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Effect of Drought Stress, Abscisic Acid, and Abscisic Acid Analogues on the Efficacy of Diclofop-methyl and Tralkoxydim

Malcolm D. Devine,[1,*] J. Curtis Harren,[1] Suzanne R. Abrams,[2] and Lawrence V. Gusta[3]

[1]Department of Crop Science and Plant Ecology and [3]Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0; and, [2]NRC Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada S7N 0W9

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Abstract. The effects of drought stress, abscisic acid (ABA), and four ABA analogues on diclofop-methyl and tralkoxydim efficacy were investigated in oat (*Avena sativa*). Drought stress conditions (6% soil moisture content) reduced the efficacy of diclofop-methyl at 350 g ha^{-1}, but not at 700 g ha^{-1}. Similarly, tralkoxydim efficacy was reduced by drought stress at 62.5 and 125 g ha^{-1}, but not at 250 g ha^{-1}. ABA (100 μM), applied as a root drench 2 days before the herbicide, protected oat plants against all rates of diclofop-methyl and against low rates of tralkoxydim. Two ABA analogues protected oat plants from diclofop-methyl injury, whereas two others had no effect. Foliage applications of ABA were much less effective than root applications in protecting against herbicide injury. Protection by ABA and the two active analogues was dependent on the relative time of application with respect to the herbicides. Optimal protection by ABA and analogue I was obtained when they were applied between 2 days before and 1 day after diclofop-methyl application. Analogue IV protected plants when applied between 3 days before and 1 day after diclofop-methyl application. Partial protection against tralkoxydim activity by ABA was observed when it was applied between 1 day before and 1 day after herbicide application. Analogue I did not afford any protection against tralkoxydim, and analogue IV afforded partial protection when applied the same day or 1 day after tralkoxydim. The results indicate that protection against these postemergence herbicides, similar to that conferred by water stress, can be induced by ABA and structural analogues that apparently mimic the action of ABA.

Diclofop-methyl and tralkoxydim are selective postemergence herbicides used to control grassy weeds such as wild oat (*Avena fatua*) and green foxtail (*Setaria viridis*) in cereal and some broadleaf crops. Both herbicides are potent inhibitors of acetyl-Coenzyme A carboxylase, a key enzyme in fatty acid biosynthesis (Burton et al. 1987, Kobek et al. 1987, Secor and Cséke 1988).

Drought stress has been reported to decrease the efficacy of diclofop and related herbicides (Akey and Morrison 1983, Dastgheib et al. 1990, Dortenzio and Norris 1980, Wilcox et al. 1987). Dortenzio and Norris (1980) reported that diclofop-methyl activity was decreased by 15–50% in wild oat grown just above the permanent wilting point, as compared with plants grown at field capacity; the maximum effectiveness of diclofop-methyl was achieved when plants were grown in high soil moisture for 2–4 days following treatment. Controlled environment experiments with wild oat revealed a 40% reduction in activity if the soil moisture content (SMC) was maintained at 10% for 5 days after treatment with diclofop-methyl compared with when the SMC was maintained at 20% (Akey and Morrison 1983). Extending the period of moisture stress after treatment further reduced diclofop-methyl activity. Similarily, Wilcox et al. (1987) observed a 20% decrease in wild oat control by diclofop-methyl at low SMC (8–12%). Increasing the duration of drought stress from 4 to 10 days led to a corresponding increase in tolerance to diclofop-methyl (Akey and Morrison 1983).

Abbreviations: SMC, soil moisture content; ABA, abscisic acid.

*Author for correspondence.

Abscisic acid (ABA) concentrations increase dramatically in drought-stressed plants. The ABA concentration in drought-stressed wheat (*Triticum aestivum*) plants increased rapidly to 40 times the nonstressed concentration (Wright and Hiron 1969). Similarly, the ABA content increased 40-fold in wilting avocado (*Persea americana*) leaves (Milborrow and Robinson 1973). ABA induces rapid stomatal closure and is implicated in the induction of chilling and freezing tolerance (Chen and Gusta 1982, Zeevaart and Creelman 1988).

