DROUGHT INDUCED TOLERANCE TO DICLOFOP-METHYL IN CULTIVATED OAT

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SUMMARY

Water stressing oats (*Avena sativa*) prior to application of diclofop-methyl afforded protection against herbicide damage. Similarly, pretreatment of plants with ABA also brought about protection against herbicide injury. Evidence suggests that protection is via the action of ABA on membranes. These data are discussed with respect to crop safeners.

INTRODUCTION

The economic and biological demands made upon new herbicides are steadily increasing. As a result, emphasis has been put upon improving the performance of compounds currently registered (Graham-Bryce 1983). To this end the use of chemicals known as 'crop safeners' has been advocated to improve herbicide selectivity. Crop safeners are agents which antagonise herbicide activity such that when applied to the crop, herbicide tolerance is improved. Crop safeners may be identified by examining situations where herbicide efficacy is reduced (Stephenson and Ezra 1982). From an understanding of the physiological mechanisms of such antagonisms, underlying principals of safener action may be realised.

Diclofop-methyl is a post emergence herbicide which acts by the inhibiting meristematic activity and disrupting membrane integrity (Brezeanu *et al* 1976; Morrison *et al* 1981). Under conditions of soil moisture stress the efficacy of diclofop-methyl against wild oats (*Avena fatua*) is reduced (Dortenzio and Norris 1980; Akey and Morrison 1983). Work by Akey and Morrison (1983) indicated that there were no significant changes in herbicide retention, uptake or translocation in droughted plants. This suggests that the antagonism of herbicide action is at the cellular level.

In this paper the timing of imposition of water stress required to induce herbicide protection is examined. In addition, the potential to substitute for droughting by using a growth regulator, abscisic acid (ABA), is assessed. For these studies cultivated oat (*Avena sativa*) was employed. It is emphasised that diclofop-methyl is not registered for use on cultivated oats. They are however susceptible to diclofop-methyl (Hoerauf and Shimabukuro 1979) and their use enables uniform plant material to be used in experiments.

MATERIALS AND METHODS

Polystyrene cups were filled with 200 g of oven dried Rapaki silt loam. All cups were weighed and water added to bring the soil moisture content to field capacity (FC) (55% water w/w). Oat seedlings (cv. Taiko) were planted individually into each cup and raised in a controlled environment cabinet (14 h photoperiod, 20 °C and 15 °C day and night temperatures respectively, 75% RH, photon density flux 350-400 μ mol/m²/s).

Water stress treatments were imposed at the expansion of the second leaf. Water was withheld sequentially from three series of pots such that soil moisture content fell in the first series of pots to half FC 2 days prior to herbicide application, on the day of herbicide application in the second series, and 4 days after herbicide treatment in the third. Half FC corresponded to wilting point. A fourth series of pots were maintained at FC throughout the experiment. All pots were weighed daily and sufficient water added to maintain the appropriate soil moisture content.

Diclofop-methyl (Hoegrass 36) was applied to plants at the appearance of leaf three. Applications were made at 1.0 kg ai/ha in 250 litres/ha. Control plants received

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water only. Twenty days after herbicide application plants were harvested, leaf necrosis was visually assessed and shoot fresh and dry weight determined.

In a second experiment plants were grown individually in 10 cm diameter pots containing potting compost/loam mix (50/50 v/v) and raised in conditions similar to above. All plants were watered daily and at no point were water stressed. At the appearance of leaf three half of the plants received abscisic acid methyl ester (ABA) (100 μ g/plant), applied as 2 x 5 microlitre droplets between the leaf sheaths of leaves one and two, and two and three. Three hours later diclofop-methyl was applied at 1.0 kg ai/ha. Control plants received water only. Plants were harvested 26 days after treatment and assessed as in the first experiment.

In all instances results presented are means from five plants. All measurements of herbicide damage are expressed as percentages of the corresponding untreated control.

RESULTS

Growth of plants was adversely affected when soil moisture content was restricted to half FC (Table 1).

TABLE 1: Effect of soil moisture on growth of unsprayed plants. Treatment abbreviations refer to time (days) of imposition of soil moisture stress relative to herbicide application. FC refers to plants maintained at field capacity. $\pm$ S.E.

Treatment	Fresh weight (mg)	Dry weight (mg)
FC	1233 $\pm$ 56	220 $\pm$ 28
-2	306 $\pm$ 26	78 $\pm$ 4
0	326 $\pm$ 16	91 $\pm$ 6
4	471 $\pm$ 49	165 $\pm$ 10

Diclofop-methyl damage to plants maintained at FC was severe. Seven days after herbicide application chlorotic regions appeared on leaves and these became more pronounced with time. At harvest all leaves were necrotic and the plants were considered dead. Shoot fresh and dry weights (8% and 28% respectively of untreated control) represented the plant material present before herbicide application (Fig. 1). Imposition of water stress 2 days before diclofop-methyl application reduced the effects of the herbicide. Leaf damage was restricted to discrete chlorotic patches totalling less than 25% of leaf area, which did not become necrotic. Little restriction in shoot growth was recorded (Fig. 1). When half FC was reached on the day of diclofop-methyl treatment herbicide protection was again observed, but not to the same degree as in plants stressed before herbicide application. Leaf damage was more extensive, but chlorosis was still less than 50% of leaf area and little necrosis was observed. Both fresh and dry weights were reduced by around 60%. When water stress was delayed until 4 days after diclofop-methyl treatment little protection was observed; leaves showed large necrotic areas (totalling over 50% of leaf area) and shoot growth was only marginally higher than treated plants grown at FC (Fig. 1).

