INFLUENCE OF PRE-EMERGENCE HERBICIDES ON GROWTH AND YIELD OF DRY BEAN CULTIVARS

by

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ABSTRACT

Variable tolerance to herbicides occurs amongst cultivars of several crop species, including: dry beans (*Phaseolus vulgaris* L. and *P. coccineus* L.), maize (*Zea maize* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L). De Beer (1988) reported that several dry bean plantings suffered from acetanilide herbicide injury during the 1982 / 83 season. Mennega, Nel & Le Court de Billot (1990) and Fouché (1996) have also reported differences in herbicide tolerance between dry bean cultivars in South Africa. Due to expected differences in herbicide tolerance between local dry bean cultivars, a study was undertaken to evaluate the influence of selected pre-emergence herbicides on: (a) the growth and yield of 10 cultivars; (b) seed yield on four soil types; and (c) the use of chlorophyll *a* fluorescence as a tool for screening the herbicide tolerance of cultivars. Dry bean morphology and cell ultrastructure were also evaluated, and guidelines were developed for routine assessments of the herbicide tolerance of dry bean cultivars. Using the parameters of plant growth and seed yield, the tolerance of dry bean cultivars to pre-emergence herbicides was compared in a field trial at the Grain Crops

Institute of the Agricultural Research Council (ARC) in Potchefstroom (North West Province) during the 1996/97 growing season. Tolerance of the dry bean cultivar Helderberg was investigated in a field trial in each of the districts Chrissiesmeer, Lichtenburg, Potchefstroom and Reitz during 1996/97. Completely unfolded leaves of cultivar Helderberg, grown in a glasshouse in the presence of herbicides for 14 days, were sampled to study the influence of herbicides on cell ultrastructure by means of transmission electronmicroscopy. Chlorophyll *a* fluorescence was measured on the primary leaves and first trifoliate leaf of cultivars Kranskop and OPS-RS1 21 days after planting, after a dark adaptation period of 25 min., with a fluorescence measuring system (Plant Efficiency Analyser, Hansatech, UK). In the field experiment at Potchefstroom, cultivars Kranskop, Monati and Cerillos were significantly less tolerant than Helderberg, Teebus, Katberg and SSN1, based on seed yield. In general, the dry bean cultivars were significantly more tolerant to dimethenamid, imazethapyr or metazachlor than to flumetsulam + metolachlor or metolachlor. The 2x-rate of herbicides caused an overall significant reduction in seed yield. In respect of foliage dry mass, Kranskop, Monati and Cerillos were less tolerant than the other seven cultivars. In the series of field experiments done at four localities, the only significant reductions in yield of cultivar Helderberg were caused by flumioxazin. In assessing the potential of chlorophyll *a* fluorescence as a tool for predicting herbicide tolerance, dimethenamid, flumioxazin, flumetsulam + metolachlor and metazachlor caused significant decreases in instantaneous fluorescence yield (Fo) of the primary leaves of Kranskop. For the same cultivar, metazachlor had a similar effect in the first trifoliate leaf. Both dimethenamid and flumioxazin caused significant decreases in Fo of the first trifoliate of cultivar OPS-RS1. The ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) of the primary leaves was significantly increased by flumioxazin, flumetsulam + metolachlor and metazachlor. Significant decreases in Fo and significant increases in Fv/Fm are typical of herbicides acting at Photosystem II of the photosynthesis process. Further research regarding the extent to which

herbicides with this site of action might be expected to influence yield is suggested. The electronmicroscopy study indicated ultrastructural changes in leaves treated with various herbicides. None of the herbicides caused drastic changes in the structure of chloroplasts. Except for imazethapyr, herbicides did cause a reduction in the number of stroma and granum lamellae. With the exception of imazethapyr-treated plants, starch granules in treated plants appeared depleted and were rounder in shape than those of control plants. Disruption of mitochondria was characterized by swollen and chaotically arranged crystae, except in imazethapyr-treated plants. These changes are probably manifestations of inhibition by imazethapyr of acetyl Co enzyme A which is needed in the formation of chlorophyll, and is an important part of the mitochondria-based Krebs cycle. As a result of this mechanism of action, both photosynthesis and respiration efficiency should be influenced negatively, which in turn would adversely affect plant growth and yield. This study confirmed the existence of differential tolerance to herbicides amongst *P. vulgaris* and *P. coccineus* cultivars. More research, especially field trials, should be conducted to identify high-risk herbicide / cultivar combinations.

INTRODUCTION

The main crops produced in the summer rainfall region of South Africa are dry beans (*Phaseolus vulgaris* L. and *P. coccineus* L.), groundnuts (*Arachis hypogaea* L.), maize (*Zea mays* L.), sorghum (Sorghum vulgare L.), soybeans (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and wheat (*Triticum aestivum* L). For the most important crop, maize, yields of approximately eight million tons have been produced annually over the last decade on ± 3.5 million hectares (ARC-Grain Crop Institute & NAMPO, 1999). The total production of dry beans in South Africa was 71 446 tons on approximately 69 052 hectares in 1999/2000, and averaged 58 000 tons on ± 56 000 hectares over the last decade.

The genus *Phaseolus* consists of more than 50 herbaceous species that range in type from annual to perennial, prostrate to erect, climbing to bush. According to Lloyd & Liebenberg (1986) three of these species are of agricultural importance in South Africa, i.e. *Phaseolus vulgaris* L. (common dry bean and green beans), *Phaseolus coccineus* L. (kidney bean) and *Phaseolus acutifolius L.* (tepary bean). The common dry bean is the most widely grown of the three species. Currently there are 51 cultivars on the dry bean variety list. There are eight seed types which differ in size, colour and shape, and within each seed type there are cultivars that differ in agronomic adaptation, disease resistance and other properties (Liebenberg & Steenekamp, 1997).

Inadequate weed control is a major problem in dry bean production. Weeds compete vigorously with dry beans and yield reductions exceeding 70% have been recorded (Parker & Fryer, 1975; Blackshaw & Esau, 1991). Dawson (1964) showed that the first five to seven weeks after planting is the most critical period for beans in terms of weed control. Weeds emerging during this period were more competitive than those germinating later. Weeds that emerged above the bean canopy were the most competitive.

Mechanical weed control has various disadvantages, e.g. weeds directly in the crop row are not removed, and weather conditions can prevent control of weeds at the most critical times (Nel, 1973). Control by early post-emergence herbicides is another alternative but could similarly be delayed, and could be more expensive than pre-emergence herbicides and should only be used in emergencies. That is, for example, when pre-emergence herbicides fail to control the weeds adequately. The use of effective pre-emergence herbicides in combination with mechanical control seems highly desirable. There is, however, a very fine balance between optimum weed control and maximum crop safety.

Cultivar tolerance to herbicides has been variable for numerous grain crops such as maize (Renner, Meggitt & Penner, 1988), rice (*Oryza sativa* L.) (Snipes, Street & Boykin, 1987), soybean (Barrentine, Hartwig, Edwards & Kilen, 1982; Buzzell & Hamill, 1988; Fourie, Rothman & de Beer, 1990; Osborne, Shaw & Ratliff, 1995), sunflower (Meissner,

Nel & Beyers, 1987), and wheat (Runyan, McNeill & Peeper, 1982; Driver, Peeper & Guenzi, 1992). Various researchers have reported differences in herbicide tolerance between dry bean cultivars (De Beer, 1988; Mennega, Nel & Le Court de Billot, 1990; Wilson & Miller, 1991; Arnold, Murray, Gregory & Smeal, 1993; Fouché, 1996; Urwin, Wilson & Mortenson, 1996).

In light of expected differences between dry bean cultivars in respect of herbicide tolerance, a study with the following objectives was undertaken to:

- Evaluate the influence of dimethenamid, imazethapyr, flumetsulam + metolachlor, metazachlor and metolachlor on the growth and yield of ten dry bean cultivars.
- Evaluate the influence of soil type (locality) on dry bean tolerance to dimethenamid, imazethapyr, flumetsulam + metolachlor, flumioxazin, metazachlor and metolachlor.
- Assess the use of chlorophyll *a* fluorescence as a tool for screening dry bean cultivars for herbicide susceptibility.
- Study the influence of dimethenamide, flumetsulam + metolachlor, metazachlor and metolachlor on dry bean morphology and cell ultrastructure.
- Set guidelines for the routine assessment of the herbicide tolerance of dry bean cultivars.

CHAPTER 1

LITERATURE REVIEW

RESPONSES OF DRY BEANS TO SOIL-APPLIED HERBICIDES

Presently, at least 19 active ingredients (Table 1) are registered in South Africa for use in common dry bean (*P. vulgaris*) and kidney bean (*P. coccineus*). Nine of these are applied pre-emergence, seven post-emergence, and the other three are pre-plant incorporated herbicides (Vermeulen, Dreyer, Grobler & Graber, 1998).

Several herbicides and herbicide combinations are used in dry beans. These herbicides effectively control many weed species but some of them have affected either dry beans or related crops negatively. Crop injury does not always culminate in measurable yield losses. The earliest and most obvious negative effects are reflected in plant morphology. Even though some changes in morphology may occur, plants may recover without any yield losses. Morphological changes caused by herbicides in beans and related crops reportedly include: shorter, larger or distorted stem growth; changes in leaf number, size and photosynthetic surface; and pruned, distorted or proliferated root growth (De Beer, 1988; Nkwen-Tamo, Jeffery, Robison & Jolley, 1989; Wilson & Miller, 1991; Johnson & Mullinix, 1996; Urwin *et al.*, 1996).

Table 1 Active ingredients and nomenclatures of herbicides registered for use in dry beans in South Africa (from Vermeulen, Dreyer, Grobler & Graber, 1998)

Active ingredient	Nomenclature
Pre-plant incorporated:	
EPTC	[s-ethyl dipropylcarbamothioate]
Pendimethalin	[N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine]
Trifluralin	[2,6-dinitro-N,N-dipropyl-4-trifluoromethyl) benzenamine]
Pre-emergence:	
Acetochlor	[2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6- methylphenyl) acetamide]
Alachlor	[2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetamide]
Dimethenamid	[2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-y1) acetamide]
Flumetsulam	[N-(2,6-difluorophenyl)-5-methyl [1,2,4]-triazolo [1,5-a] pyrimidine-2-sulfonamide]
Flufenacet	[N-(4-fluorophenyl)-N-(1-methylethyl)-2[[5(trifluoromethyl)-1,3,4-thiadiazol-2yl]oxy]acetamide]
Imazethapyr	[(±)-2-[4,5-dihidr-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-y1]-5-ethyl-3-pyridinecarboxylic acid]
Metazachlor	[2-chloro-N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl) acetamide]

Metolachlor	[2-chloro-N-(2-ethyl-6-methylphenyl)-N-(methoxy-1-methylethyl) acetamide]
Propaquizafop	[(R)-2-[[(1-methylethylidene)amino]oxy]ethyl-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy]propanoate]
Post-emergence:	
Bendioxide	[3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide]
Cycloxidim	[2-[1-(ethoxyimino)butyl]-3-hydroxy-5-(2H-tetrahydrothiopiran-3-y1)-2-cyclohexen-1-one]
Fluazifop-P-butyl	[(R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid]
Fomesafen	[5-[2-chloro-4-(trifluoromethyl)phenoxyl]-N-(methylsulfonyl)2-nitrobenzamide]
Haloxyfop-R-methyl	[methyl2-[4-[[3-chloro-5-ester(trifluoromethyl)-2-pyridinyl]oxy]phenoxy] propanoate]
Quizalofop-P-ethyl	[(±)-2-[4-[(6-chloro-2-uinozalinyl)-oxy]phenoxy]propanoic acid]
Sethoxydim	[2-[1-(ethoxyimino) butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one]

Cultivar tolerance to herbicides

Cultivar tolerance to herbicides is reportedly variable for diverse grain crops, e.g., soybean (Barrentine *et al.*, 1982; Buzzell & Hamill, 1988; Fourie *et al.*, 1990; Osborne *et al.*, 1995), sunflower (Meissner *et al.*, 1995), maize (Renner *et al.*, 1988), wheat (Runyan *et al.*, 1982; Driver *et al.*, 1992) and rice (Snipes *et al.*, 1987).

Various researchers have shown differences between dry bean cultivars with regard to herbicide tolerance. Mennega *et al.* (1990) found that the large white kidney bean (cv SSN1) is the most tolerant cultivar to atrazine in South Africa. Cultivars Maskam (painted lady) and Majuba (yellow sugar bean) also showed some degree of tolerance. According to Mennega *et al.* (1990) large-seeded cultivars and lines are the most tolerant while small-seeded cultivars and lines are the most susceptible to atrazine.

Wilson & Miller (1991) found that the light-red kidney cultivar Sacramento and the pinto cultivar Agate were injured less by imazethapyr than the great northern cultivars Beryl and GN1140 or pinto cultivars UI114 and Olathe. Bauer, Renner, Penner & Kelly (1995) found similar differences in herbicide tolerance with imazethapyr application to pinto cultivars Sierra and Olathe.

Differences in bean tolerance to dimethenamid also exist (Renner, 1997). Adzuki beans are very sensitive to most herbicides and will not tolerate alachlor, dimethenamid or

metolachlor. Dark and light red kidney beans, pinto beans and cranberry beans are tolerant to pre-emergence applications of dimethenamid. The two bean classes that appear to be least tolerant to dimethenamid are navy and black beans. Renner (1996) applied metolachlor and dimethenamid at the recommended and double recommended rates to several cultivars and classes of beans. They found no injury to any pinto, great northern, kidney or cranberry cultivars from dimethenamid or metolachlor at both rates. Some injury to the navy and black bean cultivars was observed from both dimethenamid and alachlor. Dimethenamid caused more injury than metolachlor to the navy and black bean cultivars.

Fouché (1996) reported that the small white cultivar Helderberg varied in tolerance to pre-emergence herbicides from tolerant to less tolerant in the order: dimethenamid > imazethapyr > metolachlor > metazachlor > flumetsulam + metolachlor.

Herbicide mechanisms of action

A herbicide's mechanism of action is the precise biochemical (e.g. inhibition of a specific enzyme) or biophysical (e.g. inhibition of electron flow or binding to a protein and disrupting cell division) lesion that creates the herbicide's final effect (Zimdahl, 1993). Herbicides can have a primary and secondary mechanism of action (e.g. fluometuron's primary mechanism of action is the inhibition of photosynthesis and it acts secondarily by inhibition of carotenoid synthesis). A herbicide's mode of action is the entire

sequence of events that occur from the moment it first contacts the plant until its final effect is expressed. Mechanism of action can be subsumed under mode of action, but the reverse is never true. Herbicides have three major mechanisms of action: 1) inhibition of respiration and photosynthesis, 2) inhibition of plant growth, and 3) inhibition of biosynthetic processes. The latter is accomplished in five different ways, i.e. action on cell division or mitosis, nucleic acid or protein synthesis, amino acid synthesis, carotenoid synthesis and lipid synthesis. There are, however, herbicides with a nonspecific and others with an unknown mechanism of action.

Two chemical classes of herbicides used in dry beans inhibit acetolactate synthase (ALS): imidazolinones (imazethapyr) and triazolopyrimidine sulfonanilides (flumetsulam). ALS is an essential enzyme in the biosynthesis pathway of the branched-chain amino acids valine, leucine and isoleucine in plants (Shaner, 1991). ALS inhibition stops protein synthesis and causes decreased photosynthate translocation to meristems that leads to rapid cessation of cell division and plant growth.

The acetanilides (acetochlor, alachlor, dimethenamid, flufenacet, metazachlor and metolachlor) inhibit early seedling growth. This effect is most evident on root growth. These responses appear to be associated with an interference with both cell division and cell enlargement. They do not appear to inhibit seed germination but they usually kill or affect susceptible plants before emergence from the soil (Ashton & Monaco, 1991). There are contradictory evidence on the effect of the chloroacetamides (alachlor

and metolachlor) on *de novo* fatty acid biosynthesis and thus on membranes. The primary mechanism of action has not been determined and their classification could change (Zimdahl, 1993).

The dinitroanilines inhibit growth of the entire plant. This is, however, brought about by initially limiting root growth, especially the development of lateral or secondary roots (Ashton & Crafts, 1973). The roots which do develop, often only the primary roots, are somewhat thickened, stubby and are devoid or only have a limited number of secondary roots. Talbert (1965) showed that root growth inhibition is associated with the cessation of cell division in the meristematic tissue.

The aryloxyphenoxy propionic acids include fluazifop-P-butyl, haloxyfop-R-methyl ester, propaquizafop and quizalofop-P-ethyl. The most obvious phytotoxic symptoms of fluazifop-P-butyl, haloxyfop-R-methyl ester and quizalofop-P-ethyl are foliar chlorosis and necrosis. Carr, Davies, Cobb & Pallett (1985) suggested that the mechanism of action involves altered lipid metabolism. This may be related to inhibition of acetyl-CoA carboxylase.

EPTC is a carbamothioate and symptoms include the lack of seedling emergence or grossly distorted emerging shoots. The edges of the coleoptile of the emerging shoot are often fused, and distorted young leaves may emerge through the side of the coleoptile. Carbamothioates also inhibit growth in general by interfering with cell division

and / or cell enlargement in meristems (Dawson, 1963; Parker, 1963). Cyclohexendiones (cycloxidim and sethoxydim) act by inhibiting lipid biosynthesis (acetyl-CoA carboxylase) and possibly flavonoid biosynthesis (Focke & Lichtenhaler, 1987; Rendina & Felts, 1988).

Diphenyl ethers (fomesafen) act by lipid peroxidation (light-induced oxidative breakdown of cell constituents) (Kunert, Sandmann & Boger, 1987). Further research indicated that the diphenyl ethers cause an accumulation of tetrapyroles and these compounds may in turn induce lethal photo-oxidative reactions (Witkowski & Halling, 1988; Matringe & Scalla, 1998).