ABA can mimic the effect of drought stress in protecting against herbicide injury. At 40–100 μg/plant, ABA induced rapid stomatal closure and a reduction in leaf extension rate and protected oat plants against damage from diclofop-methyl at 1.5 kg ha^{-1} (Field and Caseley 1987). Application of ABA 3 days or 3 h prior to herbicide treatment protected plants against diclofop-methyl, but ABA applied after herbicide treatment was less effective (Field and Caseley 1987). Similarly, exogenous ABA (10 μM) provided the greatest protection against the herbicide endothall when it was applied prior to the herbicide (Rikin and Rubin 1983).

Attempts to understand the molecular requirements for ABA action have led to the testing of ABA analogues for biological activity (e.g., Churchill et al. 1992, Walker-Simmons et al. 1992). Practical applications related to the growing of plants under stress conditions have also spurred an interest in ABA analogues (Blake et al. 1990, Churchill et al. 1992, Gusta et al. 1990, Flores et al. 1988). However, the biological activity of the various ABA analogues varies considerably according to the analogue used and the experimental system under investigation (Churchill et al. 1992, Gusta et al. 1990).

Since ABA had previously been shown to protect plants against diclofop-methyl activity, this study was conducted to evaluate further the biological activity of ABA and four ABA analogues as herbicide protectants. The objectives of the present study were to determine: (1) the effects of drought stress and ABA on herbicide efficacy; (2) the effects of ABA analogues on herbicide efficacy; and (3) the effect of relative time of ABA or ABA analogue application on herbicide efficacy.

Materials and Methods

General Experimental Procedures

Oat (*A. sativa* L. cv. Calibre) was used in all experiments. The plants were grown in a controlled environment chamber under a 14-h daylength (640 μE m^{-2} s^{-1} at pot height), 22/15 °C day/night temperatures, and relative humidity of 45–55% and 70–80% during the day and night, respectively.

Herbicides were applied when plants were at the three- to four-leaf stage. Two h prior to herbicide application the plants were watered as required for their stress treatments. The herbicides were applied in the equivalent of 115 L ha^{-1} using a single 80015 spray nozzle in a mechanical spray booth. The plants were returned to the controlled environment chamber immediately after herbicide treatment and harvested 21 days later. Shoot fresh weights and heights were measured immediately after harvest, and dry weights were determined after 48 h at 60 °C. All experiments were conducted at least twice, with a minimum of five plants per treatment per experiment; combined results are shown in the tables. All experiments were designed as factorials, with moisture stress/ABA treatment and herbicide dose/relative time of application as the two factors. Since there was a significant interaction in all experiments, the data for each stress or ABA treatment were analyzed separately. All statistical analyses were performed on dry weight data; LSD ($p \leq 0.05$) was used to determine treatment differences.

Chemicals

The commercial formulations of diclofop-methyl (emulsifiable concentrate, 284 g of active ingredient L^{-1}) and tralkoxydim (dry flowable, 25% active ingredient) were used (AgrEvo Canada Inc., Regina, Sask. and Zeneca Agro Inc., Stoney Creek, Ont.). Racemic ABA (>99% pure) was obtained from Sigma Chemical Company (St. Louis, MO).

The ABA analogues were synthesized as described previously (Lamb and Abrams 1990). The analogues were: I, (±)-4(*Z*)-(4α,5β)-4-hydroxy-4-(5-hydroxy-3-methylpent-3-en-1-ynyl)-3,5,5-trimethylcyclohexanone (PBI-11); II, (±)-1(*Z*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-enyl)-3-methylpent-2-en-4-ynal (PBI-16); III, (±)-1(*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohexyl)-3-methylpent-2-en-4-ynal (PBI-19); IV, (±)-methyl 1(*Z*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-enyl)-3-methylpent-2-en-4-ynoate (PBI-53). The structures of ABA and the ABA analogues are shown in Figure 1. All four analogues have the C-4, C-5 bond order changed from a *trans* double bond to a triple bond, with further changes at the C-1 functional group, the C-2′, C-3′ bond order, and the stereochemistry of the C-2′ methyl group.

