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.

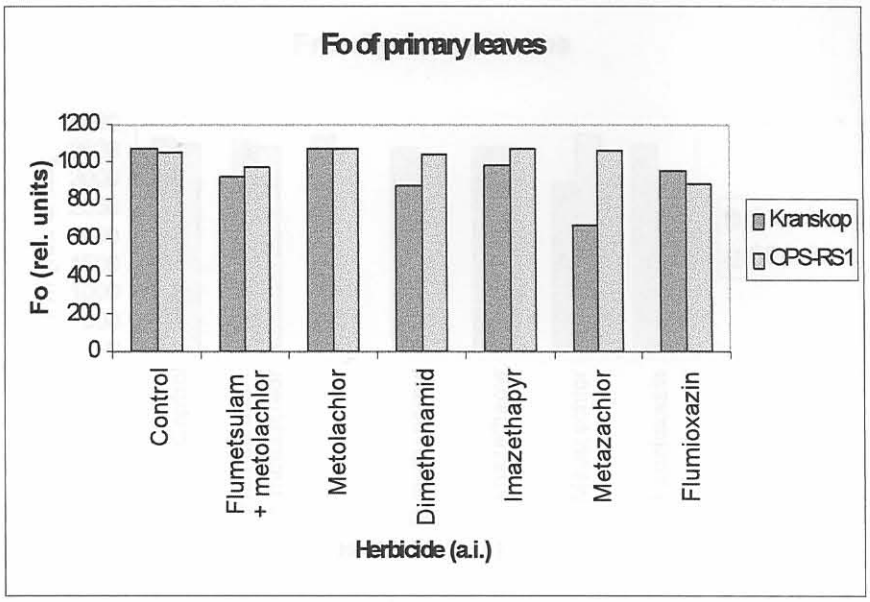

Figure 4.1 Herbicide-induced changes in the chlorophyll fluorescence parameter Fo of the primary leaf in relative units of both Kranskop and OPS-RS1 at 21 days after planting (ANOVA in Appendix B: Table 9B). LSD_T (P=0.05): Cultivar x Herbicide = 107.96.

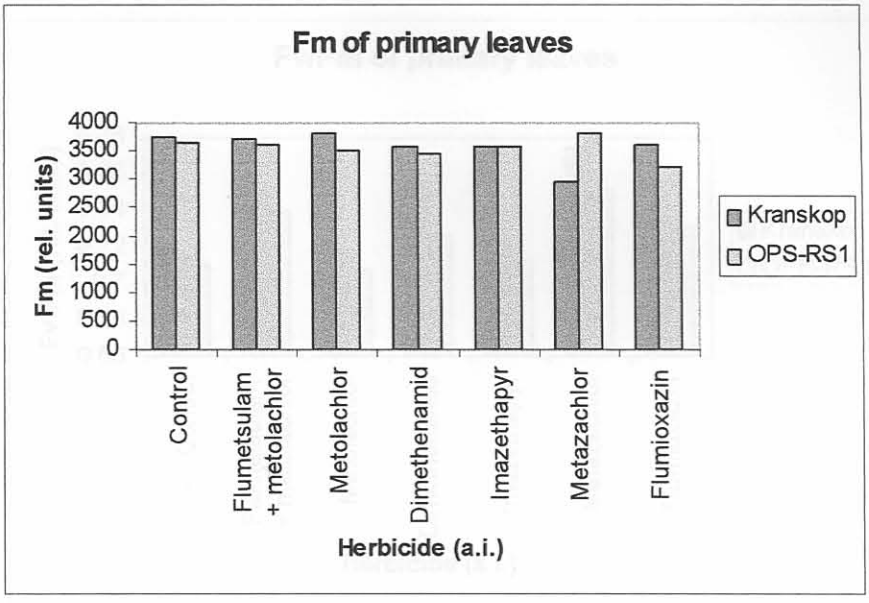

Figure 4.2 Herbicide-induced changes in the chlorophyll fluorescence parameter Fm of the primary leaf in relative units of both Kranskop and OPS-RS1 at 21 days after planting (ANOVA in Appendix B: Table 10B). LSD_T (P=0.05): Cultivar x Herbicide = 301.29.

