AN ABSTRACT OF THE THESIS OF

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Title: Some Physiological and Biochemical Aspects of Haloxyfop Herbicidal Activity Alone or Combined with Dicamba

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Soybean (Glycine max L. Merr. 'Evans') was more tolerant than red fescue (Festuca rubra L. 'Pennlawn') and this species more tolerant than tall fescue (Festuca arundinaceae Schreb. 'Houndog') to root-absorbed haloxyfop. Root tissue of the grass species was more affected by haloxyfop than foliar tissue. Amounts of ^{14}C-haloxyfop in soybean roots or shoots were higher than in red fescue or tall fescue, thus, differences in uptake and translocation of haloxyfop by roots do not account for differences in tolerance among species.

In the second phase of the research, dicamba antagonism on haloxyfop herbicidal activity in tall fescue was studied. Haloxyfop at 14 to 20 μM inhibited the activity of ACCase in cell-free extracts from tall fescue by nearly 50%. Dicamba did not prevent this effect.

Antagonism of dicamba on haloxyfop action increased with increasing dicamba concentration and occurred only when both herbicides were applied to the same foliar tissue. Applying dicamba 12 h after haloxyfop-methyl was applied reduced the antagonism but applying dicamba 12 h before haloxyfop-methyl did not. The antagonism occurred

with technical grade acid or formulated ester or salt forms of the herbicides. Actively growing foliar tissue was the main target of haloxyfop.

In the last phase of the research, attempts were made to elucidate the mechanisms involved in the antagonism. Dicamba did not change the pH of the spray solution and did not combine chemically with haloxyfop. Dicamba did not affect leaf retention or uptake of ^{14}C-haloxyfop-methyl, but it reduced haloxyfop movement to the pseudostems (leaf sheaths + crown meristem) and roots. Neither herbicide, alone or combined, changed the structure of mitochondria, phloem end walls, or cuticles. Dicamba stimulated stress-ethylene production, but treatment with ethylene gas did not affect haloxyfop phytotoxicity. Haloxyfop stopped development of proplastids into chloroplasts by arresting internal membrane synthesis when applied alone but not in presence of dicamba. A greater amount of a polar conjugate of haloxyfop was found when plants were treated with a mixture of formulated haloxyfop-methyl and dicamba than when treated only with the former. ^{14}C-haloxyfop accumulated in microsomal membrane vesicles from tall fescue etiolated coleoptiles in a pH-dependent manner, resembling results with ^{3}H-IAA. This accumulation was reduced by addition of dicamba.

Some Physiological and Biochemical Aspects of Haloxyfop Herbicidal
Activity Alone or Mixed with Dicamba

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INTRODUCTION

Haloxyfop, an aryloxyphenoxypropanoic acid herbicide is currently being developed for use in a wide variety of dicotyledoneous crops for selective control of graminaceous weeds. Although regarded as a postemergence herbicide, the compound has shown significant soil activity. Another interesting aspect of this and related compounds is that they are antagonized in their herbicidal activity by herbicides with contrasting chemistries. It appears that different mechanisms might be involved for the different mixtures. It is hoped that an understanding of how the antagonism operates would help make a better use of these herbicides without having to increase rates as an easy but environmentally unsound solution.

Research contained in Chapter 1 was conducted to compare the relative activity, uptake, and translocation of haloxyfop when applied to the nutrient solution in three species of contrasting tolerance to foliar sprays.

Research in Chapter 2 explores the possibility that dicamba, a benzoic acid derivative, was antagonistic of haloxyfop action in foliar sprays of the herbicides in tall fescue. Once such antagonism was confirmed, the interaction directly at the acetyl CoA-carboxylase level was tested. This enzyme is believed to be the site of action of haloxyfop.

Once we discarded ACCase as the site where the antagonism occurs, several aspects of the phenomenon, such as effects of different rates of dicamba, time of application, time of exposure, and age of tall fescue shoots, were examined in Chapter 3.

In Chapter 4, we tested several mechanisms that could account for the antagonism including processes occurring before the herbicides are applied as well as processes on or in the plant.

Finally, in Chapter 5, we integrate evidence that we believe may account for the antagonism of dicamba on haloxyfop activity. Much confirming data are lacking, but we hope that other groups can test our ideas to a deeper extent.

Each chapter was written as a complete and self-contained manuscript. Detailed data are presented in the Appendix except for the "in vivo leaf spectroscopy studies"; in that case the voluminous amount of data generated made it impractical to be included here. However, such data are available on request from Dr. Larry Daley's laboratory at the Department of Horticulture of Oregon State University.

CHAPTER 1. UPTAKE, TRANSLOCATION, AND PHYTOTOXICITY OF ROOT-ABSORBED HALOXYFOP IN SOYBEAN, RED FESCUE, AND TALL FESCUE

ABSTRACT

Concentrations of haloxyfop in nutrient solution required to reduce total plant dry weight of soybean (Glycine max L. Merr. 'Evans'), red fescue (Festuca rubra L. 'Pennlawn'), and tall fescue (Festuca arundinaceae Schreb. 'Houndog') by 50% (GR_{50}) were determined. The GR_{50} values for soybean, red fescue and tall fescue were 76 μM, 3 μM and 0.4 μM, respectively. Growth reduction in roots and shoots of soybean was similar. On the contrary, relative reduction in root tissue weight was greater than for foliar tissue in both grass species. Amounts of ^{14}C-haloxyfop in soybean roots or shoots were higher than in red fescue or tall fescue. Red fescue accumulated less haloxyfop in the foliage than in roots. On the other hand, similar amounts of ^{14}C-haloxyfop accumulated in both organs in either soybean or tall fescue. ^{14}C-haloxyfop appeared to be absorbed actively by roots of all species. Soybean absorbed more nutrient solution but used less of it on a per gram of dry matter produced than the grass species. Differences in uptake and translocation of haloxyfop by roots do not account for differences in tolerance among species; however, a higher retention of haloxyfop in roots of red fescue than in tall fescue may provide an additional selectivity advantage to the former in situations of significant root exposure to the herbicide.

INTRODUCTION

Haloxyfop-methyl is a relatively new herbicide under development for selective postemergence grass control in a wide range of broadleaf species (1, 10). Although this and related herbicides appear to be more effective as postemergence treatments (1, 2, 6, 10), preemergence activity has been reported (4, 11).

Buhler and Burnside (4) controlled forage sorghum with either haloxyfop-methyl or fluazifop-butyl applied preemergence. Rahman et al. (11) reported haloxyfop activity in soil up to 13 weeks after application.

Dicots, in general, are highly tolerant to postemergence applications of aryloxyphenoxypropanoic acid or cyclohexanedione herbicides (3). Red fescue is one of the few grass species with tolerance to foliar sprays of these herbicides. Butler and Appleby (6) reported that red fescue tolerated foliar rates 70 times greater than tall fescue in the greenhouse.

Recent studies with chloroplast extracts from several species suggest acetyl CoA carboxylase as the site of action of sethoxydim, haloxyfop, and related compounds. Differences in enzyme susceptibility to these herbicides among several species seemed to account for differences in tolerance (5, 8, 9, 12, 14, 15).

In most studies, susceptibility to haloxyfop with intact plants has been quantified from foliar applications. Because many field applications are made at the seedling stage and a considerable amount of the herbicide reaches the soil, and because haloxyfop is phytotoxic when applied preemergence, more information on relative uptake and selectivity from root uptake is desirable.

Objectives of this research were to a) quantify tolerance of three plant species to haloxyfop absorbed through roots, b) compare relative tolerance of species to that from foliar treatment reported in the literature, and c) test if absorption or translocation differences of root-absorbed ^{14}C-haloxyfop among species provides an additional explanation for the differences in susceptibility.

MATERIALS AND METHODS

Two-week-old soybean plants and three-week-old red fescue and tall fescue plants were used in all experiments. Plant nutrition was provided with half-strength Hoagland's #2 nutrient solution, pH adjusted to 6.5 with either NaOH or HCl.

Soybean seeds were placed in rolls of paper partially immersed in distilled water. To reduce water loss, beakers and rolls were covered with transparent plastic bags. Four days later, the most uniform seedlings were transferred to 250-ml Erlenmeyer flasks filled with nutrient solution. Plants were placed in a growth chamber adjusted to provide an average of 400 μE m^{-2}s^{-1} photosynthetic photon flux density (PPFD) from a mixture of fluorescent and incandescent lights. Temperature cycle was 27/22 C (day/night), and photoperiod was 15 h.

Seeds of both grass species were pregerminated for 48 h; then placed between double sheets of germination paper. These layers were supported against styrofoam walls fitted vertically into washing pans containing distilled water. Once plants had germinated, distilled water was replenished with nutrient solution. Sheets were kept wet by capillarity. Pans were placed in a growth chamber set to provide 300 μE m^{-2}s^{-1} of PPFD from a mixture of fluorescent and incandescent lights. Temperature cycle was 18/12 C (day/night), and the photoperiod was 15 h. Two-week-old plants (two-leaf stage) were transferred from pans to test tubes containing 50 ml of nutrient solution.

Tolerance studies

A stock solution of 75 μM haloxyfop was prepared by dissolving the appropriate amount of technical grade haloxyfop acid in 3 ml of acetone (final maximum acetone concentration was 0.05% v/v which did not affect plant growth in preliminary studies) and then slowly adding it to the nutrient solution. By successive dilution with more nutrient solution, lower concentrations needed for each experiment were prepared. Experiments were conducted twice. Rates in the first experiment were below the GR_{50} for soybean, so for that species, formulated haloxyfop-methyl was used in the second experiment to overcome the solubility limit of haloxyfop acid. Ranges of concentrations tested in both experiments were 10 to 120 μM for soybean, 0.075 to 5 μM for red fescue, and 0.05 to 1 μM for tall fescue.

Two-week-old soybean plants (second trifoliolate leaf starting to expand) and three-week-old grasses (3-leaf stage) were selected for uniformity and treated by replacing their nutrient solution with fresh nutrient solution containing desired concentrations of haloxyfop. Fifteen and twenty-one days after treatment in the first and second experiment, respectively, plants of each species were harvested, separated into roots and foliage, weighed, dried, and weighed again. Treatments were arranged in a randomized complete block design with four replications. GR_{50} values were obtained by linear regression on the average of roots, shoots and total plant dry weight values. Regression lines of the two experiments for a given species did not differ; thus, data are presented as a combination of the two experiments for which a new equation was fitted.

[14]C-haloxyfop absorption and translocation studies

The three species were grown in a single growth chamber under the conditions previously described for soybean. Each experiment was conducted as a completely randomized design with three replications and one concentration of ^{14}C-haloxyfop (0.03 μM). ^{14}C-haloxyfop in tissue of each species was assayed on five different occasions at 48-h intervals up to 10 days.

Erlenmeyer flasks (250 ml) for soybean or 50-ml tubes for grasses were filled with nutrient solution. Twenty-five μCi haloxyfop (specific activity = 9.44 mCi/ mmol) was dissolved in 10 ml of methanol (HPLC grade) and stored at 5 C until use. After allowing this stock solution to reach room temperature, aliquots were diluted with nutrient solution. From these diluted solutions, 1 ml or 0.25 ml were spiked to each flask or tube for soybean or grasses, respectively, to provide approximately 600 dpm/ml nutrient solution.

Plants were then transferred to the flasks. At each sampling time, plants were removed and roots were washed (preliminary experiments showed no need to measure ^{14}C in this rinse water because of negligible counts detected). Plants were divided into several parts and dried at 75 C for 48 h. Plant parts were weighed and samples (50 mg or less) were added to scintillation vials, wetted with 0.25 ml distilled water, and solubilized in 1.0 ml of 0.6 N plant solubilizer (NCS, Amersham, USA) for 48 h. To reduce color quenching, solubilized plant materials were bleached with 1 ml/vial of a 20% w/v solution of benzoyl peroxide in toluene (kept at 50 C). One hour later, fifteen ml of scintillation cocktail consisting of 5 g of PPO, 0.1 g of POPOP, 400 ml of methoxy-methanol, and 600 ml toluene were added to each

vial. ^{14}C was assayed with a liquid scintillation counter Beckman model LS 7500 (Beckman, USA). Nutrient solution volumes remaining at harvest time were recorded and unabsorbed ^{14}C was estimated by assaying 0.25 ml subsamples from each flask.

^{14}C-haloxyfop accumulation patterns into roots or foliage of each species were determined by regression methods using percentage accumulation of total activity added as the dependent variable and time of exposure to the herbicide as the independent variable. Herbicide accumulation rates among the species were compared by the slopes of the fitted models.

By using GR$_{50}$ values of roots and shoots, estimated haloxyfop accumulated inside the plant 10 days after exposure, and water volume inside foliage or roots of each species 10 days after exposure, the concentration of haloxyfop inside the plant was estimated.

Out of total ^{14}C-haloxyfop originally added, the present system allowed an average recovery of 90%.

RESULTS AND DISCUSSION

Tolerance studies

Soybean plants were 25 times more tolerant to haloxyfop than red fescue plants, which were 8 times more tolerant than tall fescue as indicated from GR_{50} values (Table 1.1). The order of tolerance coincides with field observations and laboratory results with haloxyfop and sethoxydim (2, 6). Root and foliage responded similarly in soybean, but roots of the grass species were inhibited more than foliage (Table 1.1).

[14]C-haloxyfop absorption and translocation studies

The model Y = B X fit all three species for haloxyfop accumulation patterns, where:

Y = amount of haloxyfop in either roots or foliage

as % of total activity originally added

B = slope (percentage haloxyfop accumulation/hour)

X = time of plant exposure to ^{14}C-haloxyfop solution

in hours

Comparison between root uptake and amount of herbicide reaching shoots showed that both soybean and tall fescue accumulated ^{14}C-haloxyfop in roots and shoots in similar amounts at any given time as shown by the respective slopes ($\underline{P}$ = 0.01); thus a single model with all data points from the two organs for each species was fitted (Fig. 1.1, A and B). On the contrary, red fescue accumulated haloxyfop in

shoots at approximately half the rate of root accumulation (Fig. 1.1, C and D; $\underline{P}$ = 0.01). The amount of ^{14}C-haloxyfop in either organ of soybean was higher than the corresponding organs of either grass.

Internal foliar concentrations of haloxyfop causing 50% reduction in growth of grass species (Table 1.2) were similar to those inhibiting 50% of acetyl CoA carboxylase activity in the same species reported by Stoltenberg $\underline{et}$ $\underline{al}$. (15). We found roots of these species to be more sensitive to a given external concentration of haloxyfop than shoots, but GR_{50} values based on estimated haloxyfop concentration inside the plant were similar in roots and shoots of either red fescue or soybean. For tall fescue, a lower internal concentration of haloxyfop in roots than in shoots was needed for an equivalent reduction in dry weight (Table 1.2). The above results suggest several possibilities: (a) acetyl CoA carboxylase (ACCase) content and/or activity in roots of tall fescue is lower than in shoots, (b) ACCases produced in shoots and roots of tall fescue differ in susceptibility to haloxyfop, (c) haloxyfop accumulates in root plastids more than in shoot plastids of tall fescue, and/or (d) constant exposure of tall fescue roots to a haloxyfop solution had secondary negative effects on their development.

Significant reductions in root growth imposed by several stresses, including herbicide treatment, often do not have an accompanying impact on foliar growth. Thus, our data showing that red fescue accumulates less than half as much haloxyfop in shoots as in roots, suggests that this species could have an additional selectivity advantage over tall fescue to preemergence treatment of this

herbicide. Such an advantage may prove useful in the selective control in red fescue of grass weeds with considerable tolerance to foliar applications of haloxyfop.

After 96 h of exposure to ^{14}C-haloxyfop solution, soybean plants absorbed 63 % of the original volume of nutrient solution supplied; yet only 21 % of the ^{14}C-haloxyfop initially added was found inside the plants. The remainder was found in unabsorbed nutrient solution indicating that the concentration of ^{14}C-haloxyfop actually increased in the remaining nutrient solution as time of exposure increased. Grasses behaved similarly with 21 and 23% nutrient solution volume absorbed but only 10 and 6% of total ^{14}C-haloxyfop inside plants of red and tall fescue, respectively, 96 h after treatment (Table 1.3). These results suggest that an active mechanism might be involved in root uptake of haloxyfop similar to the results of Donaldson (7) with 2,4-D. At a pH of 6.5 used for the nutrient solution, nearly 100% of the herbicide molecules exist as the anion form (pK_a of haloxyfop = 4.3). The same can be expected of 2,4-D with a pK_a of 2.8. Anions, in general, are actively absorbed by cells because they are strongly repelled by negative charges present inside membranes (13).

Higher volumes of nutrient solution transpired by soybean may account for more haloxyfop accumulated in either organ of this species compared to grasses on a per plant basis. Red fescue and tall fescue transpired similar volumes of nutrient solution (Table 1.3); yet red fescue retained more ^{14}C-herbicide in its roots and, as a result, somewhat less ^{14}C-haloxyfop moved into its shoots than in tall fescue (Fig. 1.1)

Soybean accumulated less ^{14}C-haloxyfop per unit of root dry tissue than grasses; besides, a reduction in the haloxyfop/foliar dry weight ratio was observed over time (Table 1.4). This could provide additional soybean tolerance to root-absorbed haloxyfop. Roots of tall fescue consistently accumulated less haloxyfop than roots of red fescue on a dry weight basis (Table 1.4); this potential advantage of tall fescue, however, was not enough to overcome its higher susceptibility (8-fold) to haloxyfop (Table 1.1).

Although the same order of tolerance among the species was mantained as reported from foliar sprays; in this study, red fescue was only 8 times more tolerant to haloxyfop than tall fescue; contrasting with the 70-fold difference reported by Butler and Appleby (6) for sethoxydim. Thus, the potential exists for selectivity patterns to be altered when high amounts of haloxyfop are available for root absorption, specially when considering species with a closer tolerance difference than the ones discussed here.

Table 1.1. GR$_{50}$ values for root-absorbed haloxyfop acid based upon plant dry weight in three species[a].

Species	Plant part	GR$_{50}$ (μM)	Regression equation	R^2
SOYBEAN	leaves	77	Y = 95 - 0.6 X	0.94
	roots	73	Y = 101 - 0.7 X	0.93
	whole plant	76	Y = 96 - 0.6 X	0.92
RED FESCUE	shoots	3	Y = 102 - 16.5 X	0.87
	roots	0.8	Y = 46 - 38.4 log X	0.89
	whole plant	3	Y = 96 - 15.4 X	0.86
TALL FESCUE	shoots	0.4	Y = 98 - 112.0 X	0.83
	roots	< 0.05[b]		
	whole plant	0.4	Y = 90 - 102.8 X	0.88

[a]Combined data from two experiments.

[b]Lowest concentration tested which produced more than 50% reduction in dry weight of this tissue. High sensitivity of root tissue of this species did not allow determination of a GR$_{50}$ value.

Table 1.2. Estimated concentrations[a] of haloxyfop inside the plant causing a 50 % reduction in weight.

Species	Haloxyfop (μM)	
	Shoots	Roots
soybean	530	450
red fescue	55	52
tall fescue	7	2[b]

[a]Based on estimated haloxyfop accumulated inside plant organ from an external concentration equivalent to the GR_{50} for dry weights of the species, and on the water volume found in roots or shoots of each species.

[b]Based on lowest concentration of haloxyfop tested which reduced dry weight of root tissue for this species by more than 50% in the GR_{50} experiments.

Table 1.3. Nutrient solution and ^{14}C-haloxyfop absorbed 48 and 96 hours after treatment.

Variable	Species		
	soybean	red fescue	tall fescue
48 HAT:			
NS[a] absorbed (ml)	57	6	5
NS absorbed as percentage of original supply	23	11	10
NS absorbed/g dry weight	81	392	229
^{14}C-haloxyfop absorbed as % of original supply	14	4	3
96 HAT:			
NS absorbed (ml)	157	10	11
NS absorbed as percentage of original supply	63	21	23
NS absorbed/g dry weight	129	381	274
^{14}C-haloxyfop absorbed as % of original supply	21	10	6

[a]NS = nutrient solution

Table 1.4. Ratio of ^{14}C-haloxyfop to dry weight accumulation in three species.

Species Organ		^{14}C-haloxyfop (DPM/g dry weight)			
		days after treatment			
		2	4	8	10
SOYBEAN	leaves	25400	20900	15100	10700
	roots	39800	40100	56800	49600
RED FESCUE	shoots	40000	43900	39900	43700
	roots	276200	426300	486100	461800
TALL FESCUE	shoots	21300	25700	42800	41600
	roots	137300	190900	350700	261300

Figure 1.1. Accumulation of ^{14}C-haloxyfop in shoots and/or roots of three species. A = combined roots and shoots of soybean, B = combined roots and shoots of tall fescue, C = roots of red fescue, and D = shoots of red fescue.

Figure 1.1
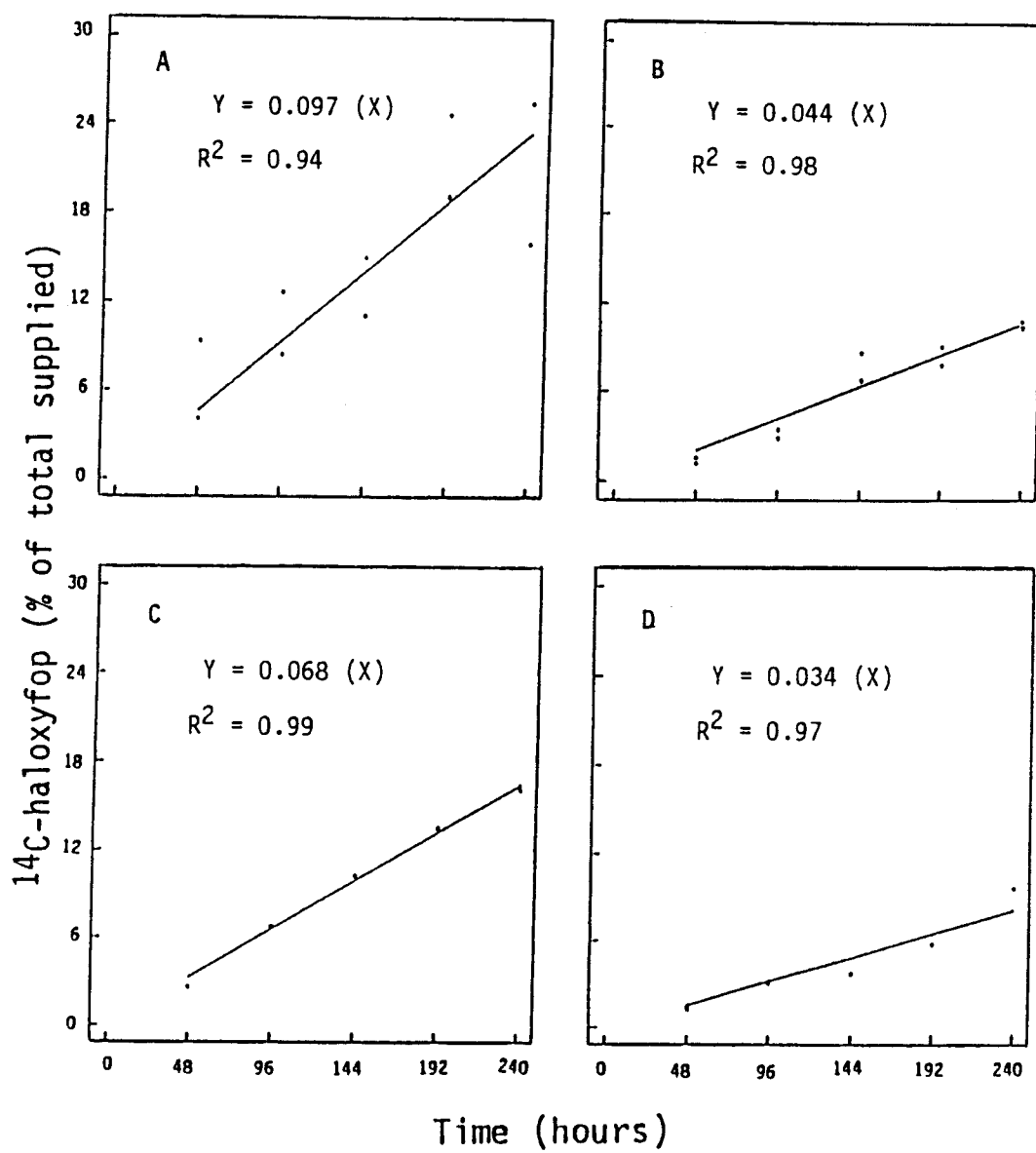
LITERATURE CITED

1. Andersen, R. N. 1982. Comparison of four herbicides for postemergence grass control. Proceedings North Central Weed Control Conference 37: 80-82.

2. Brewster, B. D. and A. P. Appleby. 1985. Annual Report 1984-85. Weed Control Research in Agronomic Crops. Crop Science Department. Oregon State University. p. 312-318.

3. Buhler, D. D. and O. C. Burnside. 1984a. Effect of application factors on postemergence phytotoxicity of fluazifop-butyl, haloxyfop-methyl, and sethoxydim. Weed Science 32: 574-583.

4. Buhler, D. D. and O. C. Burnside. 1984. Herbicidal activity of fluazifop-butyl, haloxyfop-methyl and sethoxydim in soil. Weed Science 32: 824-831.

5. Burton, J. D., J. W. Gronwald, D. A. Somers, J. A. Connely, B. G. Gengenbach, and D. L. Wyse. 1987. Inhibition of plant acetyl-coenzyme A carboxylase by the herbicides sethoxydim and haloxyfop. Biochemical and Biophysical Research Communications 148: 1039-1044.

6. Butler, J. H. B. and A. P. Appleby. 1986. Tolerance of red fescue (Festuca rubra) and bentgrass (Agrostis spp) to sethoxydim. Weed Science 34: 457-461.

7. Donaldson, T. W., D. E. Bayer, and O. A. Leonard. 1973. Absorption of 2,4-dichlorophenoxyacetic acid and 3-(p-chlorophenyl)-1,1-dimethylurea (monuron) by barley roots. Plant Physiology 52: 638-645.

8. Focke, M. and H. K. Lichtenhaler. 1987. Inhibition of the acetyl-CoA carboxylase of barley chloroplasts by cycloxydim and sethoxydim. Zeitschrift Fur Naturforschung 42c: 1361-1363.

9. Kobek, K., M. Focke, and H. K. Lichtenhaler. 1988. Fatty acid biosynthesis and Acetyl-CoA carboxylase as a target of diclofop, fenoxaprop and other aryloxy-phenoxy-propionic acid herbicides. Zeitschrift Fur Naturforsch 43c: 47-54.

10. Nalewaja, J. D., S. D. Miller, and A. G. Dexter. 1982. Postemergence grass and broadleaf herbicide combinations. Proc. North Central Weed Control Conference 37: 77-80.

11. Rahman, A., T. K. James, and J. Mortimer. 1985. Residual activity of some newly developed post-emergence herbicides for grass weeds. Proceedings New Zealand Weed and Pest Control Conference 38: 61-65.

12. Rendina, A. R. and J. M. Felts. 1988. Cyclohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses. Plant Physiology 86: 983-986.

13. Salisbury, F. B. and C. W. Ross. 1985. Plant Physiology. Wadsworth, California. p. 133.

14. Secor, J. and C. Cseke. 1988. Inhibition of acetyl-CoA carboxylase activity by haloxyfop and tralkoxydim. Plant Physiology 86: 10-12.

15. Stoltenberg, D. E., J. W. Gronwald, D. L. Wyse, J. D. Burton, D. A. Somers, and B. G. Gengenbach. 1988. Effect of sethoxydim and haloxyfop on acetyl-coenzyme A carboxylase activity in Festuca species. Weed Science 37: 512-516.

CHAPTER 2. ANTAGONISM OF HALOXYFOP ACTIVITY IN TALL FESCUE (Festuca
arundinacea) BY DICAMBA OR BENTAZON IS NOT DUE TO EFFECTS ON ACETYL-
COA CARBOXYLASE

ABSTRACT

Studies were conducted to test if dicamba (3,6-dichloro-2-
methoxybenzoic acid) reduces haloxyfop {2-[4-[[3-chloro-5-
(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid} herbicidal
activity and to determine if this interaction by auxin-type herbicides
or bentazon {3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one
2,2 dioxide} was caused by preventing the haloxyfop-induced inhibition
of acetyl-CoA carboxylase (ACCase). Addition of dicamba at
concentrations of 2.17 or 4.34 mM reduced haloxyfop activity on tall
fescue shoots. Haloxyfop at 14 to 20 μM inhibited the activity of
ACCase in cell-free extracts from tall fescue by nearly 50%. Dicamba
did not prevent the inhibitory effect of haloxyfop on ACCase activity
in these cell-free enzyme preparations. Dicamba alone at
concentrations as high as 3.2 mM had no significant effect on the in
vitro activity of the enzyme. Bentazon concentrations up to 10 mM
did not prevent haloxyfop inhibition of ACCase activity. Bentazon
alone or mixed with haloxyfop significantly inhibited ACCase activity
at bentazon concentrations starting at 1 mM with near 40% inhibition
at 10 mM. Of the numerous possible explanations for the interference
of dicamba or bentazon with haloxyfop action, interaction directly at
the enzyme level appears to have been eliminated.

INTRODUCTION

Aryloxyphenoxypropanoic acid and cyclohexanedione herbicides exhibit selective activity against grass species. This is a rather diverse group of herbicides, both chemically and in the range of species they control. However, they appear to share a common mechanism of action, the inhibition of fatty acid synthesis (2, 6, 14). Acetyl-CoA carboxylase (ACCase)[1], present in the stroma of chloroplasts (16) and in other plastids (24, 27, 30), is inhibited by these compounds in grasses but not in broadleaf species (5, 18, 19, 22, 28). Further evidence for this enzyme as the site of action of above herbicides comes from a resistant biotype in Italian ryegrass (Lolium multiflorum Lam.) in Oregon, which has a modified ACCase (9). The tolerance of this biotype to diclofop could not be explained by metabolism of the herbicide (23). However, mechanisms other than inhibition of ACCase also may be involved in haloxyfop phytotoxicity (12).