Metabolism of herbicides — detoxification as a basis of selectivity

Herbicides recommended for the selective control of weeds have been developed to exploit differential sensitivity between species, in order that competing weeds are killed or harmed without significantly reducing crop yields. In some cases the margin of selectivity may be quite modest and can be rendered inadequate when the timing of application coincides with unfavourable climatic conditions (Owen, 1989). There are a number of factors which can contribute to herbicide selectivity, including soil placement, rates of absorption and subsequent translocation, localisation (both within the plant and at the sub-cellular level) and transformation to products of modified phytotoxicity.

The selective properties of herbicides often result from a complex interaction of these factors, although there are many examples where one dominant factor has been implicated. This is certainly true in the case of herbicide uptake and movement (Hess, 1985) and differential metabolism to less phytotoxic products (Owen, 1989). The mechanisms for metabolism of herbicides in plants are varied, e.g., oxidation reactions that result in aromatic ring- and alkyl hydroxylation, *N*-dealkylation, *O*-dealkylation and sulphoxidation, hydrolytic reactions, deamination, and conjugation with carbohydrate residues (glycosidation) or the tripeptide glutathione (Jensen, 1982; Shimabukuro, Lamoureux & Frear, 1982; Cole, 1983; Shimabukuro, 1985; Hathway, 1986; Cole, Edwards & Owen, 1987).

Metabolism of herbicides by plants can occur via a three-phase process (Shimabukuro, 1985). Phase I reactions (oxidation, reduction or hydrolysis) are initial reactions that generally detoxify herbicides and predispose the resultant metabolite to conjugation (Phase II reactions) with sugars, amino acids or other natural plant constituents. Phase III reactions are unique to plants and involve secondary reactions or the formation of insoluble bound residues. In general, plants "immobilize" these metabolites whereas animals excrete them.

Detoxification of selected herbicides

Acetanilides. Grasses absorb these herbicides (dimethenamid, metazachlor, metolachlor) mainly through the emerging shoots whereas broadleaf plants absorb them through both emerging shoots and roots. Their translocation appears to be mainly in the apoplast (xylem). Limited symplastic (phloem) transport may also occur. Most evidence suggests that the acetanilides or their degradation products undergo conjugation with glutathione and / or glucose (Duke, 1985). Hydrolysis of the parent molecule may also occur. Differential rates of detoxification by conjugation with glutathione of the acetanilide herbicides may explain differences in tolerance among plant species and even cultivars.

Imidazolinones. These herbicides (imazethapyr) are readily absorbed by roots and leaves and are translocated in both the symplast and apoplast. Rapid metabolic degradation of these herbicides in tolerant crops is their basis of selectivity (Ashton & Monaco, 1991).

Triazolopyrimidine sulfonanilides (flumetsulam). Flumetsulam is absorbed by both the roots and foliage of plants and translocated to the growing points. The relative susceptibility of plant species towards the herbicide is a function of the time required for absorption and translocation, as well as the rate of metabolism within the plant (Chambers, 1997).

CHLOROPHYLL FLUORESCENCE AS A TOOL FOR PREDICTING HERBICIDE DAMAGE ON A PHYSIOLOGICAL BASIS

The photosynthetic electron transport pathway

The light energy conversion process of photosynthesis is located in the grana of the chloroplasts, while the reduction of carbon dioxide occurs within the stroma. Grana consist of stacks of thylakoids, i.e. vesicle-like structures having an internal space surrounded by a membrane. The grana are interconnected by unappressed stroma thylakoids. The thylakoid membranes contain the electron- and proton- translocating components (Figure 1).

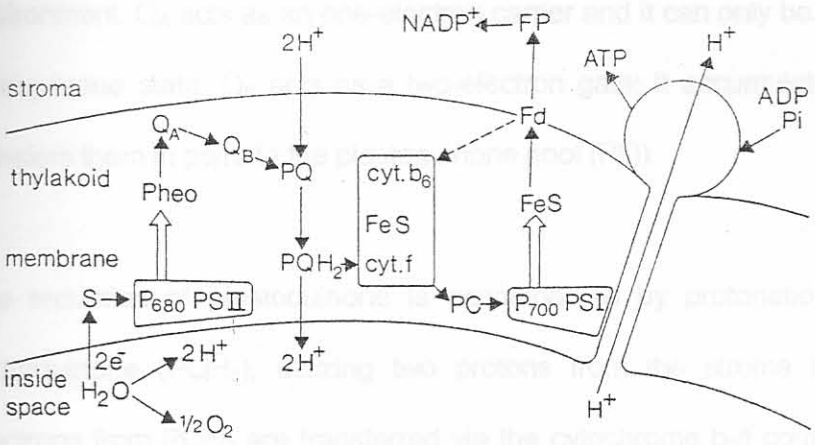


Figure 1 Electron and proton transport pathways in photosynthetic electron flow (from Van Rensen, 1989)

Photosynthesis is initiated by the absorption of light energy by the chlorophylls of both photosystems (PS II and PS I). Exitation energy is then transferred to the reaction centres: P680 in PS II and P700 in PS I. P680 and P700 are specialized chlorophyll *a* molecules which are able to accomplish a charge separation, resulting in $P680^+$ and $Pheo^-$ in light reaction II, and $P700^+$ and FeS^- in light reaction I (Pheo represents pheophytin; FeS are several iron sulphur centres) (Van Rensen, 1989). The charge separations are followed by electron- and proton- translocating reactions. The electron hole of $P680^+$ is filled via various steps, denoted in Figure 1 as S, by an electron which is ultimately derived from water. This water-splitting not only yields electrons, but also protons and oxygen. From $Pheo^-$, the electron is transported to the first stable quinone electron acceptor Q_A and then to the secondary acceptor Q_B. Because of its micro-environment, Q_A acts as an one-electron carrier and it can only be reduced as far as the semiquinone state. Q_B acts as a two-electron gate: it accumulates two electrons and transfers them in pairs to the plastoquinone pool (PQ).

The reduction of plastoquinone is accompanied by protonation to a fully reduced hydroquinone (PQH_2), utilizing two protons from the stroma (Van Rensen, 1989). Electrons from PQH_2 are transferred via the cytochrome b_6/f complex to plastoquinone (PC). During this reaction two protons from PQH_2 are liberated into the internal space of the thylakoid. PC is the primary electron donor to $P700^+$. From FeS^- the electron is transferred to Fd (soluble ferredoxin) and via ferredoxin $NADP^+$ reductase (FP) to $NADP^+$. Under certain conditions a cyclic electron flow is possible by transfer of

electrons from Fd to the cytochrome b_6/f complex. The protons which have been accumulated in the internal space of the thylakoid can flow back to the stroma through the ATP-ase, which phosphorylates ADP to ATP.

Chlorophyll *a* fluorescence

In recent years, chlorophyll *a* fluorescence measurements have been increasingly applied to various aspects of plant physiology (Krause & Weis, 1984). The concept is that the energy content of the blue and red component of light is absorbed by the chlorophyll molecules, and that this energy is then used in a variety of processes. Some are used to drive the chemical reactions of photosynthesis; the remainder is lost as heat, radiationless de-excitation and re-emission as light known as fluorescence (Lavorell & Ettiene, 1977). Chlorophyll can be regarded as an intrinsic fluorescent probe of the photosynthetic system. In the leaf or algal cell, the yield of fluorescence is influenced in a very complex manner by events that are, directly or indirectly, related to photosynthesis (Krause & Weis, 1984).

Several investigators (Daniell, Sarojini, Kumarachinnayan & Kulandaivelu, 1981; Voss, Renger, Kotter & Graber, 1984; Habash, Percival & Baker, 1985; Ahrens, 1989) have used non-destructive fluorescence techniques to measure indirectly the kinetics of absorption and metabolism of Photosystem II-inhibiting herbicides in intact leaves. Use of fluorescence for this purpose is based on the principle that fluorescence yield

increases as the PS II inhibitor binds to the 32 to 34 kDalton polypeptide associated with Q_B, consequently blocking electron transport which causes Q_A to remain in the reduced state. Q_A and Q_B are the primary and secondary quinone acceptors of PS II (Mullet & Arntzen, 1981)

A plant capable of metabolizing a herbicide would presumably reduce the cellular concentrations, thereby reducing the percentage of PS II reaction centres that will be affected. This should lower fluorescence yield. Thus, fluorescence should decline relatively rapidly for a plant species having a high rate of herbicide metabolism, but decline slowly or not at all for species having a low rate of metabolism (Ahrens, 1989).

The concequences of electron transport inhibition

The displacement of Q_B and the failure to pass electrons to the plastoquinone pool will lead to an inhibition of $NADP^+$ reduction and hence, indirectly CO_2 incorporation. Although early workers suggested that photosynthetic inhibition would lead to plant starvation, the appearance of phytotoxic symptoms such as chlorophyll bleaching was enhanced if plants were illuminated (Minshall, 1957; Ashton, 1965). This has given rise to the concept that when light energy absorbed by photosynthetic pigments is not utilized, it will overtax the normal chloroplast protective system and lead to chloroplast damage and bleaching. Duyens & Sweers (1963) first demonstrated that fluorescence emission was enhanced if algae were treated with diuron. Since that time numerous

investigators (Voss *et al.*, 1984; Habash *et al.*, 1985) have used chlorophyll fluorescence to study not only inhibition of electron flow, but also to monitor the rate of herbicide penetration into plants.

The absorption of light energy by a photosynthetic pigment such as chlorophyll will promote the generation of the singlet state with a lifetime of around 10^{-6} to 10^{-8} s. In addition to the emission of fluorescence, which indicates energy wastage, excitation energy is passed by resonance transfer to excite the acceptor P680. $P680^{+}$ accumulates because the displacement of Q_B as pheophytin is reduced, the strongly oxidizing potential could instigate damage to adjacent pigments and polypeptides. It is possible that P680 could be dissociated from the light-harvesting antennae of PS II (Cleland, Melis & Neale, 1986).

The conversion of singlet chlorophyll by intersystem crossing to the triplet state could be equally damaging. Triplet chlorophyll can directly interact in a damaging way with pigments, proteins and lipids. Furthermore, it can interact with triplet or ground state oxygen ($^3O_2$) to generate the potentially damaging singlet oxygen ($^1O_2$). Investigations with leaves (Pallet & Dodge, 1980) and isolated chloroplasts showed that photodynamic damage was enhanced by the prescence of oxygen. Recent studies with isolated PS II reaction centres (McTavish, Picorel & Seibert, 1989) have shown that they are particulurly susceptible to photodynamic damage, particularly in the presence of oxygen.

In the presence of PS II inhibitors, excitation energy generated by p680 can not be dissipated by normal electron flow beyond Q_A^-, and so fluorescence yield is dramatically enhanced and activated oxygen species generated. Under these conditions the natural protective mechanisms are rapidly overloaded, especially at increased temperatures and photon flux densities, and lipid peroxidation is initiated in thylakoids by hydrogen abstraction (Cobb, 1992).

The free radical attacks unsaturated membrane fatty acids and is quenched by hydrogen atom abstraction. Since a hydrogen atom has only one electron, it leaves behind an unpaired electron on a carbon atom. This carbon (lipid) radical rapidly reacts with oxygen to yield a hydroperoxy radical, which is itself able to abstract hydrogen atoms from other unsaturated lipid molecules, thus initiating a chain reaction of lipid peroxidation. Eventually the unsaturated fatty acids of the thylakoid are totally degraded to malondialdehyde and ethane, and the appressed thylakoid structure progressively opens up and disintegrates. Finally, cell membranes and tissues disintegrate from this chain reaction of free radical attack (Sanders & Pallett, 1986; Derrick, Cobb & Pallett, 1988; Cobb, 1992).

One paradoxical feature of PS II herbicide-induced damage is that binding of the herbicide to the D1 protein (protein of 30 kDa size in the reaction centre of PS II) actually inhibits D1 turnover. Damage to the protein has been suggested to be catalyzed by the Q_B semiquinone radical, which could induce damage directly or indirectly by

reducing oxygen to superoxide (Mattoo, Sopery, Greenberg, Callahan, Ghirardi & Edelman, 1989). In normal circumstances it would appear that D1 turnover and repair is essential for the maintenance of a fully functional photosynthetic system (Dodge, 1991).

The practical consequences of the above are that plants with PS II herbicide-induced damage will have a reduced CO_2 incorporation, and damaged and bleached chloroplasts. This will result in a lower growth rate and eventually lower yields in the case of cultivated plants.

CHAPTER 2

EFFECT OF PRE-EMERGENCE HERBICIDES ON THE GROWTH AND YIELD OF DRY BEAN CULTIVARS

Introduction

Not only does the interaction of herbicides with various environmental factors (physical, chemical and biological) impact on yield (Wood, Powell & Anderson, 1977) but the interaction with plant factors, of which genetic content is of major importance, may also influence yield negatively. Various mechanisms exist by means of which plants transform foreign compounds. These include oxidation reactions that result in aromatic ring- and alkyl hydroxylation, N-dealkylation, O-dealkylation and sulphoxidation, hydrolic reactions, deamination, and conjugation with carbohydrate residues (glycosidation) or with the tripeptide glutathione (Jensen, 1982; Shimabukuro *et al.*, 1982; Cole, 1983; Shimabukuro, 1985; Hathway, 1986; Cole *et al.*, 1987).

The acetanilides (e.g. dimethenamid, metazachlor, metolachlor) and their degradation products undergo conjugation with glutathione and / or glucose (Duke, 1985), whereas rapid metabolic degradation of the imidazolinones (e.g. imazethapyr) (Ashton & Monaco, 1991) and triazolopyrimidine sulfonamides (e.g. flumetsulam) (Chambers, 1997) may explain their differential tolerance.

Differences between dry bean cultivars with regard to herbicide tolerance have been shown by various researchers (De Beer, 1988; Mennega *et al.*, 1990; Wilson & Miller, 1991; Arnold *et al.*, 1993; Fouché, 1996; Urwin *et al.*, 1996). De Beer (1988) demonstrated the importance of environmental and genetic factors in the response of dry beans to alachlor and metolachlor. Cultivar Nuweveld was found to be less tolerant to alachlor and metolachlor than either Nep 2 or Kamberg. The tested cultivars were also less tolerant to alachlor than to metolachlor. Fouché (1996) reported that the small white cultivar Helderberg varied in tolerance to pre-emergence herbicides from tolerant to less tolerant in the order: dimethenamid > imazethapyr > metolachlor > metazachlor > flumetsulam + metolachlor.

Dimethenamid (Frontier® 900 EC), flumetsulam + metolachlor (Bateleur® 816 EC), imazethapyr (Hammer® 100 SL) and metazachlor (Pree® 400 EC) are pre-emergence herbicides used to control certain broadleaf weeds and grasses (Vermeulen *et al.*, 1998). Imazethapyr is also registered for post-emergence application. Metolachlor (Dual-S® 930 EC) is a pre-emergence herbicide registered for control of grasses in beans. Pree® 400 EC has since undergone a name change to Preecede® 400 EC and was withdrawn from the market in 1998.

The present study was conducted to evaluate the effects of these pre-emergence herbicides on the growth and yield of 10 South African dry bean cultivars. These cultivars are divided into eight seed types on the basis of size, colour and shape. Within

each seed type there are cultivars that differ in agronomic adaptation, disease resistance and other properties (Liebenberg & Steenekamp, 1997). It is envisaged that the results of this study could be used to identify yield-reducing cultivar / herbicide combinations, thus adding another dimension to the selection of cultivars.

Materials and Methods

The tolerance of 10 dry bean cultivars to five pre-emergence herbicides was investigated in a field trial at the Grain Crops Institute of the Agriculture Research Council (ARC-GCI) in Potchefstroom (North West Province) during the 1996/97 growing season.

The trial was conducted on a clay loam soil (34 % clay) with a pH(H_2O) of 6.5, and the following macronutrient concentrations: P (23.7 mg kg^{-1}), K (122.0 mg kg^{-1}), Ca (1260.0 mg kg^{-1}) and Mg (385.0 mg kg^{-1}). Standard fertilizer [2:3:4 (30) + urea] was broadcast at a rate (60 kg ha^{-1}) based on the soil analysis. The bean cultivars chosen represented the commercially important bean types in South Africa at the time. They included the small white canning beans (Helderberg and Teebus), red-speckled sugar beans (Kranskop, Enseleni and Monati), Alubia bean (Cerrillos), yellow haricot bean (Katberg), carioca bean (Mkuzi) and yellow sugar bean (Majuba). The only available large white kidney bean cultivar, SSN1, was also used.

Seeds were hand-planted at a depth of 5.5 cm with an inter-row spacing of 91 cm and within-row spacing of 8 and 15 cm for *P. vulgaris* and *P. coccineus* respectively. The experimental design was a split-plot (main-plots = cultivars, sub-plots = herbicides) with three replicates. Each plot consisted of two rows of 2.5-m length. The herbicides were applied at two rates, i.e. registered and double the registered dosage (Table 2) and were sprayed diagonally across the rows with a CO_2 field sprayer which delivered 200 L of water per hectare at 2.5 kPa. Control plants were left untreated.

Sprinkler irrigation (25 mm) was applied immediately after herbicide application and was provided throughout the growing season (Table 1C in Appendix C). All plots were kept weed-free, by hand and implement, to eliminate weed interference.

The early effect of the herbicides on plant growth was measured 21 days after planting. Three plants of each treatment were harvested and their aboveground dry mass measured. Yield was eventually determined by harvesting two 1-m rows for each treatment. In addition, the 100-seed mass for each treatment was also determined using these samples. Data for seed yield and aboveground (top growth) dry mass were expressed as percentage of the corresponding controls (zero herbicide). Percentage data were subjected to statistical analysis for which standard analysis of variance (ANOVA) procedures were used. Means were compared at the 5% level of significance by using the least significant difference test of Tukey.

Table 2 Active ingredients, commercial names and registered dosages of herbicides used in the bean cultivar tolerance trial at Potchefstroom

Active ingredient (a.i.)	A.I. Concentration ($g\ L^{-1}$)	Product	Registered dosage ($L\ ha^{-1}$)
Dimethenamid	900	Frontier®	1.25
Flumetsulam + metolachlor	16 + 800	Bateleur®	1.90
Imazethapyr	100	Hammer®	0.50
Metazachlor	400	Pree®	1.50
Metolachlor	930	Dual®	2.00

Results and Discussion

Data for seed yield and aboveground dry mass are given in Table 3 and Table 4 respectively. Data for 100-seed mass (Table 3A of Appendix A) are not discussed in detail since this parameter did not provide more information on herbicide tolerance than did the other two parameters.

