

## 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
<b>Pre-plant incorporated:</b>	
EPTC	[s-ethyl dipropylcarbamothioate]
Pendimethalin	[N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine]
Trifluralin	[2,6-dinitro-N,N-dipropyl-4-trifluoromethyl) benzenamine]
<b>Pre-emergence:</b>	
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-quinoxalinyloxy]phenoxy]propanoate]

**Post-emergence:**

Bendioxide [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide]

Cycloxdim [2-[1-(ethoxyimino)butyl]-3-hydroxy-5-(2H-tetrahydrothiopyran-3-yl)-2-cyclohexen-1-one]

Fluazifop-P-butyl [(R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid]

Fomesafen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-(methylsulfonyl)-2-nitrobenzamide]

Haloxifop-R-methyl [methyl 2-[4-[[3-chloro-5-ester(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoate]

Quizalofop-P-ethyl [(±)-2-[4-[(6-chloro-2-quinoxalinyloxy]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 (cycloxdim 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.

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).

## 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).

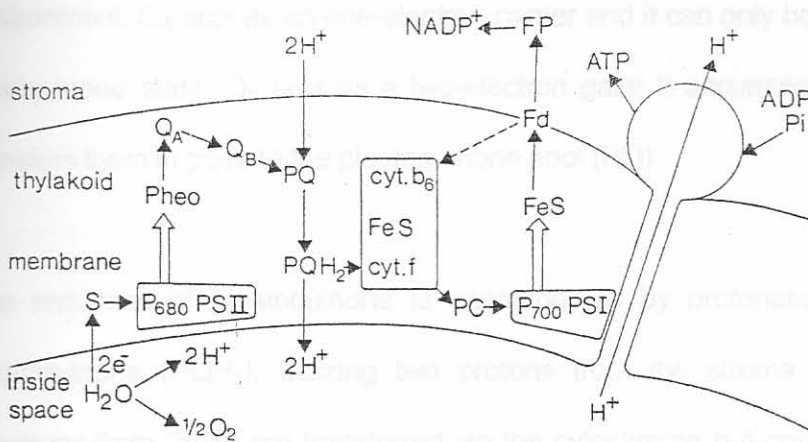


Figure 1 Electron and proton transport pathways in photosynthetic electron flow  
(from Van Rensen, 1989)

Photosynthesis is initiated by the absorption of light energy by the chlorophylls of both photosystems (PS II and PS I). Excitation 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<sup>+</sup> reductase (FP) to NADP<sup>+</sup>. 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, Kottler & 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 & Amtzen, 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 consequences 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<sup>+</sup> accumulates because the displacement of Q<sub>B</sub> 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 (<sup>3</sup>O<sub>2</sub>) to generate the potentially damaging singlet oxygen (<sup>1</sup>O<sub>2</sub>). Investigations with leaves (Pallet & Dodge, 1980) and isolated chloroplasts showed that photodynamic damage was enhanced by the presence of oxygen. Recent studies with isolated PS II reaction centres (McTavish, Picorel & Seibert, 1989) have shown that they are particularly 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, Soper, 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).

## DRY BEAN CULTIVARS

The practical consequences of the above are that plants with PS II herbicide-induced damage will have a reduced CO<sub>2</sub> incorporation, and damaged and bleached chloroplasts. This will result in a lower growth rate and eventually lower yields in the case of cultivated plants.

gen of herbicides with various environmental factors (physical, chemical and biological) based on yield (Wool, Power & Anderson, 1977) but the herbicides with good factors of water content is of high importance. The yield depends on yield regularly. Various mechanisms exist by means of which plants can tolerate foreign compounds. These include oxidation reactions that result in aromatic ring and aliphatic hydroxylation, N-acylation, O-dealkylation and sulphoxidation, hydrolysis, conjugation, and conjugation with carbohydrates residues (glycosylation) or with the tripeptide glutathione (Gantow, 1982; Shimabara et al., 1982; Cole, 1983; Srinivasan, 1985; Pathway 1986; Cole et al., 1987).

The acetanilides (e.g. dimethenamid, metolachlor) and their degradation products undergo conjugation with glutathione and / or glucose (Duke, 1985), whereas rapid metabolic degradation of the imidazopyridines (e.g. imazethapyr) (Ashton & Storey, 1991) and triazolopyridine sulfonylureas (e.g. flumetsulam) (Charters, 1987) may explain their differential tolerance.