The general lack of activity of haloxyfop and related grass herbicides on broadleaf weeds makes mixtures with other herbicides desirable to broaden the spectrum of weed control. However, the herbicidal activity of haloxyfop and similar compounds is reduced when mixed with 2,4-D {(2,4-dichlorophenoxy)acetic acid} (4, 8, 13, 15, 26), MCPA {(4-chloro-2-methylphenoxy)acetic acid} (8, 17), or bentazon (3, 7, 10, 20, 21). This effect has two practical implications: (a) because haloxyfop performance is lowered, the interaction has a

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[1]Abbreviations: ACCase, acetyl-CoA carboxylase; PPFD, photosynthetic photon flux density; HPLC, high performance liquid chromatography; DMA, dimethylamine; GR_{50}, haloxyfop concentration reducing shoot fresh weight by 50%; I_{50}, haloxyfop concentration causing a 50% reduction in ACCase activity

negative impact in weed control; and (b) the interaction may enable herbicides such as haloxyfop to be used selectively in certain grass crop species that have only moderate tolerance. Products are being developed that take advantage of these herbicidal interactions (e.g., fenoxaprop-ethyl {($\pm$)-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid} + 2,4-D for use in certain varieties of wheat). A better understanding of how and why these interactions occur would help to avoid the problems and exploit the advantages.

The nature of the antagonism of 2,4-D, MCPA, and bentazon on the grass herbicides remains unclear. Several mechanisms may be involved. Antagonism between sethoxydim and the sodium salt of bentazon has been attributed to high polarity of Na-sethoxydim formed in this mixture, which may decrease its uptake by plants (29). In another study, however, neither foliar damage by bentazon nor chemical reactivity with haloxyfop could account for the antagonism between these two herbicides, although a reduction in haloxyfop uptake also occurred (7). No reduction in uptake of diclofop-methyl by wild oats (Avena fatua L.) occurred when this herbicide was mixed with 2,4-D, but translocation was inhibited (26). Further research on antagonism of haloxyfop activity by two chemically unrelated herbicides, dicamba and bentazon, on a species not previously studied may be helpful. The present studies were undertaken to determine if dicamba antagonizes haloxyfop activity and to investigate the biochemical basis of haloxyfop antagonism by dicamba and by bentazon on tall fescue.

MATERIALS AND METHODS

<u>Plant material</u>. Seeds of tall fescue were germinated in paper rolls partially immersed in distilled water in plastic beakers and covered with plastic bags. Eight days later, rolls were opened and seedlings were transplanted.

For herbicide interaction studies, seedlings were transplanted to test tubes (2.5 cm in diameter, 15 cm long) filled with half-strength Hoagland's nutrient solution (11); pH was adjusted to 6.5 with NaOH. Plants were grown in a controlled-environment chamber set at 26/16 $\pm$ 1 C day/night temperature, with an average photosynthetic photon flux density of 350 μE m^{-2} sec^{-1} from a mixture of fluorescent and incandescent lights during the 15-h photoperiod.

Plants for enzyme experiments were transplanted to flats filled with soil, peat moss, and sand (1:1:1, by vol). The soil pH was adjusted to 6.0 with agricultural lime. Plants were grown in a painted glass house at about 26/16 $\pm$ 2 C day/night temperatures, a 16-h photoperiod, and a PPFD of 350 μE m^{-2} sec^{-1} from fluorescent lamps.

<u>Antagonism of haloxyfop activity in whole plants by the dimethylamine salt of dicamba</u>. Uniform plants were selected in the 3-leaf stage and treated by dipping in one of six concentrations of formulated haloxyfop-methyl from 0 to 332 μM, either alone or mixed with formulated dicamba dimethylamine salt at 2.17 or 4.34 mM.

The above experiment was repeated using the acid form of haloxyfop at five concentrations from 0 to 99.5 μM. A stock solution of technical grade haloxyfop (99% purity) was prepared in HPLC-grade methanol, and concentrations were prepared by successive dilutions with distilled water. Methanol concentration was adjusted in all

treatments to 0.5% (v/v), which did not affect plant growth in preliminary studies. The surfactant X-77[2] at 0.1% (v/v) was added to all treatments in both experiments.

Twelve days after plants were treated, shoot fresh weight was determined. Rates of haloxyfop (alone and mixed with dicamba) needed to reduce shoot fresh weight by 50% (GR_{50}) were estimated by linear regression on mean values.

Extraction of ACCase activity from tall fescue. ACCase was extracted from 20- to 25-day-old tall fescue shoots by macerating 15 g of fresh tissue in a pre-chilled mortar in 50 ml of cold buffer (pH 8.3) containing 100 mM tricine, 10% glycerol (by vol), 1 mM Na_2EDTA, 1 mM phenylmethyl sulfonyl fluoride, and 10 mM ß-mercaptoethanol. All subsequent procedures were conducted at about 4 C.

The supernatant from the macerate was centrifuged (15 000 g, 20 min). Polyethylene glycol (M.W. 8000) was added to the supernatant (60 g/l). The solution was stirred for 20 min and then centrifuged (15 000 g, 20 min). The polyethylene glycol concentration of the supernatant was increased to 140 g/l. This solution was stirred and centrifuged as above. The supernatant was discarded and the pellet was resuspended in 2 ml of cold 10 mM tricine-KOH (pH 7.8) containing 10% glycerol (by vol). The suspension was centrifuged (10 000 g, 5 min) and the supernatant was used as a crude ACCase preparation in enzyme assays. The protein content of ACCase preparations ranged from 1.7 to 2.4 $\mu g/\mu l$ (1). ACCase extracts were used within 5 h of preparation.

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[2]X-77, a mixture of alkylaryl polyoxyethylene glycols, free fatty acids, and isopropanol. Chevron Chemical Company, Ortho Agricultural Chemicals Division. San Francisco, California, USA.

Assay of ACCase activity. ACCase activity was assayed in reaction volumes of 250 μl containing 50.8 mM tricine-KOH (pH 8.3), 5 mM $MgCl_2$, 2 mM dithiothreitol, 2 mM adenosine 5-triphosphate, 10 mM $NaH^{14}CO_3$ (0.26 μCi/μmol), 20 μl of crude ACCase extract, and 0.32 mM acetyl-CoA. The reaction mixtures were incubated at 35 C for 15 min before initiating the reaction with acetyl-CoA. The enzyme reaction was stopped by adding 100 μl of 6 N HCl. The reaction mixtures were evaporated to dryness at 90 C to allow vaporization of unreacted $^{14}CO_2$. The solids were redissolved in 0.25 ml of double-distilled water and radioassayed in 5 ml of scintillation cocktail[3]. Enzyme activity was estimated from the radioactivity recovered as heat-stable ^{14}C-malonyl-CoA counts. ACCase activity from crude extracts was estimated to be linear for the first 20 min in preliminary experiments. All following experiments were conducted within the linear phase of the ACCase time-dependent activity.

ACCase inhibition by haloxyfop acid. To determine the concentration of haloxyfop causing a 50% reduction in ACCase activity (I_{50}), haloxyfop concentrations (0 to 316 μM) were added to reaction mixtures just before adding the enzyme. A concentrated solution of haloxyfop acid analytical grade was prepared in 95% v/v ethanol. From it, treatment stocks were prepared for each haloxyfop concentration tested, containing 50 mM tricine-KOH (pH 8.3) and 3.75% ethanol by vol. Aliquots (100-μl) were added from each treatment stock to the corresponding reaction mixture. Ethanol concentration in reaction mixtures was 1.9% (v/v), which did not affect ACCase activity in preliminary experiments. The experiment was conducted twice.

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[3]Hydrocount, J.T. Baker Chemicals B.V. Deventer, Holland.

<u>Tests of herbicide mixtures on ACCase activity</u>. Haloxyfop (20 μM) and dicamba (0 to 1.0 mM) were combined with ACCase extract 15 min before addition of acetyl-CoA to start the reaction. For this experiment, a concentrated dicamba solution was prepared by dissolving the appropriate amount of technical grade dicamba acid in double-distilled water kept at nearly boiling point. The cooled dicamba solution was made up to 50-mM tricine-KOH solution (pH 8.3). Dicamba treatment stocks were prepared by serial dilution of the above solution with 50 mM tricine-KOH (pH 8.3).

The above experiment was repeated, using the DMA salt of dicamba (0 to 3.2 mM) to overcome solubility limitations of the acid form at the higher concentrations. When conducted a third time, the DMA salt of dicamba was tested to concentrations up to 10 mM. In this experiment, dicamba was added to the reaction mixture before haloxyfop and held (with the enzyme extract and all other components) for 7 min. Haloxyfop was added, the incubation continued for an additional 8-min period and finally, the reaction was started with acetyl-CoA.

Mixtures of haloxyfop and the sodium salt of bentazon also were tested in replicated experiments similar in procedures and concentrations to those of the DMA salt of dicamba described above.

Treatment stocks for the salt forms of dicamba and bentazon were prepared by direct dilution of formulated herbicides in the tricine buffer solution.

All ACCase experiments in this section tested the hypothesis that dicamba and bentazon reverse the haloxyfop-induced inhibition of ACCase activity. However, because of the minor modifications, data are presented and discussed separately for each experiment.

RESULTS AND DISCUSSION

Antagonism of haloxyfop activity in whole plants by the dimethylamine salt of dicamba. Dicamba reduced the effect on tall fescue shoot fresh weight by haloxyfop-methyl (Figure 2.1). The GR_{50} for haloxyfop-methyl applied alone was 18 μM. In combination with 2.17 mM and 4.34 mM dicamba, the GR_{50} value for haloxyfop-methyl was increased to 155 μM and 190 μM, respectively. All plants treated with dicamba showed epinasty symptoms within 48 h after treatment. Epinasty was especially marked when haloxyfop-methyl also was present in the solution, indicating that the formulation of haloxyfop-methyl may have increased dicamba uptake. Chlorosis, typical of haloxyfop action, first appeared 6 to 8 days after dipping plants in haloxyfop-methyl alone.

Dicamba interaction also was observed with haloxyfop acid. The GR_{50} of haloxyfop acid alone was 74 μM, but up to 99.5 μM haloxyfop acid (highest concentration tested) did not reduce fresh weight in the presence of either 2.17 or 4.34 mM dicamba (Figure 2.2).

ACCase Experiments

ACCase inhibition by haloxyfop acid. The I_{50} of haloxyfop for ACCase activity as estimated by linear regression from the average of two experiments was 14 μM (Figure 2.3).

Effect of herbicide mixtures on ACCase activity. Neither dicamba acid nor the dimethylamine salt reduced the haloxyfop inhibition of ACCase activity at any of the concentrations tested (Table 2.1). Concentrations of dicamba alone as high as 3.2 mM had no significant effect on the enzyme activity.

Prior addition of dicamba to the reaction mixture did not reduce the haloxyfop inhibition of ACCase activity. Dicamba at 10 mM reduced ACCase activity by nearly 20% when mixed with haloxyfop (Table 2.1).

The sodium salt of bentazon also had no effect on haloxyfop inhibition of ACCase activity (Table 2.2). Bentazon itself, however, inhibited the enzyme activity at concentrations above 1 mM. The same results as with dicamba were obtained when adding the sodium salt of bentazon prior to haloxyfop. However, a 10-mM concentration of bentazon alone or mixed with 20 μM haloxyfop reduced the activity of ACCase by nearly 40% (Table 2.2).

Dicamba caused strong antagonism of the herbicidal activity of haloxyfop-methyl on shoots of tall fescue. The antagonism could be reduced either by increasing haloxyfop-methyl concentration or by reducing dicamba concentration (Figure 2.1). This dose-dependent relationship may be indicative of a competitive process operating between these herbicides. Though other components present in the formulation of haloxyfop-methyl may affect the magnitude of the antagonism, they do not appear essential for its occurrence, as the interaction also occurred with haloxyfop acid.

Our results are consistent with previous work on site of action of haloxyfop (2). The high degree of ACCase inhibition is consistent with susceptibility of tall fescue to this herbicide. Similar results with another variety of tall fescue were reported by Stoltenberg et al. (25).

Haloxyfop inhibition of ACCase activity was not reversed by either bentazon or dicamba, regardless of their concentration or order of addition to the enzyme preparation. This lack of interaction of

haloxyfop and dicamba or bentazon at the enzyme level indicates that the antagonism may be due to mechanisms that reduce the amount of haloxyfop reaching its site of action.

The fact that the antagonizers did not alter haloxyfop inhibition of ACCase activity, even when put in contact with the enzyme extract prior to haloxyfop, indicates that these herbicides either bind to a different site in the enzyme, or that they do not bind at all; the inhibitory effects observed at the higher concentrations probably are the result of secondary physiological effects.

Table 2.1. Effect of dicamba on haloxyfop inhibition of ACCase activity.

Dicamba Concentration	------- ^{14}C-malonyl-CoA formed --------	
	No haloxyfop acid[a]	20 μM haloxyfop acid[a]
(mM)	-- % of non-treated check --	

Experiment 1: dicamba acid (means of 3 replications)

0	100 a	46 a
0.001	92 a	44 a
0.01	99 a	44 a
0.1	96 a	45 a
1	97 a	47 a

Experiment 2: DMA salt of dicamba (means of 2 replications)

0	100 a	52 a
0.1	104 a	53 a
0.32	99 a	54 a
1	111 a	51 a
3.2	101 a	52 a

Experiment 3: DMA salt of dicamba added to reaction mixture 7 min prior to haloxyfop (means of 3 replications)

0	100 a	57 a
0.1	104 a	57 a
1	103 a	56 a
10	88 a	42 b

[a]Means in columns followed by the same letter are not significantly different at the 0.01 level according to Fisher's Protected LSD test.

Table 2.2. Effect of Na-bentazon on haloxyfop inhibition of ACCase activity.

Bentazon concentration	^{14}C-malonyl-CoA formed	
	No haloxyfop acid[a]	20 μM haloxyfop acid[a]
(mM)	---- % of non-treated check ----	
Experiment 1:		
0	100 a	42 a
0.1	98 a	42 a
0.32	98 a	39 a
1	92 b	37 a
3.2	76 c	30 b
Experiment 2: bentazon added to reaction mixture 7 min prior to haloxyfop		
0	100 a	45 a
0.1	101 a	46 a
1	99 a	45 a
10	66 b	24 b

[a]Means in columns followed by the same letter are not significantly different at the 0.01 level according to Fisher's Protected LSD test. All data points are averages of three replications.

Figure 2.1. Effects of haloxyfop-methyl and DMA-dicamba combinations on shoot fresh weight of tall fescue. Lines were drawn by connecting raw data points (as averages of three replications). SE averaged $\pm$ 12% of the means.

Figure 2.1

Figure showing Shoot fresh weight (% of control) versus Haloxyfop concentration (µM).

Legend:
- ● Haloxyfop-methyl alone ($Y = 100 - 40 \log X$; $R^2 = 0.97$)
- ✳ Haloxyfop-methyl + 2.17 mM dicamba ($Y = 86 - 0.23 X$; $R^2 = 0.80$)
- ○ Haloxyfop-methyl + 4.34 mM dicamba ($Y = 84 - 0.18 X$; $R^2 = 0.92$)
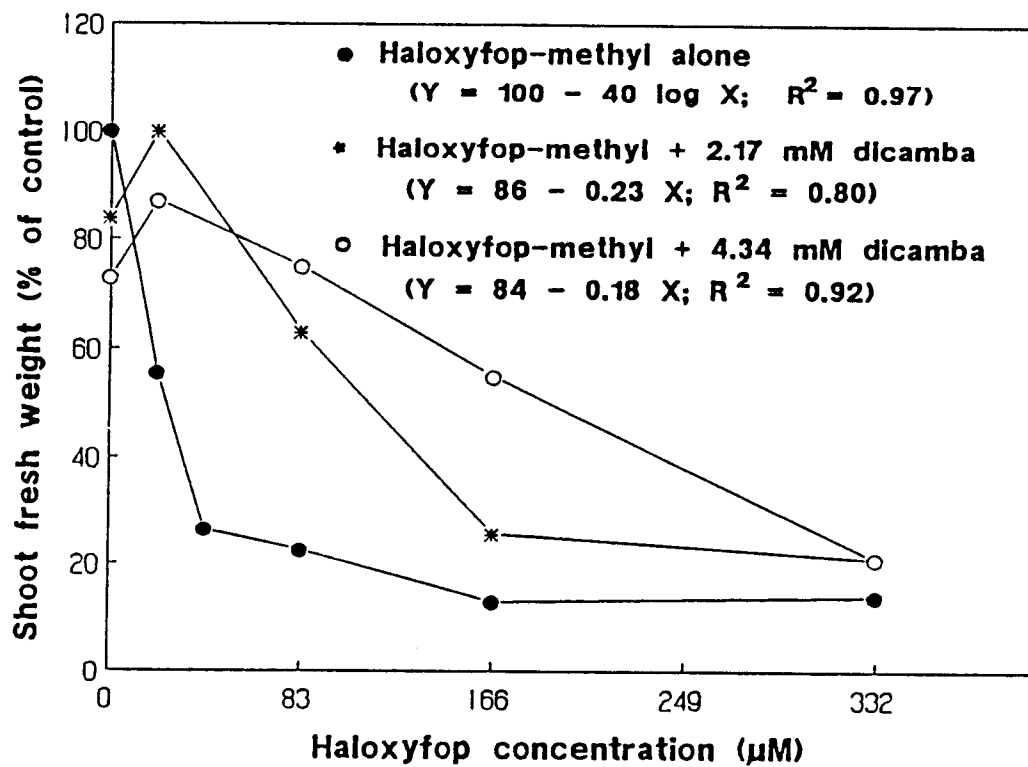
Figure 2.2. Effects of haloxyfop acid and DMA-dicamba combinations on shoot fresh weight of tall fescue. Lines were drawn by connecting raw data points (as averages of five replications). SE averaged ± 9% of the means. No GR_{50} values were calculated for the mixtures with DMA-dicamba because no significant shoot fresh weight reduction was observed.

Figure 2.2

Y-axis: Shoot fresh weight (% of control)

X-axis: Haloxyfop acid concentration (µM)

● Haloxyfop acid alone
  (Y = 106.4 − 0.76 X; R^2 = 0.97)

○ Haloxyfop acid + 2.17 mM dicamba

✴ Haloxyfop acid + 4.34 mM dicamba
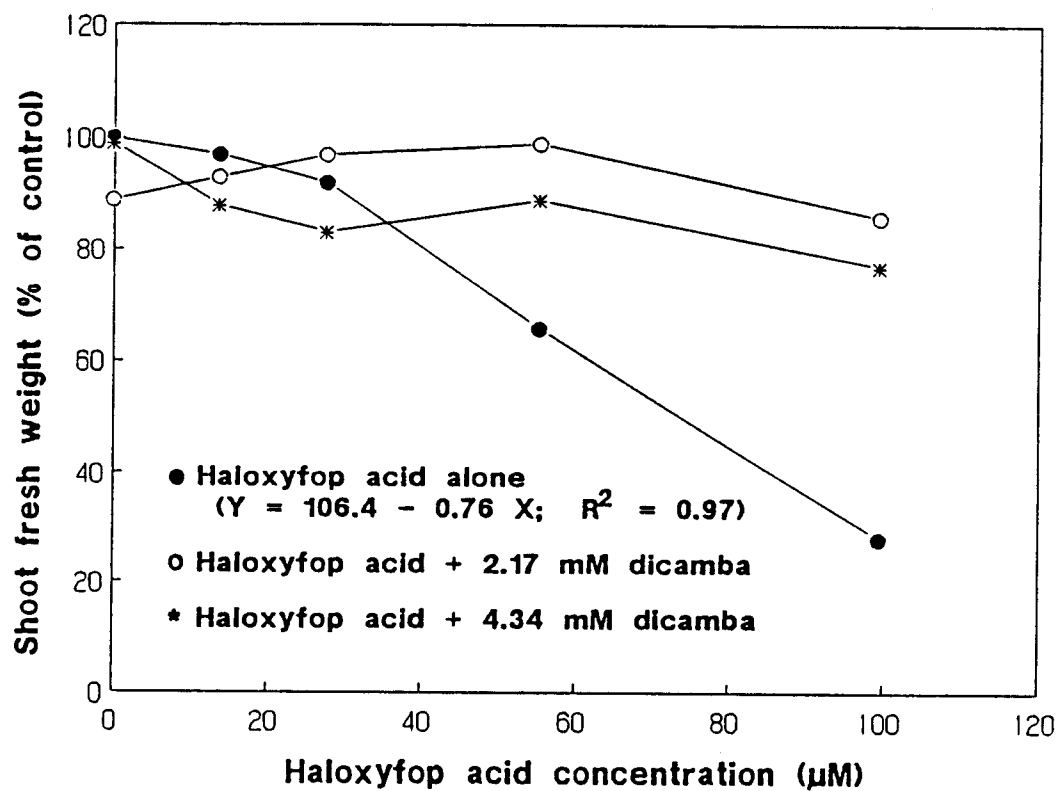
Figure 2.3. Haloxyfop inhibition of ACCase activity extracted from tall fescue shoots. Regression was run on mean values of two experiments. SE values averaged ± 1.3% of the means.

Figure 2.3

Y = 98.7 − 22.6 log (10X)

$R^2 = 0.99$ $I_{50} = 14\ \mu M$
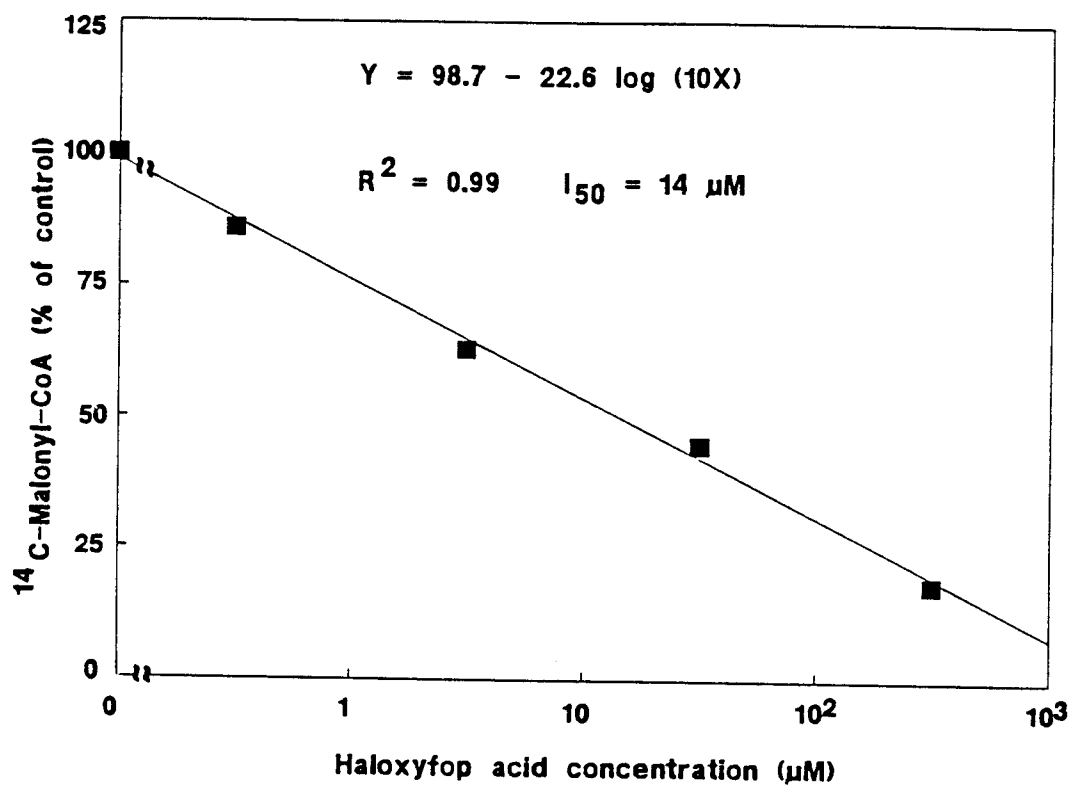
LITERATURE CITED

1. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248.

2. Burton, J. D., J. W. Gronwald, D. A. Somers, J. A. Connelly, B. G. Gegenback, and D. L. Wise. 1987. Inhibition of plant acetyl-CoA carboxylase by the herbicides sethoxydim and haloxyfop. Biochem. and Biophys. Res. Comm. 148: 1039-1044.

3. Campbell, J. R. and D. Penner. 1982. Compatibility of diclofop and BAS 9052 with bentazon. Weed Sci. 30: 458-462.

4. Fletcher, R. A. and D. M. Drexler. 1980. Interactions of diclofop-methyl and 2,4-D in cultivated oats (Avena sativa). Weed Sci. 28: 363-366.

5. Focke, M. and H. K. Lichtenthaler. 1987. Inhibition of the acetyl-CoA carboxylase of barley chloroplasts by cycloxydim and sethoxydim. Z. Naturforsch 42c: 1361-1363.

6. Focke, M., K. Kobek, and H. K. Lichtenthaler. 1988. Fatty-acid biosynthesis and acetyl-CoA carboxylase as a target of diclofop, fenoxaprop and other aryloxy-phenoxy-propionic acid herbicides. Z. Naturforsch 43c: 47-54.

7. Gerwick, B. C. 1988. Potential mechanisms for bentazon antagonism with haloxyfop. Weed Sci. 36: 286-290.

8. Gillespie, G. R. and J. D. Nalewaja. 1989. Influence of 2,4-D and MCPA formulations and oil on diclofop phytotoxicity. Weed Sci. 37: 380-384.

9. Gronwald, J. W., C. V. Eberlein, K. J. Betts, K. M. Rosow, N. J. Ehlke, and D. L. Wyse. 1989. Diclofop resistance in a biotype of Italian ryegrass. Plant Physiol. 89(Suppl.): 115.

10. Hartzler, R. G. and C. L. Foy. 1983. Compatibility of BAS 9052 OH with acifluorfen and bentazon. Weed Sci. 31: 597-599.

11. Hoagland, D. R. and D. I. Arnon. 1938. The water-culture method of growing plants without soil. California Agricultural Experiment Station Circular 347.

12. Irzyk, G. P. and N. C. Carpita. 1989. Relationship between haloxyfop sensitivity and acetyl-CoA carboxylase activity in cultures of proso millet. Plant Physiol. 89(Suppl.): 3.

13. Kafiz, B., J. P. Caussanel, R. Scalla, and P. Gaillardon. 1989. Interaction between diclofop-methyl and 2,4-D in wild oat (Avena fatua L.) and cultivated oat (Avena sativa L.), and fate of diclofop-methyl in cultivated oat. Weed Res. 29: 299-305.

14. Kobek, K., M. Focke, H. K. Lichtenthaler, R. Günther, and B. Würzer. 1988. Inhibition of fatty acid biosynthesis in isolated chloroplasts by cycloxydim and other cyclohexane-1,3-diones. Physiol. Plant. 72: 492-498.

15. Mueller, T. C., W. W. Witt, and M. Barrett. 1989. Antagonism of johnsongrass (Sorghum halepense) control with fenoxaprop, haloxyfop, and sethoxydim by 2,4-D. Weed Tech. 3: 86-89.

16. Ohlrogge, J. B., D. N. Kuhn, and P. K. Stumpf. 1979. Subcellular localization of acyl carrier protein in leaf protoplasts of Spinacia oleracea. Proc. Natl. Acad. Sci. USA. 76: 1194-1198.

17. Olson, W. and J. D. Nalewaja. 1982. Effect of MCPA on ^{14}C-diclofop uptake and translocation. Weed Sci. 30: 59-63.

18. Rendina, A. R. and J. M. Felts. 1988. Cyclohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses. Plant Physiol. 86: 983-986.

19. Rendina, A. R., J. M. Felts, J. D. Beaudoin, A. C. Craig-Kennard, L. L. Look, S. L. Paraskos, and J. A. Hagenah. 1988. Kinetic characterization, stereoselectivity, and species selectivity of the inhibition of plant acetyl-CoA carboxylase by the aryloxyphenoxypropionic acid grass herbicides. Arch. Biochem. Biophys. 265: 1-7.

20. Rhodes, G. N. and H. D. Coble. 1984. Influence of application variables on antagonism between sethoxydim and bentazon. Weed Sci. 32: 436-441.

21. Rhodes, G. N. and H. D. Coble. 1984. Influence of bentazon on absorption and translocation of sethoxydim in goosegrass (Eleusine indica). Weed Sci. 32: 595-597.

22. Secor, J. and C. Cséke. 1988. Inhibition of acetyl-CoA carboxylase activity by haloxyfop and tralkoxydim. Plant Physiol. 86: 10-12.

23. Shimabukuro, R. H. and B. L. Hoffer. 1989. Metabolism of diclofop-methyl in annual ryegrass, Lolium rigidum, and its relationship to recovery of membrane potential. Abstr. Weed Sci. Soc. Amer., No. 186. p. 82.

24. Simcox, P. D., E. E. Reid, D. T. Canvin, and D. T. Dennis. 1977. Enzymes of the glycolytic and pentose phosphate pathways in pro-plastids from the developing endosperm of Ricinus communis L. Plant Physiol. 59: 1128-1132.

25. Stoltenberg, D. E., J. W. Gronwald, D. L. Wyse, J. D. Burton, D. A. Somers, and B. G. Gengenbach. 1988. Effect of sethoxydim and haloxyfop on acetyl-coenzyme A carboxylase activity in Festuca species. Weed Sci. 37: 512-516.

26. Todd, B. G. and E. H. Stobbe. 1980. The basis of the antagonistic effect of 2,4-D on diclofop-methyl toxicity to wild oat (_Avena_ _fatua_). Weed Sci. 28: 371-377.

27. Vick, B. and H. Beevers. 1978. Fatty acid synthesis in endosperm of young castor bean seedlings. Plant Physiol. 62: 173-178.

28. Walker, K., S. M. Ridley, T. Lewis, and J. L. Harwood. 1988. Fluazifop, a grass-selective herbicide which inhibits acetyl-CoA carboxylase in sensitive plant species. Biochem. J. 254: 307-310.

29. Wanamarta, G., D. Penner, and J. J. Kells. 1989. The basis of bentazon antagonism on sethoxydim absorption and activity. Weed Sci. 37: 400-404.

30. Zilkey, B. F. and D. T. Canvin. 1971. Localization of oleic acid biosynthesis enzymes in the proplastids of developing castor endosperm. Can. J. Bot. 50: 323-326.

CHAPTER 3. CHARACTERIZATION OF HALOXYFOP HERBICIDAL ACTIVITY ALONE OR IN COMBINATION WITH DICAMBA IN TALL FESCUE

ABSTRACT

Dicamba at concentrations from 0.54 to 4.34 mM antagonized haloxyfop-induced reduction of tall fescue shoot weight. Antagonism increased with increasing dicamba concentration and occurred only when both herbicides were applied to the same foliar tissue. Applying dicamba 12 h after haloxyfop-methyl was applied reduced the antagonism but applying dicamba 12 h before haloxyfop-methyl did not. The antagonism occurred with formulated and acid forms of the herbicides. Actively growing foliar tissue was the main target of haloxyfop, dicamba antagonism appears to occur by preventing haloxyfop from reaching and/or disrupting this tissue.