Seed yield. In the case of seed yield only the main effects were significant ($P = 0.05$) (Table 1B of Appendix B). The red-speckled sugar bean cultivars (Kranskop and Monati) and Alubia cultivar (Cerillos) were significantly less tolerant than the small white

(Helderberg and Teebus), yellow haricot (Katberg) and large white kidney (SSN1) bean cultivars (Table 3). The latter cultivar was the most tolerant. Mennega *et al.* (1990) showed that the large white kidney bean is the most tolerant dry bean cultivar to atrazine in South Africa while the small-seeded cultivars are the most susceptible. They ascribed these differences in tolerance to seed size. This seems not to have been the case in the results presented here since both the red-speckled sugar bean and Alubia cultivars have larger seed sizes than the small white, yellow haricot and yellow sugar cultivars.

The dry bean cultivars were significantly more tolerant to dimethenamid, imazethapyr or metazachlor than metolachlor or flumetsulam + metolachlor. This is in accordance with findings by Fouché (1996) who concluded that Helderberg is less tolerant to flumetsulam + metolachlor and metolachlor than dimethenamid or imazethapyr. As expected, the 2x-rate of herbicides caused an overall significant reduction in seed yield (Table 3).

Renner (1997) reported differences in tolerance towards dimethenamid of dry bean cultivars grown in Michigan (USA). Dark- and light-red kidney beans were most tolerant to dimethenamid. Wilson & Miller (1991) and Bauer *et al.* (1995) have also demonstrated differences in dry bean cultivar tolerance towards imazethapyr. Renner (1996) demonstrated differences in dry bean cultivar tolerance towards metolachlor. He concluded that metolachlor caused less injury to bean cultivars than dimethenamid. De

Beer (1988) also reported that South African dry bean cultivars varied in tolerance to alachlor and metolachlor. He concluded that the tested dry bean cultivars were less tolerant to alachlor than metolachlor at the recommended rates.

Table 3 Seed yield expressed as percentage of the control for ten dry bean cultivars exposed to five herbicides at both the recommended (1x) and 2x-rates on one soil (ANOVA in Table 1B of Appendix B)

Herbicides (a.i. g ha $^{-1}$)		Cultivar										Mean (Herbicide)
		Helderberg	Teebus	Kranskop	Enseleni	Monati	Cerillos	Katberg	Mkuzi	Majuba	SSN1	
Dimethenamid												
	1125	113.8	92.1	75.5	93.0	83.0	88.6	124.6	101.4	107.1	106.6	97.5
	2250	99.3	89.9	70.3	100.6	99.2	68.5	134.0	99.5	91.8	110.6	
Flumetsulam + metolachlor												
	1550	82.5	107.4	87.6	84.6	86.5	86.5	83.6	87.7	83.6	128.6	84.5
	3100	80.9	100.8	84.2	79.1	61.4	77.9	73.3	48.2	66.9	99.1	
Imazethapyr												
	50	119.7	98.1	91.8	102.2	89.7	104.3	132.1	108.5	110.2	116.1	101.8
	100	94.0	92.7	71.0	98.9	101.2	98.1	101.0	100.7	80.2	124.0	
Metazachlor												
	800	125.8	98.2	102.1	86.8	94.2	89.8	106.6	98.4	120.0	108.6	96.3
	1600	92.3	90.0	83.0	73.9	84.0	80.0	103.5	100.3	84.5	103.8	
Metolachlor												
	1860	99.0	88.0	92.5	106.4	82.4	90.2	109.1	96.9	105.0	90.8	91.7
	3720	86.5	82.9	80.6	79.8	79.5	86.4	100.7	77.5	68.8	131.6	
Mean (Cultivars)		99.4	94.0	83.9	90.5	86.1	87.0	106.8	91.9	91.8	112.0	
Mean	**Rate** **1**	99.3										
Mean	**Rate** **2**	89.3										
LSD_T (P = 0.05)		Herbicide = 4.39		Rate = 3.25		Cultivar = 7.25						

Aboveground dry mass. For aboveground dry mass all the main effects, except herbicide rate, were significant (Table 2B of Appendix B). Except for the herbicide x cultivar interaction, the other interaction effects for this parameter were significant also. Variation in plant size between the cultivars would have contributed to the significance of the herbicide x rate x cultivar interaction, and therefore, percentage data are appropriate for discussion (Table 4).

For dimethenamid, aboveground DM was not significantly reduced for cultivars Helderberg, Monati, Majuba and SSN 1 (Table 4). Kranskop was the least and Monati the most tolerant to dimethenamid. This parameter was significantly reduced by flumetsulam + metolachlor for cultivars Kranskop and Katberg. Kranskop was again the least and Helderberg and SSN 1 the most tolerant to flumetsulam + metolachlor.

Imazethapyr caused no significant growth reductions. Kranskop was, however, the least and SSN 1 the most tolerant to imazethapyr. For metazachlor, aboveground DM was significantly reduced for cultivars Kranskop, Enseleni, Cerillos and Majuba. Kranskop was the least tolerant and SSN 1 the most tolerant to metazachlor. In the case of metolachlor this parameter was significantly reduced for cultivars Kranskop, Enseleni, Cerillos and Mkuzi. Mkuzi was the least and SSN 1 the most tolerant to metolachlor.

At the recommended rate, the two red-speckled sugar bean cultivars (Kranskop and Monati) and the Alubia bean cultivar (Cerillos) were more susceptible than the other

seven cultivars (Table 4). The large white kidney bean was once again the most tolerant cultivar. Imazethapyr caused the least reduction in aboveground DM across cultivars.

At the 2x-rate, dimethenamid significantly reduced the DM of Kranskop, Enseleni and Cerillos (Table 4). At the same rate, flumetsulam + metolachlor significantly reduced the DM of Helderberg, Kranskop, Enseleni, Cerrillos and Katberg. Also at that rate, imazethapyr caused a significant reduction in DM of Enseleni, and metazachlor inhibited the growth of all cultivars, except Teebus, Monati and SSN1. Metolachlor (2x-rate) significantly reduced the DM of Kranskop, Enseleni and Katberg. The phenomena that in some cases the 1x-rate reduced DM more than the 2x-rate (Table 4) could be partly due to experimental error.

Wilson & Miller (1991) found that the light-red kidney cultivar Sacramento and the pinto cultivar Agate were injured less by imazethapyr than the great northern cultivars Beryl and GN1140 or pinto cultivars UI114 and Olathe. Bauer *et al.* (1995) found similar differences in herbicide tolerance with imazethapyr application to pinto cultivars Sierra and Olathe. Differences in bean tolerance to dimethenamid also exist. Adzuki beans are very sensitive to most herbicides and will not tolerate alachlor, dimethenamid or metolachlor. Dark and light red kidney beans, pinto beans and cranberry beans are tolerant to pre-emergence applications of dimethenamid (Renner 1997). The two bean classes that appear to be least tolerant to dimethenamid are navy and black beans. Renner (1996) applied metolachlor and dimethenamid at the recommended rate and

double the rate to several cultivars and classes of beans. No injury was found to any pinto, great northern, kidney or cranberry cultivars from dimethenamid or metolachlor at both application rates. Some injury to the navy and black bean cultivars was observed from both herbicides. Dimethenamid caused more injury than metolachlor to the navy and black bean cultivars.

Table 4 Aboveground dry mass expressed as percentage of the control for ten dry bean cultivars exposed to five herbicides at both the recommended (1x) and 2x-rates on one soil (ANOVA in Table 2B of Appendix B)

	Cultivar									
Herbicides (a.i. g ha $^{-1}$)	Helderberg	Teebus	Kranskop	Enseleni	Monati	Cerillos	Katberg	Mkuzi	Majuba	SSN1
Dimethenamid										
1125	90.4	66.0	50.6	72.7	101.6	74.3	79.0	64.4	94.4	94.4
2250	97.2	94.3	56.9	79.6	107.8	68.8	88.6	125.3	91.2	121.6
Flumetsulam + metolachlor										
1550	121.1	86.1	66.6	100.1	106.2	84.6	78.5	105.1	99.1	120.3
3100	75.1	102.9	80.2	76.2	112.7	72.7	56.0	99.2	98.3	112.7
Imazethapyr										
50	102.1	106.5	84.9	90.8	98.3	100.3	93.0	104.3	109.6	118.1
100	103.7	99.6	91.5	76.9	141.1	94.0	86.0	84.0	111.9	136.8
Metazachlor										
800	84.4	103.8	57.7	79.7	103.9	69.8	92.0	95.1	75.4	107.5
1600	81.4	82.9	72.0	74.2	108.8	73.7	77.6	62.8	77.8	103.4
Metolachlor										
1860	121.9	111.0	81.4	80.8	128.9	77.1	102.7	76.0	84.0	122.5
3720	105.8	87.1	66.9	74.8	125.8	104.2	80.8	91.4	96.7	135.9

LSD_T (P = 0.05)

Herbicide x Rate x Cultivar = 17.51

100-Seed mass. The herbicide x cultivar interaction effect and both main effects were significant for 100-seed mass (Table 3B of Appendix B). Helderberg and Monati were the only cultivars where 100-seed mass was reduced significantly (Table 3A of Appendix A). This was probably due to the large variation in seed size of these two cultivars. As indicated earlier, 100-seed mass was not considered to be a particularly good indicator of herbicide tolerance in this study, probably due to the large variation in seed size within cultivars.

Visual bean injury. Early stunting (30 days after planting) of seedlings was the major injury symptom observed. Imazethapyr caused less stunting than dimethenamid, metolachlor or metazachlor. The cultivars showed stunted growth in response to flumetsulam + metolachlor. Most of the cultivars, except Kranskop, recovered from the stunting effect. Cupping and crinkling of primary leaves were also observed in some cases but those plants eventually recovered fully.

Several herbicides and herbicide combinations are used in dry beans. These herbicides effectively control many weed species but some of them have affected either dry beans or related crops negatively. Crop injury does not always culminate in measurable yield losses. The earliest and most obvious negative effects are reflected in plant morphology. Some of the morphological changes which have been evoked in beans or related crops by various herbicides are: shorter, larger or distorted stem growth; changes in leaf number, size and photosynthetic surface; pruned, distorted or

proliferated root growth (De Beer, 1988; Nkwen-Tamo *et al.*, 1989; Wilson & Miller, 1991; Johnson & Mullinix, 1996; Urwin *et al.*, 1996). Even though some changes in morphology may occur, plants could recover without any yield losses, or losses might be incurred.

Conclusions

Results of this study show that South African dry bean cultivars vary in tolerance to selected registered herbicides. Differences in tolerance also exist within dry bean types. The tested cultivars were generally more tolerant to dimethenamid, imazethapyr and metazachlor than to flumetsulam + metolachlor or metolachlor. If one regards 10% as a "economically significant " yield loss, certain existing herbicide / cultivar combinations should be avoided. The most important yield-reducing combinations were for those cultivars (Helderberg, Kranskop, Enseleni, Monati, Cerrillos, Katberg, Mkuzi and Majuba) treated with flumetsulam + metolachlor. However, all the herbicides tested are very effective in controlling weeds, and therefore, acceptance of small losses in yield must be considered.

Discovering significant differential tolerances only as late as harvesting could be catastrophic, and therefore it would be very helpful if this could be done during screening tests in a glasshouse as will be discussed in Chapter 4.

CHAPTER 3

TOLERANCE OF A DRY BEAN CULTIVAR TO PRE-EMERGENCE HERBICIDES AT DIFFERENT LOCALITIES

Introduction

Not all injury caused by certain herbicides and herbicide combinations result in measured yield losses (Wilson & Miller, 1991; Johnson & Mullinix, 1996; Urwin *et al.*, 1996). This depends not only on plant factors but also on external factors, i.e. physical processes (adsorption, volatility, leaching, soil erosion by wind and water), chemical processes (photochemical decomposition, chemical reactions with soil constituents, biological factors (uptake by plants and microorganisms) and microbial decomposition (Wood *et al.*, 1977).

The physical processes of volatility and leaching result in the loss, to a greater or lesser extent, of the chemically unchanged herbicides from soils. Erosion (wind and water) of the soil surface may also be considered a physical process contributing to the loss of chemically unchanged herbicides from the soil surface. Leaching is enhanced by soils with low clay contents. Leaching tends to increase in soils with an increasing permeability and volume of water moving past a given point. Herbicides that are water soluble and remain dissolved in the soil solution are readily subjected to leaching.

Adsorption is the most important factor affecting the leachability of herbicides in soils. Herbicides that are adsorbed to soil particles do not leach unless the soil particles move with the flow of water. The effect of pH on leaching of herbicide molecules lies primarily in its influence on the adsorption of these molecules to soil colloids and on chemical reactions between the herbicide molecules and various soil constituents. The influence of soil colloids on leaching of herbicide molecules in soils is primarily one of adsorption of these molecules. Increases in the soil colloid content of a soil results in increased adsorption of herbicide molecules, accompanied by decreased leachability (Lambert, Porter & Schieferstein, 1965; Harris, 1969; Kearney & Helling, 1969).

Adsorption in soils is the most important factor by which herbicides become unavailable for uptake (absorption) by plants and microorganisms. All soil-applied herbicides are adsorbed to some extent and their herbicidal activity is reduced in direct proportion to the amount adsorbed. In soils the adsorption of herbicide ions and molecules occurs on both the inorganic (clay) and organic (humus) colloidal fractions. In general, herbicides are much more strongly adsorb in dry than wet soils, and they are more strongly adsorbed to particles of humus than those of clay. Most adsorbed herbicides are readily displaced from their adsorptive sites on clays by the competitive action of water molecules for these sites. Herbicides are not readily displaced from adsorption to humus. The organic colloids (humus) are considered to be the most important single factor affecting herbicide adsorption in soils. Humus has a high adsorptive capacity for herbicides (Stevenson, 1972).

Dimethenamid, imazethapyr, flumetsulam + metolachlor and metazachlor are registered for the pre-emergence control of certain broadleaf weeds. All of these herbicides, except imazethapyr, also give a degree of grass control. Metolachlor controls mainly annual grasses (Vermeulen *et al.*, 1998). Flumioxazin controls broadleaf weeds and is not registered for use in dry bean fields.

This study was conducted to evaluate the effect of six herbicides on the yield of one dry bean cultivar on four soil types with different clay contents, organic matter contents and water regimes. The main aim will be, therefore, to determine to which extent organic matter and clay content influence the effect of herbicides on dry bean cultivar Helderberg.

Materials and Methods

Tolerance of the dry bean cultivar Helderberg to six herbicides (Table 5) was investigated in a field trial in each of the districts Chrissiesmeer, Lichtenburg, Potchefstroom and Reitz during the 1996/97 growing season.

Fertilizer was applied at rates recommended from a soil analysis: Chrissiesmeer - 160 kg 3:2:4 (31) + Zn; Lichtenburg - 53 kg 2:3:4 (30) + urea, Potcheftsroom - 60 kg 2:3:4 (30) + urea and Reitz - 300 kg 6:2:3 (33). The dry bean cultivar was chosen due to high disease resistance and potential susceptibilty to herbicide damage due to small seed

size (Mennega *et al.*, 1990). Seeds were planted at a depth of 5.5 cm with an inter-row spacing of 91 cm and within-row spacing of 8 cm.

A randomized block design with three replicates was used. Each plot consisted of four rows of 2.5-m length. The herbicides (Table 5) were sprayed across the rows with a CO_2-field sprayer that delivered 200 L of water per hectare at 2.5 kPa. Control plots were left untreated, and were kept weed-free, by hand and implement, to eliminate weed interference. Sprinkler irrigation was provided at Lichtenburg and Potchefstroom throughout the growing season. Selected soil characteristics at each trial site are given in Table 6. Rainfall received is given in Appendix C.

Yield was determined by harvesting two 2-m rows for each treatment. In addition, the mass of 100 seeds for each treatment was also determined using the same samples. Data was subjected to analysis of variance and means were compared at the 5% level of significance using the LSD test of Tukey.

Table 5 Active ingredients and commercial names of herbicides used in the dry bean (cv Helderberg) tolerance trial at four Sites: Chrissiesmeer, Lichtenburg, Potchefstroom and Reitz

Active ingredient (a.i.)		**Commercial name**	**Dosage (l ha^{-1})**			
Name	**Concentration (g l^{-1})**		**Chrissiesmeer**	**Lichtenburg**	**Potchefstroom**	**Reitz**
Dimethenamid	900	Frontier® 900 EC	0.75	0.75	1.25	0.75
Flumetsulam + metolachlor	16 + 800	Bateleur® 816 EC	1.3	1.3	1.9	1.3
Imazethapyr	100	Hammer® 100 SL	0.4	0.3	0.5	0.4
Metazachlor	400	Pree® 400 SC	1.25	1.25	1.65	1.25
Metolachlor	930	Dual® 930 EC	1.1	1.1	2	1.1
Flumioxazin*	500 g kg^{-1}	Sumimax® 500 WP	0.1	0.1	0.1	0.1

* Flumioxazin is not registered for use in dry bean

Table 6 Selected characteristics of the soil at the four localities

Locality	Clay (%)	% C	pH(H_2O)	P	K	Ca	Mg
				(mg kg^{-1})			
Chrissiesmeer	17	0.053	6.5	35	90	796	199
Lichtenburg	15	0.049	6.2	23	94	238	56
Potchefstroom	34	0.754	6.5	24	122	1260	385
Reitz	18	0.040	5.1	41	111	305	49

Results and Discussion

Data for seed yield are given in Table 7, and data for 100-seed mass, as influenced by the herbicides, are given in Table 8.

All main effects and the herbicide x locality effect were significant (P = 0.05) for seed yield. The fact that a yield loss of 100% was recorded for one of the treatments at Reitz, probably contributed most to the herbicide x locality interaction being significant. Locality was the only main effect to influence 100-seed mass significantly.