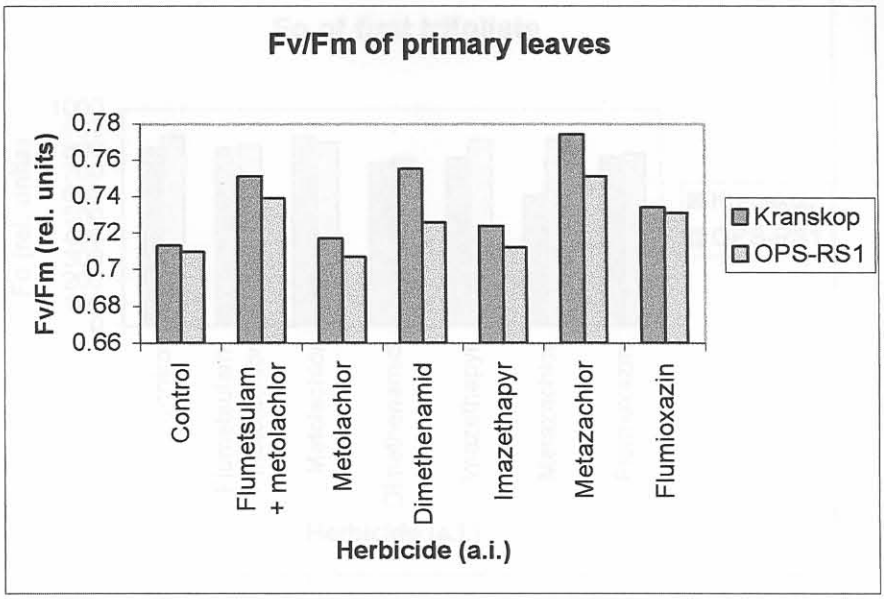

Figure 4.3 Herbicide-induced changes in the chlorophyll fluorescence parameter Fv/Fm of the primary leaf in relative units of both Kranskop and OPS-RS1 at 21 days after planting (ANOVA in Appendix B: Table 11B). LSD_T (P=0.05): Cultivar = 0.009; Herbicide = 0.018.

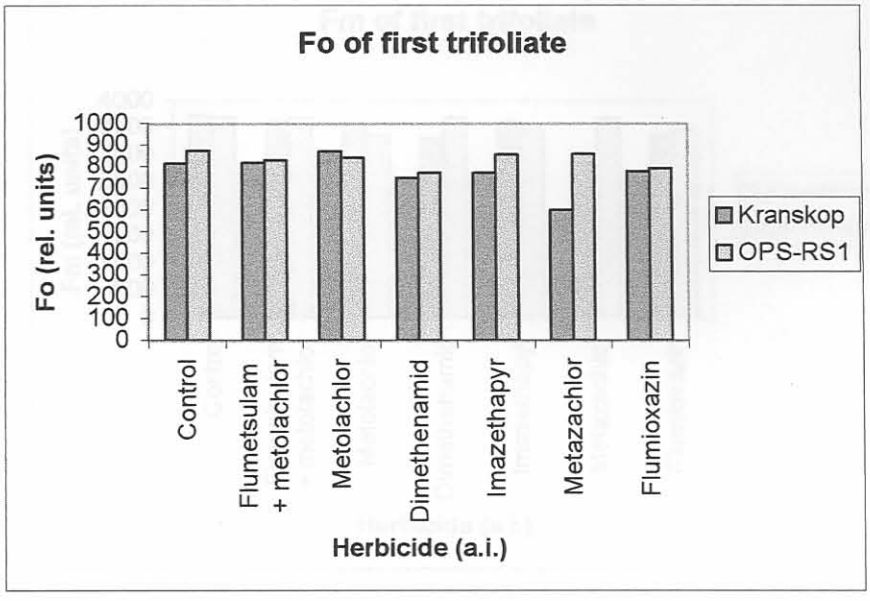

Figure 4.4 Herbicide-induced changes in the chlorophyll fluorescence parameter Fo of the first trifoliate in relative units of both Kranskop and OPS-RS1 21 days after planting (ANOVA in Appendix B: Table 12B). LSD_T (P=0.05): Cultivar x Herbicide = 79.51.