ABA and ABA analogue solutions were prepared by dissolving the parent compound in 90% aqueous ethanol and then diluting in double-distilled water to give a final concentration of 100 μM ABA or ABA analogue in 1% ethanol. Treatment with 1% ethanol had no effect on plant growth or herbicide activity (data not shown).

Effects of Drought Stress on Herbicide Activity

Four oat seeds were sown in 9 × 9 × 10-cm pots containing 425 g of a 3:1:1 (soil:silica sand:peat) potting mixture. Soil moisture treatments consisted of nonstressed and stressed conditions, equivalent to 18% SMC and 6% SMC, respectively. Soil moisture content of 18% was equivalent to field capacity, whereas 6% SMC was just enough to prevent the onset of permanent wilting. The nonstressed treatments were maintained at 18% SMC. In the stressed pots the soil was kept at 18% SMC until plants were in the two-leaf stage, at which time the soil was allowed to dry down to 6% SMC.

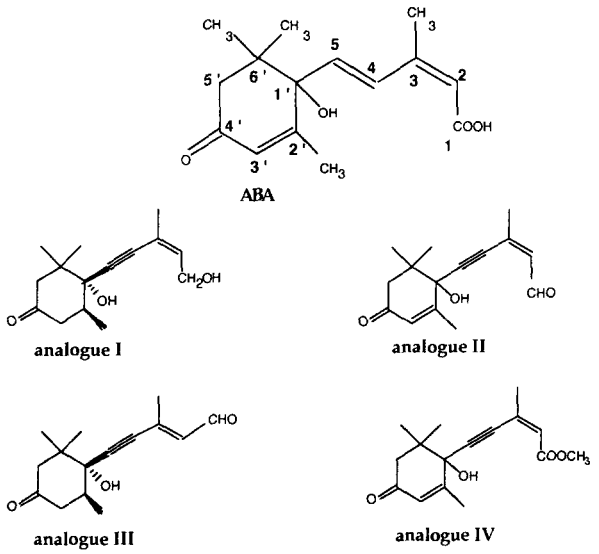

Fig. 1. Structures of ABA and the four ABA analogues used in this study. All compounds were racemic mixtures. Hydrogen atoms are omitted for clarity.

To maintain a consistent SMC in each treatment, each pot was weighed daily and water added until the desired SMC was obtained. The weight required to reach field capacity was determined by watering five pots until puddling occurred on the surface. These five pots were allowed to drain for 24 h before being reweighed. The mean of the five weights was taken and used as the weight at which the soil was at field capacity. The unwatered, stressed treatment pots were watered after wilting became apparent (indicated by loss of leaf turgor). When wilting was observed the five pots were weighed and the mean weight determined. Ten g of water was added to each pot, and the plants were observed 24 h later for signs of wilting. This cycle was repeated until a weight of water was determined at which wilting would just start to become apparent after a 24-h period. Diclofop-methyl was applied at 0, 175, 350, or 700 g (acid equivalent) ha^{-1} and tralkoxydim at 0, 62.5, 125, or 250 g (active ingredient) ha^{-1}. Plants were harvested 21 days after herbicide application, as described above.

Effect of ABA on Herbicide Activity

The effect of foliage-applied and root-applied ABA on herbicide efficacy was examined. In the foliage-applied ABA experiments, the plants were grown as described above for the nonstressed treatment and sprayed with a solution of ABA (100 μM) 2 h prior to herbicide treatment. Diclofop-methyl and tralkoxydim were applied as described above.

