ABSCISIC ACID PROTECTION AGAINST DICLOFOP-METHYL DAMAGE IN CULTIVATED OAT

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Summary. The efficacy of diclofop-methyl against cultivated oats, *Avena sativa*, was shown to be antagonised by exogenous application of abscisic acid (ABA). The resulting protection was rate related. Furthermore, it was demonstrated that diclofop-methyl induced electrolyte leakage from leaf tissue was significantly reduced by pre-treatment of ABA. The uptake and translocation of radiolabelled diclofop-methyl was not altered by pre-treatment with ABA. This evidence suggests that protection afforded by ABA against diclofop-methyl is not due to any changes in lethal doses of the herbicide reaching the normally susceptible plasmalemma, but due to increasing membrane tolerance to diclofop-methyl.

INTRODUCTION

The concept of crop safening, namely actively protecting a crop against herbicide damage, is not new (10). Although widespread use of crop safeners has not occurred, as the registration of new herbicides becomes both more difficult and expensive the idea of crop safeners is increasingly attractive. The identification of crop safeners is not easy; the empirical screening of large numbers of compound combinations is not practical. Stephenson and Ezra (10) suggested that by examining reported situations of herbicide antagonism, and from a physiological understanding of such interactions, rational safener development might be possible. Field and Caseley (3), and Foreman and Field (4) identified an antagonism where the efficacy of the herbicide diclofop-methyl was reduced against wild oat and cultivated oat grown under conditions of soil moisture stress. Furthermore, it was demonstrated that an exogenous application of the phytohormone abscisic acid (ABA) to non-water stressed plants could prevent herbicide damage in a similar manner to water stress. From this evidence it has been suggested that there may be potential for ABA, or its chemical analogues, to act as an effective crop safener (4, 5).

In this paper experiments designed to investigate the physiological basis for ABA protection are described.

METHODS

Plant material. Seedlings of Oat, *Avena sativa* cv. Taiko, were planted individually into 11 cm pots containing loan/potting mix (1:1 v/v) and raised in an environmental cabinet (14h photoperiod, photon flux 350-400 $\mu E\ m^{-2}\ s^{-1}$, day/night temperatures 20/15°C respectively, and 75% RH). Plants were watered regularly and were never water stressed.

Growth analysis. At the 2-3 leaf stage plants were treated with diclofop-methyl (as the commercially formulated Hoegrass^R 36EC) at 1.0 kg a.i. ha^{-1}, applied in 250 L ha^{-1}. Control plants received water only. Three hours prior to herbicide application half of the plants received abscisic acid methyl ester at 10, 20, 50 and 100 $\mu g\ plant^{-1}$, applied between leaf sheaths as decribed elsewhere (3). Twenty-six days after herbicide application plants were harvested: leaf necrosis was visually assessed, and shoot dry weight determined. Six replicate plants were used for each treatment.

Electrolyte leakage. Membrane integrity of oat leaves was assessed six and

ten days after application of diclofop-methyl at 1.0 kg ha^{-1}, with and without pretreatment of ABA at 100 *ug* $plant^{-1}$. Membrane integrity was assessed using the electrolyte leakage technique previously described by Foreman *et al.* (5). Nine replicate plants were used for each treatment and membrane integrity expressed as the herbicide induced electrolyte leakage as a precentage of total potential electrolyte leakage.

Diclofop-methyl uptake and translocation. At the 2-3 leaf stage half the plants were treated with 100 *ug* ABA three hours before radiolabelling. Radiolabelled ^{14}C-diclofop-methyl was applied to the upper lamina of the second leaf as 10x0.5 *ul* droplets to give at total activity of 0.1 *uCi*. Twenty-four hours after application the treated leaf was detached from the plant and dissected into three portions: the lamina and sheath below the site of application, the site of application, and the lamina above the site of application. The lamina at the site of ^{14}C-diclofop-methyl was rinsed with unlabelled diclofop-methyl to ascertain the amount of radiolabelled compound adhering to the leaf surface and not absorbed. This sample was termed the "wash off". All three leaf portions were then ground in liquid nitrogen prior to being solubilised in Soluene.350 (Packard Instrument Co.). The radioactivity of all samples was determined by scintillation counting. Ten replicate plants were used for each treatment.

RESULTS AND DISCUSSION

Diclofop-methyl was highly phytotoxic to oats; all leaves were necrotic after 26 days and shoot dry weight was reduced by over 90% (Table 1). Application of ABA to control plants was associated with small depressions in shoot dry weights but no leaf damage. Treatment with ABA prior to diclofop-methyl application significantly reduced herbicide injury. Generally, damage to leaves was restricted to local chlorosis, while shoot growth, even though reduced, was maintained. This protection was rate related; little protection was evident with 10 *ug* ABA in comparison to pre-treatment with 100 *ug*, where leaf chlorosis totalled less than 25% of leaf area and shoot dry weight was reduced by only 47%.