Seed yield. The only significant reductions in yield were caused by flumioxazin at both Lichtenburg and Reitz (Table 7). Yield losses of 100% and 42% were recorded at Reitz

and Lichtenburg respectively. It seems that flumioxazin application is dependent on the soil clay and organic material contents.

Table 7 Seed yield expressed as percentage of the control of one dry bean cultivar (Helderberg) exposed to six herbicides at the respective recommended rates on four soils (ANOVA in Table 4B of Appendix B)

	Locality			
Herbicides (a.i.)	**Chrissiesmeer**	**Lichtenburg**	**Potchefstroom**	**Reitz**
Dimethenamid	101.3	90.5	103.6	100.4
Flumetsulam + metolachlor	95.1	79.0	92.7	102.3
Imazethapyr	87.3	85.8	104.8	86.4
Metazachlor	93.2	96.2	93.1	108.8
Metolachlor	97.7	90.5	96.4	94.1
Flumioxazin	83.9	58.0	90.2	0
LSD_T (P = 0.05)	Herbicide x locality = 16.43			

In Chapter 2 it was reported that flumioxazin, at both the recommended and 2x-rates, did not lead to significant reductions in yield of cv Helderberg in the cultivar tolerance trial at Potchefstroom. The recommended application rate for flumioxazin is the same for all soils irrespective of clay content, and therefore, it is suggested that diffences in soil characteristics at the Potchefstroom and Reitz sites probably explain most of the

differential response of cv Helderberg. The clay and organic matter contents at the two sites were: Potchefstroom 34 % clay and 0.754 % C, and Reitz 18 % and 0.04 % respectively.

Dimethenamid caused slight, but not significant, reductions in yield at one of the four localities (Table 7). Metazachlor also caused slight reductions in yield at all the loacalities, except Reitz. Slight yield reductions were also obtained with application of metolachlor. All these reductions were not significant. Ketchersid, Norton & Merkle (1981) found that metolachlor is more strongly adsorbed to dry soil than to wetter soil. They found that irrigation or rain immediately after metolachlor application damaged grain sorghum. Wetter soil conditions probably explains why metolachlor and flumetsulam + metolachlor caused high yield reductions at Lichtenburg (Table 3C of Appendix C). Imazethapyr caused non-significant reductions in yield at all the localities, except at Potchefstroom where no reduction was recorded. Non-significant reductions in yield were also caused on all localities, except Reitz, by application of flumetsulam + metolachlor. Results confirm that organic matter and clay content are major determinants of the influence of herbicides on plants (Stevenson, 1972).

100-Seed mass. The herbicide effect for 100-seed mass was not significant (P = 0.05), but locality had a significant influence on 100-seed mass (ANOVA in Table 5B of Appendix B). Locality seems to have a greater influence on 100-seed mass than

herbicides. This is probably due to differences in growth conditions during the growing season (i.e. rainfall) and the moisture content of the grain at harvest.

Table 8 100-seed mass expressed as a percentage of the control of dry bean cultivar Helderberg exposed to six herbicides at the respective recommended rates on four soils (ANOVA in Table 5B of Appendix B)

	Locality				
Herbicides (a.i.)	**Chrissiesmeer**	**Lichtenburg**	**Potchefstroom**	**Reitz**	**Mean:**
Dimethenamid	100.8	95.9	104.3	102.2	100.8
Flumetsulam + metolachlor	99.7	92	103.3	103.2	99.6
Imazethapyr	98.8	93.9	103.5	100.4	99.2
Metazachlor	105.3	95.3	101.2	--	100.6
Metolachlor	97.8	96	104.5	100.4	99.7
Flumioxazin	97.2	99.3	100.5	103.4	100.1
Mean	99.9	95.4	102.9	101.9	
LSDT (P = 0.05)	Locality = 1.52				

Conclusions

Results suggest that the tolerance of dry bean cultivar Helderberg to selected herbicides is determined by soil organic matter content, clay content and rainfall received. It is suggested that the future use of flumioxazin in dry beans should be examined with close scrutiny, since excessive crop injury was incurred at two of the four trial sites.

Dry beans as a crop seems to be sensitive to certain herbicides. This must be taken into account when planning cultivar trials to evaluate agronomic characteristics of new cultivars. One must also keep in mind that other factors, i.e. climate (adverse weather conditions, light intensity), physical (planting date, planting depth, fertilizer placement), plant (seed quality, vigour) and soil (soil temperature, plant nutrient status) may interact with the applied registered herbicide to decrease yield.

CHAPTER 4

USE OF CHLOROPHYLL *a* FLUORESCENCE AS A TOOL FOR SCREENING THE HERBICIDE TOLERANCE OF DRY BEAN CULTIVARS

Introduction

In recent years, chlorophyll *a* fluorescence measurements have been increasingly applied to various aspects of plant physiology. The technique is based on the principle that the energy content of the blue and red component of light is absorbed by chlorophyll and that this energy is then used in a variety of processes. Some is used to drive the chemical reactions of photosynthesis; the remainder is lost as heat, radiationless de-excitation and re-emission as light known as fluorescence (Lavorell & Etienne, 1977). Chlorophyll can be regarded as an intrinsic fluorescent probe of the photosynthetic system. In the leaf or algal cell, the yield of fluorescence is influenced in a very complex manner by events that are, directly or indirectly, related to photosynthesis (Krause & Weis, 1984).

Due to its non-intrusive nature and the fact that physiological disturbances can be detected before any visible symptoms can be detected, fluorescence detection would seem to satisfy the requirements for screening herbicides for potential yield-reducing modes of action (Van Rensburg, Kruger & Nolte, 1994). The latter contention is

supported by results (Moreland, 1967) which seem to indicate that most herbicides modify the growth of an intact plant by influencing more than one biochemical process. It can also be visualized that the subcellular concentrations of the herbicide in a given organ of an intact plant may increase with time after application until the external source is depleted, and then decrease as degradation increases. Ashton, De Villiers, Glenn & Duke (1977) proposed that initial studies on a new herbicide should give priority to a process such as photosynthesis which covers several biochemical reactions. Their rationale being that in the absence of knowing the primary site of herbicide action, a concept of metabolic sites of action rather than a single primary site of action may contribute to a better understanding of how a given herbicide alters the growth of an intact plant.

Selective herbicides do, however, usually have a sufficient safety margin, but if this threshold is exceeded by incorrect herbicide / cultivar combinations, injury to the crop plants will inevitably occur. Mennega, Nel & Le Court De Billot (1990) have also demonstrated that chlorophyll fluorescence can be used as a rapid method for assaying low concentrations of atrazine in soil. The primary objective of this investigation was, therefore, to determine whether chlorophyll fluorescence measurements could be used to determine the differential sensitivities of two dry bean cultivars to six chemically diverse herbicides for which photosynthesis inhibition is not regarded as a primary effect.

Materials and Methods

The dry bean cultivars Kranskop and OPS-RS1 were used in this glasshouse trial because Kranskop was one of the least tolerant cultivars to registered herbicides in previous field trials; and OPS-RS1 is a new red-speckled sugar bean cultivar to be released by the ARC in the near future.

This experiment was conducted with a clay loam soil (31% clay) in 7.5-L pots with plastic linings to prevent leaching of the herbicides. Six seeds were planted per pot and upon emergence seedlings were thinned to four per pot. Fertilizer (2:3:4 (30) 60 kg ha^{-1}) were mixed into the soil according to a soil analysis. Dimethenamid (Frontier® 900 EC), flumetsulam + metolachlor (Bateleur® 816 EC), imazethapyr (Hammer® 100 SL), metolachlor (Dual-S® 930 EC), metazachlor (Pree® 400 SC) and flumioxazin (Sumimax® 500 WP) were applied to the soil surface at the recommended rates for the particular soil (Table 9).

Water levels were replenished on alternate days to a level approximating the water holding capacity of the growth medium. Treatments were replicated three times and pots were arranged according to the completely randomized design. Positions of pots were changed on alternate days. Data was subjected to analysis of variance and means were compared at the 5% level of significance using the LSD of Tukey.

Table 9 Active ingredients, recommended rates and active ingredient (g a.i. ha^{-1})

Active ingredient	Recommended rate (l ha^{-1})	Active ingredient (g a.i. ha^{-1})
Dimethenamid	1.3	1125
Flumetsulam + metolachlor	1.9	1550.4
Imazethapyr	0.5	50
Metazachlor	1.5	600
Metolachlor	2.0	1860
Flumioxazin	100 g ha^{-1}	50

The temperature regime in the mechanically heated and cooled glasshouse was 25/18 ± 2^{0}C day/night at 12 hours day length. Chlorophyll fluorescence measurements were conducted on the primary leaves and first trifoliate leaf 21 days after planting, after a dark adaptation period of 25 min., with a fluorescence measuring system (Plant Efficiency Analyser, Hansatech, UK). The instantaneous fluorescence yield (Fo), maximum fluorescence yield (Fm) and the ratio of variable (Fv) to maximum fluorescence yield (Fv/Fm = 1 - Fo/Fm) were determined. After fluorescence was measured the plants were cut off at the soil surface and their top growth dry matter determined.

Results and Discussion

Data for herbicide-induced changes in chlorophyll fluorescence parameters of the primary and trifoliate leaves of both cultivars are given in Figures 4.1 to 4.6. Data for the influence of selected herbicides on aboveground dry mass are given in Table 10.

In the case of primary leaf data (Figures 4.1 – 4.3) the cultivar x herbicide interaction was significant for both Fo and Fm, and for Fv/Fm the main effects were significant. For trifoliate leaf data (Figure 4.4) the cultivar x herbicide interaction was only significant for Fo. Dimethenamid, flumioxazin, flumetsulam + metolachlor, and metazachlor caused significant decreases in Fo of the primary leaves of Kranskop (Figure 4.1). For the same cultivar, metazachlor had a similar effect in the first trifoliate leaf (Figure 4.4). Flumioxazin was the only herbicide to decrease the Fo of the primary leaves of OPS-RS1 significantly (Figure 4.1). Both dimethenamid and flumioxazin caused significant decreases in Fo of the first trifoliate leaf of OPS-RS1 (Figure 4.1). Slight increases in Fo caused by imazethapyr, metazachlor or metolachlor were recorded on the primary leaves of OPS-RS1, but these increases were not significant. The Fv/Fm ratio of the primary leaves was significantly increased by flumioxazin, flumetsulam + metolachlor or metazachlor (Figure 4.3). These herbicides failed to have the same effect on the first trifoliate leaves (Figure 4.6).

For aboveground dry mass both main effects, cultivar and treatment, as well as the treatment x cultivar interaction effect were significant. Aboveground dry mass was significantly reduced by all herbicides, except flumetsulam + metolachlor or imazethapyr (Table 10). These results are consistent with findings in Chapter 5 where all the herbicides, except imazethapyr, caused a significant reduction in DM. Dimethenamid, metazachlor and metolachlor were responsible for the largest reduction. These findings are in accordance with that in Chapter 2 where aboveground DM was reduced by all the herbicides at the 2x-rate, except for imazethapyr and metolachlor.

When comparing the results of the herbicide-induced changes in the fluorescence parameters Fo, Fv/Fm and Fm (Figures 4.1 – 4.6) for both cultivars, it becomes clear that those plants for which Fo values were significantly decreased, were characterized by Fv/Fm ratios which were significantly higher than the controls. This was the case for all herbicides, except dimethenamid. Furthermore, irrespective of herbicide / cultivar combination used, and despite perturbation in the Fo and / or Fv/Fm fluorescence ratios, as long as the latter was statistically significant, the Fm values stayed essentially the same or decreased. These significant decreases in Fo and significant increases in Fv/Fm are typical of herbicides acting at Photosystem II of the photosynthesis process. It is, however, generally accepted that dimethenamid, flumioxazin, flumetsulam + metolachlor and metazachlor do not influence photosynthesis as their primary mechanism of action (Zimdahl 1993). Theoretically, these herbicides must have interrupted the photosynthetic electron flow after the plastoquinone pool (which explains

the significantly higher Fv/Fm ratio) for a binding site at the FeS-Rieske centre (Trebst, 1980). A possible explanation as to why this occurred may be found in several studies on the physiological adaptation of plants treated with sub-lethal doses of herbicides (Lichtenthaler, Burkard, Grumbach & Meier, 1980; Meier, Lichtenthaler & Burkard, 1980), which indicated that additional physiological effects induce a shade-type growth response. These physiological effects include changes in chloroplast ultrastructure, pigment composition, the enzyme and metabolic contents of the chloroplasts (Pfister & Urbach, 1983), and other metabolic reactions (Fedtke, 1979).

As exceptions do occur, it is advisable to use both fluorescence parameters in combination, since a change in the Fo level would seem merely to indicate that a herbicide influences the metabolism of a specific cultivar. But the extent to which this disturbance might be expected to influence yield can probably best be explained by examining the Fv/Fm ratio (Van Rensburg *et. al.*, 1994).

According to Van Rensburg *et al.* (1994) the perturbations caused in the fluorescence parameters Fo and Fv/Fm could be used to quantify the extent to which herbicides might be expected to influence yield. Further research regarding this aspect was envisaged by Van Rensburg *et al.* (1994). In the present study, Fo was significanly reduced and the Fv/Fm ratio significantly increased by selected herbicides, indicating that these herbicides did influence the metabolism of the dry bean cultivars but would not necessarily have a negative effect on seed yield.

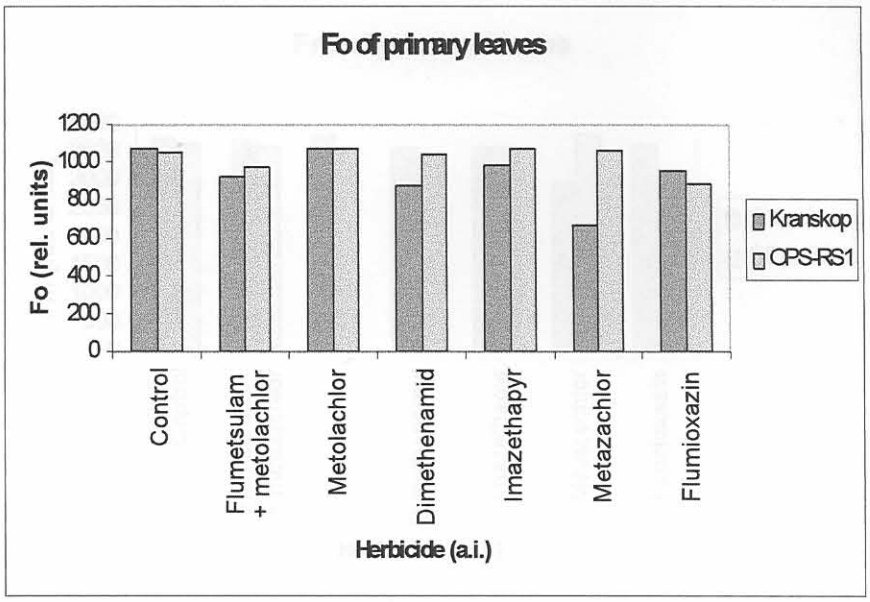


Figure 4.1 Herbicide-induced changes in the chlorophyll fluorescence parameter Fo of the primary leaf in relative units of both Kranskop and OPS-RS1 at 21 days after planting (ANOVA in Appendix B: Table 9B). LSD_T (P=0.05): Cultivar x Herbicide = 107.96.

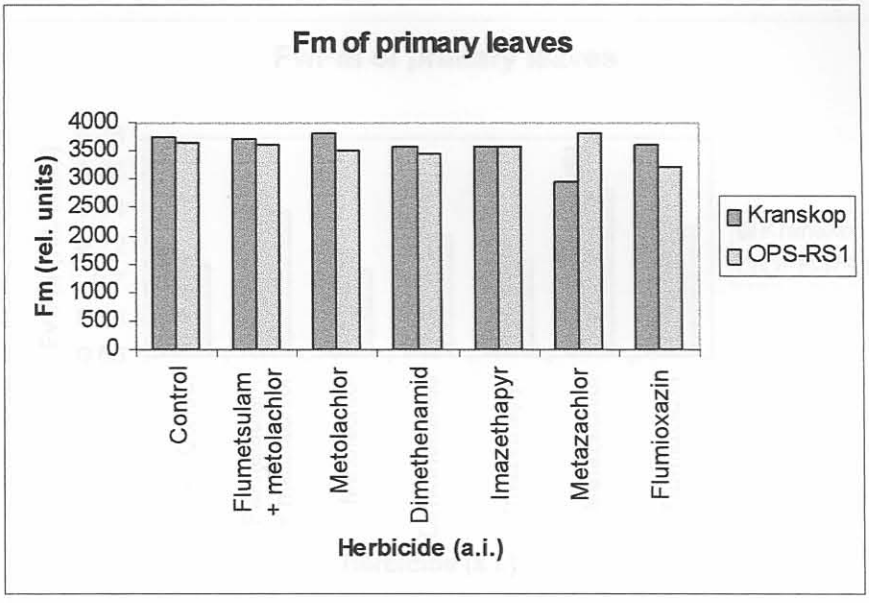


Figure 4.2 Herbicide-induced changes in the chlorophyll fluorescence parameter Fm of the primary leaf in relative units of both Kranskop and OPS-RS1 at 21 days after planting (ANOVA in Appendix B: Table 10B). LSD_T (P=0.05): Cultivar x Herbicide = 301.29.