Application of ABA to plants prior to diclofop-methyl treatment also reduced herbicide damage (Table 2). Leaf damage was restricted to local chlorotic patches

TABLE 2: Effect of ABA on diclofop-methyl injury in cultivated oats.

ABA g/plant	Dry weight (mg)		Leaf number	
	0	Diclofop-methyl kg ai/ha 1.0	0	1.0
0	1077 $\pm$ 41	106 $\pm$ 17	7.0 $\pm$ 0.0	3.4 $\pm$ 0.5
100	991 $\pm$ 40	523 $\pm$ 24 $\pm$ S.E.	7.0 $\pm$ 0.0	6.0 $\pm$ 0.3

Fig. 1: Shoot freshweight following diclofop-methyl treatment.

covering less than 25% of leaf area and no necrosis was recorded. Furthermore a suppression of leaf production observed in diclofop-methyl treated plants was countered by ABA. Although the depression in shoot growth associated with herbicide injury was also ameliorated by ABA pretreatment (Table 2), protection was less than that observed from droughting 2 days prior to diclofop-methyl application.

DISCUSSION

When oats were water stressed prior to diclofop-methyl application, herbicide damage was reduced. This result is consistent with observations by Akey and Morrison (1983) and Field and Caseley (1986). Application of ABA prior to diclofop-methyl treatment also protected oats against injury although for shoot growth this protection was incomplete. This failure of ABA to completely counteract the effects of herbicide damage is cultivar specific; for example with cv. Amuri, ABA protection is complete with respect to both leaf damage and shoot growth (Foreman and Field, unpublished). This is being further investigated. Field and Caseley (1986) also report on the capacity of ABA to ameliorate diclofop-methyl damage to wild oats.

As plants experience increasing water stress a series of physiological events commence (Levitt 1980; Hsiao *et al* 1976). Leaf expansion, being a turgor driven process, is highly sensitive to even mild water stress; decreased leaf turgor results in reductions in leaf area development which in turn has profound effects on plant productivity (Hsiao *et al* 1976). As water stress approaches wilting point there is a

marked rise in ABA levels within leaves (Wright and Hiron 1969). Application of ABA can thus be considered analogous in physiological terms to water stress, protecting against diclofop-methyl damage in a similar manner. It is generally accepted that high ABA levels are instrumental in the stomatal closure associated with water stress (Davies and Mansfield 1983).

Uptake of diclofop-methyl by the shoot is via either cuticular or stomatal pathways. Since stomatal closure would occur in plants at wilting point or following exogenous ABA treatment, entry of diclofop-methyl into leaves would be through the cuticle. However Akey and Morrison (1983) reported no difference in diclofop-methyl uptake into droughted plants. Furthermore this experiment has shown that water stressing plants after herbicide treatment afforded some protection. No drought induced stomatal closure would occur in these plants during the 24 h following diclofop-methyl application, the period in which most of the herbicide is absorbed (Todd and Stobbe, 1980; Akey and Morrison, 1983). Thus protection via restricted herbicide uptake appears unlikely.

A second potential mechanism for herbicide protection is through the reduced meristematic activity of water stressed plants (Hsiao *et al* 1976). Reduction in the frequency of mitosis would restrict the "opportunity" for diclofop-methyl to exert phytotoxic activity. Leaf production in plants protected by exogenous ABA was maintained (Table 2), indicating continued meristematic activity in these plants. This argument is therefore discounted.

Riken and Rubin (1983) have reported that pre-treatment of cotton leaves with ABA can also confer protection against endothall injury. They suggested that ABA modifies or 'hardens' membrane structure, thereby preventing injury. ABA induced hardening of membranes occurs in response to environmental stresses (Levitt 1980), and is likely to be non-specific i.e. once hardened, membranes are resistant to a range of stresses (Boussiba *et al* 1975). It is therefore possible that protection against diclofop-methyl injury could be due to membrane hardening.

The timing of water stress required to procure protection is therefore likely to depend on three factors:

- * The time taken for water stress to reach the threshold point at which high ABA levels result
- * The time taken for ABA to effect membrane hardening
- * The time taken for diclofop-methyl to reach and cause injury to membranes

If ABA is found to have a central role in safening against diclofop-methyl injury, or against other herbicides which act upon membranes, then the possibility for the development of a broad spectrum herbicide safener may exist. Currently investigations are being undertaken to confirm the action of ABA on hardening membranes against diclofop-methyl damage.

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