INTRODUCTION

Haloxyfop, an aryloxyphenoxypropanoic acid herbicide with potent activity in most grass species (2), currently is under development for use in a wide range of dicot crops. Careful handling of field rates also could allow selective use of this and similar herbicides in certain grass species with moderate tolerance (18).

In many situations, mixing haloxyfop with a dicot-active herbicide would be desirable to broaden the spectrum of weed control. However, some dicot-active herbicides of different chemical families have been found to antagonize haloxyfop action (14, 16). One such compound, dicamba, has not been studied in depth in this respect. We have previously reported that high concentrations of the dimethylamine salt of dicamba strongly antagonized the activity of haloxyfop acid and formulated haloxyfop-methyl on shoots of tall fescue (1).

The present studies were undertaken to characterize haloxyfop herbicidal activity, alone or mixed with several concentrations of dicamba, on shoots of tall fescue. Additionally, effects of formulation of both herbicides, time interval between applications, physical placement on the plant, and the growth stage of foliar tissue at time of application were examined.

MATERIALS AND METHODS

Tall fescue plants were grown in plastic cones (3 cm in diameter, 14 cm long; filled with soil, peat moss, and sand, 1:1:1 v/v) in a growth chamber set to conditions described in Chapter 2. Experiments were conducted twice unless otherwise indicated, and data are presented as the average of the two experiments. All concentrations are expressed as acid equivalent.

Haloxyfop activity in whole plants

Tolerance studies. The rates of haloxyfop required to reduce fresh weight of tall fescue shoots by 50% (GR_{50})[1], alone or mixed with dicamba concentrations ranging from 0.54 to 4.34 mM, were determined by linear regression methods. Variables evaluated include: formulation, days after treatment to harvest and application times. Effects of separate applications of each herbicide to different tissues of the same plant also were evaluated.

Regrowth studies. Tall fescue plants (second leaf fully developed, third leaf actively growing) were dip treated with the herbicides. The concentration of formulated haloxyfop-methyl (FHM) was 33 μM (GR_{50} = approx. 17 μM). DMA-dicamba was used at 1.08 mM, a concentration that reversed approximately 50% of FHM action on shoot weight in previous experiments. Ten days after treatment, plant shoots were cut at the soil level, allowed to regrow for 20 days, and harvested again, and fresh weights were recorded.

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[1]Abbreviations: GR_{50}, haloxyfop concentration reducing shoot fresh weight by 50 %; FHM, formulated haloxyfop-methyl; DMA, dimethylamine; CPX, chlorophyll-protein complexes

Haloxyfop activity in mature and actively growing foliar tissue

Effect of removing mature leaves on the antagonism. Mature leaves (first and second leaves) of plants in the 3-leaf stage, were removed and discarded immediately after dipping plants in FHM, DMA-dicamba or a mixture of both. Plants with remaining tissue were allowed to grow for twenty days and then shoot fresh weights were recorded.

Mature leaf segments study. Segments approximately 15-cm long from the second leaf of tall fescue plants (3-leaf stage) were cut under water and immediately transferred to glass vials partially filled with 5 ml of deionized water. Vial openings were sealed with parafilm. The segments were introduced through small slits in the parafilm, which provided support to segments and prevented water evaporation. Water did not evaporate from vials without leaf segments but with parafilm and slits.

To allow recovery from cutting stress, leaf segments were left untreated for 2 h after cutting. Then, the segments, still in their vials, were individually inverted and dipped up to the parafilm layer in herbicide solutions ranging from 0 to 36 μM FHM alone or mixed with 1.08 mM DMA-dicamba, all containing 0.05% X-77[2] v/v. Segments were kept in a growth chamber under conditions previously described (1). Seven days later, fresh and dry weights of leaf segments were recorded.

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[2]X-77, a mixture of alkylaryl polyoxyethylene glycols, free fatty acids, and isopropanol. Chevron Chem. Co., Ortho Agric. Chem. Div. San Francisco, CA, USA.

Intact leaf-lamina spectroscopy studies. Plants were grown in the greenhouse and treated at the 3 to 4-leaf stage. Accordingly, a higher FHM concentration than the one giving a GR_{50} in previous studies with younger plants was needed to achieve a similar response. Thus, shoots were dipped in a 36-μM solution of FHM alone or combined with 1.08 mM DMA-dicamba. A 1.08-mM DMA-dicamba check and a non-treated control were included.

Eight days after treatment, intact leaves (third leaf for one series of experiments and the second leaf for others) were harvested in about 5-cm segments and inmediately analyzed spectroscopically (7, 8) with a Shimadzu 260 spectrophotometer with an integrating sphere attachment[3] as described by Fisher et al. (11).

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[3]Shimadzu Scient. Instr., Columbia, MD

RESULTS AND DISCUSSION

Haloxyfop activity in whole plants

Tolerance studies. FHM at 18 μM reduced tall fescue shoot fresh weight by 50%. Phytotoxicity was reduced by all concentrations of dicamba tested (Table 3.1). The antagonism occurred with commercial formulations as well as with high-purity acid forms of both herbicides (Table 3.2). Higher rates of the acid forms needed to observe haloxyfop activity and dicamba antagonism were due to lower penetration of the acid than of the ester into leaves as determined in preliminary experiments with ^{14}C-haloxyfop (data not shown). Antagonism did not occur when DMA-dicamba was applied to shoots and haloxyfop acid to the nutrient solution for root uptake, nor when both herbicides were added to the nutrient solution (Table 3.3). Work in our laboratory[4] has shown that antagonism of haloxyfop activity by dicamba in tall fescue is not due to a process operating outside the cuticle; thus, the above results suggest that the herbicides need to be together inside foliar tissue for the antagonism to occur.

Harvesting tall fescue shoots 20 days after treatment instead of 10 did not alter the antagonistic response, indicating that this is not a temporary effect (Table 3.4). Twenty days after treatment, growth had almost completely ceased in haloxyfop-treated plants as indicated by the presence of fewer tillers (Table 3.4).

The magnitude of antagonism diminished as time of application of DMA-dicamba following FHM was increased. On the other hand, applying DMA-dicamba 12 h before FHM did not change the antagonism (Table 3.5).

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[4]For details see Chapter 4.

<u>Regrowth studies</u>. Plants treated with 33 μM FHM did not regrow 20 days after clipping, indicating death of the shoot apical meristem. When the same concentration of haloxyfop was mixed with 1.08 mM DMA-dicamba, plants regrew (Table 3.6). These results led us to conclude that haloxyfop kills tall fescue plants by targeting meristematic regions. This view agrees with several reports in the literature for this and similar herbicides (3, 6, 13, 15). In addition, crown sections from treated tall fescue plants, excised and observed under a light microscope, confirmed total death of crown tissue in FHM-treated plants but not in plants treated with FHM mixed with DMA-dicamba.

<u>Haloxyfop activity in mature and actively growing foliar tissue</u>

<u>Effect of removing mature leaves on the antagonism</u>. Eliminating mature leaves immediately after dipping plants in the different treatments and allowing the remaining tissue to grow for 20 days, illustrated two main aspects of the antagonism. First, that haloxyfop exported from mature leaves to sink areas has an impact on concentration needed to inhibit growth of this tissue, as higher concentrations of FHM were needed to produce an equivalent growth inhibition in the absence of mature leaves (Tables 3.1 and 3.7). Second, that antagonism occurs by mechanism(s) triggered by dicamba that reduce haloxyfop activity in meristematic tissue of tall fescue (Table 3.7). Since we have shown that uptake through cuticle is not involved in the antagonism[4], data in table 3.7 indicates that both long distance and short distance transport of haloxyfop to highly sensitive regions on the plant are reduced in the presence of dicamba.

<u>Mature leaf segments study</u>. No significant reduction in fresh or dry weight of leaf segments was observed in any of the treatments ($\underline{P}$ = 0.01). Segments were harvested 7 days after treatment because all started showing symptoms of senescence. However, before that period, they remained healthy and transpired similar amounts of water (an average of 600 μl segment^{-1} for the 7-day period).

<u>Intact leaf-lamina spectroscopy studies</u>. Results from studies with detached leaf segments should be interpreted with reservation; thus, to confirm results from the previous section, we used spectroscopy analysis of intact plant tissue. Validity of the method has been proven extensively in recent years (7, 8, 9, 10, 11). The method allowed us to compare herbicidal effects in leaves of different ages on the same plant.

Haloxyfop reduced the <u>in vivo</u> light absorption maxima of the actively growing third leaves of tall fescue (Figure 3.1). Dicamba did not affect this parameter, but when mixed with haloxyfop it prevented haloxyfop reduction of absorption maxima (Figure 3.1). None of the treatments affected light absorption in leaf segments that were fully developed at application time (second leaf) (Figure 3.1). These results support our starting hypothesis and previous reports in the literature (3, 6, 13, 15) that haloxyfop targets actively growing tissue, and that death of such tissues (including the apical meristem), accounts for the later death of the whole plant.

We have observed in our laboratory[4] that proplastids from meristematic tissue of haloxyfop-treated plants do not form the thylakoidal membrane system in the process of developing into chloroplasts, thus remaining as non-functional, chlorophyll-lacking

entities. Our results are consistent with present knowledge that haloxyfop inhibits activity of acetyl CoA carboxylase (5), an enzyme involved in one of the initial steps of fatty acid synthesis; however, the effect of such enzymatic inhibition appears to be much more dramatic in actively growing tissue than in mature tissue, probably due to differences in the relative rates of fatty acid synthesis in those tissues.

Spectroscopic evidence in the present study is consistent with haloxyfop-mediated changes in the lipid membranes that support chlorophyll-protein complexes (CPX). It was observed early (17) that when chlorophyll is extracted, spectral properties change and peak maxima move to shorter wavelengths. Spectral properties of in situ CPX were worked out in decades of effort by the French and the Butler research groups (4, 12). Recently, these in situ data has been correlated to specific chloroplast components, and these data to in vivo CPX signals (9).

In vivo CPX maxima are highly conserved in higher plants (9). Changes in these maxima occur only with profound changes in CPX function (7, 8, 10). Blue shifts (to shorter wavelengths) indicate disruption of CPX. Haloxyfop treatment causes dramatic blue shifts in actively growing leaves of tall fescue[5]. This is consistent with damage to membrane lipids associated with CPX. This, together with an effect on proplastids discussed previously, might explain haloxyfop herbicidal properties on new tissue of grasses with a susceptible ACCase.

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[5]Larry S. Daley. Department of Horticulture, Oregon State University. Personal communication.

Table 3.1. Concentration of formulated haloxyfop-methyl reducing shoot fresh weight by 50 % (GR_{50}) as affected by concentration of DMA-dicamba.

DMA-dicamba	FHM[a] GR_{50}[b]
---- mM ----	--- μM ---
0.00	17
0.54	28
1.08	38
2.17	155[c]
4.34	190[c]

[a]FHM = formulated haloxyfop-methyl

[b]GR_{50} values determined by linear regression methods from a single experiment performed for each dicamba concentration; and from the average of three experiments for the control.

[c]Source: Agüero, Appleby, and Armstrong (1990)

Table 3.2. Effect of formulation on haloxyfop interaction
with dicamba.

Treatment	Shoot fresh weight	
	--- % of control[a]---	
control	100	A
18 μM FHM[b]	62	B
18 μM FHM + 2 mM dicamba acid	98	A
70 μM haloxyfop acid	69	B
70 μM haloxyfop acid + 1 mM DMA[c]-dicamba	87	A
70 μM haloxyfop acid + 2 mM dicamba acid	89	A
1 mM DMA-dicamba	98	A
2 mM dicamba acid	96	A

[a]Means followed by the same letter are not significantly
different at the 0.01 level according to Fisher's Protected
LSD test.

[b]FHM = formulated haloxyfop-methyl

[c]DMA = dimethylamine

Table 3.3. Effect of herbicide initial location on the antagonism of dicamba on haloxyfop activity.

Initial location	Shoot fresh weight[a]	
--- μM ---	--- % of control ---	
I. Both herbicides[b] in the nutrient solution (NS)[c]		
0.01 dicamba	94	C
0.1 dicamba	93	C
1.0 dicamba	81	BC
0.1 haloxyfop	54	AB
0.1 haloxyfop + 0.01 dicamba	83	BC
0.1 haloxyfop + 0.1 dicamba	55	AB
0.1 haloxyfop + 1.0 dicamba	30	A
II. Haloxyfop acid in NS; DMA-dicamba on shoots[c]		
1085 DMA-dicamba	95	A
2170 DMA-dicamba	108	A
0.1 haloxyfop	62	B
0.1 haloxyfop + 1085 DMA-dicamba	66	B
0.1 haloxyfop + 2170 DMA-dicamba	58	B

[a]Means in columns of each experiment followed by the same letter are not significantly different at the 0.05 level according to Fisher's Protected LSD test.

[b]acid forms of herbicides were used.

[c]each experiment conducted once.

Table 3.4. Leaf and tiller number 20 DAT[a], and comparison of two dates of harvest after treatment on the antagonism of dicamba on haloxyfop activity[b].

Treatment	Shoot fresh weight		Leaf Number	Tiller Number
	10 DAT	20 DAT		
Shoot fresh weight	-- % of control) --		-- No./plant --	
control	100	100	10 A	3.0 A
18 μM FHM	55 A	34 A	6 B	1.7 B
18 μM FHM + 1.08 mM DMA-dicamba	94 B	88 B	11 A	3.4 A

[a]DAT = days after treatment

[b]Means followed by the same letter within a column are not significantly different at the 0.01 level for shoot weight and leaf number, and at the 0.05 level for tiller number, according to Fisher's Protected LSD test.

Table 3.5. Effect of separating dicamba and haloxyfop-
methyl application on the antagonism.

Treatment	Shoot fresh weight[a]	
--- μM ---	-- % of control --	
1085 D[b]	109	D
18 H[b]	49	B
18 H + 1085 D (at same time)	94	CD
18 H ; 1085 D (3 h apart)	77	C
18 H ; 1085 D (12 h apart)	59	B
18 H ; surfactant[c] (3 h apart)	30	A
1085 D ; 18 H (3 h apart)	77	C
1085 D ; 18 H (12 h apart)	91	CD
1085 D ; surfactant[c] (3 h apart)	98	CD

[a]Means followed by the same letter are not significantly
different at the 0.05 level according to Fisher's Protected
LSD test.

[b]H = formulated haloxyfop-methyl, D = dimethylamine salt of
dicamba.

[c]0.025 % X-77 by vol.

Table 3.6. Shoot regrowth of tall fescue clipped 10 days after herbicide treatment.

Treatment[a]	Shoot regrowth
	-- % of control[b] --
33 μM FHM	5
33 μM FHM + 1.08 mM DMA-dicamba	58

[a]Herbicides applied to 3-leaf tall fescue by dipping entire shoot. Plants excised at soil level 10 days after treatment, and shoot regrowth measured 20 days after clipping.

[b]Means are significantly different at the 0.01 level according to Fisher's protected LSD test.

Table 3.7. Effect of dicamba on haloxyfop activity in tall
fescue plants in which mature leaves were removed immediate-
ly after dipping entire shoots in herbicide solutions.

Treatment	Shoot fresh weight 20 days after treatment[a]
--- μM ---	-- % of control --
control	100 C
11 FHM	99 C
16.5 FHM	83 CB
33 FHM	25 A
1085 DMA-dicamba	104 C
16.5 FHM + 1085 DMA-dicamba	98 C
33 FHM + 1085 DMA-dicamba	83 CB
44 FHM + 1085 DMA-dicamba	60 B

[a]Means followed by the same letter are not significantly
different at the 0.05 level according to Fisher's Protected
LSD test.

Figure 3.1. In vivo light absorption spectra of tall fescue leaf segments that were actively expanding at time of treatment (third leaf), as affected by haloxyfop-methyl and/or the dimethylamine salt of dicamba. C = control, D = DMA-dicamba, and H = formulated haloxyfop-methyl; insert in left corner corresponds to the same treatments but from leaf segments that were fully expanded (second leaf) at time of treatment.

Figure 3.1
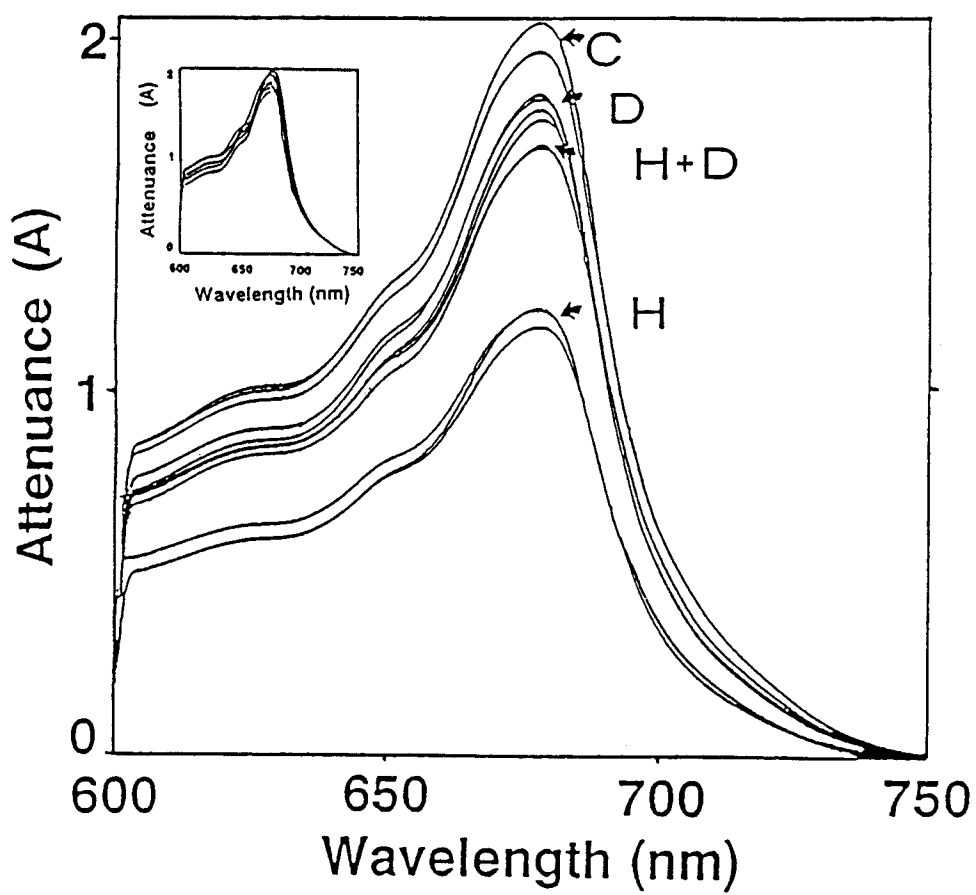
LITERATURE CITED

1. Agüero Alvarado, R., A. P. Appleby, and D. J. Armstrong. 1990. Antagonism of haloxyfop activity in tall fescue (Festuca arundinaceae) by dicamba or bentazon is not due to effects on acetyl-CoA carboxylase. Weed Science (in review)

2. Andersen, R.N. 1982. Comparisson of four herbicides for postemergence grass control. Proceedings North Central Weed Control Conference 37: 80-82.

3. Asare-Boamah, N. K. and R. A. Fletcher. 1983. Physiological and cytological effects of BAS-9052-OH on corn (Zea mays) seedlings. Weed Sci. 31: 49-55.

4. Butler, W. L. and K. H. Norris. 1960. The spectrophotometry of dense light scattering material. Arch. Biochem. Biophys. 87: 31-40.

5. Burton, J. D., J. W. Gronwald, D. A. Somers, J. A. Connely, B. G. Gegenback, and D. L. Wise. 1987. Inhibition of plant acetyl-CoA carboxylase by the herbicides sethoxydim and haloxyfop. Biochim. and Biophys. Res. Comm. 148:1039-1044.

6. Chow, P. N. P. and D. E. LaBerge. 1978. Wild oat studies. 2. Physiological and chemical changes in barley and wild oat treated with diclofop-methyl herbicide in relation to plant tolerance. J. Agric. Food Chem. 26: 1134-1137.

7. Daley, L. S., O. Jahn, and C. Guttridge. 1987. Differences between Fragaria clones by fourth derivative room temperature spectroscopy of intact leaves. Photosynth. Res. 11: 183-188.

8. Daley, L. S., J. R. Powell, G. A. Strobel, R. B. Boone, J. Postman, M. Willie, and J. H. Brown. 1988. Spectroscopic methods of detection of differences in photosynthetic apparatus and function of plants. Plant Physiol. Biochem. 1988: 683-694.

9. Daley, L. S. 1990. French's nomenclature use: In vivo chlorophyll complexes. Plant Physiol. Bioch. 28: 271-282.

10. Eskelsen, S. R., G. D. Crabtree, R. B. Boone and L. S. Daley. 1989. Determination of herbicide damage to light harvesting apparatus of radishes (Raphanus sativus). Proc. Western Soc. Weed Sci. 42: 44-51.

11. Fisher, H. H., R. A. Menendez, L. S. Daley, D. Robb-Spencer, and G. D. Crabtree. 1987. Biochemical characterization of itchgrass (Rottboellia exaltata) biotypes. Weed Sci. 42: 44-51.

12. French, C. S., J. S. Brown, and M. C. Lawrence. 1972. Four universal forms of chlorophyll a. Plant Physiol. 49: 421-429.

13. Gealy, D. R. and F. W. Slife. 1983. BAS 9052 effects on leaf photosynthesis and growth. Weed Sci. 31: 457-461.

14. Gerwick, B. C. 1988. Potential mechanisms for bentazon antagonism with haloxyfop. Weed Sci. 36: 286-290.

15. Harwood, J. L. 1988. The site of action of some selective graminaceous herbicides is identified as acetyl-CoA carboxylase. TIBS 13: 330-331.

16. Mueller, T. C., W. W. Witt, and M. Barrett. 1989. Antagonism of johnsongrass (Sorghum halepense) control with fenoxaprop, haloxyfop, and sethoxydim by 2,4-D. Weed Tech. 3: 86-89.

17. Smith, E. L. 1938. Solutions of chlorophyll-protein compounds (pyllochlorins) extracted from spinach. Science 88: 170-171.

18. Soto, A., R. Agüero, and N. Zúñiga. 1986. Rice tolerance to fenoxaprop-ethyl and fluazifop-butyl: rates and timing of application. Turrialba 36: 381-388. (in spanish).

CHAPTER 4. DICAMBA REDUCES THE AMOUNT OF HALOXYFOP THAT REACHES MERISTEMATIC TISSUES IN TALL FESCUE

ABSTRACT

Dicamba reduces haloxyfop toxicity in tall fescue. Research was conducted to clarify the reasons for this antagonism. Dicamba did not change the pH of the spray solution and did not combine chemically with haloxyfop. Dicamba did not affect leaf retention or uptake of ^{14}C-haloxyfop-methyl, but it reduced haloxyfop movement to the pseudostems (leaf sheaths + crown meristem) and roots. Neither herbicide, alone or combined, changed the structure of mitochondria, phloem end walls, or cuticles. Dicamba stimulated stress-ethylene production, but treatment with ethylene gas did not affect haloxyfop phytotoxicity. Haloxyfop stopped development of proplastids into chloroplasts by arresting internal membrane synthesis when applied alone. Addition of dicamba prevented this effect by reducing the amount of haloxyfop that reaches actively growing tissue.

INTRODUCTION

Cyclohexanedione herbicides such as sethoxydim, and aryloxyphenoxypropanoic acid herbicides (e.g. haloxyfop), exhibit potent activity on most grasses and are selective in broadleaf species (2, 4, 6, 16). Selectivity apparently results from differences in susceptibility of the enzyme acetyl CoA carboxylase to these compounds (5, 13, 18, 20).

Lack of phytotoxicity to broadleaf plants, however, can be a disadvantage unless a second herbicide can be added to control broadleaf weeds. Some broadleaf herbicides have been found to antagonize the activity of the "grass herbicides" (8, 10, 11, 14, 15, 17, 19).

Bentazon, a herbicide used against broadleaf weeds (23), appears to reduce uptake of haloxyfop and related compounds (10, 24). The mechanism for this reduction, however, is not clear. The mechanism by which growth regulator herbicides such as 2,4-D and MCPA antagonize the grass herbicides is even less understood with some researchers indicating an effect on translocation (22) while others question the importance of translocation and believe that the antagonism occurs directly at the apical meristem (12). Furthermore, Taylor, Loader and Norris (21) found that picloram and triclopyr did not antagonize diclofop-methyl or flamprop-methyl. Thus, different mechanisms might account for the antagonism observed between the different mixtures of grass and broadleaf herbicides.

We reported that dicamba, a benzoic acid derivative, strongly antagonizes the activity of haloxyfop-methyl and haloxyfop acid in shoots of tall fescue (1). We also determined that initially,

haloxyfop affects actively growing tissue and that antagonism results from dicamba preventing damage to that tissue[1]. We also found that antagonism of haloxyfop activity on tall fescue by bentazon or dicamba does not result from a direct interaction on acetyl CoA carboxylase (1).

Potential effects of DMA-dicamba on the herbicide solution, such as changes in pH, or formation of a chemical complex with FHM, as well as on processes on or in the plant, including FHM leaf retention, FHM uptake, FHM translocation, stress ethylene production, and structural changes in meristematic tissue were examined.

[1]For details see Chapter 3.

MATERIALS AND METHODS

In all experiments discussed herein, haloxyfop was used at 18 μM, a concentration that reduced tall fescue shoot fresh weight by 40 to 60% in several previous experiments, and dicamba at 1.08 mM, a concentration antagonizing haloxyfop activity by about 50%. The herbicides were applied alone or combined. For all experiments involving dipping of shoots, the adjuvant X-77[2] was added to the herbicide solutions at 0.05% v/v. Tall fescue plants were grown as previously described[1].

Interactions in the herbicide solution

pH of herbicide solution. Herbicide solutions were prepared, including addition of X-77. pH values were obtained with a pH meter by standard procedures.

Chemical reaction. Herbicide solutions of haloxyfop alone or mixed with dicamba were prepared. To each solution, sufficient ^{14}C-haloxyfop-methyl (specific activity = 19.98 mCi mmol^{-1}) was added to provide about 1200 CPM μl^{-1}. From each of these solutions, 5 μl were spotted 3 cm from the origin onto a thin-layer chromatography (TLC)[3] silica gel plate[4], 20 by 20 cm and 250 μm in thickness. Approximately 100000 CPM of pure ^{14}C-haloxyfop-methyl in "HPLC grade" methanol were placed in a separate spot. The plate was developed up to 18 cm with a

[2]X-77, a mixture of alkylaryl polyethylene glycols, free fatty acids, and isopropanol. Chevron Chemical Company, Ortho Agric. Chem. Div. San Francisco, CA, USA.
[3]Abbreviations: TLC, thin layer chromatography; FHM, formulated haloxyfop-methyl; DMA-dicamba, dimethylamine salt of dicamba.
[4]J.T. Baker Inc., Phillipsburg, New Jersey 08865, USA.

solvent system consisting of 20:16:4:1:1 hexane: chloroform: ethyl acetate: methanol: acetic acid (by vol). ^{14}C-haloxyfop-methyl in two identically developed plates was located with a TLC radioscanner[5].

Processes on or in the plant

Herbicide retention. Retention of a solution of FHM[3] alone or a mixture of FHM + DMA-dicamba[3] by tall fescue shoots was compared. Each of these solutions contained about 37000 CPM ml^{-1} of ^{14}C-haloxyfop-methyl. Tall fescue shoots (3-leaf stage) were dipped in the above solutions (10 plants/ herbicidal solution), dried for about 10 min, separated from the rest of the plant, placed inside individual vials containing 10 ml methanol:water (80:20 by vol), and shaken vigorously for about 5 min. Aliquots (1-ml) were taken from these vials, mixed with scintillation cocktail A[6] and radioassayed. Herbicide retained by shoots of tall fescue was estimated from the radioactivity recovered. The experiment was conducted twice.

Stress-ethylene production. In preliminary experiments, we often observed epinasty symptoms 24 to 48 h after treatments at the highest dicamba concentrations tested. Thus, we measured stress-ethylene production induced by dicamba and determined the effect of this gas on haloxyfop phytotoxicity.

Shoots of whole plants in the 3-leaf stage were dipped in FHM, DMA-dicamba, or a mixture of the two. Plants were allowed to dry for about 10 min. The two newest leaves from each treated plant joined by part of the pseudostem were cut and placed in test tubes of 1.5 cm diameter, 15 cm long (containing a piece of wet paper at the bottom),

[5]Bioscan System 400 Imaging Scanner; Bioscan Inc., 4590 McArthur Blv. NW, Washington D.C., USA.
[6]Scintillation cocktail A consisted of 60% toluene, 40% ethylene glycol monomethyl ether, 5 g PPO/l, and 0.1 g POPOP/l.

tubes were then sealed with serum stoppers. Ethylene production was monitored by taking 1-ml air samples from sealed test tubes with disposable syringes at time zero and at daily intervals for up to 3 days. Within minutes after each sampling, samples were injected into a gas chromatograph[7] programmed to detect ethylene with a standard (6 ft by 1/8 inch) stainless steel column packed with Porapak R 80-100 mesh[8]. The experiment was conducted twice.

In additional experiments, shoots were treated with FHM or FHM + DMA-dicamba and treated whole plants were sealed inside test tubes with serum stoppers. Ethylene at 7 and 14 picomoles ml^{-1} (concentrations above highest ethylene concentration detected from experiments described in previous section) was added to some of the FHM-treated plants. Plants were removed from the tubes after 48 h, allowed to grow for 8 additional days, and shoot fresh weights were recorded. The experiment was conducted twice.

Uptake and translocation studies. Experiments were conducted seven times. Plants were harvested 48, 72, or 96 h after treatment, depending on the experiment. No differences between experiments were observed; thus, means from the seven experiments were analyzed as blocks and data are presented as the average of the seven blocks. Plants were dipped in FHM or FHM + DMA-dicamba. Ten minutes later, 5 or 10 μl (when using a 50% diluted stock) of ^{14}C-haloxyfop-methyl was applied evenly on the middle section of the adaxial surface of the second leaf to provide approximately 97000 CPM/plant. Plants were then placed back in the growth chamber. Plants were excised and

[7]Hewlett Packard model 5830 A; 1501 Page Mill Road, Palo Alto, CA 94304, USA.
[8]Waters Assoc. Inc. Framingham, Massachussets, USA

divided into treated leaf, leaf above treated leaf, leaf below treated leaf, pseudostems (leaf sheaths + crown meristem), and roots. Treated leaves were placed in 20-ml vials and washed twice in 10 ml methanol (80% by vol) by shaking for about 1 min each time to remove any unabsorbed ^{14}C-haloxyfop-methyl. Washes were combined and 1-ml aliquots were radioassayed. In the last two experiments, a third treated-leaf wash with pure chloroform was performed, but no significant additional radioactivity recovery was observed. Plant parts were kept at -20 C until oxidized in an automatic sample oxidizer[9]. The ^{14}CO$_2$ evolved was trapped and radioassayed in scintillation cocktail B[10]. Absorbed herbicide was calculated as the total counts recovered inside the plant. Translocation was considered to be the specific counts recovered in tissues other than the treated leaf blade. The ^{14}C-recovery ranged from 50 to 70%; recovery did not vary among treatments. In other experiments with similar procedures, absorption and movement of haloxyfop-methyl was studied using plants of which separate leaves on the same plant were treated with ^{14}C-haloxyfop-methyl and DMA-dicamba.

