, 2009).

Tolerance of maize to acetochlor was also affected by temperature, with plants being more susceptible to the herbicide at the optimal growing temperatures. However, the effect in the sensitive cultivar (DKC 73-76R) was more marked than in the acetochlor tolerant cultivar (PAN 6Q521R). Susceptibility of maize seedlings grown under supra-optimal temperatures (35/28°C) was not significantly different from that at sub-optimal temperatures (15/8°C). These results are similar to those found on alachlor toxicity by

Rice (1977) and Allemann (1993) in that toxicity increased with an increase in temperature until a certain point where further increase in temperature did not result in toxicity. Maize seedlings growing in the lowest temperature regime (15/8°C) were shorter and thick unlike those from super optimal temperature (35/28°C) which were tall and slender, probably due to differences in the growth rate at the different temperatures.

The main contributions of this study were the finding that maize cultivars differ in their tolerance to acetochlor, as well as the determination of the influence of planting depth and possibly seed size on acetochlor activity. These findings could assist in explaining certain anomalies in acetochlor sensitivity that have been noted in the field.

A major shortcoming of the study was the inability to extrapolate the results of the glasshouse studies to the field situation. It is recommended that at least the cultivar sensitivity trial be repeated in the field to ascertain if seedling sensitivity to the herbicide translates into yield reductions.

This study has suggested a number of aspects that require further research in order to clarify questions raised. A screening of all commercially available maize cultivars to identify those that are sensitive to acetochlor. This would assist in making recommendations regarding weed management practices to be adopted when using these cultivars. Further investigation of the effect of planting depth needs to be conducted, particularly in the light of planting depths of up to 100 mm being used by farmers. This should also be coupled with soil type and amount of rainfall following herbicide application. The relative importance of shoot and root absorption of acetochlor in maize should be investigated, as this will determine the value of future pot trials to evaluate the use of this herbicide on maize. A great deal of research is still required in order to elucidate the effect of seed size and temperature on acetochlor activity in maize.

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SUMMARY

A number of the commercially available maize cultivars were screened in order to establish their tolerance to acetochlor. These cultivars demonstrated significant differences in their tolerance, with some cultivars being tolerant and others sensitive to the herbicide. The cultivar PAN6Q521R was the most tolerant and DKC 73-76R was the most sensitive, while most other cultivars were intermediate in their sensitivity. This indicates that maize tolerance to acetochlor is genetically controlled.

The influence of planting depth on maize tolerance to acetochlor was investigated using these two cultivars. Both cultivars were found to be more sensitive as the planting depth increased to 45 mm. However, seedlings of DKC 73-76R exhibited more phytotoxicity than PANQ6521R at all planting depths. The effect of seed size on acetochlor tolerance could not be determined with any clarity. Cultivar sensitivity to the herbicide as well as seed shape appeared to also play a role. In DKC 73-76R it appeared as though large flat seeds produced plants more tolerant to acetochlor than smaller flat seeds.

The effect of soil type on acetochlor phytotoxicity on a tolerant (PANQ6521R) and sensitive (DKC 73-76R) maize cultivar was examined in two trials, one using an average application rate on all soils, and the second utilizing the recommended application rates for each soil. Acetochlor activity was found to be lowest on the clay loam soil (38% Clay) and highest on the sandy soil (8% Clay) in the first case, with severe phytotoxicity occurring on the latter soil. In the second trial bioactivity was similar on all soils, although greater on the sand. This indicated that the recommended application rate on sandy soils might need adjustment.

The influence of temperature on maize tolerance to acetochlor was investigated at sub-optimal (15/8°C), optimal (25/18°C) and supra-optimal (35/28°C) temperature regimes (day/night temperature respectively). Seedling growth of both cultivars was inhibited significantly by acetochlor at the optimum temperature regime (25/18°C). All results confirmed that DKC 73-76R was more sensitive to acetochlor than PAN6Q521R.

Keywords: Acetochlor, clay percentage, maize cultivar, phytotoxicity, planting depth, seed size, temperature, tolerance

OPSOMMING

‘n Aantal mielie cultivars wat in die handel beskikbaar is, is getoets vir hulle sensitiwiteit teenoor asetochlor. Betekenisvolle verskille m.b.t. sensitiwiteit van hierdie cultivars is gevind, met sommige cultivars wat verdraagsaam was teenoor die onkruidodder, terwyl ander sensitief was. Die cultivar PAN6Q521R het die hoogste verdraagsaamheid getoon, terwyl DKC 73-76R die sensitiefste was. Die meeste ander cultivars was intermediêr in hulle verdraagsaamheid. Hierdie gee ‘n aanduiding dat die verdraagsaamheid van mielies teenoor asetochlor geneties beheer word.

Die invloed van plantdiepte op die sensitiwiteit van mielies teenoor asetochlor is ondersoek met beide cultivars. Dit is gevind dat beide cultivars meer sensitief geraak het na mate die plantdiepte na 45 mm toegeneem het. Saailinge van DKC 73-76R het grootter fitotoksisiteit getoon as die van PAN6Q521R by alle plantdieptes. Die invloed van saadgrootte op asetochlor verdraagsaamheid kon nie met sekerheid bepaal word nie. Cultivar sensitiwiteit teenoor die onkruidodder asook saadvorm het skynbaar ‘n rol hier gespeel. Dit blyk dat groot plat sade saailinge geproduseer het wat meer verdraagsaam teenoor asetochlor was as die van klein plat sade in DKC 73-76R.

Die invloed van grondtipe op asetochlor fitotoksisiteit op ‘n verdraagsame (PAN6Q521R) en sensitiewe (DKC 73-76R) cultivar is in twee proewe getoets. Die eerste proef het ‘n gemiddelde toedienings hoeveelheid van die onkruidodder op alle gronde gebruik, terwyl die tweede proef die aanbevole toedieningshoeveelheid vir elke grond gebruik het. Asetochlor aktiwiteit was die laagste op die klei-leem grond (38% klei) en die hoogste op die sand grond (8% klei) in die eerste proef, en ernstige fitotoksisiteit het op die sandgrond voorgekom. Bioaktiwiteit was soortgelyk op alle gronde in die tweede proef, alhoewel dit strawwer was op die sandgrond. Hierdie het ‘n aanduiding gegee dat die aanbevole toedieningspeil op die sanderige grond moontlik aangepas behoort te word.

Temperatuur se invloed op die verdraagsaamheid van mieliecultivars teenoor asetochlor is getoets by sub-optimale (15/8°C), optimale- (25/18°C) en supra-optimale (35/28°C) temperatuur regimes (dag-/nagtemperatuur). Saailinggroei van beide cultivars is betekenisvol onderdruk deur die onkruidoder by die optimale temperatuur regime. Alle resultate het aangedui dat die cultivar DKC 73-76R meer sensitief is teenoor asetochlor as PAN6Q521.

Sleutelwoorde: Asetochlor, fitotoksiteit, kleipersentasie, mieliecultivar, plantdiepte, saadgrootte, temperatuur, verdraagsaamheid.