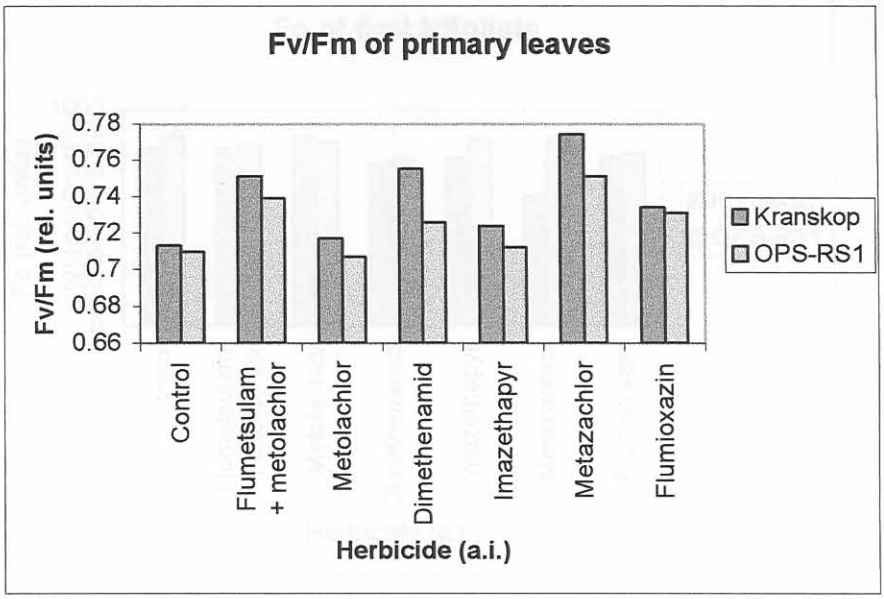


Figure 4.3 Herbicide-induced changes in the chlorophyll fluorescence parameter Fv/Fm of the primary leaf in relative units of both Kranskop and OPS-RS1 at 21 days after planting (ANOVA in Appendix B: Table 11B). LSD_T (P=0.05): Cultivar = 0.009; Herbicide = 0.018.

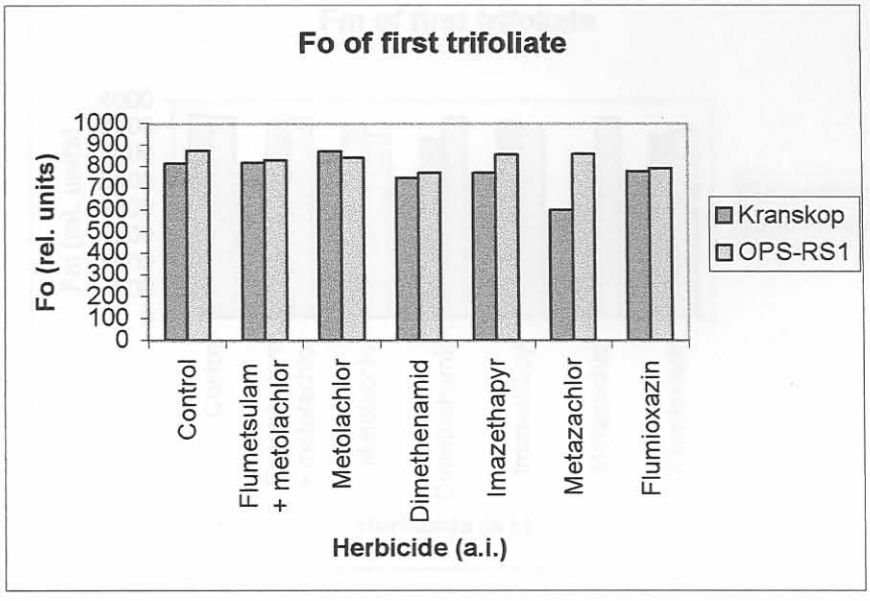


Figure 4.4 Herbicide-induced changes in the chlorophyll fluorescence parameter Fo of the first trifoliate in relative units of both Kranskop and OPS-RS1 21 days after planting (ANOVA in Appendix B: Table 12B). LSD_T (P=0.05): Cultivar x Herbicide = 79.51.

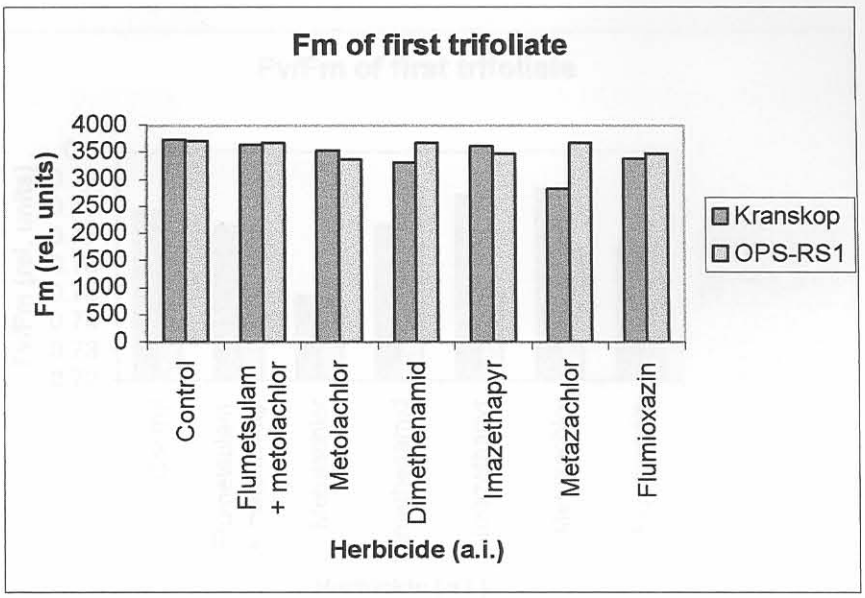


Figure 4.5 Herbicide-induced changes in the chlorophyll fluorescence parameter Fm of the first trifoliate in relative units of both Kranskop and OPS-RS1 21 days after planting (ANOVA in Appendix B: Table 13B). LSD_T (P=0.05): Cultivar x Herbicide = ns.

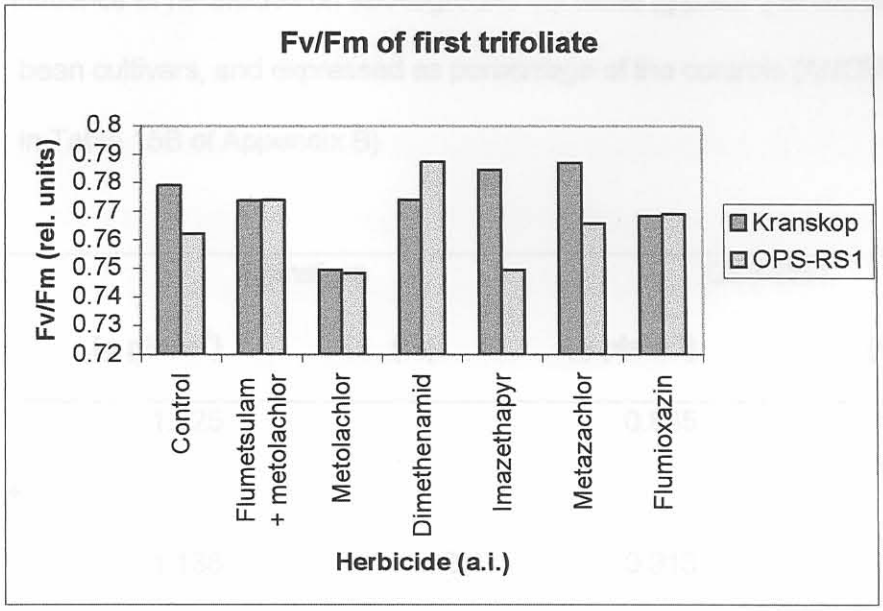


Figure 4.6 Herbicide-induced changes in the chlorophyll fluorescence parameter Fv/Fm of the first trifoliate in relative units of both Kranskop and OPS-RS1 21 days after planting (ANOVA in Appendix B: Table 14B). LSD_T (P=0.05): Cultivar x Herbicide = ns.

Table 10 Influence of herbicides on aboveground dry mass (g plant^{-1}) of two dry bean cultivars, and expressed as percentage of the controls (ANOVA in Table 15B of Appendix B)

Treatment	Kranskop		OPS-RS1	
	(g plant^{-1})	(%)	(g plant^{-1})	(%)
Control	1.225	-	0.835	-
Flumetsulam + Metolachlor	1.138	92.9	0.913	109.3
Metolachlor	0.638	52.1	0.715	85.6
Dimethenamid	0.937	76.5	0.478	57.2
Imazethapyr	1.150	93.9	0.697	83.5
Metazachlor	0.630	51.4	0.333	39.9
Flumioxazin	0.475	38.8	0.600	71.9
Mean	0.885	72.2	0.653	78.2
LSD_T (P = 0.05)				
Cultivar x Herbicide = 0.152				

Conclusions

Changes in response to herbicide application of the two fluorescence parameters (Fo and Fv/Fm), as measured for the primary and trifoliate leaves, indicate that primary leaves are more likely to give a reliable indication of inherent tolerance to herbicides than older leaves. Measurements on older leaves would be influenced by the ability of the plant to recover from initial herbicide injury. Results indicate that the metabolism (photosynthetic electron flow) of the primary leaves of Kranskop is significantly influenced by flumetsulam + metolachlor, flumioxazin and metazachlor. This finding suggests that this cultivar is less tolerant than OPS-RS1.

Selectivity can not be considered an inherent feature of a particular chemical because the trait is strongly dependent on the amount of herbicide applied in a specific environment (Pfister & Urbach, 1983). Therefore, the results presented at best underline the potential value of using chlorophyll fluorescence to screen for herbicide tolerance. Further research regarding the influence of degree of the direction of change in the fluorescence parameters on dry bean yield should be conducted.

Chloropalsts and mitochondria of herbicide-treated plants will be studied in the following chapter on the ultrastructural level, by means of transmission electronmicroscopy, to determine in what way these organelles are influenced, if at all.

CHAPTER 5

INFLUENCE OF DIMETHENAMID, FLUMETSULAM + METOLACHLOR, IMAZETHAPYR, METAZACHLOR AND METOLACHLOR ON DRY BEAN MORPHOLOGY AND CELL ULTRASTRUCTURE

Introduction

Ultrastructural studies with herbicides are typically limited to observing or detecting structural changes caused by several biochemical alterations originating from one primary site of action (Bartels, 1985). If one step (primary site) of a process is inhibited by a herbicide, subsequent steps in that pathway or in others of the integrated, complex system may not be able to operate or to remain stable.

The majority of herbicides have been developed to act on respiration and photosynthesis due to the importance of these life processes in plant growth (Black, 1985). Those herbicides inhibit various reactions of the respiration and photosynthesis processes and can also have an indirect influence on these plant functions *via* their effect on chloroplasts and mitochondria (Bartels, 1985).

Herbicides that inhibit the enzyme acetyl-CoA carboxylase include: fluazifop-P-butyl, haloxyfop-R-methyl ester, propaquizafop, quizalofop-P-ethyl, cycloxydim and

sethoxydim. Those that inhibit acetolactate synthase are, *inter alia*, flumetsulam and imazethapyr. Pendimethalin and trifluralin inhibit tubulin formation; EPTC disrupts mitosis; and acetochlor, alachlor, dimethenamid, metazachlor and metolachlor are regarded as having multiple sites of action (Zimdahl, 1993). Four chemical classes of herbicides inhibit acetolactate synthase (ALS): sulfonylureas (e.g. chlorsulfuron), imidazolinones (e.g. imazethapyr), triazolopyrimidine sulfonanilides (e.g. flumetsulam) and pyrimidinyl thiobenzoates. ALS is an essential enzyme in the biosynthesis pathway of the branched-chain amino acids: valine, leucine and isoleucine in plants (Shaner, 1991). ALS inhibition stops protein synthesis and causes decreased photosynthate translocation to meristems, which leads to rapid cessation of cell division and plant growth.

The acetanilides (metolachlor, metazachlor and dimethenamid) inhibit early seedling growth. This effect is most evident on root growth. These responses appear to be associated with an interference with both cell division and cell enlargement. They do not appear to inhibit seed germination but they usually kill or affect susceptible plants before they emerge from the soil (Ashton & Monaco, 1991). Reinhardt & Nel (1986) reported that the "non-photosynthesis-inhibiting" herbicide alachlor, which belong to the acetanilide group, disrupts the chloroplast membranes of grain sorghum plants *(Sorghum bicolor* L.). This and other changes in membrane integrity were observed when alachlor-treated plants were studied on an ultrastructural level by means of electronmicroscopy. There are more apparently contradictory evidence on the effect of

the acetanilides on *de novo* fatty acid biosynthesis and thus on membrane integrity. The primary mechanism of action of the acetanilides has not been determined and their classification could change (Zimdahl, 1993).

The purpose of this study was to compare the effects of certain "non-photosynthesis-inhibiting" herbicides on the ultrastructure of chloroplasts, mitochondria and starch grains in a dry bean cultivar. Growth responses of the crop were also monitored.

Materials and Methods

The dry bean cultivar Helderberg was used in this glasshouse trial because it was identified in field trials (Fouché, 1996) as one of the least tolerant cultivars to the registered herbicides used. The growth medium was washed river sand in 7.5-L pots with plastic linings to prevent leaching of the herbicides. Six seeds were planted per pot and upon emergence plants were thinned to five seedlings. Dimethenamid (Frontier® 900 EC, 1350 g a.i. ha^{-1}), flumetsulam + metolachlor (Bateleur® 816 EC, 2202 g a.i. ha^{-1}), imazethapyr (Hammer® 100 SL, 90 g a.i. ha^{-1}), metolachlor (Dual-S® 930 EC, 2100 g a.i. ha^{-1}) and metazachlor (Pree® 400 SC, 900 g a.i. ha^{-1}) were applied to the soil surface at three times the recommended rate to force injury symptoms. Excessive amounts of herbicide in practice could be due to over application or conditions that promote high concentrations in the plant (e.g. climate, soil type, activity enhancers, etc.).

Water levels were replenished on alternate days to a level approximating the water-holding capacity of the growth medium. All pots received a commercial nutrient solution (Chemicult® and Multifeed-P®) after seedling emergence. There were three replications and pots were arranged according to the completely randomized design. Positions of pots were changed on alternate days.

The temperature regime in the mechanically heated and cooled glasshouse was 25/18 ± 2^0C day/night at 12 hours daylength. Seedling emergence, plant height and dry mass were measured 14 days after planting. Data was subjected to analysis of variance and means were compared at the 5% level of significance using the LSD test of Tukey.

One of the completely unfolded leaves at the primary (unifoliate) leaf node from one replication was sampled for each treatment 14 days after planting. These leaves were used to prepare ultra-thin sections to study cell structure by means of transmission electronmicroscopy.

Fresh tissue segments (1 mm by 2 mm) were cut from the leaves of control and treated seedlings with a surgical blade. The tissue was fixed in 2.5% glutaraldehyde in 0.075 M phosphate buffer (pH 7.5) for one hour. Another fixation with 0.25% aqueous osmium tetroxide in the same buffer followed for two hours. Specimens were dehydrated in a graded series of acetone (from 30 to 100%) whereafter specimens were infiltrated with 30 and 60% quetol in acetone, each for one hour. Another infiltration with pure quetol

followed for four hours. The specimens were then polimerised at 65 °C for a 24-hour period (van der Merwe & Coetzee, 1992).

Sections were cut on an ultramicrotome and stained with 4% aqueous uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). The specimens were observed and photographed with an electronmicroscope equipped with a 35-mm camera.

Results and Discussion

Data for seedling emergence, plant height and aboveground dry mass (DM) are given in Table 11.

Emergence. Imazethapyr was the only herbicide that caused a significant reduction in the emergence of cv. Helderberg (Table 11).

Plant height. Significant reductions in plant height were caused by all herbicides. Imazethapyr caused the smallest reduction; and dimethenamid, metazachlor and metolachlor the largest.

Aboveground dry mass. All the herbicides, except imazethapyr, caused a significant reduction in DM. Dimethenamid, metazachlor and metolachlor were responsible for the largest reduction. These findings are in accordance with that in Chapter 2 where aboveground DM was reduced by all the herbicides at the 2x-rate, except for imazethapyr and metolachlor.

Table 11 Influence of excessive amounts (3x-rate) of herbicides on emergence (number of seedlings), plant height (cm) and aboveground dry mass (g) of cv Helderberg (ANOVA in Appendix B: Tables 6B, 7B & 8B)

Treatment	**Emergence**	**Plant height (cm)**	**Plant height (% of control)**	**Aboveground DM (g)**	**Aboveground DM (% of control)**
Control	4.67	8.00		0.327	
Dimethenamid	4.00	3.33	41.6	0.044	13.5
Flumetsulam + metolachlor	5.00	4.67	58.4	0.077	23.5
Imazethapyr	3.00	6.50	81.3	0.239	73.1
Metazachlor	4.67	2.67	33.4	0.047	14.4
Metolachlor	5.00	3.97	49.6	0.063	19.3
Mean	4.39	4.86	52.9	0.132	40.4
LSDT (P=0.05)	Emergence = 1.23	Plant height = 1.40	DM = 0.10		

Morphological changes

Except for imazethapyr, all the herbicides altered the morphological appearance of the dry bean plants. The acetanilides (dimethenamid, metolachlor and metazachlor) stunted the plants severely. Main stems of all the treated plants were shorter and had more and shorter internodes than those of the control plants. The most prominent effect was the crinkling of primary leaves. Leaves started dying back from the leaf tips. No chlorosis, typical of photosynthetic inhibitors, was observed. Flumetsulam + metolachlor showed similar results but were less prominent. Visual damage varied from high to low in the order: metazachlor > dimethenamid > metolachlor > flumetsulam + metolachlor > imazethapyr. De Beer (1988) found similar results with excessive amounts of alachlor and metolachlor. He concluded that alachlor caused more pronounced phytotoxic symptoms than metolachlor on dry beans.

The acetanilides inhibit early seedling growth, and the effect is most evident on root growth (Ashton & Monaco, 1991). These responses appear to be associated with interference in both cell division and cell enlargement. Acetanilides do not appear to inhibit seed germination but they usually kill or affect susceptible plants before emergence from the soil. The imidazolinones (imazethapyr), on the other hand, inhibit acetolactate synthase with subsequent inhibition of protein synthesis and decreased photosynthate translocation to meristems. This leads to rapid cessation of cell division and plant growth (Shaner, 1991). Findings in this study are in accordance with Fouché

(1996) who concluded that the small white cultivar Helderberg varied in tolerance to pre-emergence herbicides from tolerant to less tolerant in the order: dimethenamid > imazethapyr > metolachlor > metazachlor > flumetsulam + metolachlor.