Structural changes induced by the herbicide treatments in meristematic tissue. Results from the previous section showed that dicamba reduces the amount of haloxyfop reaching the growing point in tall fescue. Leaf sheath sections were examined by electron microscopy[11] to detect possible structural changes in this tissue that could account for the above effect.

[9]Packard Oxidizer 306; Packard Instrument Company, 2200 Warrenville Road, Downers Grove Ill 60515.
[10]Scintillation cocktail B, a mixture of 9 ml of methoxyethylamine (^{14}CO$_2$ absorber) and 11 ml of toluene/methanol (9/1 by vol) per vial.
[11]Philips em 12/Stem; Philips Analytical, International Business Center-Electron Optics, 5600 MD Eindhoven, The Netherlands.

Plants selected for uniformity at the 3-leaf stage were dipped in FHM, DMA-dicamba, or a mixture of both. Seventy-two h after dipping, leaf blades and roots were discarded. Pseudostems were used to examine structural features. Pseudostems were fixed in 2.5% glutaraldehyde for 2 h; then the specimens were buffer-washed overnight. Following the buffer wash, samples were placed in a 1% osmium solution for 1 h. Pseudostems were dehydrated, starting with a 50% acetone solution for 15 min, then immersed for 20 min in a 70% acetone solution that had been saturated with uranyl acetate, and ending with three changes in absolute acetone for 15 min each. Pseudostems were then infiltrated beginning with a 1/1 solution by vol of acetone and Spurr's plastic for 4 to 5 h. Enough Spurr's was then added to the samples to make a 2:1 Spurr's:acetone mixture, and the samples were left overnight. They were then flat embedded in 100% Spurr's and placed in a 70-C oven overnight.

Sectioning was done on a Sorvall Porter-Blum, model MT2, ultramicrotome to a thickness of 800-1100 Å. Reynolds' lead citrate was used as a post-stain. Outside cuticle layers, phloem end-walls, mitochondria, and proplastids were examined.

RESULTS AND DISCUSSION

Interactions in the herbicide solution

pH of herbicide solution. None of the herbicides influenced the pH of the treatment dipping solution, which averaged 5.

Chemical reactivity. ^{14}C-haloxyfop-methyl standard in developed plates was found at an R_f of 0.87. Dicamba did not alter the R_f at which ^{14}C-haloxyfop-methyl was found, with 96% located at the same R_f as the standard when mixed with FHM and 94% when mixed with FHM + DMA-dicamba; thus, dicamba does not appear to form a chemical complex with haloxyfop-methyl.

Processes on or in the plant

Herbicide retention. The dimethylamine salt of dicamba did not affect haloxyfop-methyl herbicidal solution volumes retained by shoots of tall fescue ($\underline{P}$ = 0.01).

Stress ethylene production. Dicamba stimulated ethylene production in tall fescue. Haloxyfop did not promote release of ethylene up to 72 h after treatment (Table 4.1). Exogenous ethylene at concentrations above those induced by dicamba, did not influence haloxyfop activity on tall fescue shoots (Table 4.2).

Uptake and translocation studies. Dicamba did not affect total ^{14}C-haloxyfop-methyl absorption in any of the seven experiments. Consistently, a reduction in the amount of ^{14}C-haloxyfop moving from the treated leaf into the pseudostem was observed in the presence of dicamba. Also, less ^{14}C-haloxyfop was detected in roots of dicamba-treated plants (Table 4.3). Clearly, less haloxyfop reached the apical meristem, a sensitive tissue (3, 7, 9, 13), when mixed with dicamba. Death of this meristem and surrounding tissues would explain

the cessation of new leaf expansion that we commonly observe in haloxyfop-methyl-treated plants. We have noted a lack of significant activity of haloxyfop in old leaves[1], which require a longer time for the appearance of chlorosis. Old leaves may be affected indirectly when death of the crown region halts water and nutrient uptake.

When the herbicides were placed on separate leaves, the effect of dicamba on haloxyfop translocation was much less obvious. Only in one experiment, and at a dicamba concentration twice that previously tested, was a reduction in haloxyfop movement into the pseudostem detected (Table 4.4).

Structural changes induced by the herbicide treatments in meristematic tissue. Proplastids from tangential sections of pseudostems from plants treated with haloxyfop-methyl appeared small, round, dense, and osmophilic and lacked nearly all internal membrane development. When mixed with dicamba, this effect was not apparent (Figure 4.1). This is consistent with previous conclusions that less haloxyfop reaches pseudostems of tall fescue when mixed with dicamba. Furthermore, this result supports the view that haloxyfop and related grass herbicides act by reducing fatty acid synthesis through inhibition of acetyl CoA carboxylase activity (5, 13, 18, 20). Lack of thylakoid membrane development explains why chlorosis is first observed in developing leaves. Even though a reduction in fatty acid synthesis also could eventually affect old tissue, by the time this happens, the plant would be severely injured because of death of the crown tissue. No distinctive structural features of cuticles, phloem end-walls, or mitochondria were affected by any of the herbicide treatments (Figures 4.2, 4.3, and 4.4).

That dicamba does not reduce uptake of haloxyfop-methyl in tall fescue shoots is now clear. Likewise, the herbicide solution pH was not changed and there was no chemical reaction in the spray solution. Evidence shows that the antagonism results from dicamba reducing the amount of haloxyfop reaching meristematic regions of the plant. Because in our experimental system we dipped shoots in the herbicide solutions, dicamba must halt both long-distance and short-distance movement of haloxyfop to meristematic regions to account for the antagonism. We have accumulated evidence for potential mechanisms accounting for dicamba reduction of haloxyfop basipetal movement. These results are discussed in Chapter 5.

Table 4.1. Stress-ethylene production by tall fescue shoots
treated with haloxyfop-methyl, dicamba, or both.

Treatment	Stress-ethylene		
	24 HAT[a]	48 HAT	72 HAT
Experiment 1:	---- pmoles/plant sample/h[b]----		
control	0.49 A	0.45 A	0.30 A
FHM	0.95 A	0.64 A	0.45 A
DMA-dicamba	3.27 B	1.90 B	1.32 B
FHM + DMA-dicamba	3.58 B	2.05 B	1.44 B
DMA-dicamba in FHM blank	3.58 B	2.20 B	1.51 B
Experiment 2:			
control	0.20 A	0.08 A	0.10 A
FHM	0.30 A	0.25 A	0.27 A
DMA-dicamba	3.40 B	2.40 B	1.72 B
FHM + DMA-dicamba	3.20 B	2.30 B	1.60 B
DMA-dicamba in FHM blank	3.00 B	1.90 B	1.30 B

[a]HAT = hours after treatment

[b]Means followed by a different letter within a column for
each of the experiments, are significantly different accord-
ing to the Fisher's Protected LSD test at the 0.01 level.

Table 4.2. Shoot fresh weight of plants kept in sealed glass tubes with ethylene for 48 h and then grown outside tubes for 8 days.

treatment	Shoot fresh weight	
	Experiment 1	Experiment 2
	----- % of control[a]-----	
control	100 A	100 A
18 μM FHM	26 B	44 B
18 μM FHM + 7 pmol ethylene/ml	32 B	39 B
18 μM FHM + 14 pmol ethylene/ml	35 B	39 B
18 μM FHM + 1085 μM DMA-dicamba	87 A	92 A

[a]Means within a column followed by a different letter are significantly different according to the Fisher's Protected LSD test at the 0.01 level.

Table 4.3. ^{14}C-haloxyfop-methyl absorption and movement in tall fescue plants previously treated with formulated haloxyfop-methyl alone or mixed with the dimethylamine salt of dicamba.

Plant part	18 μM FHM	18 μM FHM + 1085 μM DMA-dicamba	
	- counts per minute[a]-		LSD$_{0.05}$
outside treated leaf	4987	6350	NS
1st leaf	72	59	NS
2nd leaf (^{14}C-treated)	45677	44635	NS
third leaf	902	522	NS
pseudostem	4164	2118	1098
roots	448	248	92
total inside plant	51256	47571	NS

[a]Data points are the average of seven experiments.

Table 4.4. Uptake and movement of ^{14}C-haloxyfop-methyl when the dimethylamine salt of dicamba was applied to separate leaves of the same plant.

Plant part	^{14}C-H-m on second leaf[a]			^{14}C-H-m on first leaf		
	First leaf dipped in			Second leaf dipped in		
	X-77[b]	1 mM D	2 mM D	X-77	1 mM D	2 mM D
Experiment one:	---------- counts per minute[c] ----------					
treated leaf	22460	26020	18070	7004	11180	10520
pseudostem	1532	2267	1311	873	1165	693
roots	353	294	300	219	257	192
total inside plant	24660	28960	20080	8589	13180	11960
Experiment two:						
treated leaf	30450	36590	30670	15370	11550	9831
pseudostem	2484	2142	1905	1512 B	1155 AB	890 A
roots	361	378	325	347 B	230 AB	165 A
total inside plant	33760	39470	33320	17880	13450	11430

a ^{14}C-H-m = ^{14}C-haloxyfop-methyl

b X-77 at 0.05% by vol; D = dimethylamine salt of dicamba.

c Means followed by a different letter for two rows of Experiment two (pseudostems and roots) are different according to Fisher's Protected LSD test at the 0.05 level. All other means within a row for each experiment were not different.

<u>Figure 4.1</u>. Electron micrograph of tangential sections from tall
fescue pseudostems showing proplastids where A = control, 25000 X; B =
FHM treated, 42000 X; C = FHM + DMA-dicamba treated, 37000 X; and D =
DMA-dicamba treated, 22000 X. All bars are equivalent to 0.2 μm.
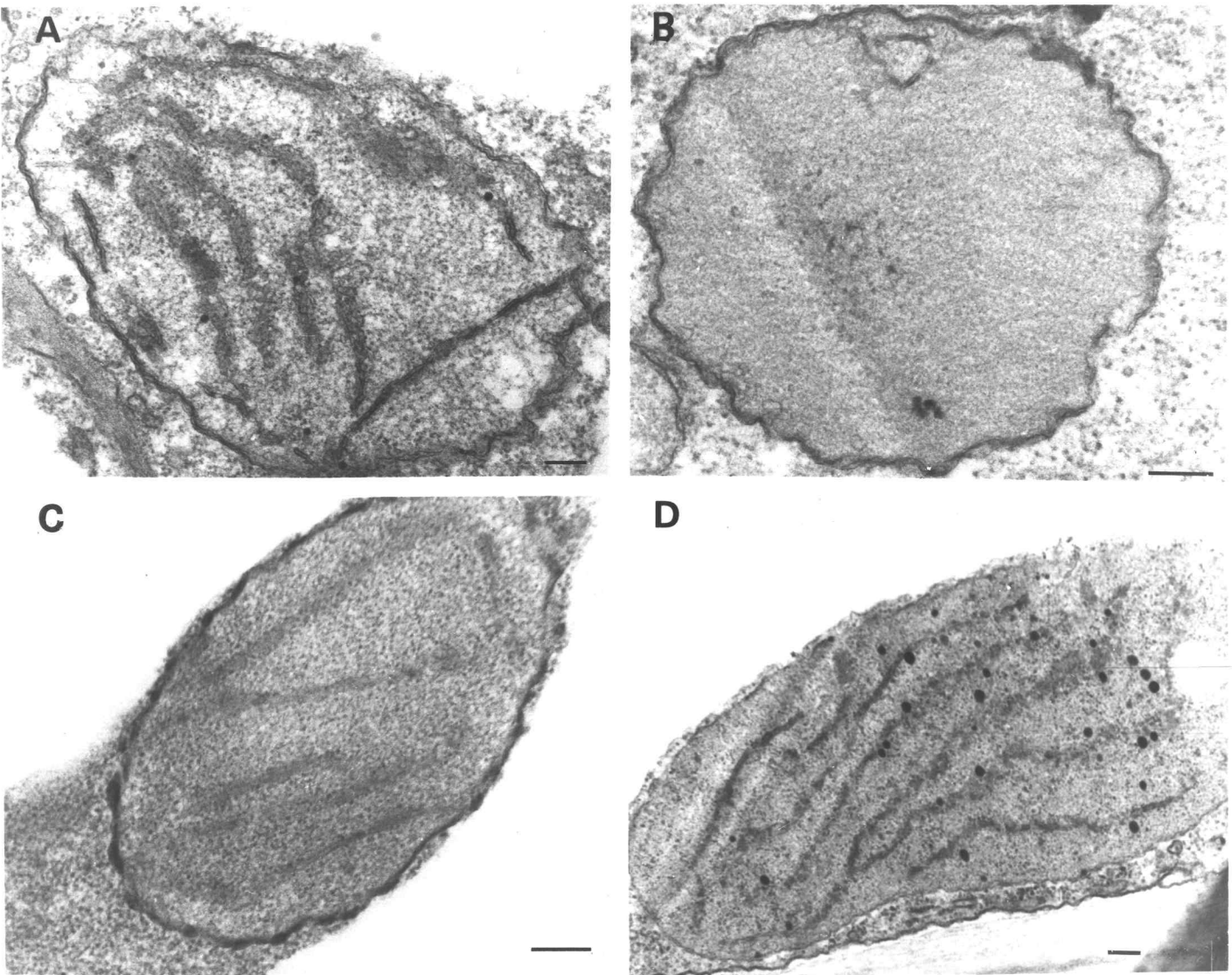
Figure 4.1

Figure 4.2. Electron micrograph of tangential sections from tall
fescue pseudostems showing mitochondria where A = control, 33000 X; B
= FHM treated, 30000 X; C = FHM + DMA-dicamba treated, 28000 X; and D
= DMA-dicamba treated, 28000 X. All bars are equivalent to 0.2 μm.
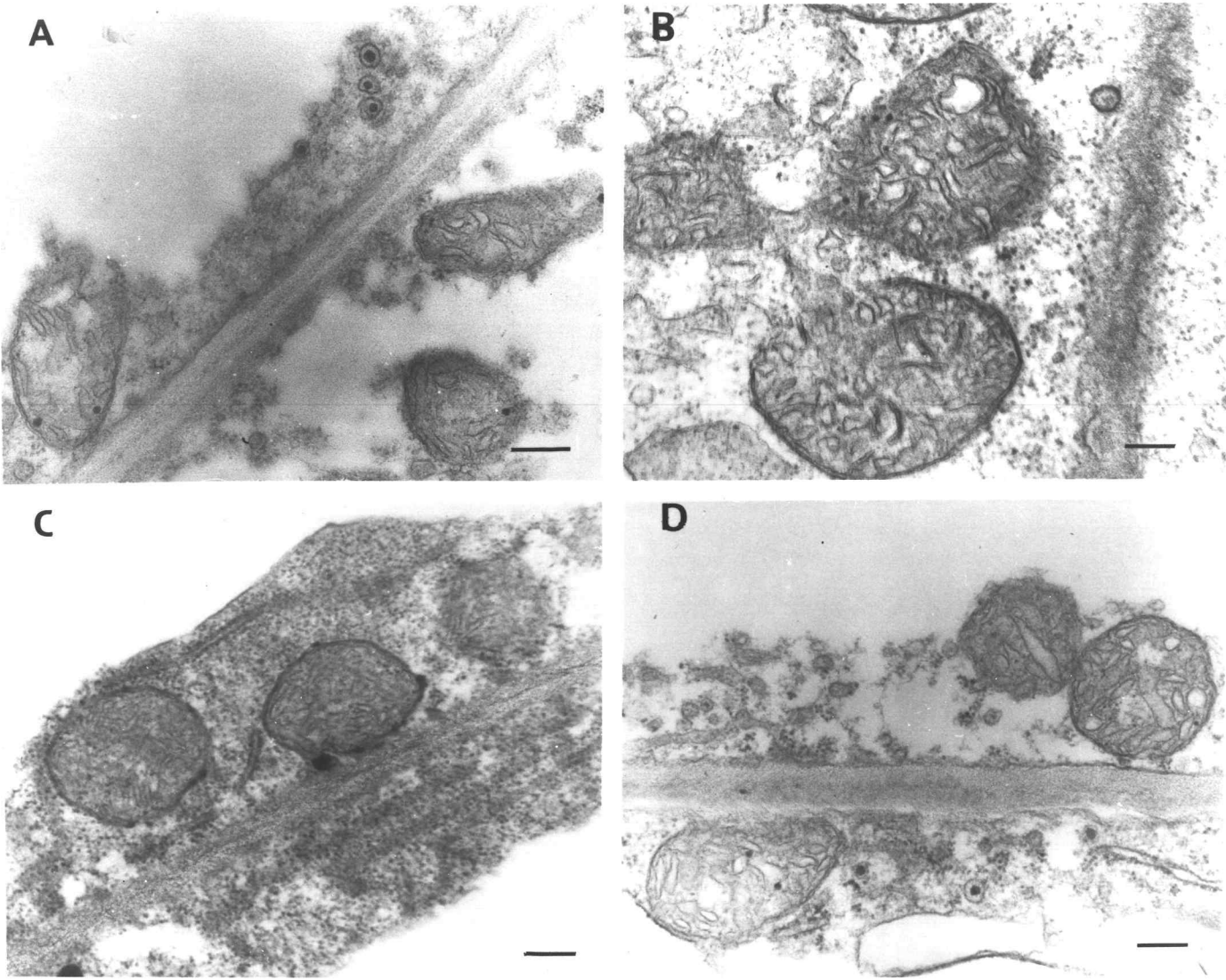
Figure 4.2

Figure 4.3. Electron micrograph of tangential sections from tall fescue pseudostems showing phloem end walls where A = control, 8000 X; B = FHM treated, 9500 X; C = FHM + DMA-dicamba treated, 12000 X; and D = DMA-dicamba treated, 7550 X. All bars are equivalent to 1 μm.
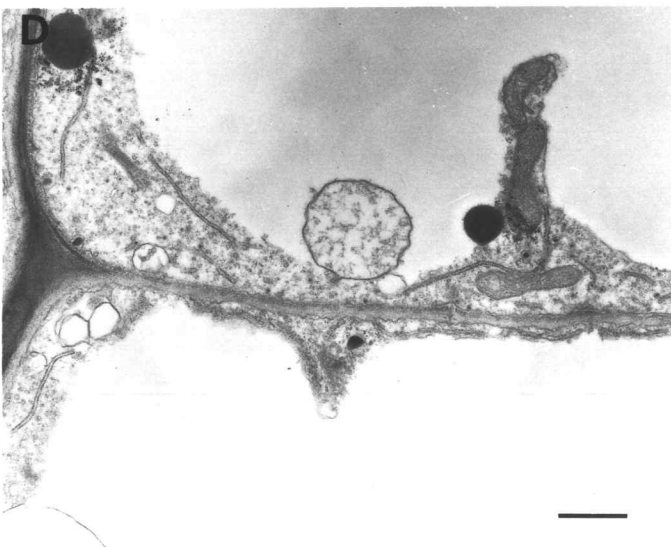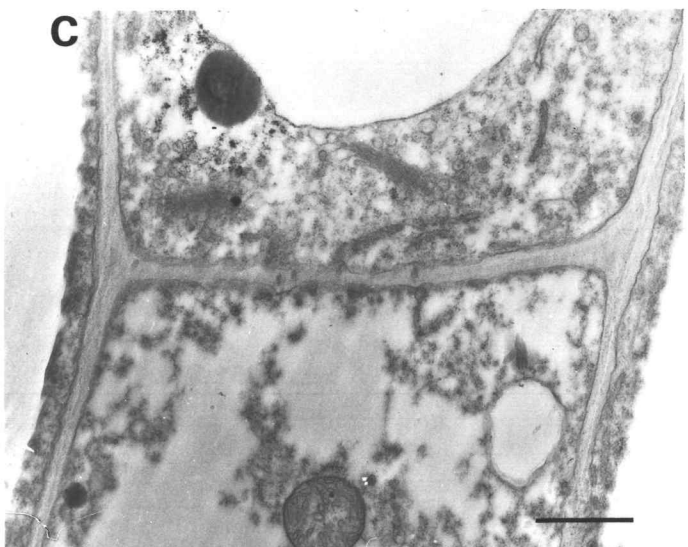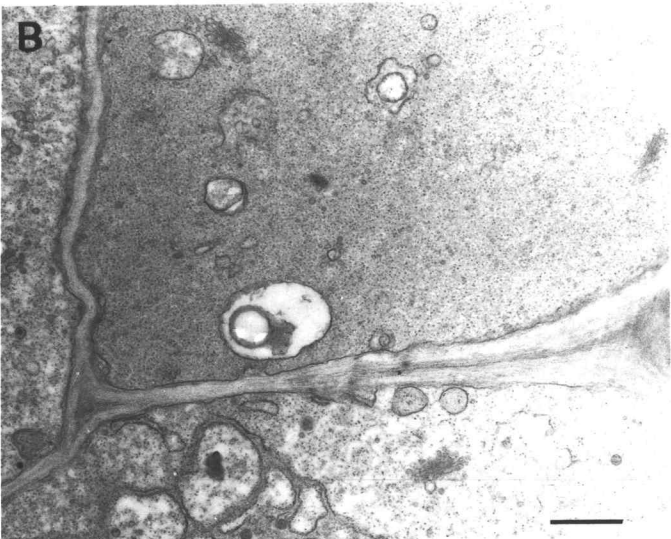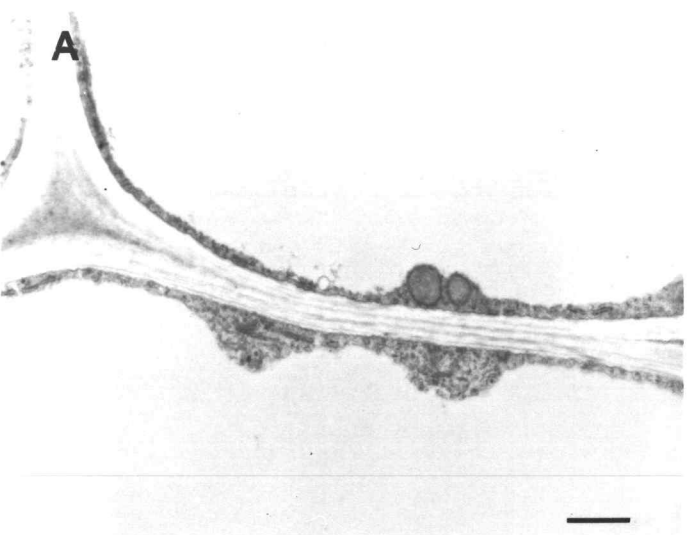
Figure 4.3

Figure 4.4. Electron micrograph of tangential sections from tall fescue pseudostems showing cuticular edge of adaxial epidermal cell wall where A = control, 4500 X; B = FHM treated, 4500 X; C = FHM + DMA-dicamba treated, 4500 X; and D = DMA-dicamba treated, 4500 X. All bars are equivalent to 1 μm. One of the cuticles is indicated with an arrow.
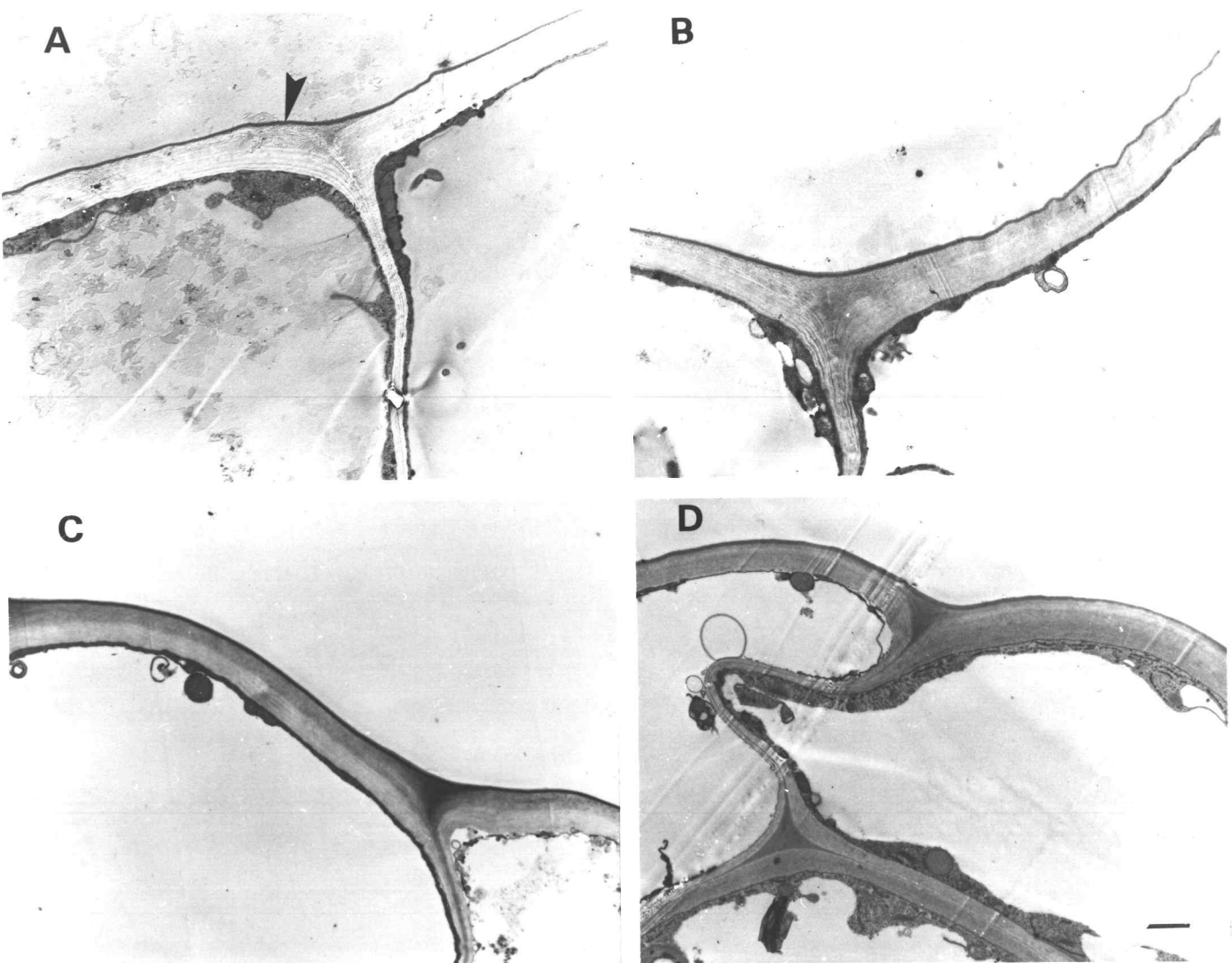
Figure 4.4

LITERATURE CITED

1. Agüero Alvarado, R., A. P. Appleby, and D. J. Armstrong. 1990. Antagonism of haloxyfop activity in tall fescue (Festuca arundinaceae) by dicamba or bentazon is not due to effects on acetyl-CoA carboxylase. Weed Science (in review)

2. Andersen, R. N. 1982. Comparisson of four herbicides for postemergence grass control. Proceedings North Central Weed Control Conference 37: 80-82.

3. Asare-Boamah, N. K. and R. A. Fletcher. 1983. Physiological and cytological effects of BAS-9052-OH on corn (Zea mays) seedlings. Weed Sci. 31: 49-55.

4. Buhler, D. D., B. A. Swisher, and O. C. Burnside. 1985. Behavior of ^{14}C-haloxyfop-methyl in intact plants and cell cultures. Weed Sci. 33: 291-299.

5. Burton, J. D., J. W. Gronwald, D. A. Somers, J. A. Connely, B. G. Gegenback, and D. L. Wise. 1987. Inhibition of plant acetyl-CoA carboxylase by the herbicides sethoxydim and haloxyfop. Biochim. and Biophys. Res. Comm. 148: 1039-1044.

6. Butler, J. H. B. and A. P. Appleby. 1986. Tolerance of red fescue (Festuca rubra) and bentgrass (Agrostis spp) to sethoxydim. Weed Sci. 34: 457-461.

7. Chow, P. N. P. and D. E. LaBerge. 1978. Wild oat studies. 2. Physiological and chemical changes in barley and wild oat treated with diclofop-methyl herbicide in relation to plant tolerance. J. Agric. Food Chem. 26: 1134-1137.

8. Fletcher, R. A. and D. M. Drexler. 1980. Interactions of diclofop-methyl and 2,4-D in cultivated oats (Avena sativa). Weed Sci. 28: 363-366.

9. Gealy, D. R. and F. W. Slife. 1983. BAS 9052 effects on leaf photosynthesis and growth. Weed Sci. 31: 457-461.

10. Gerwick, B. C. 1988. Potential mechanisms for bentazon antagonism with haloxyfop. Weed Sci. 36: 286-290.

11. Gillespie, G. R. and J. D. Nalewaja. 1989. Influence of 2,4-D and MCPA formulations and oil on diclofop phytotoxicity. Weed Sci. 37: 380-384.

12. Hall, C., L. V. Edgington, and C. M. Switzer. 1982. Translocation of different 2,4-D, bentazon, diclofop, or diclofop-methyl combinations in oat (Avena sativa) and soybean (Glycine max). Weed Sci. 30: 676-682.

13. Harwood, J. L. 1988. The site of action of some selective graminaceous herbicides is identified as acetyl-CoA carboxylase. TIBS 13: 330-331.

14. Kafiz, B., J. P. Caussanel, R. Scalla, and P. Gaillardon. 1989. Interaction between diclofop-methyl and 2,4-D in wild oat (Avena fatua L.) and cultivated oat (Avena sativa L.), and fate of diclofop-methyl in cultivated oat. Weed Res. 29: 299-305.

15. Mueller, T. C., W. W. Witt, and M. Barrett. 1989. Antagonism of johnsongrass (Sorghum halepense) control with fenoxaprop, haloxyfop, and sethoxydim by 2,4-D. Weed Tech. 3: 86-89.

16. Nalewaja, L. D., S. D. Miller, and A. G. Dexter. 1982. Postemergence grass and broadleaf herbicide combinations. Proceedings North Central Weed Control Conference 37: 77-80.