In the root-applied ABA experiments, 10 oat plants were sown 2 cm deep in silica sand and watered with half-strength Hoagland's solution (Hoagland and Arnon 1950). Pots were lined with plastic bags and filled with 300 ml of silica sand. Nutrient solution was added as required to maintain nonstressed growing conditions. Two days prior to herbicide application, the plants were treated with 25 ml of 100 μM ABA instead of the nutrient solution. Watering with nutrient solution was resumed on the day of spraying, prior to herbicide application. Diclofop-methyl was applied at rates of 0, 350, 700, or 1,050 g ha^{-1} and tralkoxydim at rates of 0, 25, 75, 125, 250, or 375 g ha^{-1}. Plants were not watered for another 2 days, to avoid washing the herbicide off the leaves, then watered regularly until harvest.

Effect of ABA Analogues on Herbicide Activity

The four ABA analogues (100 μM) were applied as described for root application of ABA in the previous section. Diclofop-methyl was applied at 0, 350, 700, or 1,050 g ha^{-1}, and tralkoxydim at 0, 125, 250 or 375 g ha^{-1}. All other procedures were as for the root-applied ABA experiment.

Effect of Relative Time of Application of ABA, Analogue I, or Analogue IV on Herbicide Activity

Plants were treated with ABA, analogue I, or analogue IV (100 μM) at various times before or after herbicide application (see Tables 4 and 5 for details). Check treatments included: (1) plants that were not treated with herbicide or ABA (or ABA analogue); (2) plants that were treated with herbicide on day 0 but not treated with ABA (or ABA analogue); and (3) plants treated with ABA (or ABA analogue) on each day but not treated with herbicide. Diclofop-methyl was applied at 0 or 700 g ha^{-1} and tralkoxydim at 0 or 75 g ha^{-1}.

Results

Shoot dry weights of untreated plants ranged from 1.3 to 1.8 g, depending on the experiment. In all cases, dry weight data, converted to percent of appropriate controls, are presented in the Tables.

Effects of Drought Stress on Herbicide Activity

Drought stress markedly reduced plant dry weights (1.3 and 0.3 g for the nonstressed and drought-stressed plants, respectively) and afforded some protection against the lower rates of diclofop-methyl and tralkoxydim. Under nonstressed conditions, diclofop-methyl at 350 and 700 g ha^{-1} significantly reduced oat shoot dry weights (Table 1). Under stressed conditions, however, shoot dry weights were reduced only by 700 g ha^{-1} diclofop-methyl. Nonstressed plants were chlorotic/necrotic at harvest following treatment with all rates of diclofop-methyl; in contrast, the drought-stressed plants did not show any herbicide injury symptoms. All rates of tralkoxydim reduced plant dry weight under nonstressed conditions, but only the higher rates (125 and 250 g ha^{-1}) reduced the dry weight of drought-stressed plants (Table 1).

Table 1. Effect of drought stress (18 versus 6% SMC) on the dry weights of diclofop-methyl- and tralkoxydim-treated oat plants. The data are expressed as percent of the dry weights of untreated plants in the corresponding moisture treatment. Mean dry weights of nonstressed and drought-stressed untreated plants were 1.3 and 0.3 g, respectively.

Herbicide rate (g ha^{-1})	Mean shoot dry weight (%)[a]	
	Nonstressed	Drought-stressed
Diclofop-methyl		
0	100a	100a
175	103a	119a
350	77b	97[b]a
700	49c	52b
Tralkoxydim		
0	100a	100a
62.%	68b	114[b]a
125	52c	82[b]b
250	49c	59c

[a] Data within each column/herbicide grouping followed by the same letter are not significantly different ($p \leq 0.05$).
[b] Significantly different ($p \leq 0.05$) from corresponding nonstressed treatment.

Table 2. Effect of root-applied ABA (100 μM) on the dry weights of diclofop-methyl- and tralkoxydim-treated oat plants. The data are expressed as percent of the dry weights of untreated plants in the corresponding treatment.