Ultrastructural changes

The chloroplasts and mitochondria of the treated plants differed in some respects from those of the control plants. These differences are shown in electronmicroscope photographs presented as Figures 5.1-5.12.

Chloroplasts. None of the herbicides caused drastic changes in the structure of chloroplasts. However, all the herbicides except imazethapyr (Figure 5.3), caused a reduction of the number of stroma and granum lamellae (metolachlor: Figure 5.5; flumetsulam + metolachlor: Figure 5.6; dimethenamid: Figure 5.7; metazachlor: Figure 5.8). Starch granules of treated plants, with the exception again of imazethapyr-treated plants (Figure 5.3), appeared depleted and were rounder in shape than those of control plants (Figure 5.1). The chloroplast is surrounded by a limiting double membrane. Its internal structure is a complex membrane system embedded in a granular matrix, the stroma (Grunning & Steer, 1975). The pigments involved in photosynthesis are found in the membranes, whereas the enzymes involved in carbon fixation, 70S ribosomes, strands of naked DNA fibrils, and often starch grains and lipid globules, are located in

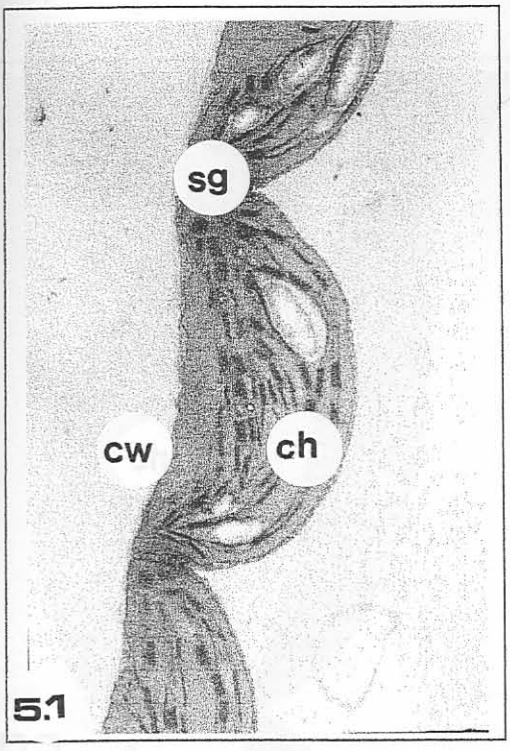
the stroma. The membranes form flattened discs or sacs (lamellae or thylakoids) that may be layered into stacks called grana.

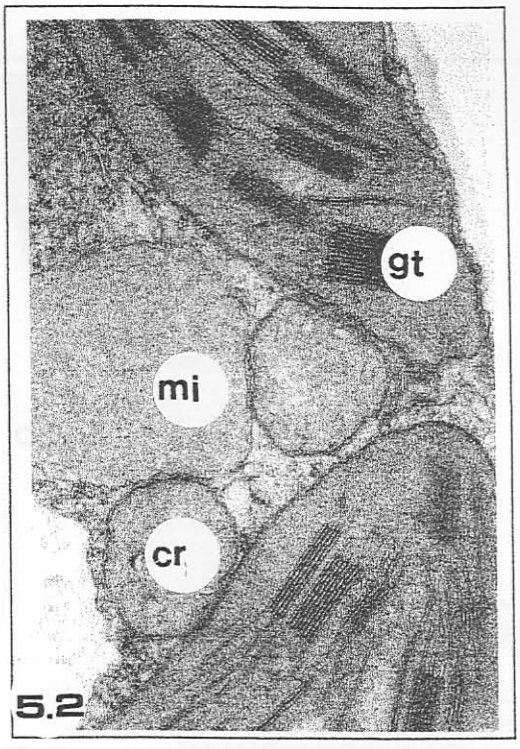
Mitochondria. A comparison of mitochondria of control plants (Figure 5.2) with those in treated plants (metolachlor: Figure 5.9; flumetsulam + metolachlor: Figure 5.10; dimethenamid: Figure 5.11 and metazachlor: Figure 5.12) revealed signs of degradation of the mitochondria, with imazethapyr-treated plants again the exception (Figure 5.4). Mitochondria move freely in streaming cytoplasm, appear to divide by fission, and coalesce with transient adherence to chloroplasts and other organelles (Tzagoloff, 1982). Mitochondria tend to be spherical in shape, but size may vary and transient changes in shape are exhibited (Tzagoloff, 1982). Mitochondria have a sophisticated membrane system within which are incorporated the enzymes that mediate cellular respiration. Cristae of mitochondria in control plants are attached to the inner wall and appear even-sized. The arrangement of cristae in treated plants (Figures 5.9, 5.10, 5.11, 5.12) was chaotic and they were swollen.

The electronmicroscope photographs show definite differences in chloroplasts and mitochondria between treated and control plants. Similar effects were reported by De Beer (1988) for dry beans treated with alachlor and metolachlor. These ultrastructural effects are probably manifestations of acetyl-Co enzyme A (CoA) inhibition as reported by Molin, Anderson & Porter (1985). According to Bidwell (1974) CoA is needed in the formation of chlorophyll, and is an important part of the Krebs cycle that is based in the

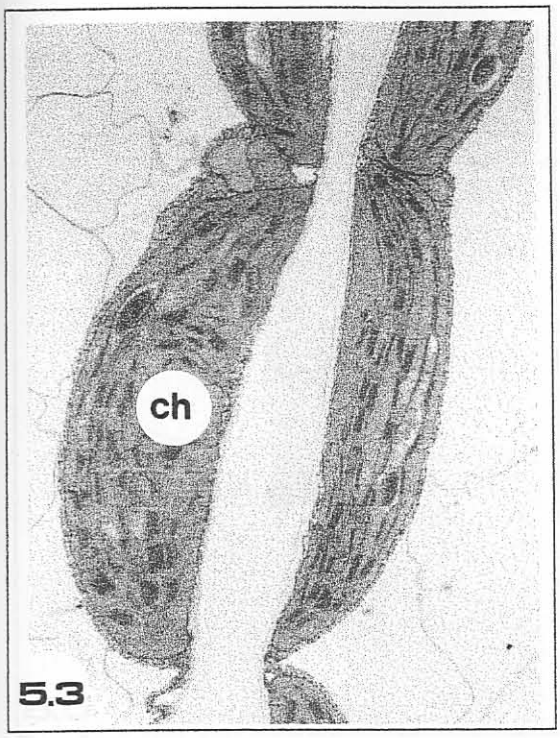
mitochondria. Changes in cristae suggest a slower respiration tempo in affected plants. Reinhardt & Nel (1986) worked with grain sorghum and reported that alachlor not only influences the stroma lammellae but also causes disintegration of cell vacuole membranes, double membranes of chloroplasts and membranes of nuclei in grain sorghum. The present study did not show any influence on chloroplast membranes.

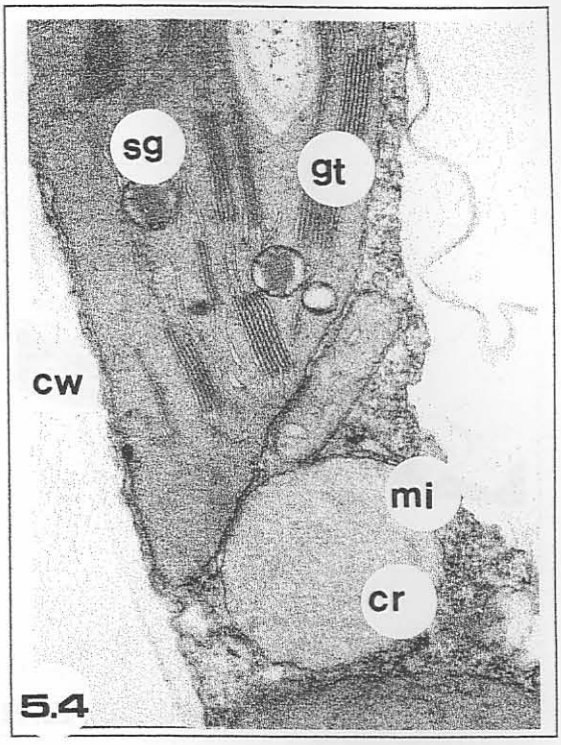
Effects on cloroplasts and mitochondria suggest that both photosynthesis and respiration efficiency will be influenced negatively, which in turn will have a negative influence on plant growth and yield.



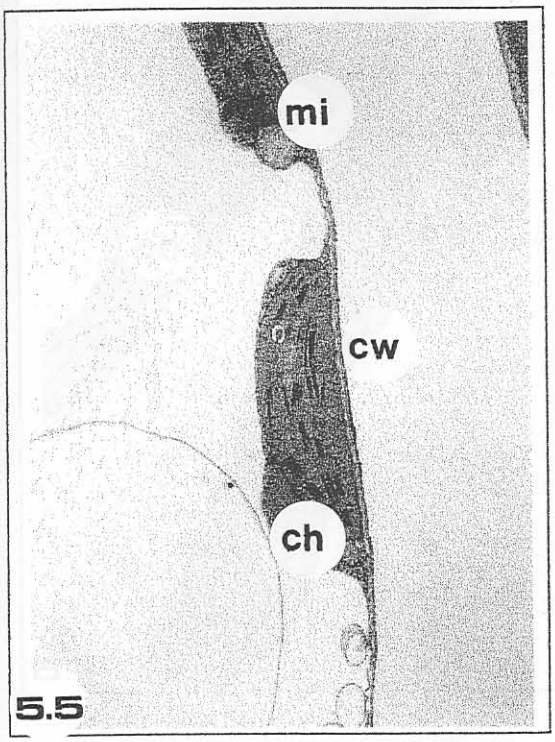


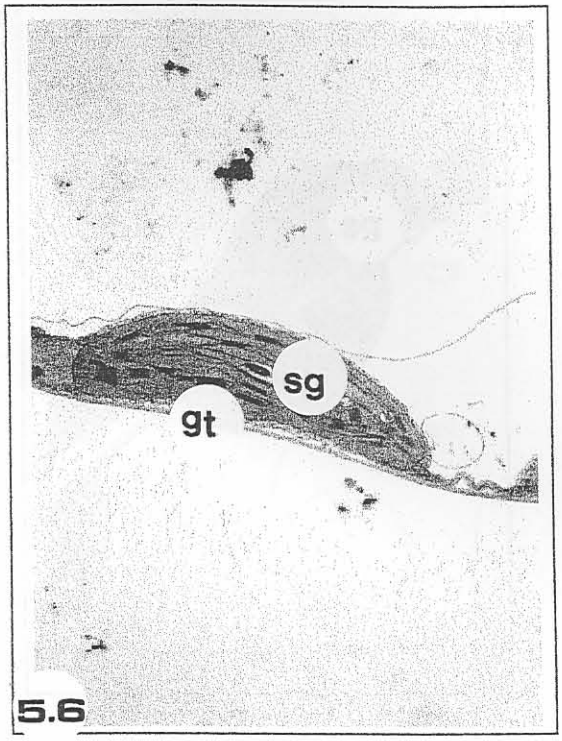
Figures 5.1 & 5.2 Micrographs of cross sections through the leaf of an untreated dry bean plant. (5.1) A chloroplast (7500 x) with starch granules and thylakoids, mitochondria and cell wall. (5.2) Mitochondria (36 000 x). Note that cristae of mitochondria of control plants are attached to the inner wall and appear even-sized. (ch - chloroplast, cr - cristae, cw - cell wall, gt - grana with thylakoids, mi - mitochondria, sg - starch granule)



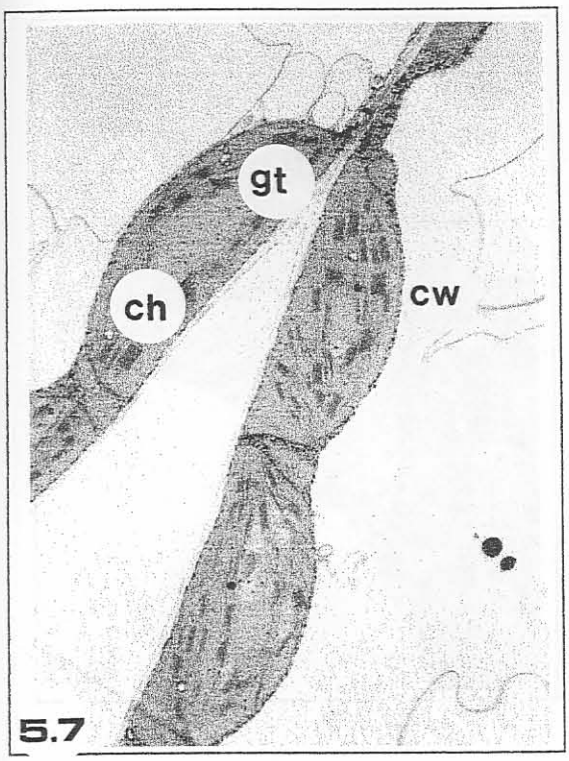


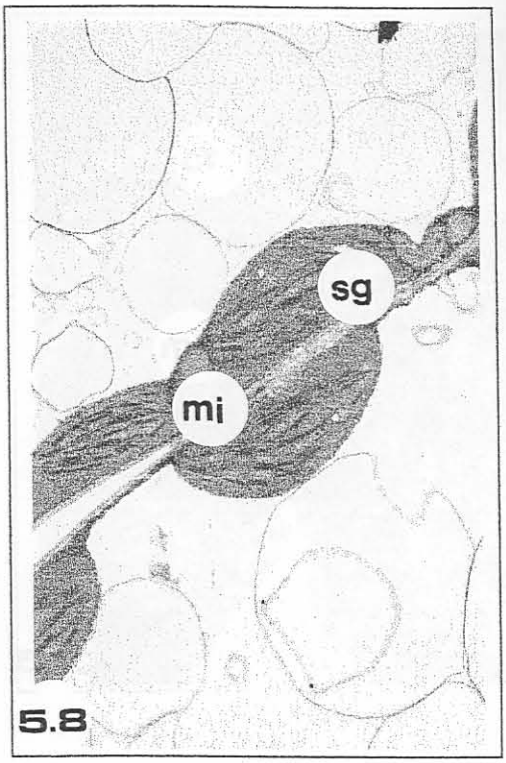
Figures 5.3 & 5.4 Micrographs of cross sections through the leaf of imazethapyr-treated dry bean plants. (5.3) A chloroplast (7 500 x) with normal starch granules and the same quantity of thylakoids, mitochondria and cell wall. (5.4) Mitochondria (36 000 x). Note that cristae of mitochondria of imazethapyr-treated plants are attached to the inner wall and appear even-sized. (ch - chloroplast, cr - cristae, cw - cell wall, gt - grana with thylakoids, mi - mitochondria, sg - starch granule)



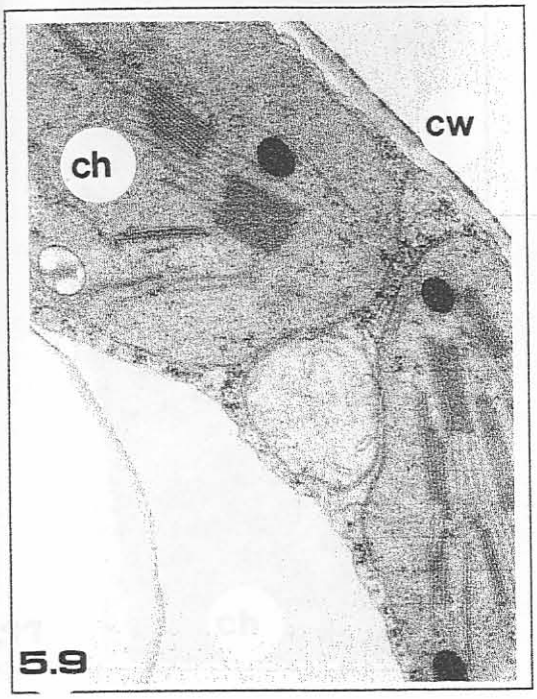


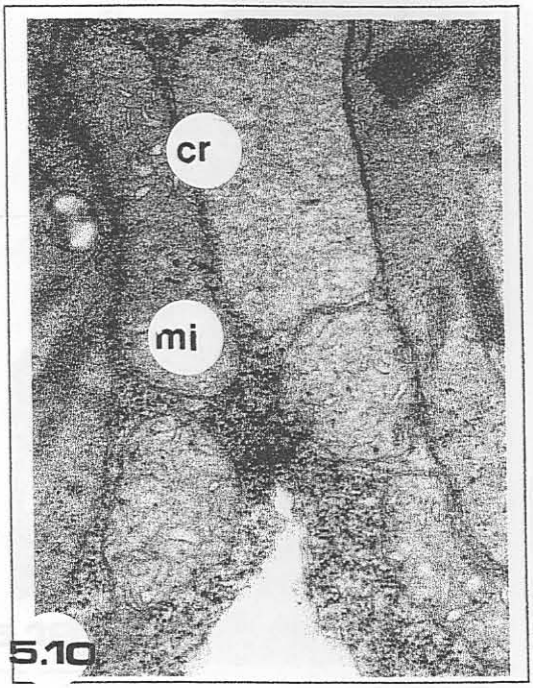
Figures 5.5 & 5.6 Micrographs of cross sections through the leaf of herbicide-treated dry bean plants demonstrating effects on chloroplasts (7 500 x). (5.5) Metolachlor, (5.6) flumetsulam + metolachlor. Starch granules appear depleted and are rounder in shape than control plants and show a reduced number of stroma and granum lamellae. (ch - chloroplast, cw - cell wall, gt - grana with thylakoids, mi - mitochondria, sg - starch granule)