17. Olson, W. and J. D. Nalewaja. 1982. Effect of MCPA on ^{14}C-diclofop uptake and translocation. Weed Sci. 30: 59-63.

18. Rendina, A. R. and J. M. Felts. 1988. Cyclohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses. Plant Physiol. 86: 983-986.

19. Rhodes, G. N. and H. D. Coble. 1984. Influence of bentazon on absorption and translocation of sethoxydim in goosegrass (Eleusine indica). Weed Sci. 32: 595-597.

20. Secor, J. and C. Cséke. 1988. Inhibition of acetyl-CoA carboxylase activity by haloxyfop and tralkoxydim. Plant Physiol. 86: 10-12.

21. Taylor, H. F., M. P. C. Loader, and S. J. Norris. 1983. Compatible and antagonistic mixtures of diclofop-methyl and flamprop-methyl with herbicides used to control broad-leaved weeds. Weed Res. 24: 185-190.

22. Todd, B. G. and E. H. Stobbe. 1980. The basis of the antagonistic effect of 2,4-D on diclofop-methyl toxicity to wild oat (Avena fatua). Weed Sci. 28: 371-377.

23. Weed Science Society of America. 1989. Herbicide Handbook. Sixth ed. WSSA, Illinois. 301 pp.

24. Wanamarta, G., D. Penner, and J. J. Kells. 1989. The basis of bentazon antagonism on sethoxydim absorption and activity. Weed Sci. 37: 400-404.

CHAPTER 5. A BASIS FOR THE ANTAGONISM OF DICAMBA ON HALOXYFOP ACTIVITY IN SHOOTS OF TALL FESCUE

ABSTRACT

The effect of dicamba on haloxyfop metabolism and cellular uptake in shoots of tall fescue was studied. A greater amount of a polar conjugate of haloxyfop was found when plants were treated with a mixture of formulated haloxyfop-methyl and dicamba than when treated with haloxyfop alone. The conjugate was broken by alkaline reaction. The conjugate yielded several products when treated with pyridine + acetic anhydride. ^{14}C-haloxyfop accumulated in microsomal membrane vesicles from tall fescue etiolated coleoptiles in a pH-dependent manner, resembling results with ^{3}H-IAA. This accumulation was reduced by addition of dicamba. Based upon present results and findings reported earlier, potential mechanisms to account for the antagonism of dicamba on haloxyfop activity in tall fescue are discussed.

INTRODUCTION

The mechanism(s) by which growth-regulator herbicides antagonize the activity of aryloxyphenoxypropanoic acid herbicides such as haloxyfop has been difficult to elucidate. Reports that seem contradictory (7, 22) may actually indicate that the mechanism for the antagonism is multifaceted and more complex than anticipated.

Understanding the antagonistic mechanisms not only has scientific relevance but also potential practical applications. Once we know how a specific antagonistic response operates, it might be possible to determine whether it can be avoided. For example, antagonism due to a change in polarity of the spraying solution (23) might be avoided by adjusting the pH of the herbicide solution.

We previously learned that dicamba, a benzoic acid derivative, antagonizes haloxyfop by reducing short and long distance transport of haloxyfop without affecting its uptake through the cuticle[1]. In the research reported here, we tested potential changes in the plant metabolism of haloxyfop in the presence of dicamba and the effect of dicamba on penetration of haloxyfop into cells. We have attempted to integrate present and previous results into potential mechanisms that may explain how this particular interaction occurs.

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[1]For details see Chapter 4.

MATERIALS AND METHODS

Metabolism studies

Plant material and haloxyfop metabolism in tall fescue leaves. Tall

fescue plants in the 3-leaf stage, grown as described previously (1),

were used for metabolism studies. Plants were dipped in either 18 μM

formulated haloxyfop-methyl (FHM) (concentration that reduced tall

fescue shoot weight by about 50% in previous experiments), or 18 μM

FHM + 1085 μM formulated dimethylamine salt of dicamba (DMA-dicamba).

This dicamba concentration antagonized FHM activity by nearly 50% in

previous experiments. Plants were allowed to dry for about 10 min,

then 10 μl of ^{14}C-haloxyfop methyl (specific activity = 19.98 mCi

mmol^{-1}) dissolved in methanol (80% by vol) to provide about 25000

CPM/μl were placed on the adaxial surface in the middle region of the

second leaf of each plant. Plants were placed in a growth chamber set

at 350 μE m^{-2}sec^{-1} photosynthetic photon flux density from a mixture

of fluorescent and incandescent lights, with a day/night temperature

of 26/16 $\pm$ 1 C and a 15-h photoperiod. Twenty-four hours after

treatment, the second leaf from each plant was detached and washed

twice in methanol (80% by vol) to remove any unabsorbed ^{14}C-haloxyfop-

methyl. Washes were combined and aliquots were taken to estimate

radioactivity present outside the treated leaves. Each leaf was

immediately cut into several pieces and macerated with a mortar and

pestle in 0.75 ml of HPLC-methanol. Aliquots (50 μl) from extracts

were mixed with scintillation cocktail A[2] to estimate total ^{14}C-

haloxyfop inside the treated leaf. Total recovery of ^{14}C-haloxyfop-

methyl was nearly 70% and was similar among treatments. This low

[2]Scintillation cocktail A consisted of 60% toluene, 40% ethylene
glycol monomethyl ether, 5 g PPO/l, and 0.1 g POPOP/l.

recovery has been associated with volatilization losses of ^{14}C-haloxyfop-methyl (17). Within an hour after extraction, 30 μl of the extract from each treatment were spotted 3 cm from the origin onto a thin-layer chromatography (TLC) silica gel plate[3], 20 by 20 cm and 250 μm in thickness. The remaining extract was kept refrigerated at about 7 C for future use. Experiments showed that refrigeration of extracts did not alter results. Aliquots (50 μl) from the solution used to measure radioactivity remaining outside treated leaves also were spotted onto a TLC silica gel plate. Spots were developed up to 18 cm in a five-component solvent system consisting of 20:16:4:1:1 hexane: chloroform: ethyl acetate: methanol: acetic acid (by vol). Radioactivity on developed plates was detected with a radioscanner[4]. The experiment was repeated. R_f values for ^{14}C-haloxyfop-methyl and ^{14}C-haloxyfop standards were determined in preliminary experiments.

Some chemical properties of unknown product. Leaf macerates from plants treated with either FHM or FHM + DMA-dicamba contained an unknown compound which remained at near the origin on TLC plates. To determine whether the unknown was a metabolite or a conjugate of haloxyfop with a plant component, plates were re-developed a second time, using methanol (90% by vol) up to 6.5 cm to avoid potential contamination of the unknown with the haloxyfop acid spot located at 8 cm from first development. Above 90% of the unknown moved with the methanol front. It was scraped off and separated from the silica gel by filtrating five times with 1-ml of methanol through Whatman #1 filter paper. Half of the recovered unknown was mixed with 100 μl of

[3]J.T. Baker Inc., Phillipsburg, New Jersey 08865, USA.
[4]Bioscan System 400 Imaging Scanner; Bioscan Inc., 4590 McArthur Blv. NW, Washington D.C., USA.

1 N NaOH and left overnight. The following day, the NaOH-treated and non-treated unknowns were spotted onto a TLC plate and developed with the five-component solvent system previously described.

The possibility that the unknown compound was a complex of haloxyfop with dicamba salt was examined. ^{14}C-haloxyfop standard was spotted several times on a TLC plate. To some of the spots, 10 μl of a 0.44 or 4.4 μg/μl solution of the dimethylamine salt of dicamba was added, and the plate was developed in the five-component solvent system. DMA-dicamba was localized on developed plates by UV light absorption at 254 nm.

Studies were conducted to test if formation of the unknown was time-dependent. Plants were dipped in solutions of 18 μM FHM or 18 μM FHM + 1085 μM DMA-dicamba and placed in a growth chamber for 24 h. Then, the second leaf was macerated with 0.75 ml methanol with a mortar and pestle, ^{14}C-haloxyfop acid was added to the macerate, and 30-μl aliquots were spotted onto a TLC plate. In another experiment, plants were dipped in the same non-radioactive herbicide solutions as above, but this time, 10 μl of ^{14}C-haloxyfop acid was added to the second leaf of each plant immediately. About 20 min later, the second leaf from each plant was detached and macerated in 0.75 ml methanol without previously washing it. Thirty-μl aliquots were spotted onto a TLC plate. Plates were developed as before in the five-component solvent system.

Identification of unknown. The unknown from previous experiments was recovered from plates by filtration with methanol as described in the preceding section. The methanol was evaporated under a hood with a gentle flow of N_2 gas inside the vials and a warm air current striking

the outside of the vials. Solids were redissolved in acetone and methylated by treatment with diazomethane in ether to allow volatilization of haloxyfop in the GC column. Excess diazomethane was removed with dry air, and 3-μl aliquots were injected into an automated gas chromatograph/EI-CI mass spectrometer system[5] with a SE 54 silica capillary column (0.32 mm x 30 m)[6] programmed from 50 to 325 C, 10 degrees/min with cold-on-column injector. Unknown peaks were examined by electron ionization.

In additional attempts at identifying the unknown, we hypothesized that it was a sugar conjugate of haloxyfop. The hypothetical sugar conjugate solids were acetylated by treatment with 50 μl acetic anhydride + 50 μl pyridine and heated to 100 C for 30 min. Aliquots (3 μl) were injected into the GC-Mass spectrometer. Unknown peaks were examined by ammonia positive chemical ionization.

Cellular uptake of haloxyfop

Plant material and preparation of microsomal membrane vesicles. Seeds of tall fescue were germinated and kept in the dark at 28 C for 8 to 10 days. Etiolated coleoptiles were excised about 10 mm above the seed and kept on ice at 4 C until used. Microsomes were obtained as described by Hicks et al. (8) with minor modifications. Coleoptiles were homogenized for 30 sec with a Polytron[7] in 1 ml/g of fresh weight of ice-cold buffer 1 (0.25 M sucrose/10 mM Tris/HCl, pH 7.5/1 mM Na_2EDTA/1 mM dithiothreitol/0.1 mM $MgSO_4$/0.2 mM phenylmethylsulfonyl fluoride with pepstatin and leupeptin at 1 μg/ml). The resulting slurry was filtered through four layers of cheesecloth. Solids

[5]Finnigan model 4023; 355 River Oaks Parkway, San Jose, CA 95134-1991 USA.
[6]Alltech Assoc. Inc., 2051 Waukegan Road, Deerfield, Il 60015 USA.
[7]Brinkmann, used at setting 6-7.

remaining in the cheesecloth were homogenized again with buffer 1 for 30 sec. The combined filtrates were pooled and centrifuged at 4 C for 20 min at 3000 x g (GPRH3.7 rotor, Beckman) and the pellets were discarded. The supernatant was centrifuged at 100,000 x g for 30 min at 4 C (33,000 rpm, Ti50.2 rotor, Beckman). The microsomal pellet was suspended in buffer 2 (5 mM potassium phosphate, pH 7.8/0.25 M sucrose/4 mM KCl) with a resulting protein concentration of 2.8 to 4 μg/μl (20), depending on the extract.

[3]H-IAA association experiments. The association of labeled IAA with microsomes was assayed as described by Hicks et al (8). ^{3}H-IAA was diluted to 4 nM in 10 mM disodium citrate/citric acid, pH 5.5/0.25 M sucrose/5 mM $MgSO_4$. Ultracentrifuge tubes of 1.5 ml were prefilled with 0.9 ml of radiolabeled auxin solution (with or without ION$_3$[8] or unlabeled IAA) to which microsomes were added in 100-μl aliquots of buffer 1 which provided about 0.14 μg/μl protein in the final reaction mixture. After 5 min, samples were centrifuged at 200,000 x g for 5 min at 4 C (Beckman TLA 100.3 rotor). The supernatants were discarded and the bottom tips of tubes containing the pellets were cut and placed in 5-ml glass-tubes; these tubes were then filled with 5 ml of Beckman Ready-Safe scintillant and radioassayed 24 h later. Microsomes for additional experiments were divided into 0.3-ml aliquots in plastic tubes, frozen in liquid nitrogen, and kept refrigerated at -70 C until used.

[14]C-haloxyfop association experiments. Following similar procedures as described in the previous section, the association of ^{14}C-haloxyfop (with or without ION$_3$, or unlabeled dicamba) was studied.

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[8]ionophores (a mixture of valinomycin, nigericin, and carbonyl cyanide m-chlorophenylhydrazone).

RESULTS AND DISCUSSION

<u>Metabolism studies</u>.

All labeled haloxyfop remaining on the outside of leaves from both treatments (either FHM or FHM + DMA-dicamba) 24 h after treatment was detected at an R_f value of 0.87, which corresponds to the same R_f as standard ^{14}C-haloxyfop-methyl, indicating that haloxyfop is not altered outside the leaves. In leaf extracts, less than 5% of labeled haloxyfop was in the ester form (Table 5.1), and even this low amount may be due to esterification of the acid form in methanol during extraction.

A polar product of unknown nature (either a metabolite or a conjugate of ^{14}C-haloxyfop) was found in leaf macerates of both treatments. However, a greater amount of the unknown was found in macerates from plants treated with the mixture of FHM + DMA-dicamba than in those treated with FHM alone. Consequently, about 50% less phytotoxic haloxyfop acid was present in leaf macerates of the latter (Table 5.1). The dimethylamine salt of dicamba placed on top of haloxyfop in the TLC plate at a concentration far above that used to treat plants, remained near the origin as two adjacent spots (ionized dicamba, and dimethyl amine), whereas all ^{14}C-haloxyfop moved to the same R_f as the ^{14}C-haloxyfop standard without dicamba. Thus, dicamba and haloxyfop did not form a complex <u>in vitro</u>.

The above result, together with our observation that the unknown polar compound was also observed in macerates from plants treated with FHM alone suggest that this particular spot does not correspond to a complex of these herbicides forming inside the plant.

We also found that the formation of the unknown was time-dependent. It occurred 24 hours after treatment but not when ^{14}C-haloxyfop-methyl was added a few minutes prior to extraction.

The five-component solvent system used did not alter the ^{14}C-haloxyfop directly as confirmed by the mass spectrum of the standard scraped from the TLC plates (Figure 5.1), which matched the mass spectrum of haloxyfop reported by others (2). When the polar unknown was treated with strong base for 10 h, ^{14}C-haloxyfop was recovered, as indicated by an identical R_f with the standard. Thus, the unknown is not a metabolite of haloxyfop but rather a conjugate, probably with a plant component. Glycosidic bonds are readily hydrolyzed by acid but resist cleavage by base (15); thus our results indicate that the conjugate was not the result of haloxyfop hydroxylation and subsequent glycosidic bonding between a hydroxyl group and a plant sugar. If haloxyfop was covalently bound to the plant component, it probably occurred through the carbonyl end of haloxyfop, possibly forming an alcohol ester, a thioester, or an amide by reaction with an amine. A glycoside ester has been reported to occur with diclofop (6, 21), and fenoxaprop-ethyl (14); compounds with similar structure and herbicidal properties to haloxyfop.

Attempts to elucidate the chemical nature of the conjugate by mass spectroscopy failed because of low amounts available, which were beyond the resolution of the apparatus. However, when it was hypothesized that the conjugate could be with a sugar, and the acetylated sample was spotted on a TLC plate and developed with the five-component solvent system, radioactivity was scanned at five positions on the plate. One spot remained at the unknown R_f, another

one corresponded to ^{14}C-haloxyfop as verified by the mass spectrum (Figure 5.2); the others may be the result of different levels of acetylation in the plant-component part of the conjugate. The possibility of several conjugates also should not be discarded.

Cellular uptake of haloxyfop

^{3}H-IAA association experiments. Tall fescue microsomal membrane vesicles were able to accumulate ^{3}H-IAA in the presence of a pH gradient. That isolated microsomes mantain a pH gradient has been experimentally proven (16). Ionophores (ION$_3$), which dissipate both the pH gradient and any membrane potential present (8) and effectively eliminate carrier uptake, reduced the accumulation of ^{3}H-IAA into microsomes. Nonradioactive IAA competed well for association (Table 5.2).

^{14}C-haloxyfop association experiments. ^{14}C-haloxyfop also accumulated inside tall fescue microsomal membrane vesicles in a manner similar to ^{3}H-IAA; that is, accumulation was reduced by addition of ionophores. Addition of dicamba above 100 μM reduced ^{14}C-haloxyfop accumulation into vesicles by 40 to 60% when considering only carrier-mediated uptake (total counts - counts in ionophore treatment). Even though there was a high non-specific binding of ^{14}C-haloxyfop to microsomes (above 50%, as indicated by counts in ionophore treatment, Tables 5.3 and 5.4), the effect of dicamba was on net uptake as deduced from Table 5.4 where dicamba mixed with the ionophores did not alter the counts as compared to addition of ionophores alone. It is possible that haloxyfop and dicamba compete for the same protein carrier during cellular uptake. Research testing this hypothesis is underway.

A basis for the antagonism of dicamba on haloxyfop herbicidal activity
in tall fescue. We have found in this and previous studies that
haloxyfop-methyl and dicamba do not interact outside the plant, uptake
of haloxyfop-methyl through the cuticle was not reduced by dicamba,
and the two herbicides did not interact at the acetyl-CoA carboxylase
level. These results suggest that the antagonism is more complex than
anticipated. Potential mechanisms to explain the antagonism, based on
our findings, are discussed next. Haloxyfop-methyl crosses the plant
cuticle at a similar rate in the presence or absence of dicamba. Once
in the apoplast continuum, haloxyfop uptake into cells (including
companion cells of phloem tissue) is reduced by dicamba (Tables 5.3
and 5.4).

Assuming a pH in the range of 5 to 6 in the wall space (16), 4 to 40
times anion/acid haloxyfop (pk_a = 4.3) can be predicted; as such,
simple diffusion inside the cell is unfavored. A lower uptake into
the cells has two main effects, less haloxyfop reaches plastids, and
less haloxyfop is loaded into the phloem for transport to highly
sensitive tissue such as the crown meristem. A specific carrier for
cellular uptake of haloxyfop would explain the small amounts of the
compound that we and others (3, 19) detected outside of the treated
tissue. The higher formation of the conjugate in the presence of
dicamba could be related to the effect on uptake previously discussed.
Our data shows that high non-specific binding of haloxyfop occurs with
microsomes (Tables 5.3 and 5.4). The possibility exists that
haloxyfop is tightly bound in at least some of these non-specific
sites. Dicamba had a net effect on uptake without competing for the
non-specific binding sites of haloxyfop (Table 5.4); thus, as less

haloxyfop enters the cell in the presence of dicamba, more is available to become bound to those other sites. Whether the conjugate of haloxyfop is formed in the apoplast or in the cytoplasm, the fact that dicamba increased its formation (Table 5.1) can account for the antagonism. Certain herbicide conjugates are thought to be inactive forms that operate as a detoxifying mechanism in plants (13, 14). Above processes also may be independent of each other, and each contributing to the antagonism.

A higher amount of haloxyfop remaining in the apoplast in the presence of dicamba would explain results by others (22) with similar mixtures indicating an increase in acropetal movement of the grass herbicide in the treated leaf when mixed with the antagonist. We observed frequent necrosis of tall fescue mature leaf tips when haloxyfop and dicamba were applied mixed, probably because the transpiration stream accumulates the herbicides on the acropetal edges of the leaf.

Dicamba also may favor haloxyfop entrapment in the cytoplasm. Since both herbicides could be competing for the same uptake carrier (Tables 5.3 and 5.4) it can be speculated that they might share the same efflux carrier. An IAA-efflux carrier is even better characterized than the IAA-uptake carrier. It is a saturable, specific site of molecular secretion found mainly at the basal end of each cell (4, 5, 9, 10, 11, 12). However, whether haloxyfop and dicamba compete for this efflux carrier remains to be proved.

As seen, several possibilities remain to be clarified; however, our results and work by others on antagonism between the postemergence grass herbicides and growth regulator herbicides (18, 19, 22) indicate

that the antagonism results from a reduction in the amount of haloxyfop translocated. We provide evidence that such effect might involve complex processes such as differential conjugation of haloxyfop to a plant metabolite and cellular uptake. It is not clear at the moment, how these processes are interrelated.

Table 5.1. Proportions of polar unknown, haloxyfop acid,
and haloxyfop-methyl recovered from tall fescue leaf
macerates 24 h after treatment with either formulated
haloxyfop-methyl (FHM) or FHM + dimethylamine dicamba
(DMA-dicamba).

Treatment	Polar unknown	Haloxyfop acid	Haloxyfop methyl
	-% of total radioactivity scanned[a]-		
FHM	45 A	50 B	4.4 A
FHM + DMA-dicamba	69 B	27 A	3.4 A

[a]Means followed by a different letter within a column, are
significantly different according to the Fisher's Protected
LSD test at the 0.01 level.

Table 5.2. Accumulation of ^{3}H-IAA in tall fescue microsomal membrane vesicles.

Treatment	^{3}H-IAA accumulation[a]	
	---- CPM[c] ----	
ethanol check[b]	2643	A
4 μM ionophore	1390	B
10^{-8} M IAA	2359	A
10^{-6} M IAA	2243	A
10^{-4} M IAA	2075	AB

[a] ^{3}H-IAA added to all treatments at 4×10^{-9} M .

[b] All assays contained 0.1 % ethanol by vol.

[c] Means are the average of three experiments; those followed by the same letter are not significantly different according to Fisher's Protected LSD test at the 0.05 level.

Table 5.3. Accumulation of ^{14}C-haloxyfop in tall fescue microsomal membrane vesicles.

Treatment	^{14}C-haloxyfop accumulation[a]
	----- CPM[c] -----
ethanol check[b]	1626 A
4 μM ionophore	900 C
10^{-4} M dicamba	1348 AB
10^{-3} M dicamba	1318 B

[a] ^{14}C-haloxyfop added to all treatments at 1.5 x 10^{-6} M.

[b] All assays contained 0.1% ethanol by vol.

[c] Means are the average of three experiments each with two replications; those followed by the same letter are not significantly different according to Fisher's Protected LSD test at the 0.05 level.

Table 5.4. Effect of adding dicamba and ionophores together on the ^{14}C-haloxyfop accumulation in tall fescue microsomal membrane vesicles.

Treatment	^{14}C-haloxyfop accumulation[a]	
	----- CPM[c] -----	
ethanol check[b]	1494	A
4 μM ionophore	818	C
10^{-3} M dicamba	1035	B
10^{-3} M dicamba + 4 μM ionophore	829	C

[a] ^{14}C-haloxyfop added to all treatments at 1.5 x 10^{-6} M.

[b] All assays contained 0.1% ethanol by vol.

[c] Means are the average of two experiments, each with two replications; those followed by the same letter are not significantly different according to Fisher's Protected LSD test at the 0.01 level.

Figure 5.1. Mass spectrum of ^{14}C-haloxyfop standard recovered from a TLC plate at R_f = 0.3

Figure 5.1

Mass Spectrum   Data: MESAMP3B #606   Base m/z: 316
03/08/90 11:44:00 + 10:06   Cali: CAL20 #6   RIC: 241408.
Sample: SAMPLE 3 STD HALOXY.
Conds.: EI SE 30 OC
 #604 to #609 Summed - #594 to #597 - #645 to #651 X1.00
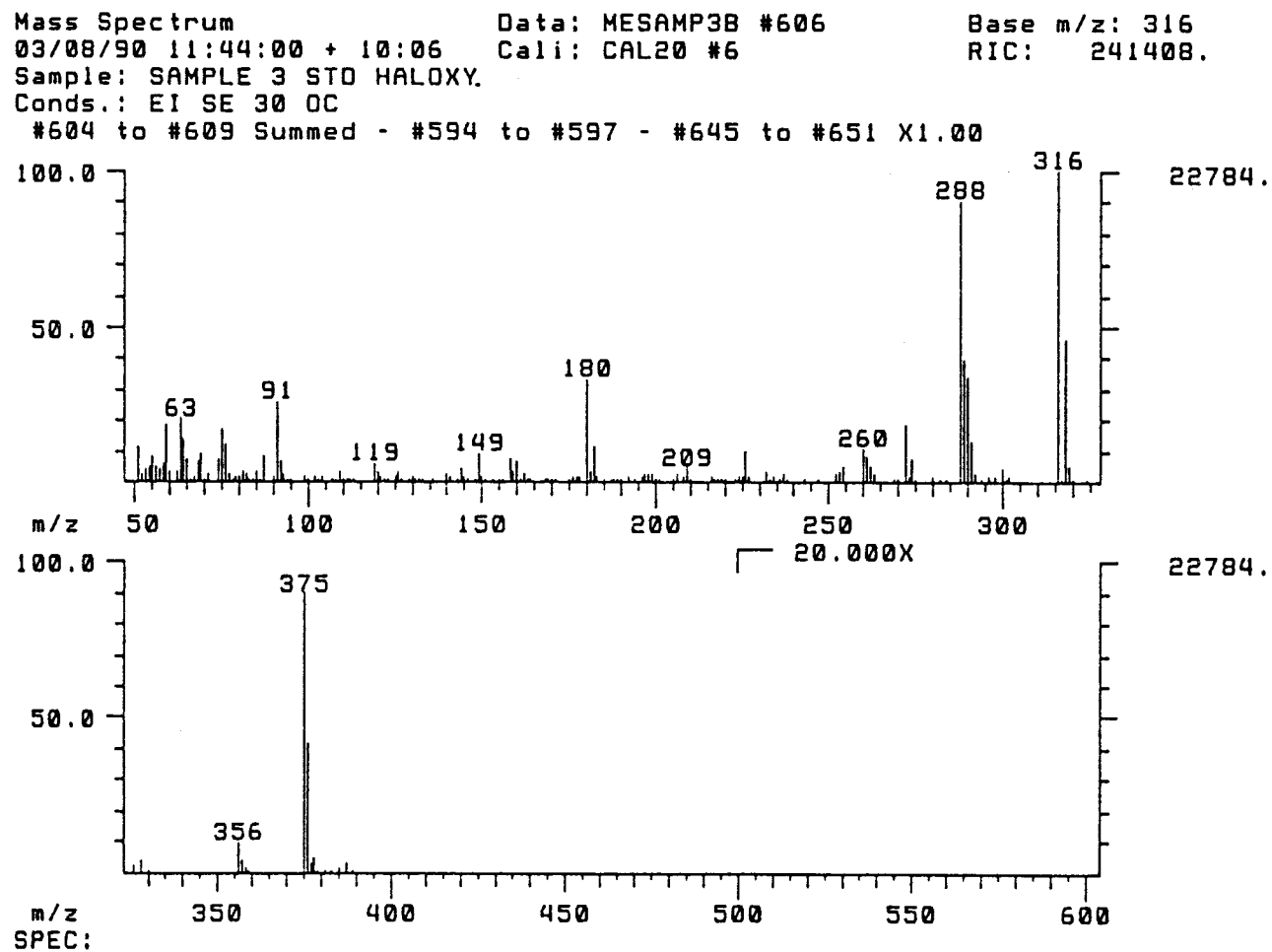
Figure 5.2. Mass spectrum of spot corresponding to same Rf as ^{14}C-haloxyfop standard after treatment of polar unknown with pyridine + acetic anhydride.

Figure 5.2

Mass Spectrum   Data: SAMPLE1 #1086   Base m/z: 87
04/03/90 9:55:00 + 18:06   Cali: CAL20 #6   RIC: 221952.
Sample: ACETYLATED GLUCO CONJUGATES
Conds.: EI GCMS 55-325@10
 #1083 to #1089 Summed - #1075 to #1077 - #1093 to #1094 X1.00
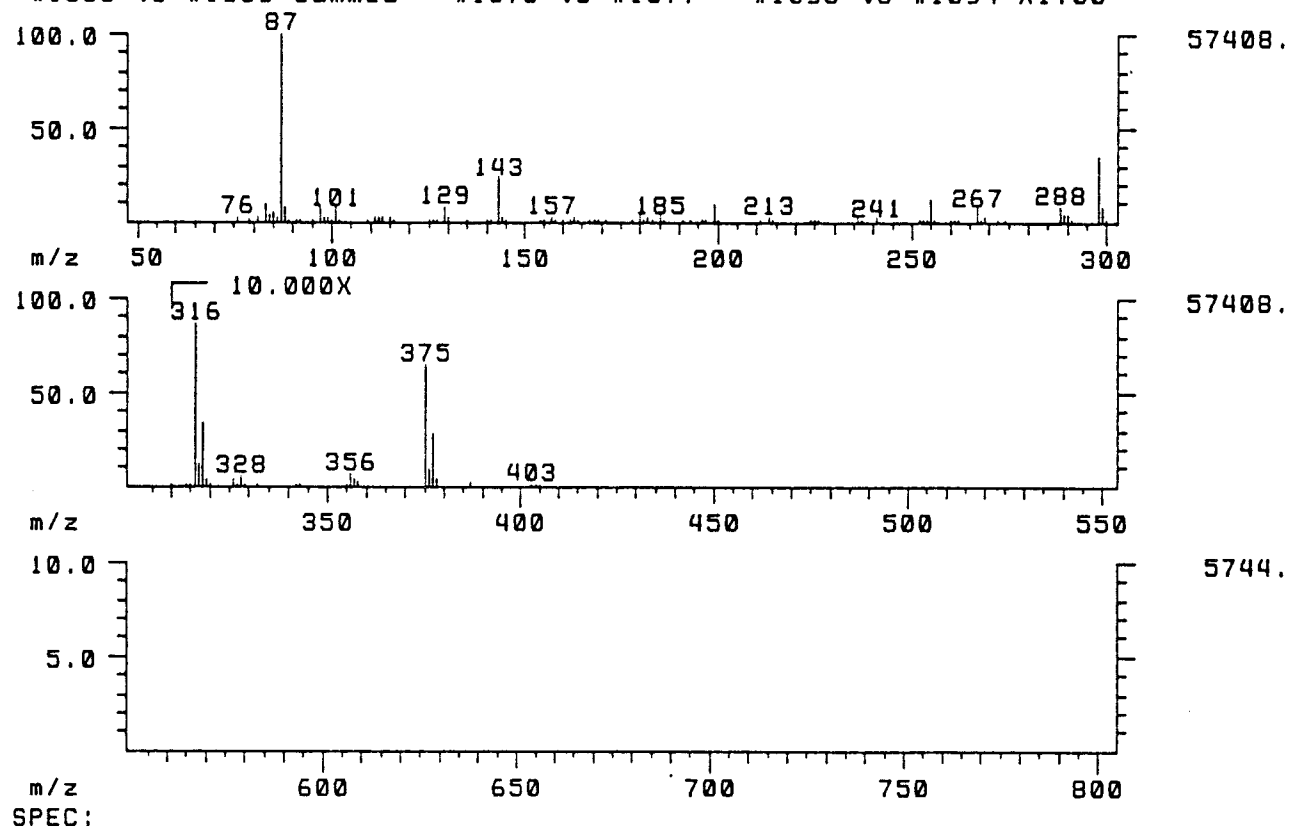
LITERATURE CITED

1. Agüero Alvarado, R., A. P. Appleby, and D. J. Armstrong. 1990. Antagonism of haloxyfop activity in tall fescue (<u>Festuca arundinacea</u>) by dicamba or bentazon is not due to effects on acetyl-CoA carboxylase. Weed Sci. (in review).