Herbicide rate (g ha^{-1})	Mean shoot dry weight (%)[a]	
	Control (no ABA)	ABA-treated
Diclofop-methyl		
0	100a	100a
350	63b	81[b]b
700	46c	66[b]bc
1,050	40c	62[b]c
Tralkoxydim		
0	100a	100a
25	56b	78[b]ab
75	43b	64[b]bc
125	45b	60bc
250	45b	49c
375	42b	46c

[a] Data within each column/herbicide grouping followed by the same letter are not significantly different ($p \leq 0.05$).
[b] Significantly different ($p \leq 0.05$) from corresponding control (no ABA) treatment.

Effect of ABA on Herbicide Activity

Foliage-applied ABA had no effect on the activity of diclofop-methyl or tralkoxydim (data not shown). Plant dry weights were not affected, and all plants were dead 21 days after herbicide application, regardless of ABA treatment.

Root-applied ABA provided significant protection against diclofop-methyl at all rates, although dry weight was also reduced in the ABA-treated plants (Table 2). The typical herbicide injury symptoms (chlorosis and necrosis) did not appear in the ABA-treated plants, whereas they were evident in plants not treated with ABA. Some chlorosis was apparent in plants treated with ABA and diclofop-methyl at 1,050 g ha^{-1}, but all plants survived. ABA alone had no effect on plant dry weight or survival (data not shown).

Tralkoxydim significantly reduced shoot dry weights in both ABA-pretreated and nontreated plants (Table 2). At the lower rates (25 and 75 g ha^{-1}), the reduction in shoot dry weights was less in ABA-pretreated plants than in non-ABA-treated plants, indicating some protection by ABA. All plants sprayed with tralkoxydim eventually became necrotic and died, except for several plants pretreated with ABA which survived treatment with tralkoxydim at 25 or 75 g ha^{-1}.

Effect of ABA Analogues on Herbicide Activity

In these experiments, all herbicide treatments in the absence of ABA analogues resulted in death of the plants. The dry weight data (Table 3) do not present a complete picture of the protection induced; in some cases the analogue-treated plants survived herbicide treatment, although the dry weight data did not indicate a protective effect of the analogue.

According to the dry weight data, analogue I reduced the effect of diclofop-methyl at the highest rate only (Table 3). However, none of the plants treated with diclofop-methyl and analogue I developed the typical herbicide injury symptoms, and all of the plants were alive at the end of the experiment. Similar results were obtained with diclofop-methyl and analogue IV (Table 3), with a protective effect on dry weight observed only at the highest herbicide rate. However, all plants treated with diclofop-methyl and analogue IV survived herbicide treatment.

In contrast, analogues II and III provided no protection against diclofop-methyl activity. This was evident both in the dry weight data (Table 3) and in the appearance of the plants. All of the plants, including those treated with diclofop-methyl and either of these two analogues, were dead by the end of the experiment.

None of the ABA analogues afforded any protection against tralkoxydim activity (Table 3). All of the tralkoxydim-treated plants eventually died, regardless of the tralkoxydim rate or whether or not they were pretreated with the ABA analogues.

Table 3. Effect of root-applied ABA analogues (100 μM) on the dry weights of diclofop-methyl- and tralkoxydim-treated oat plants. The data are expressed as percent of the dry weights of untreated plants in the corresponding treatment.

	Mean shoot dry weight (%)[a]				
Herbicide rate (g ha^{-1})	No analogue	Analgoue I	Analogue II	Analogue III	Analogue IV
Diclofop-methyl					
0	100a	100a	100a	100a	100a
350	77ab	76ab	86a	80b	79ab
700	63b	67b	71b	65bc	72b
1,050	52b	73[b]b	61b	59c	71[b]b
Tralkoxydim					
0	100a	100a	100a	100a	100a
125	57b	50b	67b	56b	67b
250	51b	46b	55bc	55b	52bc
375	47b	40b	43c	54b	39c

Data within each column/herbicide grouping followed by the same letter are not significantly different ($p \leq 0.05$).