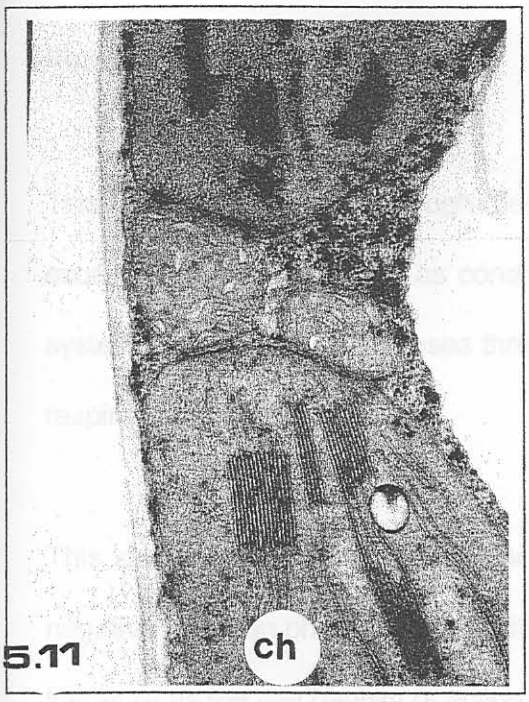


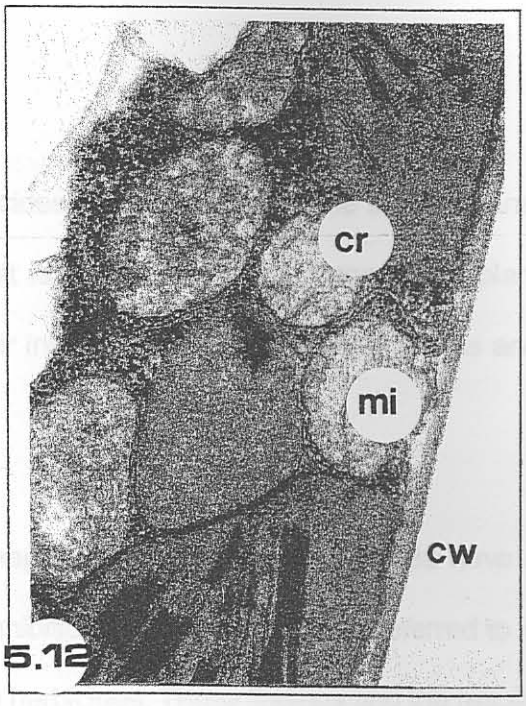
Figures 5.7 & 5.8 Micrographs of cross sections through the leaf of herbicide-treated dry bean plants demonstrating effects on chloroplasts (7 500 x). (5.7) Dimethenamid, (5.8) metazachlor. Starch granules appear depleted and are rounder in shape than control plants and show a reduced number of stroma and granum lamellae. (ch - chloroplast, cw - cell wall, gt - grana with thylakoids, mi - mitochondria, sg - starch granule)





Figures 5.9 & 5.10 Micrographs of cross sections through the leaf of herbicide-treated dry bean plants demonstrating effects on the mitochondria (36 000 x). (5.9) Metolachlor, (5.10) flumetsulam + metolachlor. Note that the arrangement of cristes in treated plants is chaotic and they are swollen. (ch - chloroplast, cr - cristae, cw - cell wall, mi - mitochondria)





Figures 5.11 & 5.12 Micrographs of cross sections through the leaf of herbicide-treated dry bean plants demonstrating effects on the mitochondria (36 000 x). (5.11) Dimethenamid, (5.12) metazachlor. Note that the arrangement of cristes in treated plants is chaotic and they are swollen. (ch - chloroplast, cr - cristae, cw - cell wall, mi - mitochondria)

Conclusions

This study showed that although these herbicides are registered for use in dry beans, excessive amounts as well as conditions that favour high concentrations in the plant system could cause yield losses through their interference in both photosynthesis and respiration efficiency.

This study indicated that, except for imazethapyr, the herbicides tested could have a negative effect on photosynthesis, although inhibition of this process is not referred to in literature as the mechanism of action of these herbicides. These findings and the results in Chapter 4 indicate that some of the tested herbicides may have an effect (probably secondary) on photosynthesis. According to Zimdahl (1993) the classification of the acetanilide group of herbicides according to their mechanism of action could change since the primary mechanism of action has not yet been determined.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

In this study the influence of selected pre-emergence herbicides on the growth and yield of ten dry bean cultivars was investigated. The potential of chlorophyll *a* fluorescence as a tool for timeous identification of differential cultivar tolerances was assessed, as well as the relationship between these responses and herbicide effects on the cell ultrastructural level. It is hoped that the findings will contribute towards more reliable identification of differences in herbicide tolerance between local dry bean cultivars. This work builds on that done earlier by De Beer (1988), Mennega *et al.* (1990) and Fouché (1996).

Effect of pre-emergence herbicides on the growth and yield of dry bean cultivars

The most important yield-reducing combinations are those cultivars (Helderberg, Kranskop, Enseleni, Monati, Cerrillos, Katberg, Mkuzi and Majuba) in combination with flumetsulam + metolachlor. All these herbicides, however, are very effective as regards to weed control and one might therefore consider accepting small losses in yield.

Tolerance of one dry bean cultivar to six pre-emergence herbicides on four soils

The only significant reductions in yield were caused by flumioxazin at both Lichtenburg and Reitz. Yield losses of 100% and 42% were recorded at Reitz and Lichtenburg respectively. It seems that flumioxazin application is dependent on the soil clay and organic material contents. This study confirms that organic matter and clay content influence the effect of herbicides on plants (Stevenson, 1972). It is suggested that the future use of flumioxazin in dry beans should be examined with close scruteny, since excessive crop injury was incurred at two of the four trial sites.

Chlorophyll *a* fluorescence screening

Changes in response to herbicide application of the two fluorescence parameters (Fo and Fv/Fm), as measured for the primary and trifoliate leaves, indicate that primary leaves are more likely to give a reliable indication of inherent tolerance to herbicides than older leaves. Measurements on older leaves would be influenced by the ability of the plant to recover from initial herbicide injury. Results indicate that the metabolism (photosynthetic electron flow) of the primary leaves of Kranskop is significantly influenced by flumetsulam + metolachlor, flumioxazin and metazachlor. This finding suggests that this cultivar is less tolerant than OPS-RS1.

Since selectivity cannot be considered an inherent feature of a particular chemical, being strongly dependent on the amount of herbicide applied under a given condition (e.g. plant species, soil type and climatic conditions) (Pfister & Urbach, 1983), the results presented underline the potential value of using chlorophyll *a* fluorescence to screen for herbicide tolerance. Further research regarding the influence of the degree of direction of change in the fluorescence parameters on dry bean yield should be conducted.

Morphological and cell-ultrastructural changes caused by selected herbicides

None of the herbicides caused drastic changes in the structure of the chloroplast. However, all of the herbicides, except imazethapyr, led to a reduction of the number of stroma and granum lamellae. Starch granules of treated plants, except for imazethapyr, appeared depleted and were rounder in shape than at control plants.

A comparison of mitochondria of control plants with those in treated plants revealed signs of degradation of the mitochondria, except for imazethapyr-treated plants. The arrangement of cristae in treated plants was chaotic and they were swollen.

The electronmicroscope photographs show definite differences between chloroplasts and mitochondria of treated and control plants. Similar differences were also reported for alachlor and metolachlor by De Beer (1988). These changes are probably

manifestations of acetyl-Co enzyme A (CoA) inhibition as reported by Molin, Anderson & Porter (1985). According to Bidwell (1974) CoA is needed in the formation of chlorophyll, and is an important part of the Krebs cycle which is based in the mitochondria. Changes in cristae suggest a slower respiration tempo in affected plants. Reinhardt & Nel (1986) did work with alachlor on grain sorghum earlier than 1988, and reported effects on chloroplasts. They found that alachlor not only influences the stroma lammelae but also causes disintegration of cell vacuole membranes, double membranes of chloroplasts and membranes of nuclei. This study did not show any influence on chloroplasts membranes. Effects on cloroplasts and mitochondria suggest that both photosynthesis and respiration efficiency will be influenced negatively, which in turn will have a negative influence on plant growth and yield.

This study indicated that the tested herbicides, except imazethapyr, could have a negative effect on photosynthesis although photosynthesis is not regarded as the mechanism of action of these herbicides. This and the results in Chapter 4 could indicate that some of the tested herbicides do have an effect on photosynthesis (secondary). According to Zimdahl (1993) the classification of the acetanilide group of herbicides can change since the primary mechanism of action has not yet been determined.

General

This study confirmed that dry bean cultivars vary in tolerance to selected pre-emergence herbicides. Chlorophyl *a* fluorescence meausurments could be used as a tool to establish differential tolerances at a very early stage. Since this is a non-destructive and inexpensive process it could be used as a routine assesment of the herbicide tolerance of different cultivars. Further research regarding the influence of the degree in direction of change in fluorescence parameters on dry bean yield should be conducted.

The responsibility of screening should primarily be the function of the seed and chemical companies. Ideally, prior to registration of a new herbicide, all the available cultivars should be screened by the relevant chemical company. On the other hand, it seems reasonable to expect that if a new cultivar is released after a particular herbicide has been registered, tolerance assessment must be the responsibility of the relevant seed company.

INFLUENCE OF PRE-EMERGENCE HERBICIDES ON GROWTH AND YIELD OF *PHASEOLUS VULGARIS* L. AND *P. COCCINEUS* L.

by

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Summary

Variable tolerance to herbicides has been reported amongst cultivars of several crop species, including: dry beans (*Phaseolus vulgaris* L. and *P. coccineus* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). De Beer (1988) reported that several dry bean plantings suffered from acetanilide herbicide injury during the 1982/83 season. Mennega, Nel & Le Court de Billot (1990) and Fouché (1996) have also reported differences in herbicide tolerance between dry bean cultivars grown in South Africa. Due to these expected differences in herbicide tolerance between local dry bean cultivars a study was undertaken to evaluate the influence of selected pre-emergence herbicides

on: a) the growth and yield of ten dry bean cultivars; b) seed yield on four soil types; c) to evaluate the use of chlorophyll *a* fluorescence as a technique for screening the herbicide tolerance of dry bean cultivars; d) dry bean morphology and cell ultrastructure, and e) to set guidelines for routine assessments of the herbicide tolerance of dry bean cultivars. The tolerance (plant growth and seed yield) of dry bean cultivars to pre-emergence herbicides was investigated in a field trial at the Grain Crops Institute of the Agriculture Research Council (ARC) in Potchefstroom (North West Province) during the 1996/97 growing season. Tolerance (based on seed yield) of the dry bean cultivar Helderberg to pre-emergence herbicides was investigated in a field trial in each of the districts Chrissiesmeer, Lichtenburg, Potchefstroom and Reitz during 1996/97. Chlorophyll *a* fluorescence was measured on the primary leaves and first trifoliate leaf of cultivars Kranskop and OPS-RS1 21 days after planting, after a dark adaptation period of 25 min., with a fluorescence measuring system (Plant Efficiency Analyser, Hansatech, UK). Cultivar Helderberg was grown in a glasshouse for 14 days. Completely unfolded leaves from one replicate were sampled and cut into ultra-thin sections to study cell structure by means of transmission electronmicroscopy. The two red speckled sugar bean cultivars (Kranskop and Monati) and Alubia cultivar (Cerillos) were significantly less tolerant than the small white (Helderberg and Teebus), yellow haricot (Katberg) and large white kidney bean (SSN1) cultivars. The latter cultivar was the most tolerant. The dry bean cultivars were significantly more tolerant to dimethenamid, imazethapyr and metazachlor than to flumetsulam + metolachlor or metolachlor. The 2x-rate caused an overall significant reduction in seed yield. For aboveground dry mass the two red-

speckled sugar bean cultivars (Kranskop and Monati) and the Alubia cultivar (Cerillos) were less tolerant than the other seven cultivars. Cultivar SSN1 was once again the most tolerant. The only significant (P = 0.05) reductions in yield of cultivar Helderberg were caused by flumioxazin at field sites Lichtenburg and Reitz. Dimethenamid, flumioxazin, flumetsulam + metolachlor and metazachlor caused significant decreases in Fo of the primary leaves of Kranskop. For the same cultivar, metazachlor had a similar effect in the first trifoliate leaf. Flumioxazin was the only herbicide to decrease the Fo of the primary leaves of OPS-RS1 significantly. Both dimethenamid and flumioxazin caused significant decreases in Fo of the first trifoliate leaf of OPS-RS1. Slight increases in Fo by imazethapyr, metazachlor and metolachlor were recorded on the primary leaves of OPS-RS1, but these increases were not significant. The Fv/Fm ratio of the primary leaves was significantly increased by flumioxazin, flumetsulam + metolachlor and metazachlor. Furthermore, irrespective of herbicide / cultivar combination used, or perturbation caused in the Fo and / or Fv/Fm fluorescence ratios, as long as the latter was statistically significant, the Fm values stayed essentially the same or decreased. These significant decreases in Fo and significant increases in Fv/Fm are typical of herbicides acting at site 2 of photosynthesis. Further research regarding the extent to which herbicides might be expected to influence yield is suggested. The electronmicroscopy study indicated ultrastructural changes in leaves treated with various herbicides. None of the herbicides caused drastic changes in the structure of chloroplasts. Except for imazethapyr, herbicides did cause a reduction in the number of stroma and granum lamellae. With the exception of imazethapyr-treated plants, starch

granules in treated plants appeared depleted and were rounder in shape than those of the control plants. Disruption of mitochondria was characterized by swollen and chaotically arranged crystae, except in imazethapyr-treated plants. These changes are probably manifestations of acetyl-Co enzyme A (CoA) inhibition which is needed in the formation of chlorophyll, and is an important part of the mitochondria-based Krebs cycle. As a result both photosynthesis and respiration efficiency will be influenced negatively, which in turn will have an adverse effect on plant growth and yield. Morphological changes included shorter and distorted stem growth; smaller leave size, and hence, less photosynthetic surface area. As expected, imazethapyr caused the least changes; and dimethenamid, metazachlor or metolachlor the largest. This study confirmed the existence of differential tolerance to herbicides amongst *P. vulgaris* and *P. coccineus* cultivars. More research, especially field trials, should be conducted to identify high risk herbicide / cultivar combinations.

INVLOED VAN VOOROPKOM-ONKRUIDDODERS OP GROEI EN OPBRENGS VAN *PHASEOLUS VULGARIS* L. EN *P. COCCINEUS* L.

deur

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DEPARTEMENT: Plantproduksie en Grondkunde

GRAAD: M Sc (Agric) Onkruidwetenskap

Opsomming

Verskeie graangewasse, droëbone (*Phaseolus vulgaris* L. en *P. coccineus* L.), koring (*Triticum aestivum* L.), mielies (*Zea mays* L.), sojabone (*Glycine max* L.), sonneblom (*Helianthus annuus* L.) en rys (*Oryza sativa* L.) varieer t.o.v. cultivargevoeligheid teenoor onkruiddoders. Gedurende die 1982/83 seisoen is verskeie droëboonaanplantings deur asetanilied onkruiddoders beskadig (De Beer, 1988). Mennega, Nel & Le Court de Billot (1990) en Fouché (1996) het ook gevind dat plaaslike droëbooncultivars varieer in gevoeligheid teenoor onkruiddoders. Na aanleiding van verwagte verskille in gevoeligheid tussen plaaslike droëbooncultivars is 'n studie onderneem om die invloed van sekere vooropkomonkruiddoders te ondersoek op: a) die groei en opbrengs van droëbooncultivars; b) die graanopbrengs op vier

grondtipes; c) om die gebruik van chlorofil *a* fluoressensie as 'n tegniek te evalueer vir gebruik in die identifisering van cultivargevoeligheid t.o.v. onkruiddoders; d) droëboon morfologie en sel-ultrastruktuur, en e) om riglyne te stel vir roetine ondersoeke na droëboon-cultivargevoeligheid teenoor onkruiddoders. Die gevoeligheid (groei en opbrengs) van droëbooncultivars teenoor vooropkomonkruiddoders is ondersoek in 'n veldproef by die Graangewas Instituut van die Landbounavorsingsraad (LNR) in Potchefstroom (Noordwes) gedurende die 1996/97 groeiseisoen. Die gevoeligheid (gemeet in saadopbrengs) van cultivar Helderberg teenoor vooropkomonkruiddoders is gedurende 1996/97 ondersoek in veldproewe in die distrikte Chrissiesmeer, Lichtenburg, Potchefstroom en Reitz. Chlorofil-fluoressensiemetings is 21 dae na plant uitgevoer op die primêre en eerste trifoliaat blare van cultivars Kranskop en OPS-RS1, na 'n donker aanpassingsperiode van 25 min., met 'n fluoressensiemeter (Plant Efficiency Analyser, Hansatech, UK). Volledig ontvoude primêre blare van cultivar Helderberg, van een herhaling, is 14 dae na plant geoes. Die blare is gebruik om ultra-dun seksies te sny wat gebruik is om selstrukture te bestudeer m.b.v. elektron transmissie mikroskopie. Die twee rooi gespikkelde suikerbone (Kranskop en Monati) en die Alubia cultivar (Cerillos) was betekenisvol meer gevoelig as die klein wit inmaakbone (Helderberg en Teebus), carioca (Mkuzi), geel haricot (Katberg) en groot wit nierboon (SSN1) cultivars. Die groot wit nierboon was die mees verdraagsame cultivar. Die cultivars was betekenisvol meer verdraagsaam teenoor dimethenamied, imazethapir of metazachlor teen die aanbevole dosis as flumetsulam + metolachlor of metolachlor. Die dubbeldosis het 'n betekenisvolle verlaging in opbrengs tot gevolg

gehad. Kranskop en Monati en die Alubia cultivar (Cerillos) was meer gevoelig as die ander sewe cultivars wat betref bogrondse DM. Cultivar SSN1 was die mees tolerante cultivar. Die enigste betekenisvolle (P = 0.05) verlaging in opbrengs is veroorsaak deur flumioxazien op veldpersele by Lichtenburg en Reitz. Geen onkruiddoder het fluoressensie opbrengs (Fo) betekenisvol (P = 0.05) verhoog nie. Dimethenamied, flumioxazien, flumetsulam + metolachlor en metazachlor het die Fo van die primêre blare van cultivar Kranskop betekenisvol verlaag. Metazachlor het 'n soortgelyke effek gehad op die tifoliaat blare van hierdie cultivar. Flumioxazien was die enigste onkruiddoder wat die Fo van die primêre blare van cultivar OPS-RS1 betekenisvol verlaag het. Beide dimethenamied en flumioxazien het die Fo van die trifoliaat blare van OPS-RS1 betekenisvol verlaag. Imazethapir, metazachlor en metolachlor het geringe stygings (nie betekenisvol) in in die Fo van die primêre blare van OPS-RS1 tot gevolg gehad. Die Fv/Fm verhouding van die primêre blare is betekenisvol verhoog deur flumioxazin, flumetsulam + metolachlor en metazachlor. Hierdie betekenisvolle verlaging in Fo en betekenisvolle verhoging in Fv/Fm is tipies van onkruiddoders wat 'n effek het op posisie 1 van fotosintese. Verdere navorsing oor die mate waartoe onkruiddoders opbrengs kan beïnvloed, word voorgestel. Die elektronmikroskoopstudie dui op ultrastrukturele veranderinge in die blare van behandelde plante. Geen behandeling het egter drastiese veranderings in chloroplasstruktuur tot gevolg gehad nie. Al die onkruiddoders, behalwe imazethapir, het egter 'n afname in die getal stroma- en granum-lamellae tot gevolg gehad. Die styselkorrels van die behandelde plante, behalwe die aan imazethapir blootgestel, het uitgeput voorgekom en het 'n ronder vorm

gehad as die onbehandelde kontrole. Die crystae in die mitochondria van behandelde plante, behalwe imazethapir, was geswolle en het chaoties voorgekom. Hierdie veranderings is waarskynlik die gevolg van manifestasies van die inhibering van asetiel-Ko-ensiem A (CoA) wat benodig word vir chlorofil vervaardiging en ook baie belangrik is in die mitochondria gebaseerde Krebssiklus. Die doeltreffendheid van beide fotosintese en respirasie sal gevolglik negatief beïnvloed word. Dit sal plantgroei en -opbrengs benadeel. Morfologiese veranderings het ingesluit korter en verwronge stingelgroei; kleiner blare en gevolglik 'n kleiner fotosintetiese oppervlak. Imazethapyr het soos verwag geringe veranderings veroorsaak, terwyl dimethenamied, metazachlor en metolachlor die grootste veranderings tot gevolg gehad het. Hierdie studie bevestig die bestaan van differensiële verdraagsaamheid teenoor onkruiddoders by *P. vulgaris* en *P. coccineus* cultivars. Verdere navorsing, veral veldproewe, behoort nog gedoen te word om ongunstige onkruiddoder / cultivar kombinasies te identifiseer.