2. Campbell, R. A., P. E. Kastl, B. E. Kropscott, and M. J. Bartels. 1989. Quantitative determination of haloxyfop in human urine by gas chromatography-mass spectroscopy. In: Wang, R., C. A. Franklin, R. C. Honeycutt, and J. C. Reinert (eds.). 1989. Biological monitoring for pesticide exposure: measurement, estimation, and risk reduction. 194th Meeting of the Amer. Chem. Soc. New Orleans, Louisiana, Aug 30-Sept 4, 1987. 387 p.

3. Derr, J. F., T. J. Monaco, and T. J. Sheets. 1985. Uptake and translocation of fluazifop by three annual grasses. Weed Sci. 33: 612-617.

4. Goldsmith, M. H. M. and P. M. Ray. 1973. Intracellular localization of the active process in polar transport of auxin. Planta 111: 297-314.

5. Goldsmith, M. H. M. 1982. A saturable site responsible for polar transport of indole-3-acetic acid in sections of maize coleoptiles. Planta 155: 68-75.

6. Gorbach, S. G., K Kuenzler, and J. Asshauer. 1977. On the metabolism of HOE 23408 OH in wheat. J. Agric. Food Chem. 25: 507-511.

7. Hall, C., L. V. Edgington, and C. M. Switzer. 1982. Translocation of different 2,4-D, bentazon, diclofop, or diclofop-methyl combinations in oat (<u>Avena</u> <u>sativa</u>) and soybean (<u>Glycine</u> <u>max</u>). Weed Sci. 30: 676-682.

8. Hicks, G. R., D. L. Rayle, A. M. Jones, and T. L. Lomax. 1989. Specific photoaffinity labeling of two plasma membrane polypeptides with an azido auxin. Proc. Natl. Acad. Sci. 86: 4948-4952.

9. Hertel, R. and A. C. Leopold. 1963. Versuche zur analyse des auxintransports in der koleoptile von <u>Zea</u> <u>mays</u> L. Planta 59: 535-562.

10. Hertel, R. and R. Flory. 1968. Auxin movement in corn coleoptiles. Planta 82: 123-144.

11. Hertel, R., M. L. Evans, A. C. Leopold, and H. M. Sell. 1969. The specificity of the auxin transport system. Planta 85: 238-249.

12. Hertel, R., T. L. Lomax, and W. R. Briggs. 1983. Auxin transport in membrane vesicles from <u>Cucurbita</u> <u>pepo</u> L. Planta 157: 193-201.

13. Jacobson, A. and R. H. Shimabukuro. 1984. Metabolism of diclofop-methyl in root-treated wheat and oat seedlings. J. Agric. Food Chem. 32: 742-746.

14. Lefsrud, C. and J. C. Hall. 1989. Basis for sensitivity differences among crabgrass, oat, and wheat to fenoxaprop-ethyl. Pestic. Biochem. Physiol. 34: 218-227.

15. Lehninger, A. L. 1982. Principles of biochemistry. Worth, New York. p. 284.

16. Lomax, T. L., R. J. Mehlhorn, and W. R. Briggs. 1985. Active auxin uptake by zucchini membrane vesicles: Quantitation using ESR volume and Δ-pH determinations. Proc. Natl. Acad. Sci. 82: 6541-6545.

17. Maroder, H. L., I. A. Prego, and M. A. Cairoli. 1987. Behavior of ^{14}C-haloxyfop in common bermudagrass (<u>Cynodon</u> <u>dactylon</u>) stolons. Weed Sci. 35: 599-603.

18. Mueller, T. C., M. Barrett, and W. W. Witt. 1990. A basis for the antagonistic effect of 2,4-D on haloxyfop-methyl toxicity to johnsongrass (<u>Sorghum</u> <u>halepense</u>). Weed Sci. 38: 103-107.

19. Olson, W. and J.D. Nalewaja. 1982. Effect of MCPA on ^{14}C-diclofop uptake and translocation. Weed Sci. 30: 59-63.

20. Schaffner, W. and C. Weissmann. 1973. A rapid, sensitive, and specific method for the determination of protein in dilute solution. Anal. Biochem. 56: 502-514.

21. Shimabukuro, R. H., W. C. Walsh, and R. A. Hoerauf. 1979. Metabolism and selectivity of diclofop-methyl in wild oat and wheat. J. Agric. Food Chem. 27: 615-623.

22. Todd, B. G. and E. H. Stobbe. 1980. The basis of the antagonistic effect of 2,4-D on diclofop-methyl toxicity to wild oat (<u>Avena</u> <u>fatua</u>). Weed Sci. 28: 371-377.

23. Wanamarta, G., D. Penner, and J. J. Kells. 1989. The basis of bentazon antagonism on sethoxydim absorption and activity. Weed Sci. 37: 400-404.

GENERAL DISCUSSION

Haloxyfop root-absorbed activity. Root-absorbed haloxyfop activity was similar to that of foliar sprays in the order of tolerance, with soybean being more tolerant than red fescue, which was more tolerant than tall fescue. Root and foliage responded similarly in soybean, but roots of the grass species were inhibited more than foliage. This could provide soybean with even more tolerance to preemergence applications of haloxyfop. Diclofop-methyl toxicity to certain graminaceous weed species varies depending on whether it is applied preemergence or postemergence[1]. Haloxyfop has shown significant soil activity (7, 56).

Our finding, that both soybean and tall fescue accumulate similar amounts of ^{14}C-haloxyfop in roots and shoots, whereas red fescue accumulated twice as much haloxyfop in the roots as in shoots deserves attention. In situations where red fescue is infested by a given graminaceous weed of similar tolerance to foliar sprays, preemergence applications of haloxyfop may prove selective.

There was similarity between our estimated internal foliar concentrations of haloxyfop causing 50% reduction in growth of red and tall fescue with in vitro concentrations inhibiting 50% of acetyl CoA carboxylase activity in the same species reported by Stoltenberg et al. (69). However, for tall fescue, a lower internal concentration of haloxyfop in roots than in shoots was needed for an equivalent reduction in dry weight, which suggests several possibilities: (a) acetyl CoA carboxylase (ACCase) content and/or activity in roots of

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tall fescue is lower than in shoots, (b) ACCases produced in shoots and roots of tall fescue differ in susceptibility to haloxyfop, and (c) haloxyfop accumulates in root plastids more than in shoot plastids of tall fescue.

In our studies, red fescue was only 8 times more tolerant to haloxyfop than was tall fescue when applied through the roots; contrasting with the 70-fold difference reported by Butler and Appleby (10) for foliar application of sethoxydim. Thus, the potential exists for selectivity patterns to be altered when high amounts of haloxyfop are available for root absorption, specially when considering species with a closer tolerance difference than the ones discussed here. The fact that soybean absorbed and translocated more ^{14}C-haloxyfop than the grasses indicates that differential uptake and/or translocation do not account for differences in tolerance among these species. ACCases of different susceptibility to the grass herbicides have recently been proposed to account for differences in tolerance among these species (9, 23, 35, 45, 57, 63).

Haloxyfop activity alone or mixed with dicamba in shoots of tall fescue. Dicamba caused strong antagonism of the herbicidal activity of haloxyfop-methyl in shoots of tall fescue. The antagonism could be reduced either by increasing haloxyfop-methyl concentration or by reducing dicamba concentration. This dose-dependent relationship, together with a reduction in the antagonism when haloxyfop application preceded that of dicamba, may be indicative of a competitive process operating between these herbicides. Though other components present in the formulation of haloxyfop-methyl may affect the magnitude of the

antagonism, they do not appear essential for its occurrence, as the interaction also occurred with haloxyfop or dicamba in their acid forms.

The high degree of ACCase inhibition by haloxyfop that we observed is consistent with susceptibility of tall fescue to this herbicide. Similar results with another variety of tall fescue were reported by Stoltenberg et al. (69). The lack of interaction of haloxyfop and dicamba or bentazon at the enzyme level indicated at this stage of the research that the antagonism may be due to mechanisms that reduce the amount of haloxyfop reaching its site of action.

The fact that dicamba or bentazon did not alter haloxyfop inhibition of ACCase activity, even when put in contact with the enzyme extract prior to haloxyfop, indicates that these herbicides either bind to a different site in the enzyme, or that they do not bind at all; the inhibitory effects observed at the higher concentrations of these antagonists probably are the result of secondary physiological effects.

In following studies, our results indicated that haloxyfop mainly targets actively growing tissue; a view shared by others (3, 14, 26, 35). Ultrastructural evidence showed that haloxyfop affects chloroplast development in meristematic tissue by arresting the formation of the thylakoidal membrane system. No chlorophyll forms if chloroplast development is not completed. This accounts well for chlorosis in new emerging leaves as well as the base of expanding leaves. That haloxyfop activity as well as dicamba antagonism of this activity was observed in actively growing leaves but not in mature ones, can be explained by a higher rate of fatty acid synthesis, and

therefore chloroplast formation, in the newer leaves. Even though older leaves could be eventually affected by inhibition of fatty acid synthesis, by the time this occurs, probably the lack of water and nutrient uptake resulting from the death of the crown region is more significant in causing death of the whole plant.

The relatively long time it takes for haloxyfop-treated plants to show symptoms could well be the result of the slow rate of translocation of the herbicide to meristematic tissues. It was also apparent from our studies, that dicamba reduced haloxyfop translocation to those areas. Two patterns to account for the antagonism of broadleaf herbicides on the postemergence grass herbicides such as haloxyfop appear to be emerging; an effect on translocation by growth regulator herbicides (52, 55, 71), and a reduction in uptake by bentazon (27, 74).

Different mechanisms might explain the reduction in translocation of the grass herbicides by growth regulator herbicides, ranging from a reduction in ester hydrolysis and promotion of differential metabolism by 2,4-D (52), to increase in haloxyfop conjugation to plant metabolites as well as reduction in cellular uptake by dicamba found at our laboratory. We are not certain, at the moment, if the increase in haloxyfop conjugation to a plant component promoted by dicamba is the result of its reduction of haloxyfop cellular uptake, or whether both processes are independently contributing to the antagonism. Clearly, both could account for a reduction in the haloxyfop basipetal translocation in tall fescue that we consistently observed.

BIBLIOGRAPHY

1. Agüero Alvarado, R., A. P. Appleby, and D. J. Armstrong. 1990. Antagonism of haloxyfop activity in tall fescue (Festuca arundinaceae) by dicamba or bentazon is not due to effects on acetyl-CoA carboxylase. Weed Science (in review)

2. Andersen, R. N. 1982. Comparison of four herbicides for postemergence grass control. Proceedings North Central. Weed Control Conference 37: 80-82.

3. Asare-Boamah, N. K. and R. A. Fletcher. 1983. Physiological and cytological effects of BAS-9052-OH on corn (Zea mays) seedlings. Weed Sci. 31: 49-55.

4. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248.

5. Brewster, B. D. and A. P. Appleby. 1985. Annual Report 1984-85. Weed Control Research in Agronomic Crops. Crop Science Department. Oregon State University. p. 312-318.

6. Buhler, D. D. and O. C. Burnside. 1984a. Effect of application factors on postemergence phytotoxicity of fluazifop-butyl, haloxyfop-methyl, and sethoxydim. Weed Science 32: 574-583.

7. Buhler, D. D. and O. C. Burnside. 1984. Herbicidal activity of fluazifop-butyl, haloxyfop-methyl and sethoxydim in soil. Weed Science 32: 824-831.

8. Buhler, D. D., B. A. Swisher, and O. C. Burnside. 1985. Behavior of ^{14}C-haloxyfop-methyl in intact plants and cell cultures. Weed Sci. 33: 291-299.

9. Burton, J. D., J. W. Gronwald, D. A. Somers, J. A. Connely, B. G. Gengenbach, and D. L. Wyse. 1987. Inhibition of plant acetyl-coenzyme A carboxylase by the herbicides sethoxydim and haloxyfop. Biochemical and Biophysical Research Communications 148: 1039-1044.

10. Butler, J. H. B. and A. P. Appleby. 1986. Tolerance of red fescue (Festuca rubra) and bentgrass (Agrostis spp) to sethoxydim. Weed Sci. 34: 457-461.

11. Butler, W. L. and K. H. Norris. 1960. The spectrophotometry of dense light scattering material. Arch. Biochem. Biophys. 87: 31-40.

12. Campbell, J. R. and D. Penner. 1982. Compatibility of diclofop and BAS 9052 with bentazon. Weed Sci. 30: 458-462.

13. Campbell, R. A., P. E. Kastl, B. E. Kropscott, and M. J. Bartels. 1989. Quantitative determination of haloxyfop in human urine by gas chromatography-mass spectroscopy. In: Wang, R., C. A. Franklin, R. C. Honeycutt, and J. C. Reinert (eds.). 1989. Biological monitoring for pesticide exposure: measurement, estimation, and risk reduction. 194th Meeting of the Amer. Chem. Soc. New Orleans, Louisiana, Aug 30-Sept 4, 1987. 387 p.

14. Chow, P. N. P. and D. E. LaBerge. 1978. Wild oat studies. 2. Physiological and chemical changes in barley and wild oat treated with diclofop-methyl herbicide in relation to plant tolerance. J. Agric. Food Chem. 26: 1134-1137.

15. Daley, L. S., O. Jahn, and C. Guttridge. 1987. Differences between _Fragaria_ clones by fourth derivative room temperature spectroscopy of intact leaves. Photosynth. Res. 11: 183-188.

16. Daley, L. S., J. R. Powell, G. A. Strobel, R. B. Boone, J. Postman, M. Willie, and J. H. Brown. 1988. Spectroscopic methods of detection of differences in photosynthetic apparatus and function of plants. Plant Physiol. Biochem. 1988: 683-694.

17. Daley, L. S. 1990. French's nomenclature use: In vivo chlorophyll complexes. Plant Physiol. Bioch. 28: In press.

18. Derr, J. F., T. J. Monaco, and T. J. Sheets. 1985. Uptake and translocation of fluazifop by three annual grasses. Weed Sci. 33: 612-617.

19. Donaldson, T. W., D. E. Bayer, and O. A. Leonard. 1973. Absorption of 2,4-dichlorophenoxyacetic acid and 3-(p-chlorophenyl)-1,1-dimethylurea (monuron) by barley roots. Plant Physiology 52: 638-645.

20. Eskelsen, S. R., G. D. Crabtree, R. B. Boone and L. S. Daley. 1989. Determination of herbicide damage to light harvesting apparatus of radishes (_Raphanus_ _sativus_). Proc. Western Soc. Weed Sci. 42: 44-51.

21. Fletcher, R. A. and D. M. Drexler. 1980. Interactions of diclofop-methyl and 2,4-D in cultivated oats (_Avena_ _sativa_). Weed Sci. 28: 363-366.

22. Fisher, H. H., R. A. Menendez, L. S. Daley, D. Robb-Spencer, and G. D. Crabtree. 1987. Biochemical characterization of itchgrass (_Rottboellia_ _exaltata_) biotypes. Weed Sci. 42: 44-51.

23. Focke, M. and H. K. Lichtenhaler. 1987. Inhibition of the acetyl-CoA carboxylase of barley chloroplasts by cycloxydim and sethoxydim. Z. Naturforsch 42c: 1361-1363.

24. Focke, M., K. Kobek, and H. K. Lichtenthaler. 1988. Fatty-acid biosynthesis and acetyl-CoA carboxylase as a target of diclofop, fenoxaprop and other aryloxy-phenoxy-propionic acid herbicides. Z. Naturforsch 43c: 47-54.

25. French, C. S., J. S. Brown, and M. C. Lawrence. 1972. Four universal forms of chlorophyll a. Plant Physiol. 49: 421-429.

26. Gealy, D. R. and F. W. Slife. 1983. BAS 9052 effects on leaf photosynthesis and growth. Weed Sci. 31: 457-461.

27. Gerwick, B. C. 1988. Potential mechanisms for bentazon antagonism with haloxyfop. Weed Sci. 36: 286-290.

28. Gillespie, G. R. and J. D. Nalewaja. 1989. Influence of 2,4-D and MCPA formulations and oil on diclofop phytotoxicity. Weed Sci. 37: 380-384.

29. Goldsmith, M. H. M. and P. M. Ray. 1973. Intracellular localization of the active process in polar transport of auxin. Planta 111: 297-314.

30. Goldsmith, M. H. M. 1982. A saturable site responsible for polar transport of indole-3-acetic acid in sections of maize coleoptiles. Planta 155: 68-75.

31. Gorbach, S. G., K Kuenzler, and J. Asshauer. 1977. On the metabolism of HOE 23408 OH in wheat. J. Agric. Food Chem. 25: 507-511.

32. Gronwald, J. W., C. V. Eberlein, K. J. Betts, K. M. Rosow, N. J. Ehlke, and D. L. Wyse. 1989. Diclofop resistance in a biotype of Italian ryegrass. Plant Physiol. 89(Suppl.): 115.

33. Hall, C., L. V. Edgington, and C. M. Switzer. 1982. Translocation of different 2,4-D, bentazon, diclofop, or diclofop-methyl combinations in oat (_Avena_ _sativa_) and soybean (_Glycine_ _max_). Weed Sci. 30: 676-682.

34. Hartzler, R. G. and C. L. Foy. 1983. Compatibility of BAS 9052 OH with acifluorfen and bentazon. Weed Sci. 31: 597-599.

35. Harwood, J. L. 1988. The site of action of some selective graminaceous herbicides is identified as acetyl-CoA carboxylase. TIBS 13: 330-331.

36. Hertel, R. and A. C. Leopold. 1963. Versuche zur analyse des auxintransports in der koleoptile von _Zea_ _mays_ L. Planta 59: 535-562.

37. Hertel, R. and R. Flory. 1968. Auxin movement in corn coleoptiles. Planta 82: 123-144.

38. Hertel, R., M. L. Evans, A. C. Leopold, and H. M. Sell. 1969. The specificity of the auxin transport system. Planta 85: 238-249.

39. Hertel, R., T. L. Lomax, and W. R. Briggs. 1983. Auxin transport in membrane vesicles from Cucurbita pepo L. Planta 157: 193-201.

40. Hicks, G. R., D. L. Rayle, A. M. Jones, and T. L. Lomax. 1989. Specific photoaffinity labeling of two plasma membrane polypeptides with an azido auxin. Proc. Natl. Acad. Sci. 86: 4948-4952.

41. Hoagland, D. R. and D. I. Arnon. 1938. The water-culture method of growing plants without soil. California Agricultural Experiment Station Circular 347.

42. Irzyk, G. P. and N. C. Carpita. 1989. Relationship between haloxyfop sensitivity and acetyl-CoA carboxylase activity in cultures of proso millet. Plant Physiol. 89(Suppl.): 3.

43. Jacobson, A. and R. H. Shimabukuro. 1984. Metabolism of diclofop-methyl in root-treated wheat and oat seedlings. J. Agric. Food Chem. 32: 742-746.

44. Kafiz, B., J. P. Caussanel, R. Scalla, and P. Gaillardon. 1989. Interaction between diclofop-methyl and 2,4-D in wild oat (Avena fatua L.) and cultivated oat (Avena sativa L.), and fate of diclofop-methyl in cultivated oat. Weed Res. 29: 299-305.

45. Kobek, K., M. Focke, and H. K. Lichtenhaler. 1988. Fatty acid biosynthesis and Acetyl-CoA carboxylase as a target of diclofop, fenoxaprop and other aryloxy-phenoxy-propionic acid herbicides. Z. Naturforsch 43c: 47-54.

46. Kobek, K., M. Focke, H. K. Lichtenthaler, R. Günther, and B. Würzer. 1988. Inhibition of fatty acid biosynthesis in isolated chloroplasts by cycloxydim and other cyclohexane-1,3-diones. Physiol. Plant. 72: 492-498.

47. Lefsrud, C. and J. C. Hall. 1989. Basis for sensitivity differences among crabgrass, oat, and wheat to fenoxaprop-ethyl. Pestic. Biochem. Physiol. 34: 218-227.

48. Lehninger, A. L. 1982. Principles of biochemistry. Worth, New York. p. 284.

49. Lomax, T. L., R. J. Mehlhorn, and W. R. Briggs. 1985. Active auxin uptake by zucchini membrane vesicles: Quantitation using ESR volume and Δ-pH determinations. Proc. Natl. Acad. Sci. 82: 6541-6545.

50. Maroder, H. L., I. A. Prego, and M. A. Cairoli. 1987. Behavior of ^{14}C-haloxyfop in common bermudagrass (Cynodon dactylon) stolons. Weed Sci. 35: 599-603.

51. Mueller, T. C., W. W. Witt, and M. Barrett. 1989. Antagonism of johnsongrass (Sorghum halepense) control with fenoxaprop, haloxyfop, and sethoxydim by 2,4-D. Weed Tech. 3: 86-89.

52. Mueller, T. C., M. Barrett, and W. W. Witt. 1990. A basis for the antagonistic effect of 2,4-D on haloxyfop-methyl toxicity to johnsongrass (Sorghum halepense). Weed Sci. 38: 103-107.

53. Nalewaja, J. D., S. D. Miller, and A. G. Dexter. 1982. Postemergence grass and broadleaf herbicide combinations.Proc. North Central Weed Control Conference 37: 77-80.

54. Ohlrogge, J. B., D. N. Kuhn, and P. K. Stumpf. 1979. Subcellular localization of acyl carrier protein in leaf protoplasts of Spinacia oleracea. Proc. Natl. Acad. Sci. USA. 76: 1194-1198.

55. Olson, W. and J. D. Nalewaja. 1982. Effect of MCPA on ^{14}C-diclofop uptake and translocation. Weed Sci. 30: 59-63.

56. Rahman, A., T. K. James, and J. Mortimer. 1985. Residual activity of some newly developed post-emergence herbicides for grass weeds. Proceedings New Zealand Weed and Pest Control Conference 38: 61-65.

57. Rendina, A. R. and J. M. Felts. 1988. Cyclohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses. Plant Physiology 86: 983-986.

58. Rendina, A. R., J. M. Felts, J. D. Beaudoin, A. C. Craig-Kennard, L. L. Look, S. L. Paraskos, and J. A. Hagenah. 1988. Kinetic characterization, stereoselectivity, and species selectivity of the inhibition of plant acetyl-CoA carboxylase by the aryloxyphenoxypropionic acid grass herbicides. Arch. Biochem. Biophys. 265: 1-7.

59. Rhodes, G. N. and H. D. Coble. 1984. Influence of application variables on antagonism between sethoxydim and bentazon. Weed Sci. 32: 436-441.

60. Rhodes, G. N. and H. D. Coble. 1984. Influence of bentazon on absorption and translocation of sethoxydim in goosegrass (Eleusine indica). Weed Sci. 32: 595-597.

61. Salisbury, F. B. and C. W. Ross. 1985. Plant Physiology. Wadsworth, California. 540 pp.

62. Schaffner, W. and C. Weissmann. 1973. A rapid, sensitive, and specific method for the determination of protein in dilute solution. Analytical Biochem. 56: 502-514.

63. Secor, J. and C. Cséke. 1988. Inhibition of acetyl-CoA carboxylase activity by haloxyfop and tralkoxydim. Plant Physiol. 86: 10-12.

64. Shimabukuro, R. H., W. C. Walsh, and R. A. Hoerauf. 1979. Metabolism and selectivity of diclofop-methyl in wild oat and wheat. J. Agric. Food Chem. 27: 615-623.

65. Shimabukuro, R. H. and B. L. Hoffer. 1989. Metabolism of diclofop-methyl in annual ryegrass, Lolium rigidum, and its relationship to recovery of membrane potential. Abstr. Weed Sci. Soc. Amer., No. 186. p. 82.

66. Simcox, P. D., E. E. Reid, D. T. Canvin, and D. T. Dennis. 1977. Enzymes of the glycolytic and pentose phosphate pathways in pro-plastids from the developing endosperm of Ricinus communis L. Plant Physiol. 59: 1128-1132.

67. Smith, E. L. 1938. Solutions of chlorophyll-protein compounds (pyllochlorins) extracted from spinach. Science 88: 170-171.

68. Soto, A., R. Agüero, and N. Zúñiga. 1986. Rice tolerance to fenoxaprop-ethyl and fluazifop-butyl: rates and timing of application. Turrialba 36: 381-388. (in spanish).

69. Stoltenberg, D. E., J. W. Gronwald, D. L. Wyse, J. D. Burton, D. A. Somers, and B. G. Gengenbach. 1988. Effect of sethoxydim and haloxyfop on acetyl-coenzyme A carboxylase activity in Festuca species. Weed Science 37: 512-516.

70. Taylor, H. F., M. P. C. Loader, and S. J. Norris. 1983. Compatible and antagonistic mixtures of diclofop-methyl and flamprop-methyl with herbicides used to control broad-leaved weeds. Weed Res. 24: 185-190.

71. Todd, B. G. and E. H. Stobbe. 1980. The basis of the antagonistic effect of 2,4-D on diclofop-methyl toxicity to wild oat (Avena fatua). Weed Sci. 28: 371-377.

72. Vick, B. and H. Beevers. 1978. Fatty acid synthesis in endosperm of young castor bean seedlings. Plant Physiol. 62: 173-178.

73. Walker, K., S. M. Ridley, T. Lewis, and J. L. Harwood. 1988. Fluazifop, a grass-selective herbicide which inhibits acetyl-CoA carboxylase in sensitive plant species. Biochem. J. 254: 307-310.

74. Wanamarta, G., D. Penner, and J. J. Kells. 1989. The basis of bentazon antagonism on sethoxydim absorption and activity. Weed Sci. 37: 400-404.

75. Weed Science Society of America. 1989. Herbicide Handbook. Sixth ed. WSSA, Illinois. 301 pp.

76. Zilkey, B. F. and D. T. Canvin. 1971. Localization of oleic acid biosynthesis enzymes in the proplastids of developing castor endosperm. Can. J. Bot. 50: 323-326.