Table 1. Effect of ABA on shoot dry weight and leaf damage of oats treated with diclofop-methyl

ABA ($ug\ plant^{-1}$)	Shoot D.W. (mg) Diclofop-methyl (kg ha^{-1}) 0	1.0	Leaf damage (% of total leaf area)
0	1.077	0.106	100% necrosis
10	0.972	0.148	>75% chlorosis
20	1.001	0.365	>50% chlorosis
50	0.961	0.481	>25% chlorosis
100	0.991	0.523	<25% chlorosis
l.s.d. (P=0.05)	0.124		

Negligible electrolyte leakage was recorded from control leaves in contrast to the significant efflux of electrolytes from diclofop-methyl treated leaves (Table 2). Within six days from treatment leaf segments exhibited electrolyte leakage which became more pronounced by day 10. Application of ABA at 10 *ug*

before herbicide treatment had limited effect on electrolyte leakage, contrasting to the high level of protection afforded at 100 *ug* ABA.

Table 2. Diclofop-methyl induced electrolyte efflux from leaf tissue, (% of total potential efflux)

	Day 6			Day 10		
	ABA (*ug* plant^{-1})					
	0	10	100	0	10	100
Control	2.5	0.7	1.1	2.4	2.6	0.8
Diclofop-methyl	15.5	14.5	3.2	36.6	22.1	7.1

pooled s.e.m. = 0.3

Over 95% of total applied radioactivity was recovered from the treated leaf and wash off solution, indicating minimal translocation of diclofop-methyl away from the treated leaf and small experimental losses. The partitioning of radioactivity (Table 3) is expressed as a percentage of recovered radioactivity from the treated leaf and wash off. Approximately one third of the applied ^{14}C-diclofop-methyl was absorbed by the leaf, with negligible translocation away from the site of application. Pre-treatment with ABA to plants did not alter the uptake or translocation of radioactivity.

Table 3. The effect of ABA on uptake and translocation of ^{14}C-diclofop-methyl (% of total recovered activity)

ABA (*ug* plant^{-1})	Wash off	Site of application	Below application	Above application
0	66.5	31.9	1.1	0.5
100	65.4	32.9	1.0	0.7
s.e.m	1.5	1.1	0.1	0.1

The phytotoxic effects of diclofop-methyl observed in this study were consistent with those described elsewhere (2, 4, 7). Furthermore, the ability of ABA pre-treatment to restrict shoot damage (Table 1) was comparable to that found by others (3, 4), although it is noted that higher rates of ABA were required to afford protection than those required for wild oat, *A. fatua*. The high level of electrolyte efflux from leaves is characteristic of diclofop-methyl damage (7). The ability of ABA to restrict such leakage does however contrast with other data where antagonistic interactions of diclofop-methyl and hormone type herbicides exist, but where no protection of the plasmalemma has been observed (7).

The current data, although not establishing firm physiological mechanisms for ABA protection, does negate one possible mechanism and supports another. Firstly, the failure to detect any change in either the uptake or translocation of diclofop-methyl following ABA pre-treatment, precludes that protection is a purely rate-related phenomenon (Table 3). Akey and Morrison

(1), and Todd and Stobbe (11) have produced similar evidence to demonstrate that loss of diclofop-methyl efficacy under drought conditions and 2,4-D antagonism respectively, was not due to changes in uptake or translocation.

The ability of ABA to reduce electrolyte leakage from leaf tissues is a possible indication of the site at which ABA induces protection (Table 2). The primary site of action for diclofop-methyl is the plasmalemma (2) leading to leaf chlorosis. Thus accepting that electrolyte leakage is a viable measure of membrane integrity, ABA appears to influence the susceptibility of the plasmalemma, and possibly other cell membranes, to diclofop-methyl damage. It has been proposed (4, 8) that ABA may "harden" membranes against herbicide damage. Although the mechanism of hardening is still unclear, the concept of ABA conferring membrane tolerance against environmental stress is widely recognised (6). Such hardening is non-specific and might well increase membrane tolerance to herbicides which are disruptive to the plasmalemma (8).

An alternative to the hypothesis of membrane hardening being the mechanism of ABA protection is that ABA may alter the metabolism of diclofop-methyl. The selectivity of diclofop-methyl is a function of the rate of hydrolysis of the ester to the acid and conjugates (9). It must therefore be established as to whether ABA has any effect upon the metabolic pathway of diclofop-methyl.

In conclusion, the identification of ABA as a crop safener against diclofop-methyl merits the continued investigation of the physiological mechanism of protection. Resolving the effect of ABA on membrane integrity and diclofop-methyl metabolism will be an important step in acheiving this aim.

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