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APPENDIX A

Contents: Field data for DM, grain yield

Table 1A Effect of various herbicides on grain yield (g per plant) of ten dry bean cultivars

	Cultivar									
Herbicides (a.i. g ha $^{-1}$)	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**
Control	20.97	22.02	36.99	34.28	21.16	29.13	11.88	22.18	12.94	55.57
Dimethenamid										
1125.0	23.74	20.13	27.10	32.03	17.85	24.47	14.95	22.26	14.24	59.36
2250.0	20.54	19.91	25.62	34.29	21.47	19.64	15.92	21.32	12.18	61.62
Flumetsulam + metolachlor										
1550.0	17.13	23.38	32.36	28.94	18.15	25.05	10.03	19.51	10.79	66.90
3100.0	16.75	22.06	31.39	26.83	13.05	23.09	8.49	11.04	8.56	53.39
Imazethapyr										
50.0	24.72	21.43	34.02	34.56	18.89	30.08	15.78	23.79	14.64	61.73
100.0	19.69	20.24	26.19	33.50	21.54	28.53	12.19	21.83	10.58	68.46
Metazachlor										
800.0	26.11	21.53	37.25	29.53	20.00	25.81	12.80	21.76	15.57	60.04
1600.0	19.61	19.76	30.76	25.35	17.63	22.99	12.41	21.48	10.87	58.58
Metolachlor										
1860.0	20.17	19.26	33.92	36.38	17.51	26.42	12.91	20.40	13.38	50.25
3720.0	17.44	18.03	29.54	27.55	16.94	25.22	11.73	16.44	8.80	70.14

Table 2A Effect of various herbicides on aboveground dry mass (g per plant) of ten dry bean cultivars

	Cultivar									
Herbicides (a.i. g ha $^{-1}$)	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**
Control	0.55	0.87	1.63	1.59	0.73	1.53	0.79	0.87	1.27	1.19
Dimethenamid										
1125.0	0.49	0.58	0.83	1.16	0.73	1.18	0.62	0.57	1.19	1.10
2250.0	0.53	0.81	0.95	1.27	0.79	1.07	0.70	1.10	1.16	1.43
Flumetsulam + metolachlor										
1550.0	0.67	0.73	1.07	1.59	0.77	1.30	0.62	0.92	1.26	1.43
3100.0	0.41	0.89	1.29	1.21	0.82	1.11	0.46	0.87	1.24	1.35
Imazethapyr										
50.0	0.57	0.92	1.39	1.44	0.72	1.56	0.73	0.91	1.39	1.40
100.0	0.58	0.86	1.48	1.22	1.01	1.43	0.68	0.73	1.42	1.61
Metazachlor										
800.0	0.47	0.88	0.94	1.27	0.76	1.06	0.73	0.83	0.95	1.27
1600.0	0.45	0.71	1.17	1.18	0.79	1.12	0.61	0.55	0.98	1.22
Metolachlor										
1860.0	0.68	0.94	1.31	1.28	0.94	1.16	0.81	0.67	1.07	1.46
3720.0	0.59	0.74	1.09	1.19	0.90	1.61	0.64	0.80	1.23	1.61

Table 3A Effect of various herbicides on 100-seed mass (g) of ten dry bean cultivars

	Cultivar									
Herbicides (a.i. g ha $^{-1}$)	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**
Control	19.67	24.73	57.47	64.07	30.07	55.40	20.93	20.97	40.73	116.73
Dimethenamid										
1125.0	18.67	25.60	59.09	63.60	26.93	57.60	22.47	22.13	42.87	117.80
2250.0	18.13	25.07	59.80	65.53	28.53	57.87	22.20	22.40	42.67	116.80
Flumetsulam + metolachlor										
1550.0	18.13	24.87	59.80	65.60	29.07	56.73	22.27	20.40	40.27	110.85
3100.0	18.20	24.47	60.98	64.73	27.40	56.67	21.60	21.67	42.00	105.73
Imazethapyr										
50.0	19.07	24.67	61.13	67.47	29.80	57.67	21.17	23.47	42.00	124.73
100.0	19.13	24.33	58.13	63.60	27.00	58.27	21.40	21.93	42.53	124.80
Metazachlor										
800.0	19.33	23.87	59.07	64.47	29.73	57.77	22.53	21.33	40.13	124.80
1600.0	17.40	22.00	57.00	66.07	28.80	57.33	21.73	20.60	39.73	110.40
Metolachlor										
1860.0	18.50	25.33	61.67	66.93	28.20	57.00	21.77	22.00	42.73	126.27
3720.0	18.67	25.57	56.33	65.60	28.27	57.33	21.30	20.93	41.70	128.67

Table 4A Effect of various herbicides on seed yield (kg ha^{-1}) of one dry bean cultivar

	Locality			
Herbicides (a.i.)	**Chrissiesmeer**	**Lichtenburg**	**Potchefstroom**	**Reitz**
Control	2544	1888	2343	1661
Dimethenamid	2585	1709	2425	1652
Flumetsulam+ metolachlor	2406	1485	2167	1682
Imazethapyr	2217	1618	2453	1442
Metazachlor	2371	1819	2177	1818
Metolachlor	2488	1710	2256	1572
Flumioxazin	2140	1114	2102	0

Table 5A Effect of various herbicides on 100-seed mass (g) of one dry bean cultivar

	Locality			
Herbicides (a.i.)	**Chrissiesmeer**	**Lichtenburg**	**Potchefstroom**	**Reitz**
Control	15.63	18.40	19.33	16.50
Dimethenamid	15.73	17.63	20.17	16.87
Flumetsulam+ metolachlor	15.17	18.27	19.43	17.07
Imazethapyr	15.40	17.27	20.00	16.57
Metazachlor	15.57	16.93	19.97	17.03
Metolachlor	15.27	17.67	20.23	16.57
Flumioxazin	16.40	17.53	19.57	–

APPENDIX B

Contents: Abbreviated analysis of variance (ANOVA) tables

Table 1B Analysis of variance of seed yield of ten dry bean cultivars exposed to different herbicides

		Seed yield at harvest (% of control data)		
Source	**DF**	**MS**	**F-value**	**PR>F**
Replicate	2	164.1		
Herbicide(H)	4	2571.9	8.54	0.005
Error	8	301.0		
Rate(R)	1	7513.4	18.29	<0.001
Cultivar(C)	9	2502.8	15.61	<0.001
HxR	4	368.1	0.9	0.468
CxH	36	508.6	1.24	0.183
RxC	9	522.9	1.27	0.254
HxR xC	36	282.1	0.69	0.909
Error	186	410.9		
Total	295			
C.V (%)	21.5			

Table 2B Analysis of variance of the aboveground dry mass 21 days after planting of ten dry bean cultivars exposed to different herbicides

	Top growth dry mass 21 days after planting (% of control data)			
Source	**DF**	**MS**	**F-value**	**PR>F**
Replicate	2	182.8		
Herbicide(H)	4	3354.5	26.36	<0.001
Error	8	127.3		
Rate(R)	1	3.4	0.01	0.906
Cultivar(C)	9	6331.0	25.80	<0.001
HxR	4	1140.5	5.65	<0.001
CxH	36	337.3	1.37	0.091
RxC	9	492.2	2.01	0.041
HxR xC	36	468.9	1.91	0.003
Error	190	245.4		
Total	299			
C.V (%)	16.9			

Table 3B Analysis of variance of the 100-seed mass of ten dry bean cultivars exposed to different herbicides

	100-Seed mass (% of control data)		
Source	**DF**	**MS**	**F-value**	**PR>F**
Replicate	2	110.05		
Herbicide(H)	4	113.22	2.41	0.135
Error	8	47.01		
Rate(R)	1	223.35	8.39	0.004
Cultivar(C)	9	415.61	15.61	<0.001
HxR	4	48.38	1.82	0.127
CxH	36	51.34	1.93	0.003
RxC	9	11.50	0.43	0.917
HxRxC	36	23.19	0.87	0.680
Error	186	26.63		
Total	295			
C.V (%)	5.1			

Table 4B Analysis of variance of the seed yield of one dry bean cultivar (Helderberg) exposed to different herbicides on four soils

	Seed yield (% of control data)			
Source	**DF**	**MS**	**F-value**	**PR>F**
Herbicide(H)	5	2839.5	14.34	<0.001
Locality(L)	3	952.8	4.81	0.006
HxL	15	991.5	5.01	<0.001
Block	2	116.5	0.59	0.782
Error	46	198.1		
Total	71			
C.V (%)	15.8			

Table 5B Analysis of variance of the 100-seed mass of one dry bean cultivar (Helderberg) exposed to different herbicides on four soils

	100-Seed mass (% of control data)			
Source	**DF**	**MS**	**F-value**	**PR>F**
Treatment	5	7.84	0.78	0.574
Locality	3	205.04	20.29	<0.001
TxL	14	17.85	1.77	0.082
Replicate/L	8	100.56	9.95	<0.001
Error	38	10.11		
Total	68			
C.V (%)	3.2			

Table 6B Analysis of variance of the herbicide-induced changes in Fo of the primary leaves of Kranskop and OPS-RS1

		Fo of primary leaves		
Source	**DF**	**MS**	**F-value**	**PR>F**
Cultivar	1	105531	9.21	0.004
Herbicide	6	47977	4.19	0.002
Cultivar x Herbicide	6	47781	4.17	0.002
Error	42	11454		
Total	55			
C.V. (%)	11			

Table 7B Analysis of variance of the herbicide-induced changes in Fm of the primary leaves of Kranskop and OPS-RS1

		Fm of primary leaves		
Source	**DF**	**MS**	**F-value**	**PR>F**
Cultivar	1	6579	0.07	0.787
Herbicide	6	127255	1.43	0.227
Cultivar x Herbicide	6	326891	3.67	0.005
Error	42	89116		
Total	55			
C.V. (%)	8.4			

Table 8B Analysis of variance of the herbicide-induced changes in Fv/Fm of the primary leaves of Kranskop and OPS-RS1

		Fv/Fm of primary leaves		
Source	**DF**	**MS**	**F-value**	**PR>F**
Cultivar	1	0.00949	15.62	<0.001
Herbicide	6	0.00215	3.54	0.006
Cultivar x Herbicide	6	0.00073	1.21	0.321
Error	42	0.00061		
Total	55			
C.V. (%)	3.4			

Table 9B Analysis of variance of the herbicide-induced changes in Fo of the trifoliate of Kranskop and OPS-RS1

		Fo of trifoliate		
Source	**DF**	**MS**	**F-value**	**PR>F**
Cultivar	1	51062	8.24	0.006
Herbicide	6	16932	2.73	0.025
Cultivar x Herbicide	6	17692	2.85	0.020
Error	42	6198		
Total	55			
C.V. (%)	9.8			

Table 10B Analysis of variance of the herbicide-induced changes in Fm of the trifoliate of Kranskop and OPS-RS1

		Fm of trifoliate		
Source	**DF**	**MS**	**F-value**	**PR>F**
Cultivar	1	311559	2.64	0.111
Herbicide	6	193710	1.64	0.159
Cultivar x Herbicide	6	248933	2.11	0.072
Error	42	117874		
Total	55			
C.V. (%)	9.8			

Table 11B Analysis of variance of the herbicide-induced changes in Fv/Fm of the trifoliate of Kranskop and OPS-RS1

		Fv/Fm of trifoliate		
Source	**DF**	**MS**	**F-value**	**PR>F**
Cultivar	1	0.00102	1.20	0.279
Herbicide	6	0.00084	0.98	0.448
Cultivar x Herbicide	6	0.00054	0.64	0.697
Error	42	0.00085		
Total	55			
C.V. (%)	3.8			

Table 12B Analysis of variance of the above ground dry mass of Kranskop and OPS-RS1 exposed to the recommended herbicide dosages

	Aboveground dry mass			
Source	**DF**	**MS**	**F-value**	**PR>F**
Cultivar	1	0.75214	33.14	<0.001
Herbicide	6	0.40851	18.00	<0.001
Cultivar x Herbicide	6	0.11772	5.19	<0.001
Error	42	0.02269		
Total	55			
C.V. (%)	19.6			

Table 13B Analysis of variance of the germination of cv Helderberg exposed to three times the recommended herbicide dosages

	Germination			
Source	**DF**	**MS**	**F-value**	**PR>F**
Herbicide	5	1.7889	3.93	0.031
Error	10	0.4556		
Total	17			
C.V. (%)	15.4			

Table 14B Analysis of variance of the plant height of cv Helderberg exposed to three times the recommended herbicides dosages

		Plant height		
Source	**DF**	**MS**	**F-value**	**PR>F**
Herbicide	5	12.3156	20.74	<0.001
Error	10	0.5939		
Total	17			
C.V. (%)	15.9			

Table 15B Analysis of variance of the above ground dry mass of cv Helderberg exposed to three times the recommended herbicide dosages

		Aboveground dry mass		
Source	**DF**	**MS**	**F-value**	**PR>F**
Herbicide	5	0.043208	13.77	<0.001
Error	10	0.003138		
Total	17			
C.V. (%)	42.2			

APPENDIX C

Contents: Rainfall, temperatures and composition of nutrient solutions tables

Table 1C Rainfall and mean daily maximum and minimum temperatures recorded at Potchefstroom for the period November 1996 to March 1997 (Chapter 2 & 3)

Period	Rainfall (mm)	Temperature (° C)	
		Max.	Min.
Nov. 1996	97.6	27	13.3
Dec.	145.8	29	15.8
Jan. 1997	48.9	28.2	16.2
Feb.	38.6	30.2	15.9
Mar.	173.4	24.2	14.2
Total rainfall	504.3		

Table 2C Rainfall and mean daily maximum and minimum temperatures recorded at Chrissiesmeer for the period November 1996 to March 1997 (Chapter 3)

Period	Rainfall (mm)	Temperature (° C)	
		Max.	Min.
Nov. 1996	22	23.4	11.2
Dec.	158	24.3	12.8
Jan. 1997	68	23.8	13.5
Feb.	38	26.2	13.4
Mar.	115	24.3	12.1
Total rainfall	401		

Table 3C Rainfall and mean daily maximum and minimum temperatures recorded at Lichtenburg for the period November 1996 to March 1997 (Chapter 3)

Period	Rainfall (mm)	Temperature (° C)	
		Max.	Min.
Nov. 1996	104	26.1	12.9
Dec.	109	27.8	15.3
Jan. 1997	97	27.4	16.1
Feb.	93	28.5	15.1
Mar.	238	23.0	14.0
Total rainfall	641		

Table 4C Rainfall and mean daily maximum and minimum temperatures recorded at Reitz for the period November 1996 to March 1997 (Chapter 3)

Period	Rainfall (mm)	Temperature (° C)	
		Max.	Min.
Nov. 1996	105	23.9	10.8
Dec.	96	26.4	13.5
Jan. 1997	194	26.1	14.7
Feb.	30	27.8	14.0
Mar.	149	22.8	13.3
Total rainfall	574		

Table 5C Composition of Multifeed - P® and Chemicult® nutrient solutions used in pot experiments

Solution	Element	Concentration
Multifeed-P®	N	19%
	P	8.20%
	K	15.80%
	Mg	900 g L
	Zn*	350 g L
	B	1000 g L
	Mo	70 g L
	Fe*	750 g L
	Mn*	300 g L
	Cu*	75 g L
Chemicult®	N	6.50%
	P	2.70%
	K	13%
	Ca	7%
	Mg	2.20%
	S	7.50%
	Fe	0.15%

Mn	0.024%
B	0.024%
Zn	0.005%
Cu	0.002%
Mo	0.001%

* chelated