[b] Significantly different ($p \leq 0.05$) from corresponding "No analogue" treatment.

Effect of Relative Time of Application of ABA, Analogue I, or Analogue IV on Herbicide Activity

ABA applied between 8 and 3 days before diclofop-methyl did not reduce the effectiveness of the herbicide (Table 4); however, application of ABA to oat plants 1 or 2 days before, the same day, or 1 day after diclofop-methyl significantly reduced its activity (Table 4). In this case the dry weight data were supported by the plant survival data; plants treated with ABA between 2 days before to 1 day after diclofop-methyl application were still healthy at the end of the experiment, whereas the other ABA-treated plants were necrotic. Again, ABA alone had no effect on plant shoot dry weight (data not shown).

ABA prevented tralkoxydim injury when the two compounds were applied on the same day (Table 4). The data also indicated partial protection when the plants were treated with ABA 1 day before or 1 day after tralkoxydim application, although the plant dry weights were reduced with respect to the control. At all other times of ABA application, tralkoxydim greatly reduced plant dry weights and survival.

Since analogues I and IV showed some protective activity against diclofop-methyl in a previous experiment (Table 3), they were included in the experiments on the relative time of ABA/analogue application. Treatment with analogue I or IV alone had no effect on oat dry weight or appearance (data not shown). Treatment with diclofop-methyl at 700 g ha^{-1} or tralkoxydim at 75 g ha^{-1} alone significantly reduced oat shoot dry weight (Table 5). Analogue I afforded some protection against diclofop-methyl when applied between 2 days before and 1 day after the herbicide (Table 5). Survival was also greatest when the plants were treated with analogue I within 2 days of diclofop-methyl application.

Analogue I application did not afford any protection against tralkoxydim, according to the dry weight data (Table 5). However, approximately half of the plants survived when treated with analogue I 1 day after tralkoxydim; in all other treatments, all the plants were dead at the end of the experiment.

Diclofop-methyl efficacy was reduced by the application of analogue IV at all times (Table 5). Plant height and survival data were in agreement with the shoot dry weight data. Analogue IV treatment the same day or 1 day after tralkoxydim application reduced its efficacy, although the plant dry weights were significantly reduced compared with the untreated controls (Table 5). Again, the plant height and survival data supported these results (data not shown).

Discussion

Previous results have shown that the herbicidal activity of diclofop-methyl and structurally related herbicides can be reduced significantly under drought stress conditions (Akey and Morrison 1983, Dastgheib et al. 1990, Dortenzio and Norris 1980). The results presented here confirm this point and indicate that the activity of tralkoxydim, a member of a second group of acetyl-Coenzyme A carboxylase-inhibiting herbicides, can also be reduced under moisture-stress conditions.

ABA and the ABA analogues provided varying

Table 4. Effect of relative timing of root application of ABA (100 μM) on the dry weight of oat plants treated with diclofop-methyl (700 g ha^{-1}) or tralkoxydim (75 g ha^{-1}). The data are expressed as percent of the dry weights of untreated plants (no herbicide or ABA treatment).

Relative time of ABA application	Mean shoot dry weight (%)[a]	
	Diclofop-methyl	Tralkoxydim
No ABA/no herbicide	100a	100a
Untreated (herbicide, no ABA)	53b	23d
8 DBH[b]	56b	25d
6 DBH	67b	24d
4 DBH	48b	30d
3 DBH	68b	26d
2 DBH	91a	35d
1 DBH	117a	59c
0 DBH	104a	82ab
1 DAH	109a	68bc

[a] Data within each column followed by the same letter are not significantly different ($p \leq 0.05$).
[b] DBH, DAH indicate days before or after herbicide application, respectively.