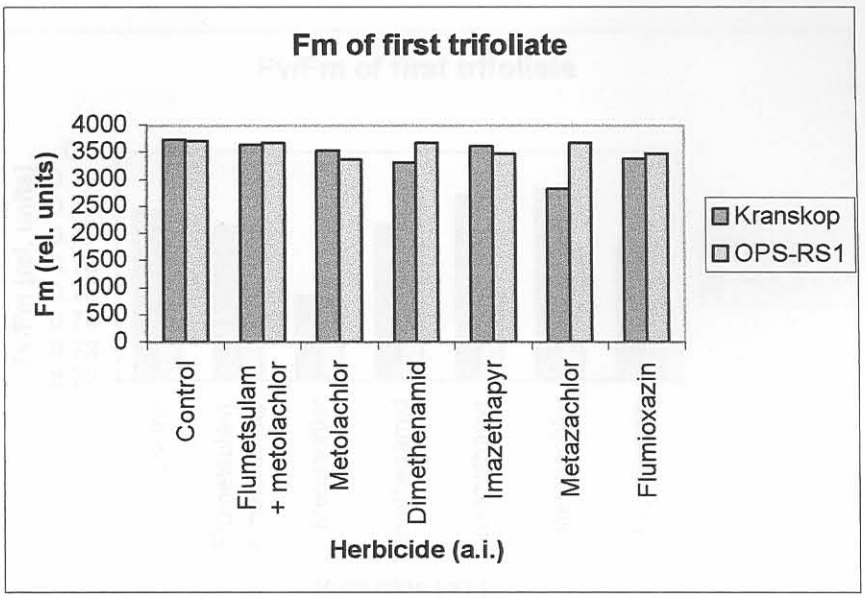

Figure 4.5 Herbicide-induced changes in the chlorophyll fluorescence parameter Fm of the first trifoliate in relative units of both Kranskop and OPS-RS1 21 days after planting (ANOVA in Appendix B: Table 13B). LSD_T (P=0.05): Cultivar x Herbicide = ns.

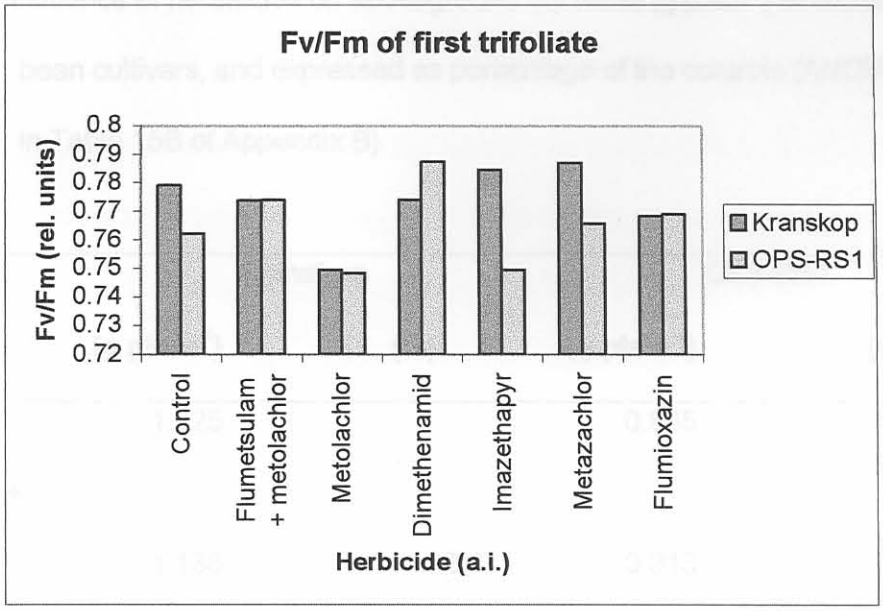

Figure 4.6 Herbicide-induced changes in the chlorophyll fluorescence parameter Fv/Fm of the first trifoliate in relative units of both Kranskop and OPS-RS1 21 days after planting (ANOVA in Appendix B: Table 14B). LSD_T (P=0.05): Cultivar x Herbicide = ns.

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.