APPENDIX

Appendix table 1. Plant parts fresh and dry weights collected for GR_{50} determination of haloxyfop root-absorbed in soybean. Experiment 1.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --	-- g --					
I						
75	9.2856	5.8314	15.117	1.8721	0.5197	2.3918
50	11.1075	6.6102	17.718	1.9998	0.5639	2.5637
40	13.1025	8.8368	21.939	2.1126	0.7476	2.8602
25	16.0932	9.7290	25.822	2.4224	0.8311	3.2535
10	14.5804	6.9437	21.524	2.3262	0.5579	2.8841
Non-treated check	18.2304	11.0600	29.290	3.1600	0.9914	4.1514
II						
75	10.1734	6.2922	16.466	1.8576	0.5432	2.4008
50	12.0862	6.2816	18.368	1.9653	0.5461	2.5114
40	12.1329	7.1541	19.287	1.9324	0.5787	2.5111
25	14.2235	6.5951	20.819	2.3699	0.5500	2.9199
10	19.3778	11.1042	30.482	3.0519	0.9798	4.0317
Non-treated check	20.8000	10.5405	31.340	3.6533	0.9554	4.6087
III						
75	7.4033	5.0507	12.454	1.5362	0.4048	1.9410
50	9.3512	4.5233	13.874	1.5361	0.3679	1.9040
40	13.7281	8.5320	22.260	2.2909	0.6743	2.9652
25	16.3548	10.1675	26.522	2.9673	0.8448	3.8121
10	18.2061	9.6306	27.837	3.0472	0.8234	3.8706

Appendix table 1 continued.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --				g		
Non-treated check	17.2235	7.8913	25.115	2.6156	0.5811	3.1967
IV						
75	10.0947	6.0600	16.155	1.7945	0.4814	2.2759
50	11.5484	6.5237	18.072	1.9118	0.5127	2.4245
40	11.7811	6.9343	18.715	1.8447	0.5509	2.3956
25	12.4256	7.0069	19.433	1.9838	0.5828	2.5666
10	17.6337	7.3174	24.951	2.8632	0.6027	3.4659
Non-treated check	15.9405	10.3118	26.252	2.8758	0.9317	3.8075

Appendix table 2. Plant parts fresh and dry weights collected for GR$_{50}$ determination of haloxyfop root-absorbed in soybean. Experiment 2.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --				g		
I						
120	3.7000	2.4161	6.1161	0.7651	0.1500	0.9151
100	4.5100	2.8276	7.3376	0.8891	0.1834	1.0725
80	8.8466	7.7456	16.5922	2.0689	0.5645	2.6334
40	13.2053	10.1800	23.3853	2.4860	0.8175	3.3035
25	14.2168	10.3054	24.5222	2.5733	0.8109	3.3842
Non-treated check	17.1350	11.6955	28.8305	3.1700	0.9530	4.1230
II						
120	4.4415	4.2210	8.6625	0.8869	0.2532	1.1401
100	4.9900	3.3962	8.3862	0.8981	0.2492	1.1473
80	7.7042	7.3639	15.0681	1.7628	0.5260	2.2888
40	13.3248	10.5049	23.8297	2.2744	0.7835	3.0579
25	15.5138	12.1731	27.6869	2.6847	0.9200	3.6047
Non-treated check	18.1408	12.6300	30.7708	3.1530	0.9514	4.1044
III						
120	5.5513	3.4465	8.9978	0.9798	0.2051	1.1849
100	5.2700	2.9710	8.2410	0.9684	0.2108	1.1792
80	7.8749	6.3420	14.2169	1.7645	0.4657	2.2302
40	12.7000	10.7800	23.4800	2.4111	0.8520	3.2631
25	16.1772	15.8568	32.0340	2.9800	1.1262	4.1062

Appendix table 2 continued.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --	----------	----------	---------- g	----------	----------	----------
Non-treated check	21.8431	14.6106	36.4537	3.7377	1.2411	4.9788
IV						
120	3.9056	2.5415	6.4471	0.7683	0.1522	0.9205
100	4.1100	2.1473	6.2573	0.8110	0.1472	0.9582
80	8.7040	9.1260	17.8300	1.9766	0.6736	2.6502
40	12.1521	10.0361	22.1882	2.1614	0.7957	2.9571
25	14.6640	14.4955	29.1595	2.6169	1.1100	3.7269
Non-treated check	17.5330	13.4530	30.9860	3.1357	1.0755	4.2112

Appendix table 3. Plant parts fresh and dry weights collected for GR_{50} determination of haloxyfop root-absorbed in red fescue. Experiment 1.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --	------------------------------------ g ------------------------------------					
I						
5.000	0.0564	0.0133	0.0697	0.0153	0.0032	0.0185
2.000	0.1115	0.0183	0.1298	0.0349	0.0033	0.0382
0.750	0.1490	0.0157	0.1647	0.0358	0.0029	0.0387
0.250	0.2224	0.0344	0.2568	0.0405	0.0046	0.0451
0.075	0.3339	0.1072	0.4411	0.0589	0.0092	0.0681
Non-treated check	0.3255	0.1247	0.4502	0.0535	0.0112	0.0647
II						
5.000	0.0687	0.0147	0.0834	0.0230	0.0031	0.0261
2.000	0.1573	0.0156	0.1729	0.0362	0.0033	0.0395
0.750	0.1634	0.0238	0.1872	0.0419	0.0046	0.0465
0.250	0.2437	0.0262	0.2699	0.0437	0.0049	0.0486
0.075	0.1904	0.0726	0.2630	0.0327	0.0073	0.0400
Non-treated check	0.2805	0.1119	0.3924	0.0437	0.0089	0.0526
III						
5.000	0.0577	0.0127	0.0704	0.0169	0.0026	0.0195
2.000	0.1255	0.0186	0.1441	0.0371	0.0037	0.0408
0.750	0.1924	0.0214	0.2138	0.0448	0.0034	0.0482
0.250	0.3166	0.0782	0.3948	0.0496	0.0069	0.0565
0.075	0.3168	0.1000	0.4168	0.0507	0.0077	0.0584
Non-treated check	0.2476	0.0768	0.3244	0.0400	0.0048	0.0448

Appendix table 4. Plant parts fresh and dry weights collected for GR$_{50}$ determination of haloxyfop root-absorbed in red fescue. Experiment 2.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --			g			
I						
5.000	0.0224	0.0053	0.0277	0.0071	0.0011	0.0082
4.000	0.0344	0.0167	0.0511	0.0086	0.0029	0.0115
3.000	0.0125	0.0054	0.0179	0.0048		0.0093
2.000	0.1543	0.0273	0.1816	0.0279	0.0030	0.0309
0.750	0.2208	0.0520	0.2728	0.0383	0.0061	0.0444
0.500					0.0061	
Non-treated check	0.2200	0.0811	0.3011	0.0286	0.0056	0.0342
II						
5.000	0.0246	0.0048	0.0294	0.0088	0.0010	0.0098
4.000	0.0370	0.0090	0.0460	0.0118	0.0016	0.0134
3.000	0.1026	0.0089	0.1115	0.0214		0.0223
2.000	0.1049	0.0123	0.1172	0.0213	0.0016	0.0229
0.750	0.2461	0.0400	0.2861	0.0425	0.0038	0.0463
0.5000					0.0035	
Non-treated check	0.3290	0.1591	0.4881	0.0559	0.0123	0.0682

Appendix table 4 continued.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --				g		
III						
5.000	0.0247	0.0087	0.0334	0.0078	0.0012	0.0090
4.000	0.0810	0.0134	0.0944	0.0181	0.0019	0.0200
3.000	0.0506	0.0056	0.0562	0.0139		0.0145
2.000	0.1060	0.0163	0.1223	0.0224	0.0017	0.0241
0.750	0.2708	0.0278	0.2986	0.0482	0.0029	0.0511
0.500					0.0070	0.0511
Non-treated check	0.3504	0.1800	0.5304	0.0507	0.0127	0.0634
IV						
5.000	0.0241	0.0061	0.0302	0.0088	0.0014	0.0102
4.000	0.0132	0.0043	0.0175	0.0044	0.0007	0.0051
3.000	0.0406	0.0074	0.0480	0.0091		0.0099
2.000	0.1782	0.0438	0.2220	0.0313	0.0051	0.0364
0.750	0.1943	0.0170	0.2113	0.0375	0.0028	0.0403
0.500					0.0054	
Non-treated check	0.2211	0.1057	0.3268	0.0319	0.0068	0.0387

Appendix table 5. Plant parts fresh and dry weights collected for GR$_{50}$ determination of haloxyfop root-absorbed in tall fescue. Experiment 1.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --				g		
I						
1.000	0.1294	0.0073	0.1366	0.0364	0.0002	0.0366
0.500	0.1118	0.0109	0.1227	0.0282	0.0005	0.0297
0.300	0.2353	0.0172	0.2525	0.0549	0.0018	0.0564
0.100	0.3557	0.0135	0.3692	0.0642	0.0016	0.0660
0.075	0.5796	0.0413	0.6209	0.0917	0.0036	0.0953
Non-treated check	0.6159	0.2311	0.8470	0.0582	0.0102	0.0684
II						
1.000	0.1371	0.0277	0.1648	0.0352	0.0001	0.0353
0.500	0.1948	0.0231	0.2179	0.0481	0.0031	0.0512
0.300	0.1962	0.0187	0.2149	0.0462	0.0005	0.0467
0.100	0.3967	0.0317	0.4284	0.0670	0.0021	0.0691
0.075	0.3600	0.0178	0.3778	0.0716	0.0014	0.0730
Non-treated check	0.7524	0.3949	1.1473	0.0957	0.0261	0.1218
III						
1.000	0.1668	0.0241	0.1909	0.0382	0.0028	0.0410
0.500	0.2034	0.0216	0.2250	0.0528	0.0023	0.0551
0.300	0.2272	0.0250	0.2522	0.0572	0.0038	0.0610
0.100	0.5527	0.0288	0.5815	0.1035	0.0034	0.1069
0.075	0.5452	0.0401	0.5853	0.0842	0.0042	0.0884

Appendix table 5 continued.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --			g			
Non-treated check	0.7225	0.3135	1.0360	0.0821	0.0212	0.1033
IV						
1.000	0.0754	0.0193	0.0947	0.0218	0.0010	0.0228
0.500	0.1755	0.0227	0.1982	0.0429	0.0031	0.0460
0.300	0.2345	0.0226	0.2571	0.0599	0.0038	0.0637
0.100	0.3702	0.0257	0.3959	0.0706	0.0034	0.0740
0.075	0.6639	0.0905	0.7544	0.0845	0.0091	0.0936
Non-treated check	0.6543	0.2368	0.8911	0.0863	0.0187	0.1050

Appendix table 6. Plant parts fresh and dry weights collected for GR$_{50}$ determination of haloxyfop root-absorbed in tall fescue. Experiment 2.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --	--- g ---					
I						
0.750	0.0361	0.0048	0.0409	0.0089	0.0013	0.0102
0.500	0.0318	0.0032	0.0350	0.0091	0.0009	0.0100
0.250	0.1334	0.0069	0.1403	0.0281	0.0013	0.0294
0.100	0.2057	0.0169	0.2226	0.0389	0.0033	0.0422
0.050	0.3004	0.0090	0.3094	0.0560	0.0012	0.0572
Non-treated check	0.5329	0.1943	0.7272	0.0656	0.0161	0.0817
II						
0.750	0.0607	0.0059	0.0666	0.0119	0.0013	0.0132
0.500	0.0588	0.0049	0.0637	0.0077	0.0012	0.0189
0.250	0.1483	0.0100	0.1583	0.0279	0.0018	0.0297
0.100	0.2180	0.0111	0.2291	0.0374	0.0015	0.0389
0.050	0.2907	0.0207	0.3114	0.0525	0.0038	0.0563
Non-treated check	0.4441	0.1740	0.6181	0.0544	0.0136	0.0680
III						
0.750	0.0450	0.0060	0.0510	0.0107	0.0015	0.0122
0.500	0.0590	0.0088	0.0678	0.0141	0.0017	0.0158
0.250	0.0843	0.0049	0.0892	0.0211	0.0012	0.0223
0.100	0.1491	0.0065	0.1556	0.0276	0.0015	0.0291
0.05	0.3138	0.0174	0.3312	0.0509	0.0030	0.0539

Appendix table 6 continued.

Rep/haloxyfop concentration	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight
-- μM --	---------------------------------- g ----------------------------------					
Non-treated check	0.1656	0.0541	0.2197	0.0233	0.0045	0.0278
IV						
0.750	0.0294	0.0050	0.0344	0.0077	0.0011	0.0088
0.500	0.0957	0.0064	0.1021	0.0222	0.0014	0.0236
0.250	0.1068	0.0031	0.1099	0.0263	0.0010	0.0273
0.100	0.2586	0.0130	0.2716	0.0465	0.0034	0.0499
0.05	0.2538	0.0110	0.2648	0.0471	0.0026	0.0497
Non-treated check	0.5822	0.1684	0.7506	0.0660	0.0142	0.0802

Appendix table 7. Transpiration in three species 48 and 96 HAT[a] for the experiment on ^{14}C-haloxyfop root-absorbed accumulation patterns.

Species	Rep	Transpiration on time		Transpiration by weight	
		48 HAT	96 HAT	48 HAT	96 HAT
		------- ml/hour -----		--- ml/g dry weight ---	
Soybean	I	1.17	1.72	85.4	133.0
	II	1.19	1.58	80.2	132.9
	III	1.21	1.59	76.8	122.0
Red fescue	I	0.13	0.10	363.4	420.6
	II	0.10	0.13	359.8	327.9
	III	0.12	0.10	452.7	395.3
Tall fescue	I	0.11	0.14	280.7	325.8
	II	0.08	0.11	196.1	267.6
	III	0.12	0.11	210.6	228.2

[a]Hours after treatment.

Appendix table 8. Nutrient solution and ^{14}C-haloxyfop absorbed 48 and 96 hours after treatment.

| Species | Rep | 48 HAT[a] | | | 96 HAT | | |
		Total volume absorbed	% volume[b] absorbed	% ^{14}C-haloxyfop[c] absorbed	Total volume absorbed	% volume absorbed	% ^{14}C-haloxyfop absorbed
		- ml -			- ml -		
Soybean	I	56.00	22.4	12.0	165.50	66.2	21.8
	II	57.00	22.8	15.4	152.00	60.8	18.0
	III	58.00	23.2	13.0	153.00	61.2	23.0
Red fescue	I	6.25	12.5	4.2	9.00	18.0	8.2
	II	4.75	9.5	3.1	12.00	24.0	10.6
	III	5.75	11.5	4.7	10.00	20.0	10.5
Tall fescue	I	5.25	10.5	3.8	13.00	26.0	7.4
	II	4.00	8.0	2.2	10.25	20.5	5.2
	III	5.75	11.5	2.5	10.75	21.5	6.3

[a]HAT - hours after treatment
[b]Originally 250 ml for soybean and 50 ml for grasses were added.
[c]600 DPM/ml ^{14}C-haloxyfop were added at beginning of experiment.

Appendix table 9. Total ^{14}C-haloxyfop accumulated by roots or shoots of soybean, red fescue, or tall fescue.

Time (hours)	Replication	Soybean			Red fescue			Tall fescue		
		Foliage	Roots	Total	Foliage	Roots	Total	Foliage	Roots	Total
48	1	12192.31	5817.78	18010.09	305.34	1055.97	1361.31	534.84	707.27	1242.11
	2	16806.79	6379.23	23186.02	476.64	523.67	1000.31	285.14	428.87	714.01
	3	12830.58	6570.16	19400.74	572.84	964.17	1537.01	349.34	457.97	807.31
96	1	18218.46	14543.84	32762.30	930.94	1742.17	2673.11	1331.04	1080.97	2412.01
	2	14758.58	11877.64	26636.39	1043.64	2387.87	3431.51	625.44	1075.07	1700.51
	3	23498.92	11264.12	34763.04	1005.64	2392.57	3398.21	813.94	1222.97	2036.91
144	1	20741.88	16096.02	36837.9	1343.14	2683.87	4027.01	1571.64	2178.87	3750.51
	2	20248.63	18999.30	39247.93	1086.44	2945.67	4032.11	2276.94	2448.27	4725.21
	3	26033.29	14293.08	40326.37	1153.84	4226.27	5380.11	2739.22	3710.97	6450.19
192	1	22689.76	27907.12	50596.88	1738.94	3322.97	5061.91	2450.00	2365.47	4815.47
	2	32810.46	42963.01	75773.47	2218.24	4501.07	6719.31	3832.9	3031.57	6864.47
	3	30189.6	39713.11	69902.71	1605.14	5301.17	6906.31	2482.44	2177.87	4660.31
240	1	25003.52	38881.99	63885.51	2097.74	5744.52	7842.31	3204.65	4164.77	7369.12
	2	22289.48	38477.31	60766.79	3723.04	4309.77	8032.81	4772.35	3191.07	7963.42
	3	24156.47	36891.07	61047.54	3412.15	5558.07	8970.22	2147.07	2991.07	5138.08

Total activity applied Total activity applied
150000 DPM/flask of 250 ml for soybean 32428 DPM/vial of 50 ml for grasses

Appendix table 10. ^{14}C-haloxyfop recovered in foliage and roots of soybean, red fescue, and tall fescue as percent of total radioactivity initially applied.

Hours after application	Soybean			Red fescue			Tall fescue		
	Shoots	Roots	Total	Shoots	Roots	Total	Shoots	Roots	Total
	----------------------- % of total radioactivity initially applied[a] -------------------								
48	9.29	4.12	13.41	1.40	2.61	4.01	1.20	1.64	2.84
96	12.55	8.37	20.92	3.06	6.70	9.76	2.85	3.47	6.32
144	14.89	10.97	25.86	3.68	10.13	13.81	6.77	8.52	15.34
192	19.04	24.57	43.61	5.72	13.49	19.21	9.01	7.79	16.8
240	15.88	25.39	41.27	9.49	16.05	25.54	10.41	10.64	21.05
% recovery (including activity recovered in remaining nutrient solution)	94.0			89.5			86.2		

[a]150,000 DPM/flask for soybean, 32,400 DPM/flask for grasses.

Appendix table 11. Total ^{14}C-haloxyfop remaining in nutrient solution at each harvest time of soybean, red fescue, and tall fescue.

Time (hours)	Replication	^{14}C CPM		
		Soybean[a]	Red fescue[b]	Tall fescue[b]
48	1	133782	27645	29673
	2	116418	28882	31882
	3	135552	29306	29093
96	1	126547	25891	26502
	2	125558	25227	26576
	3	121832	27451	27141
144	1	100563	25518	22991
	2	105534	23452	21960
	3	113076	24128	20957
192	1	75329	24473	22475
	2	66617	25555	19709
	3	66150	19671	18996
240	1	75020	18343	18321
	2	74611	20400	18020
	3	68006	18624	24243

[a]Radioactivity initially added/flask = 150000 DPM.
[b]Radioactivity initially added/tube = 32400 DPM.

Appendix table 12. Total fresh and dry weights of soybean for ^{14}C-haloxyfop root uptake and translocation study. Subsample dry weights assayed to estimate radioactivity.

Tissue	Rep	Total fresh weight					Total dry weight					Dry weights assayed for ^{14}C				
		48	96	144	192	240[a]	48	96	144	192	240	48	96	144	192	240
Newer tissue	I	352	1506	2150	2938	4211	54	235	384	535	841	42.7	42.5	48.4	47.6	47.7
	II	642	957	2663	3191	4457	96	145	487	611	920	43.3	43.5	48.7	46.5	49.4
	III	381	1426	2175	3163	4002	60	230	392	623	815	45.4	46.4	45.9	45.2	49.2
First	I	724	1407	1833	2377	2422	102	228	352	501	574	45.2	43.4	46.4	46.8	47.2
trifoliate	II	716	1646	1947	2650	2132	106	257	382	580	517	40.8	42.8	41.8	48.0	44.5
leaf	III	954	1628	2103	2643	2698	147	263	408	600	630	46.6	43.7	47.8	44.2	45.3
Primary leaves	I	1721	2200	2326	2040	2110	253	319	409	406	422	42.7	45.0	44.5	45.9	42.0
+	II	1940	2132	2157	2341	1684	265	310	341	418	333	41.5	45.1	42.2	45.9	44.0
cotyledons	III	1843	2000	1839	2302	1908	284	314	312	433	377	46.5	44.7	45.8	48.9	46.6
Stem	I	704	1046	1457	1673	2066	87	139	248	279	384	41.9	50.0	47.2	47.6	47.6
	II	737	1061	1477	1913	1935	91	131	249	324	377	40.0	47.0	45.2	46.5	49.0
	III	806	1020	1372	1907	2374	105	132	214	350	476	46.6	46.7	49.1	44.6	48.4

Appendix table 12 continued.

Tissue	Rep	Total fresh weight					Total dry weight					Dry weights assayed for ^{14}C				
		48	96	144	192	240[a]	48	96	144	192	240	48	96	144	192	240
Roots	I	2484	5287	6295	8123	10334	160	323	456	592	775	46.3	46.3	45.4	40.4	44.7
	II	2592	4882	6075	9133	10225	153	300	448	670	755	47.7	46.7	46.1	50.0	43.9
	III	2587	4810	6341	8722	9971	159	315	454	684	774	48.0	41.8	50.0	48.0	48.9

		48	96[b]	144	192	240
Remaining nutrient solution	I	194	84.5	152	159	229
at time of harvest (ml)	II	193	98	142	123	232
	III	192	97	162	136	224

[a]Hours after treatment.
[b]Nutrient solution was replenished in all remaining flasks 120, 168, and 216 hours after treatment.

Appendix table 13. Total fresh and dry weights (mg) as well as remaining volume of nutrient solution (ml) for ^{14}C-haloxyfop root-uptake experiment in red fescue.

Timing[a]	Rep	Total fresh weight			Total dry weight			Remaining nutrient solution[b]
		Older leaves	Newer leaves	Roots	Older leaves	Newer leaves	Roots	
48	I	60.0	15.1	42.6	10.5	2.7	4.0	43.75
	II	47.5	20.5	34.9	8.1	2.7	2.4	45.25
	III	43.6	20.0	44.4	6.8	3.1	2.8	44.25
96	I	64.8	27.1	46.7	13.7	4.3	3.4	41.0
	II	105.7	42.2	92.7	22.6	7.4	6.6	38.0
	III	75.8	26.6	77.8	15.4	4.5	5.4	40.0[c]
144	I	93.5	46.7	65.2	21.8	6.7	4.6	44.25
	II	113.4	42.6	83.8	30.0	6.6	7.6	42.25
	III	90.8	47.2	90.6	22.5	7.5	7.6	43.5
192	I	111.0	90.0	83.3	32.7	15.2	6.9	45.5
	II	99.8	126.1	111.4	28.6	26.5	9.6	44.5
	III	62.9	100.9	130.0	15.6	20.8	10.4	44.0
240	I	124.3	76.4	104.2	35.7	16.5	8.8	47.0
	II	186.4	144.9	148.5	46.2	30.0	11.3	46.0
	III	251.8	90.0	156.2	67.0	15.3	13.7	45.75

[a]Hours after treatment.
[b]Values represent total volume remaining at time of harvest.
[c]Nutrient solution was replenished in all remaining flasks, 120, 168, and 216 hours after treatment.

Appendix table 14. Total fresh and dry weights (mg) as well as remaining volume of nutrient solution (ml) for ^{14}C-haloxyfop root-uptake experiment in tall fescue.

| Timing[a] | Rep | Total fresh weight | | | Total dry weight | | | Remaining nutrient solution[b] |
		Older leaves	Newer leaves	Roots	Older leaves	Newer leaves	Roots	
48	I	59.3	33.9	45.6	10.5	4.6	3.6	44.75
	II	91.0	41.7	44.7	12.8	4.3	3.3	46.0
	III	95.6	56.7	64.1	15.6	7.0	4.7	44.25
96	I	117.4	113.6	73.2	20.7	13.6	5.6	37.0
	II	101.3	102.5	56.2	18.7	14.7	4.9	39.75
	III	106.5	92.8	72.8	21.8	18.1	7.2	39.25[c]
144	I	261.7	47.8	97.5	50.5	6.9	10.6	41.75
	II	216.9	42.3	80.0	42.5	6.2	8.3	43.0
	III	312.2	114.1	113.5	65.2	17.2	11.6	40.75
192	I	260.0	139.5	91.9	56.2	22.2	8.6	45.25
	II	299.1	76.8	101.6	54.1	10.8	8.3	43.5
	III	161.8	98.4	44.7	42.7	19.0	4.7	42.0
240	I	295.6	178.9	162.0	64.6	26.0	15.3	46.0
	II	297.3	143.3	196.9	66.7	22.9	17.1	45.75
	III	256.9	40.0	84.0	57.1	6.0	7.1	47.25

[a]Hours after treatment.
[b]Values represent total volume remaining at time of harvest.
[c]Nutrient solution was replenished in all remaining flasks, 120, 168, and 216 hours after treatment.

Appendix table 15. Haloxyfop-methyl GR$_{50}$ in tall fescue shoots as
affected by mixture with 2.17 or 4.34 mM dicamba.

| Treatment | Shoot fresh weight | | | | % check |
	I	II	III	AVG	(as AVG)
	------------- mg ------------				
Non-treated check	649	500	443	531	100
21 H	311	227	327	285	54
41 H	165	121	127	138	26
83 H	163	94	95	117	22
166 H	86	46	70	67	13
332 H	67	80	71	73	14
0 H + 2.17 D	483	428	396	436	82
21 H + 2.17 D	600	572	619	597	112
41 H + 2.17 D					
83 H + 2.17 D	259	261	462	327	62
166 H + 2.17 D	150	104	145	133	25
332 H + 2.17 D	99	98	132	110	21
0 H + 4.34 D	482	317	339	379	71
21 H + 4.34 D	577	482	294	451	85
41 H + 4.43 D	505	385	425	438	82
83 H + 4.34 D	334	439	402	392	74
166 H + 434 D	160	344	360	288	54
332 H + 4.34 D	86	125	121	111	21

H = haloxyfop-methyl (from formulated Verdict) as micromolar.
D = dimethyl amine salt of dicamba (from formulated Banvel) as
milimolar.

Appendix table 16. Haloxyfop acid GR_{50} in tall fescue shoots as affected by mixture with 2.17 or 4.34 mM dicamba.

Treatment	Shoot fresh weight						% abs. check
	I	II	III	IV	V	AVG	(as AVG)
-- μM --	---------------- mg --------------						
Extra check	614	711	594	582	---		
0 H	578	751	---	501	455	598.2	100
14 H	468	634	723	565	522	582	97
28 H	351	748	634	576	437	549	92
56 H	250	384	445	497	---	394	66
99.5 H	138	166	129	169	243	169	28
0 H + 2.17 D	594	469	468	553	565	530	89
14 H + 2.17 D	626	715	386	541	503	554	93
28 H + 2.17 D	575	494	636	536	647	578	97
56 H + 2.17 D	500	684	675	537	561	591	99
99.5 H + 2.17 D	---	449	506	609	498	516	86
OH + 4.34 D	569	741	550	514	601	595	99
14 H + 4.34 D	499	626	377	664	454	524	88
28 H + 4.34 D	503	489	471	577	435	495	83
56 H + 4.34 D	490	523	306	703	656	535	89
99.5 H + 4.34 D	448	319	529	429	583	462	77
X-77 check	458	650	700	375	505	538	90

H = haloxyfop acid (technical material, 99.5% purity) as micromolar.
D = dimethylamine salt of dicamba (from formulated Banvel) as milimolar.
--- = missing values.

Appendix table 17. Effect of simultaneous addition of haloxyfop and dicamba acids on ACCase activity.

Dicamba acid μM	^{14}C-malonyl CoA formed (as CPM[a])					
	No haloxyfop acid			20 μM haloxyfop acid		
	Rep I	Rep II	Rep III	Rep I	Rep II	Rep III
0	3007.9	3798.2	3692.9	1513.7	1751.4	1609.7
1	3095.3	3607.6	2912.6	1391.5	1645.6	1628.7
10	2912.3	3723.3	3836.2	1361.6	1663.8	1622.1
100	3037.6	3416.1	3613.1	1459.7	1662.8	1627.1
1000	3181.2	3465.4	3514.3	1429.7	1813.5	1641.1

[a]CPM = ^{14}C counts per minute.

Appendix table 18. Effect of simultaneous addition of haloxyfop acid and the dimethylamine salt of dicamba on ACCase activity.

Dimethylamine salt of dicamba μM	^{14}C-malonyl CoA formed (as CPM)			
	No haloxyfop acid		20 μM haloxyfop acid	
	Rep I	Rep II	Rep I	Rep II
0	2252.3	2152.7	1137.1	1145.9
100	2338.1	2242.2	1175.2	1181.7
320	2213.7	2141.7	1163.5	1202.1
1000	2302.2	2569.8	1124.7	1140.9
3200	2360.9	2105.9	1137.0	1138.5

Appendix table 19. Effect of sequential addition of the dimethylamine salt of dicamba followed by haloxyfop acid on ACCase activity.

Dimethylamine salt of dicamba μM	^{14}C-malonyl CoA formed (as CPM[a])					
	No haloxyfop acid			20 μM haloxyfop acid		
	Rep I	Rep II	Rep III*	Rep I	Rep II	Rep III*
0	2065.7	2224.7	7850.4	1274.1	1271.6	4032.8
100	2159.1	2282.4	8169.8	1295.9	1234.3	4133.8
1000	2040.7	2297.9	8330.0	1198.6	1239.2	4191.0
10000	1712.7	1593.7	8567.8	841.7	839.0	3663.2

*For this replication, younger tissue was used (15-days old tall fescue shoots).

Appendix table 20. Effect of simultaneous addition of haloxyfop acid and the sodium salt of bentazon on ACCase activity.

Sodium salt of bentazon mM	^{14}C-malonyl-CoA formed (as CPM)							
	No haloxyfop				20 uM haloxyfop			
	I	II	III	IV	I	II	III	IV
0	3481.4	2950.6	2227.7	4340.1	1530.1	1275.6	885.1	2265.7
100	3532.5	2815.0	2130.6	4077.2	1503.0	1369.8	819.9	2171.7
320	3455.2	2810.4	2194.9	4191.1	1361.0	1212.0	815.6	2060.0
1000	3229.0	2674.3	2042.5	3766.4	1298.8	1146.9	759.7	1770.5
3200	2591.4	2165.7	1758.8	3211.7	974.3	922.2	712.9	1312.5

Appendix table 21. Effect of sequential addition of the Na-salt of bentazon followed by haloxyfop acid on ACCase activity.[a]

Bentazon mM	^{14}C-malonyl-CoA formed (as CPM)					
	I		II		III	
	No H	20 H[b]	No H	20 H	No H	20 H
0	9313.45	4004	8285.49	4200.4	9390.37	3780
0.1	9381.81	4014.87	8477.08	4396.2	9265.02	3825.8
1	9012.80	3874.07	8476.77	4278.2	9239.72	3812.93
10	5874.53	2328.73	6477	2074.07	5467	2193.2

[a]For this experiment, younger foliar tissue was used to extract ACCase activity (2-week old shoots).
[b]H = haloxyfop as micromolar.

Appendix table 22. Data for determination of haloxyfop concentration inhibiting ACCase activity by 50% (I_{50})

Haloxyfop concentration	ACCase activity					
	Experiment 1			Experiment 2		
	I	II	AVG	I	II	AVG
---- μM -----	--------------- ^{14}C-malonyl-CoA CPM ----------------					
0	2784	2882	2833	4200	3190	3695
0.316	2305	2387	2346	3694	2869	3281
3.16	1767	1779	1773	2628	2008	2318
31.60	1222	1177	1200	1995	1539	1767
316	456	425	441	891	638	765

Appendix table 23. Haloxyfop-methyl GR_{50} in tall fescue shoots as affected by mixture with dicamba.

Treatment	Shoot fresh weight[a]			
	I	II	III	AVG
-- μM --	------------- mg -----------			
X-77 check	1340	1390	1580	1440
Non-treated check	1240	1400	1160	1270
0 H + 543 D	1480	1400	1550	1480
2.76 H[b]	1530	1300	1390	1410
5.52 H	1420	1310	1490	1410
11.04 H	960	1100	870	980
16.58 H	1100	400	610	700
22.08 H	480	510	510	500
33.17 H	350	320	390	350
5.52 H + 543 D[c]	1310	1400	1310	1340
11.04 H + 543 D	1620	1070	1580	1420
22.08 h + 543 D	800	700	1210	900
33.17 h + 543 D	300	520	360	390
44.16 H + 543 D	370	370	380	373

[a]Three plants/replication
[b]H = formulated haloxyfop-methyl.
[c]D = dimethylamine salt of dicamba.

Appendix table 24. Data for GR$_{50}$ of haloxyfop-methyl as affected by mixture with 1085 μM dicamba.

| Treatment | Shoot fresh weight[a] | | | |
	I	II	III	AVG
-- μM --	------------- mg ------------			
X-77 check	1400	1050	1050	1170
Non-treated check	1440	1050	1220	1240
0 H + 1085 D	1340	1150	1230	1240
1.38 H[b]	1260	1370	1120	1250
2.76 H	1120	1060	1240	1140
5.52 H	1270	1320	1210	1270
11.04 H	600	1180	570	780
22.08 H	340	360	420	370
33.17 H	310	340	340	330
44.16 H	220	300	330	280
1.38 H 1085 D[c]	1150	1250	1160	1190
2.76 H + 1085 D	1180	1170	1300	1220
5.52 H + 1085 D	1150	1450	1210	1270
11.04 H + 1085 D	1060	1300	1360	1240
22.08 h + 1085 D	1170	1110	1130	1240
33.17 h + 1085 D	820	620	710	720
44.16 H + 1085 D	200	420	350	320

[a]Three plants/replication.
[b]H = formulated haloxyfop-methyl.
[c]D = dimethylamine salt of dicamba.

Appendix table 25. Haloxyfop-methyl GR$_{50}$ in tall fescue shoots as affected by mixture with dicamba.

Treatment	Shoot fresh weight					AVG
	I	II	III	IV	V	
-- μM --	------------- mg -------------					
X-77 check	533.7	663.4	610.2	525.3	385.7	543.66
Extra non-treated check	577.7	906.2	689.5	477.8	419.2	
Non-treated check	660.2	829.5	497.4	446.7	520.8	602.5
10.35 H[a]	502.1	509.8	693.2	510.3	629.2	568.9
20.70 H	504.8	590.0	612.7	584.8	307.6	519.9
41.40 H	230.2	615.1	493.3	196.2	493.4	405.64
82.80 H	---	169.7	158.0	136.9	231.7	174.07
165.60 H	67.1	128.1	134.6	102.4	143.3	115.1
10.35 H + 271.4 D[b]	337.4	788.7	711.6	564.1	436.4	567.6
20.70 H + 271.4 D	296.9	766.1	685.8	651.3	554.5	590.0
41.40 H + 271.4 D	353.7	568.7	302.4	414.2	515.9	430.98
82.80 H + 271.4 D	235.0	359.4	476.5	276.6	---	336.9
165.60 H + 271.4 D	95.3	215.2	143.9	150.5	131.8	147.34
10.35 H + 542.8 D	448.0	560.0	632.2	576.4	434.6	530.2
20.70 H + 542.8 D	517.6	651.2	605.9	567.4	503.0	569.02
41.40 H + 542.8 D	589.6	630.8	658.4	562.0	388.9	565.90
82.80 H + 542.8 D	245.6	299.2	193.8	304.2	213.7	251.3
165.60 H + 542.8 D	160.3	168.3	150.3	165.8	159.8	160.9

--- = missing values.
[a]H = formulated haloxyfop-methyl.
[b]D = dimethylamione salt of dicamba.