degrees of protection against the two herbicides. Root-applied ABA protected plants against diclofop-methyl, preventing the onset of chlorosis and allowing many of the plants to survive the herbicide treatment, particularly at low herbicide rates. Pretreatment with analogues I or IV also protected plants from diclofop-methyl injury. Based on visual observations, analogue IV was the most effective analogue, protecting plants from diclofop-methyl injury at all rates. In comparison, analogue I did not completely prevent injury following treatment with diclofop-methyl at the highest rate. In previous research, analogue I provided only a small degree of freezing tolerance in rye seedlings (Churchill 1992) and was inactive in freezing tolerance studies in bromegrass cell cultures (Churchill et al. 1992). Wilen et al. (1993) reported that at low concentrations the *R*-enantiomer of analogue 1 (PBI-51) was a competitive inhibitor of ABA (as indicated by effects on napin and oleosin gene expression) in *Brassica napus*, whereas the *S*-enantiomer (PBI-63) was ABA-like at all concentrations tested. Analogue IV is ABA-like in its action in both freezing tolerance induction (Churchill 1992) and germination inhibition tests (Walker-Simmons et al. 1992).

Protection by ABA, analogue I, or analogue IV was dependent on their relative time of application with respect to the herbicides. These results are consistent with those of Field and Caseley (1987), who reported that ABA applied 3 days or 3 h before diclofop-methyl application provided protection, whereas ABA applications after herbicide spraying were less effective. Similarly, ABA best protected plants against endothall when it was applied 5 or 10 h before the herbicide (Rikin and Rubin 1983).

Protection by ABA against tralkoxydim was marginal, with only a few plants treated at the lowest rates surviving the herbicide treatment. In addition, tralkoxydim efficacy was not affected by any of the ABA analogues if applied 2 days before the herbicide; however, some protection against tralkoxydim activity by ABA, analogue I, and analogue IV was observed when they were applied within 1 day of herbicide application. The reduced protection against tralkoxydim compared with diclofop-methyl may be related to the high herbicidal activity of tralkoxydim, even at the relatively low rates used. Rates of diclofop-methyl approaching the recommended field rate (700 g ha^{-1}) were required for consistently effective control of oat plants in these experiments; in contrast, tralkoxydim provided excellent control at rates considerably lower than its recommended field rate (250 g ha^{-1}). Much higher doses of ABA (or ABA analogues) may be required to protect against tralkoxydim at its recommended rate. Alternatively, ABA or the active ABA analogues may only provide protection against extremely low rates of tralkoxydim or when applied closer to the time of tralkoxydim application.

Analogues II and III did not confer any protection when applied 2 days before diclofop-methyl or tralkoxydim (Table 3). In studies on the effect of ABA analogues on increasing the freezing tolerance of rye seedlings, analogue II was almost as effective as ABA (Churchill 1992). Its ABA-like activity has also been shown in many other systems (Walton 1983). However, it was inactive in increasing the freezing tolerance of bromegrass cell cultures (Churchill et al. 1992). The lack of activity of ana-

Table 5. Effect of relative timing of root application of analogue I or IV (100 μM) on the dry weight of oat plants treated with diclofop-methyl (700 g ha^{-1}) or tralkoxydim (75 g ha^{-1}). The data are expressed as percent of the dry weights of untreated plants (no herbicide or ABA treatment).

Relative time of ABA analogue application	Mean shoot dry weight (%)[a]	
	Diclofop-methyl	Tralkoxydim
Analogue I		
No analogue/no herbicide	100a	100a
Untreated (herbicide, no analogue)	54c	35b
Analogue I 3 DBH*	70bc	34b
Analogue I 2 DBH	87ab	30b
Analogue I 1 DBH	85ab	27b
Analogue I 0 DBH	80ab	37b
Analogue I 1 DAH	91ab	50b
Analogue IV		
No analogue/no herbicide	100a	100a
Untreated (herbicide, no analogue)	48b	39c
Analogue IV 3 DBH	90a	35c
Analogue IV 2 DBH	92a	42c
Analogue IV 1 DBH	92a	45c
Analogue IV 0 DBH	114a	72b
Analogue IV 1 DAH	96a	63b

[a] Data within each column/analogue grouping followed by the same letter are not significantly different ($p \leq 0.05$).
[b] DBH, DAH indicate days before or after herbicide application, respectively.

logue III in this study is in agreement with the results of Churchill et al. (1992) which indicate that a *cis* conformation at the C-2, C-3 position (Fig. 1) is required for biological activity, whereas a *trans* substitution inactivates the molecule.

Plant response to ABA is dependent on the concentration of ABA in the tissue and the sensitivity of that tissue to ABA (Hetherington and Quatrano 1991). Consequently, it is likely that the biological activity of ABA or ABA analogues as herbicide protectants is influenced in part by the dose applied, the site of application (e.g., root versus shoot), and by the capacity of the plant to absorb and transport sufficient quantities of the active molecule(s) to the required tissues. The lack of effect of foliage-applied ABA in our experiments may reflect limited cuticular penetration of ABA. Higher concentrations of ABA (as in Field and Caseley 1987) may be required to get sufficient quantities into the tissue; alternatively, a more nonpolar form of the molecule could be applied, to facilitate cuticular penetration. Since esters are usually absorbed into leaves more readily than the corresponding free acids, one might speculate that analogue IV, which was active when applied to the roots, may also have been active as a foliar application; however, this was not tested in the present study.

Transport in the phloem or xylem depends on the physicochemical properties of the molecules and on the presence of carriers that may influence membrane transport of the molecules (Devine 1989). For example, weak acids are more likely to be transported in the phloem than derivatives with other functional groups; this would favor the transport of ABA over any of the analogues in the series tested here; however, deesterification of analogue IV to the free acid would also result in a more phloem-mobile compound. Other metabolic reactions may increase or decrease the activity of the analogues, either by changing their transport characteristics or by altering their interaction with an ABA receptor. The conflicting molecular requirements for the activity of ABA analogues in different systems (Churchill et al. 1992, Walton 1983) illustrate the complexity in predicting or explaining optimal activity in a new test system.

The mechanism by which ABA or the analogues protect against herbicide activity has not been determined, and it is unclear what tissue(s) they must reach and what response(s) must be induced for them to act as protectants. Although drought stress and ABA induce rapid stomatal closure, they do not reduce herbicide penetration into the leaves (Akey and Morrison 1983; Dortenzio and Norris 1980; Downey and Devine, unpublished results). However, reduced carbon assimilation and transport in stressed plants may result in less herbicide being translocated to the apical meristem, the most sensitive target tissue for these herbicides. This possibility remains to be investigated.

Alternatively, one may envisage a biochemical basis or the antagonistic activity of ABA or the active analogues, as follows: ABA/analogue enters the plant, is transported to various tissues in the plant and, before or after this, is metabolized to more or less active compounds. In certain tissues, the ABA/analogue induces protection. There may be a lag before the protection is optimal, perhaps reflecting the need for gene induction and de novo protein synthesis as part of the protection mechanism, as observed with ABA-induced protection against environmental stress (e.g., Robertson et al. 1994). In addition, protection may be of limited duration, perhaps because the ABA/analogue is degraded in the tissue. This would explain the rather narrow window for ABA/analogue activity (e.g., 1 or 2 days before herbicide application to 1 day after application). The different activities of ABA and the analogues in this study may reflect the different concentrations and biological activities of the compounds reaching specific tissues within the plant.

Based on the results presented here, ABA has the optimal combination of characteristics to induce protection against the herbicides used in this study. Analogues I and IV share some of these characteristics but are less effective. However, analogues II and III are not sufficiently ABA-like to protect against these herbicides. Further research is required to determine the mechanism of protection against these herbicides and whether other ABA derivatives may have more activity in inducing and maintaining this protection over longer time periods.

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