Appendix table 26. Antagonism of the acid forms of haloxyfop and dicamba in shoots of tall fescue.

Treatment	Shoot fresh weight				% check
	I	II	III	AVG	(as AVG)
-- μM --	------------- mg ------------				
Experiment 1: 2 plants/rep					
Non-treated check	760	650	630	680	100
18 H-m	530	400	330	420	62
18 H-m 2000 D-a	680	710	520	637	94
2000 D-a	710	710	600	673	99
70 H-a	450	580	380	470	69
70 H-a + 1000 D-s	770	580	430	593	87
70 H-a + 2000 D-a	610	680	620	637	94
1000 D-s	690	720	580	663	98
Experiment 2: 2 plants/rep					
Non-treated check	600	570	630	600	100
18 H-m	240	470	400	370	61.7
18 H-m + 2000 D-a	670	460	720	617	102.8
2000 D-a	550	630	500	560	93.3
70 H-a	420	400	400	407	67.8
70 H-a + 1000 D-s	---	550	490	515*	86.0
70 H-a + 2000 D-a	550	500	470	506.7	84.4
1000 D-s	550	620	590	586.7	97.8

--- = missing value.
*Value of Rep I estimated as missing.
H-m = haloxyfop-methyl.
H-a = haloxyfop-acid.
D-s = dimethylamine salt of dicamba.
D-a = dicamba acid.

Appendix table 27. Effect of herbicide placement on the antagonism of dicamba on haloxyfop activity.

Placement/ treatment	Shoot fresh weight			
	I	II	III	AVG
-- μM --		--------- % of control ---------		
I. Both herbicides in nutrient solution				
0 H[a]+ 0.01 D[b]	105	85	92	94
0 H + 0.1 D	130	77	73	93
0 H + 1.0 D	95	79	69	81
0.1 H + 0 D	46	67	48	54
0.1 H + 0.01 D	93	101	55	83
0.1 H + 0.1 D	66	77	23	55
0.1 H + 1.0 D	28	37	25	30
II. Haloxyfop in nutrient solution; dicamba on shoots				
0 H; 1085 D-s[c]	75	112	97	95
0 H; 2170 D-s	121	103	101	108
0.1 H; 0 D-s	64	---	60	62
0.1 H; 1085 D-s	70	65	62	66
0.1 H; 2170 D-s	37	71	66	58

--- = missing value
[a]H = haloxyfop acid.
[b]D = dicamba acid.
[c]D-s = dimethylamine salt of dicamba.

Appendix table 28.   Antagonism of haloxyfop-methyl activity by dicamba as affected by the days after treatment.   Experiment 1.

Treatments	Shoot fresh weight[a]								AVG
	I	II	III	IV	V	VI	VII	VIII	
-- μM --	-- mg --								
Non-treated check (10 DAT[b])	210	200	200	280	320	260	220	200	236
18 H (10 DAT)	100	150	130	140	140	120			130
18 H + 1085 D (10 DAT)	200	420	210	220	210	200			243
Non-treated check (20 DAT)	542.7	675.2	914.6	1291.3	754.7	514.9	293.7	341.8	666
18 H (20 DAT)	131.0	---	81.4	141.0	456.5	137.7			190
18 H + 1085 D (20 DAT)	582.3	---	294.3	685.0	1113.6	520.0			639

[a]One plant/replication.
[b]DAT = days after treatment.
8 reps used for checks.
6 reps used for treatments.
--- = missing values.

Appendix table 29. Antagonism of haloxyfop-methyl activity by dicamba 20 days after treatment, measuring leaf and tiller number, and root fresh weight. Experiment 1.

Treatments	Replication								AVG
	I	II	III	IV	V	VI	VII	VIII	

-- μM --

Leaf number:

Treatments	I	II	III	IV	V	VI	VII	VIII	AVG
Non-treated check	10	10	10	10	7	10	4	5	8.3
18 H	3	---	3	3	10	3			4.4
18 H + 1085 D	10	---	7	10	10	10			9.4

Tiller number:

Treatments	I	II	III	IV	V	VI	VII	VIII	AVG
Non-treated check	3	3	3	3	2	3	1	1	2.4
18 H	1	---	1	1	3	1			1.4
18 H + 1085 D	3	---	2	3	3	3			2.8

Root fresh weight:
 -- mg --

Treatments	I	II	III	IV	V	VI	VII	VIII	AVG
Non treated check	784.5	888.6	1325.6	1157.9	1452.1	886.0	887.0	674.1	1007
18 H	646.0	---	448.5	894.5	872.3	520.0			676
18 H + 1085 D	1279.5	---	684.8	893.5	1680.0	1173.6			1142

8 reps used for checks.   H = formulated haloxyfop-methyl.
6 reps used for treatments.   D = dimethylamine salt of dicamba
--- = missing values.

Appendix table 30. Antagonism of haloxyfop-methyl activity by dicamba as affected by the days after treatment. Experiment 2.

Treatments	Shoot fresh weight[a]								AVG
	I	II	III	IV	V	VI	VII	VIII	
-- μM --	---------------------------------- mg ----------------------------------								
Non-treated check (10 DAT[b])	313.3	255.8	491.0	406.1	340.0	302.2	343.5	285.3	342
18 H (10 DAT)	115.0	113.5	291.3	306.1	108.3				187
18 H + 1085 D (10 DAT)	248.0	280.0	192.2	371.0	---				273
Non-treated check (20 DAT)	1400	1010	1400	1000	1410	1620	1110	1140	1261
18 H (20 DAT)	170	450	70.0	910	930				506
18 H + 1085 D (20 DAT)	1350	1130	1320	1120	1110				1206

[a]One plant/replication.
[b]DAT = days after treatment.
8 reps used for checks.
5 reps used for treatments.
--- = missing value.

Appendix table 31. Antagonism of haloxyfop-methyl activity by dicamba, 20 days after application, as measured by leaf and tiller number. Experiment 2.

| Treatments | Replication | | | | | | | | AVG |
	I	II	III	IV	V	VI	VII	VIII	
-- μM --									
Leaf number:									
Non-treated check	13	11	14	10	11	12	11	11	12
18 H	3	10	3	12	7				7
18 H + 1085 D	16	11	14	10	10				12
Tiller number:									
Non-treated check	4	3	5	3	3	4	3	3	4
18 H	1	3	1	4	3				2
18 H + 1085 D	6	3	5	3	3				4

8 reps used for checks.   H = formulated haloxyfop-methyl.
5 reps used for treatments.   D = dimethylamine salt of dicamba.

Appendix table 32. Effect of sequential dicamba and haloxyfop-methyl application on the antagonism. Experiment 1.

| Treatment | Shoot fresh weight[a] | | | | % check (as AVG) |
	I	II	III	AVG	
-- μM --	---------- mg ----------				
X-77 check	1330	1320	1340	1330	87.5
Non-treated check	1560	1500	1510	1520	100.0
0 H + 1085 D	1690	1770	1790	1750	115.0
18 H	540	520	640	570	37.5
18 H + 1085 D (mixed together)	1610	1170	1250	1340	88.0
18 H + 1085 D (D 3 h later)	950	1020	1020	1000	66.0
18 H + 1085 D (D 12 h later)	760	890	860	840	55
18 H + dw (dw 3 hr later)	370	300	470	380	25
1085 D + 18 H (H 3 hr later)	880	880	1130	960	63
1085 D + 18 H (H 12 h later)	1720	1010	1330	1350	89
1085 D + dw (dw 12 hr later)	1380	1770	1680	1610	106

[a]Three plants/replication.
h = hours.
dw = distilled water with 0.025% v/v X-77.
H = formulated haloxyfop-methyl.
D = dimethylamine salt of dicamba.

Appendix table 33. Effect of sequential dicamba and haloxyfop-methyl application on the antagonism. Experiment 2.

Treatment	Foliar fresh weight[a]						AVG	% check (as AVG)
	I	II	III	IV	V	VI		
-- μM --					mg			
X-77 check	363.3	272.5	311.2	377.7	380.0	166.1	312	
Non-treated check	320.5	277.4	306.8	311.7	241.8	340.0	300	100
0 H + 1085 D	334.7	405.1	296.1	263.3	326.1	326.9	325	104
18 H	250.8	127.9	307.1	163.6	127.7	165.4	190.42	62
18 H + 1085 D (mixed together)	246.7	357.0	---	282.3	380.9	271.8	307.74	99.43
18 H + 1085 D (D 3 h later)	216.8	340.0	260.0	316.2	290.0	226.1	275	88
18 H + 1085 D (D 12 h later)	109.7	195.1	224.1	290.0	166.1	170.0	193	62
18 H + dw (dw 3 h later)	130.0	150.0	115.2	70.0	68.8	111.7	108	35
1085 D + 18 H (H 3 h later)	98.6	393.9	175.8	247.9	268.0	504.6	281	90
1085 D + 18 H (H 12 h later)	236.1	366.5	347.8	220.0	292.8	300.0	294	94
1085 D + dw (dw 3 h later)	340.0	335.6	258.2	270.0	313.7	313.3	305	98

[a]One plant/replication.
h = hours.
dw = distilled water.
H = formulated haloxyfop-methyl.
D = dimethylamine salt of dicamba.

Appendix table 34. Tall fescue shoot regrowth 20 days after treatment.

Treatment	Shoot fresh weight				AVG
	I	II	III	IV	
-- μM --	-------------------- mg --------------------				
Experiment one: 3 plants/rep					
Non-treated check	1920	1170	1000	1830	1480
33 H[a]	0	0	0	0	0
33 H + 1000 D[b]	0	800	830	880	628
Experiment two: 2 plants/rep; 3 reps					
Non-treated check	460	610	670		580
33 H	240	0	0		80
33 H + 1000 D	400	590	410		467

[a]H = formulated haloxyfop-methyl.
[b]D = dimethylamine salt of dicamba.

Appendix table 35. Effect of dicamba on haloxyfop activity in tall fescue plants in which mature leaves were removed immediately after dipping entire shoots in herbicide solutions.

| Treatment | Shoot fresh weight | | | | % check |
	I	II	III	AVG	(as AVG)
-- μM --			mg		
Experiment 1: 2 plants/rep					
Non-treated check	800	1170	1140	1037	100
11 H	1170	410	1230	937	90
16.5 H	1100	710	800	870	84
22 H	440	870	720	677	65
33 H	370	60	50	160	15
0 H + 1085 D	1060	900	1440	1133	100
16.5 H + 1085 D	1260	920	850	1010	97
22 H + 1085 D	840	1190	830	953	92
33 H + 1085 D	830	1170	750	917	88
44 H + 1085 D	770	620	650	680	66
Experiment 2: 1 plant/rep					
Non-treated check	38*	740	410	575	100
11 H	530	540	770	613	107
16.5 H	500	410	510	473	82
22 H	550	400	770	573	100
33 H	20	160	420	200	35
0 H + 1085 D	440	610	540	530	92
16.5 H + 1085 D	430	510	760	567	99
22 H + 1085 D	480	560	60	367	64
33 H + 1085 D	300	710	310	440	77
44 H + 1085 D	320	330	290	313	54

*Declared an outlier.

Appendix table 36. Effect of haloxyfop-methyl and/or dicamba on fresh weight of tall fescue mature leaf segments.

Treatments	Segment fresh weight					AVG
	I	II	III	IV	V	
-- μM --	---------------- mg ---------------					
Experiment 1:*						
Non-treated check	22.7	26.3	35.0	30.0	26.5	28
9 H[a]	25.6	30.0	28.9	28.8	26.8	28
13.5 H	26.1	27.0	36.9	34.9	20.9	29
18 H	22.2	28.6	42.0	28.7	22.2	29
36 H	20.4	28.1	30.0	36.8	30.0	29
0 H + 1085 D[b]	23.7	24.1	38.4	33.6	24.4	29
9 H + 1085	28.9	36.0	34.7	29.2	22.0	30
13.5 H + 1085 D	22.8	28.5	27.1	32.8	20.0	26
18 H + 1085 D	27.2	33.8	25.9	30.0	26.9	29
36 H + 1085 D	24.0	35.0	36.5	37.6	40.0	35
Experiment 2:**						
Non-treated check	47.6	36.1	38.9	47.4	40.0	42
9 H	40.0	45.2	41.6	46.4	48.4	44
13.5 H	37.9	42.8	43.8	38.4	44.6	42
18 H	40.0	57.9	45.9	43.9	55.0	49
36 H	38.0	44.4	41.4	43.3	40.0	41
0 H + 1085 D	46.3	41.1	47.4	34.9	37.7	41
9 H + 1085 D	46.8	27.3	52.4	38.5	44.7	42
13.5 H + 1085 D	42.6	36.1	41.5	45.6	55.9	44
18 H + 1085 D	36.3	37.8	53.7	46.5	45.7	44
36 H + 1085 D	35.3	51.4	40.0	36.9	34.6	40

*Segments of 10-12 cm length used.
**Segments of 14-15 cm length used.
Each segment transpired an average of 92 μl/day; with no differences among treatments.
[a]H = formulated haloxyfop-methyl.
[b]D = dimethylamine salt of dicamba.

Appendix table 37. Effect of dicamba on pH of herbicide solutions.

Treatment	pH				AVG
	I	II	III	IV	
Deionized water	5	5	5.1	5.1	5.05
0.05% v/v X-77	5.2	5.1	5.0	4.9	5.05
0.1% v/v X-77	4.8	4.7	4.8	4.8	4.8
18 H	5.2	5	4.9	5	5.03
1085 D	5.1	5	5.05	5	5.04
18 H + 1085 D	5	5	5	4.95	4.99

H = formulated haloxyfop-methyl.
D = dimethylamine salt of dicamba.

Appendix table 38. Radioactivity found at Rf equivalent to that of ^{14}C-haloxyfop-methyl standard for aliquots taken from herbicide solutions.

	Radioactivity found at Rf=0.87[a]	
	% of total detected	
Treatment	Plate 1	Plate 2
FHM[b] + ^{14}C-haloxyfop-methyl	97	95
FHM + DMA[c] dicamba + ^{14}C-haloxyfop-methyl	94	94

[a]Rf equivalent to that of standard ^{14}C-haloxyfop-methyl.
[b]FHM = formulated haloxyfop-methyl.
[c]DMA = dimethylamine.

Appendix table 39. Herbicidal solution retained by shoots of tall fescue. Experiment 1.

Treatment	Rep	Shoots dry weight	CPM retained plant	Estimated volume retained/plant[a]
		---- mg ---		------- μl -----
A) 18 H[b]	I	10.7	728	20
	II	7.9	572	15
	III	10.5	820	22
	IV	8.3	724	19
	V	7.0	502	13
	VI	10.0	636	17
	VII	6.6	345	9
	VIII	10.9	625	17
	AVG	9	619	17
B) 18 H + 1085 D[c]	I	8.1	477	13
	II	6.4	485	13
	III	8.5	520	14
	IV	8.2	546	15
	V	8.1	691	19
	VI	8.5	618	17
	VII	9.0	735	20
	VIII	8.9	663	18
	IX	11.9	775	21
	AVG	8.62	612	17

[a]Plants initially were dipped in 50-ml herbicidal solutions of treatments A and B each containing 1,864,900 CPM of ^{14}C-haloxyfop-methyl. Ten min. later, shoots were cut and washed in a methanol:water solution (80:20 v/v) and volume retained was estimated from ^{14}C-haloxyfop counts recovered.
[b]H = formulated haloxyfop-methyl.
[c]D = dimethylamine salt of dicamba.

Appendix table 40. Herbicidal solution retained by shoots of tall
fescue. Experiment 2.

Treatment	Rep	Shoots dry weight	CPM retained plant	Estimated volume retained/plant[a]
		---- mg ---		------- μl -----
A) 18 H[b]	I	8.9	601	16
	II	11.9	849	21
	III	7.9	500	13
	IV	11.4	959	26
	V	11.7	953	26
	VI	11.1	704	19
	VII	11.2	793	21
	VIII	8.4	510	14
	AVG	10	734	20
B) 18 H + 1085 D[c]	I	8.0	619	17
	II	10.8	892	24
	III	10.3	672	18
	IV	11.2	864	23
	V	11.5	853	23
	VI	13.3	1114	30
	VII	12.6	763	20
	VIII	9.1	640	17
	AVG	11	802	22

[a]Plants originally were dipped in 50-ml herbicidal solutions of
treatments A and B each containing 1,864,900 CPM of ^{14}C-haloxyfop-
methyl. Ten min later, shoots were cut and washed in a methanol:water
solution (80:20 v/v) and volume retained was estimated from
^{14}C-haloxyfop counts recovered.
[b]H = formulated haloxyfop-methyl.
[c]D = dimethylamine salt of dicamba.

Appendix table 41. Stress-ethylene production by shoots of tall fescue treated with haloxyfop-methyl, DMA-dicamba, or both and enclosed in glass tubes.[a] Experiment 1.

Stress-ethylene	Rep	Treatment[b]				
		1	2	3	4	5
		------- pmole/plant section[c]/h --------				
Stress-ethylene production 24 HATE[d]	I	0.77	0.77	4.28	3.88	4.98
	II	0	0.73	3.31	2.01	4.95
	III	0.83	0.70	2.14	4.31	1.87
	IV	0.47	1.30	2.34	5.11	1.64
	V	0.37	1.27	4.28	2.61	4.48
	AVG	0.49	0.95	3.27	3.58	3.58
Stress-ethylene production 48 HATE	I	0.48	0.53	2.6	2.24	3.41
	II	0.35	0.52	1.75	0.99	3.02
	III	0.62	0.99	1.55	2.87	1.00
	IV	0.60	0.55	1.27	2.39	1.04
	V	0.23	0.60	2.29	1.79	2.34
	AVG	0.46	0.64	1.9	2.06	2.16
Stress-ethylene production 72 HATE	I	0.48	0.31	1.76	1.49	2.40
	II	0.20	0.37	1.20	0.73	1.98
	III	0.33	0.71	0.96	2.08	0.69
	IV	0.39	0.38	0.73	1.47	0.98
	V	0.12	0.48	1.97	1.45	1.51
	AVG	0.30	0.45	1.32	1.44	1.51

[a]Total glass tube volume = 20 ml.
[b]Treatments: 1 = Non-treated check.
2 = 18 μM haloxyfop-methyl.
3 = 1085 μM DMA-dicamba.
4 = 18 μM haloxyfop-methyl + 1085 uM DMA-dicamba.
5 = 1085 μM DMA-dicamba in Verdict blank.
[c]Two newer leaves joined by part of the pseudostem.
[d]HATE = hours after treatment and enclosement in glass tubes.

Appendix table 42. Stress-ethylene production by shoots of tall fescue treated with haloxyfop-methyl, DMA-dicamba, or both and enclosed in glass tubes.[a] Experiment 2.

| Stress-ethylene | Rep | Treatment[b] | | | | |
		1	2	3	4	5
		-------- pmole/plant section[c]/h --------				
Stress-ethylene	I	0	0.6	2.5	4.6	3.2
production 24 HATE[d]	II	0.9	0.4	4.2	1.7	4.4
	III	0	0	2.6	3.2	1.9
	IV	0	0	4.5	6.3*	3.5
	V	0	0.5	3.3	3.2	1.8
	AVG	0.2	0.3	3.4	3.2	3.0
Stress-ethylene	I	0	0.5	1.6	3.8	2.0
production 48 HATE	II	0.2	0	3.7	1.4	2.7
	III	0.2	0.2	2.0	2.0	1.4
	IV	0	0.4	2.6	6.2*	2.4
	V	0	0.2	2.3	2.0	1.0
	AVG	0.08	0.3	2.4	2.3	1.9
Stress-ethylene	I	0.1	0.2	1.2	2.6	1.2
production 72 HATE	II	0.3	0.4	2.5	1.1	2.1
	III	0	0.2	2.5	1.1	1.1
	IV	0	0.4	1.7	5.0*	1.6
	V	0.1	0.2	1.7	1.5	0.5
	AVG	0.1	0.3	1.7	1.6	1.3

[a]Total glass tube volume = 20 ml.
[b]Treatments: 1 = Non-treated check.
　　　　　　　2 = 18 μM haloxyfop-methyl.
　　　　　　　3 = 1085 μM DMA-dicamba.
　　　　　　　4 = 18 μM haloxyfop-methyl + 1085 uM DMA-dicamba.
　　　　　　　5 = 1085 μM DMA-dicamba in Verdict blank.
[c]Two newer leaves joined by part of the pseudostem.
[d]HATE = hours after treatment and enclosement in glass tubes.
* = Declared as outliers.

Appendix Table 43. Dry weight of plant sections used to determine stress-ethylene production. Experiment 1.

| Treatment | Dry weight[a] | | | | | |
	I	II	III	IV	V	AVG
---- μM -----						
Non-treated check	8.7	6.9	6.6	5.7	5.9	6.8
18 μM H	5.9	8.7	8.5	6.4	7.3	7.4
1085 μM D	6.7	5.3	5.5	6.4	7.9	6.4
18 μM H + 1085 uM D	6.2	6.3	4.9	7.4	8.8	6.7
1085 μM D in Verdict blank	5.8	8.5	8.2	5.9	6.5	7.0

H = formulated haloxyfop-methyl.
D = dimethylamine salt of dicamba.

Appendix Table 44. Dry weight of plant sections used to determine stress-ethylene production. Experiment 2.

| Treatment | Dry weight[a] | | | | | |
	I	II	III	IV	V	AVG
----- μM -----						
Non-treated check	7.9	7.8	8.1	8.2	5.9	7.6
18 μM H	6.7	8.3	8.0	6.5	4.6	6.8
1085 μM D	7.1	9.4	9.1	8.9	8.7	8.6
18 μM H + 1085 μM D	7.7	7.5	5.9	9.8	5.3	7.2
1085 μM D in Verdict blank	9.7	7.2	6.4	10.5	8.2	8.4

H = formulated haloxyfop-methyl.
D = dimethylamine salt of dicamba.

Appendix table 45. Shoot fresh weight of plants kept in sealed glass
tubes with ethylene gas for 48 h and then grown outside tubes for 8
days.

| Treatment | Shoot fresh weight | | | | | % non-treated check |
	I	II	III	IV	AVG	
	--------- (mg) --------					
Experiment 1:						
Absolute check	320	360	380	280	335	100
18 μM H	50	100	110	90	87.5	26
18 μM H + 7 pmol/ml ethylene[a]	80	100	130	120	107.5	32
18 μM H[b] + 14 pmol/ml ethylene	60	140	120	150	117.5	35
18 μM H + 1085 μM D[c]	260	300	370	230	290	87
Experiment 2:						
Absolute check	190	220	240	260	228	100
18 μM H	80	110	90	120	100	44
18 μM H + 7 pmol/ml ethylene	100	90	70	90	88	39
18 μM H[b] + 14 pmol/ml ethylene	60	100	110	80	88	39
18 μM H + 1085 μM D	380	180	100	180	210	92

[a]Ethylene concentration was achieved by injecting the appropriate
amount of pure gas through a serum stopper once plants had been
treated and enclosed in glass tubes.
[b]H = formulated haloxyfop-methyl.
[c]D = dimethylamine salt of dicamba.

Appendix table 46. ^{14}C-haloxyfop-methyl absorption and movement in tall fescue as influenced by dicamba. Experiment 1.

Plant part	Treatment 1[a]						Treatment 2[b]					
	I	II	III	IV	V	AVG	I	II	III	IV	V	AVG
Experiment 1 (48 HAT[c])						(CPM)						
Outside treated leaf	7407	10377	5865	7375	6985	7602	10969	18247	7391	7620	12241	11294
Treated leaf	34665	33178	38188	29507	35337	34175	39408	33297	45176	47958	30891	39346
Leaf above treated leaf	375	139	140	289	451	279	216	119	276	315	301	245
Leaf below treated leaf	73	54	54	110	67	72	36	29	45	58	37	41
Pseudostem (leaf sheaths + meristem)	2872	2541	2520	2999	4412	3069	1652	814	1959	1210	1522	1431
Roots	376	314	364	245	450	350	84	172	221	258	382	223
Total inside plant	38361	36226	41266	33150	40717	37944	41396	34431	47677	49799	33133	41287
Total counts	45768	46603	47131	40525	47702	45546	52365	52678	55068	57419	45374	52581
% recovery[d]	50	51	51	44	52	50	57	57	60	62	49	57

[a]Treatment 1 = 18 μM Formulated haloxyfop-methyl; ^{14}C-haloxyfop-methyl on adaxial surface of second leaf.
[b]Treatment 2 = 18 μM formulated haloxyfop-methyl + 1085 μM dicamba (dimethylamine salt); ^{14}C-haloxyfop-methyl on adaxial surface of second leaf.
[c]HAT = Hours after treatment.
[d] = 92000 CPM/plant were originally added.

Appendix table 47. Fresh weight of plant parts used to assess ^{14}C-haloxyfop-methyl uptake and translocation. Experiment 1.

Plant part	Treatment 1[a]						Treatment 2[b]					
	I	II	III	IV	V	AVG	I	II	III	IV	V	AVG
Experiment 1 (48 HAT[c])	--- (mg) -------------------------------------											
Treated leaf	42.4	43.8	44.6	30.0	40.7	40.3	27.0	50.0	44.6	47.6	32.9	40.4
Leaf above treated leaf	16.4	17.1	8.3	15.8	27.1	16.9	17.7	14.1	20.0	12.5	16.1	16.1
Leaf below treated leaf	9.2	10.5	10.7	6.4	7.0	8.8	5.4	10.0	9.2	8.3	6.6	7.9
Pseudostem (leaf sheaths + meristem)	32.7	30.9	28.6	35.7	36.9	33.0	28.0	32.5	42.0	27.0	28.1	31.5
Roots	18.3	18.3	18.4	9.1	15.7	16.0	6.9	19.3	12.3	26.6	15.2	16.1

[a]Treatment 1 = 18 μM Formulated haloxyfop-methyl; ^{14}C-haloxyfop-methyl on adaxial surface of second leaf.
[b]Treatment 2 = 18 μM formulated haloxyfop-methyl + 1085 μM dicamba (dimethylamine salt); ^{14}C-haloxyfop-methyl on adaxial surface of second leaf.

Appendix table 48. ^{14} -C-haloxyfop-methyl absorption and movement in tall fescue as influenced by DMA-dicamba. Experiment 2.

Plant part	I	II	III	IV	V	VI	AVG

------------------------------ (CPM) ---------------------

48 HAT[a]

Treatment 1: 18 μM haloxyfop-methyl

	I	II	III	IV	V	VI	AVG
Outside treated leaf	7261	11209	6321	5027	7034	4471	6887
Treated leaf	45457	38924	38522	46033	42552	47403	43149
Leaf above treated leaf	229	327	253	1004	574	1451*	477
Leaf below treated leaf	58	39	46	56	34	48	47
Pseudostem (Leaf sheaths + meristem)	2258	4216	3313	4146	2588	6256	3796
Roots	821	416	307	302	267	343	409
Total inside plant	48823	43922	42441	51541	46015	54050	47799
TOTALS	56084	55131	48762	56568	53049	59021	54769
% recovery [b]	58	57	51	59	55	61	57

Treatment 2: 18 μM haloxyfop-methyl + 1085 μM DMA-dicamba

	I	II	III	IV	V	VI	AVG
Outside treated leaf	6747	8491	9824	10656	8955	5344	8336
Treated leaf	42999	54828	38939	40196	47632	43457	44675
Leaf above treated leaf	199	176	204	1710*	892	208	336
Leaf below treated leaf	22	22	31	58	56	66	43
Pseudostem (Leaf sheaths + meristem)	1133	1264	1060	1924	2994	1880	1709
Roots	82	104	118	103	112	152	112
Total inside plant	44435	56394	40352	42281	51686	45763	46819
TOTALS	51179	64885	50176	53237	60641	51107	55204
% recovery	53	68	52	55	63	53	57

[a]HAT = hours after treatment.
[b] = 96000 CPM/plant were added on top of second leaf.
*Declared outliers.

Appendix table 49. ^{14}C-haloxyfop-methyl absorption and movement in tall fescue as influenced by DMA-dicamba. Experiment 3.

Plant part	I	II	III	IV	V	VI	AVG

-------------------------- (CPM) ---------------------

96 HAT[a]

Treatment 1: 18 μM haloxyfop-methyl

Plant part	I	II	III	IV	V	VI	AVG
Outside treated leaf	2488	2524	2956	2668	3168	2884	2781
Treated leaf	35751	51055	55807	47085	35061	59523	47380
Leaf above treated leaf	239	1874*	234	596	240	144	291
Leaf below treated leaf	64	117	82	61	45	29	66
Pseudostem (Leaf sheaths + meristem)	4713	6601	2635	5260	4038	2579	4304
Roots	628	405	472	341	308	157	385
Total inside plant	41395	58178	59230	53343	39692	62432	52378
TOTALS	43883	60702	61714	56011	42860	65316	55081
% recovery [b]	46	63	64	58	45	68	57

Treatment 2: 18 μM haloxyfop-methyl + 1085 μM DMA-dicamba

Plant part	I	II	III	IV	V	VI	AVG
Outside treated leaf	3424	3208	3344	2776	5108	3204	3511
Treated leaf	51876	52002	50988	57616	39218	37515	48203
Leaf above treated leaf	235	131	182	443	187	257	239
Leaf below treated leaf	50	37	0*	65	18	32	40
Pseudostem (Leaf sheaths + meristem)	1389	1679	1295	2742	1952	1102	1693
Roots	140	235	112	235	141	246	185
Total inside plant	53690	54084	52577	61101	41516	39152	50353
TOTALS	57114	57292	55921	63877	46624	42356	53864
% recovery	59	60	58	67	47	44	56

[a]HAT = hours after treatment.
[b] = 96000 CPM/plant were added on top of second leaf.
*Declared outliers.

Appendix table 50. Fresh weight of plant parts used to assess ^{14}C-haloxyfop-methyl uptake and translocation. Experiments 2 and 3.

Plant part	Treatment 1[a]						Treatment 2[b]					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
	--- (mg) --											
Experiment 2 (48 HAT[c]):												
Treated leaf	51.8	33.0	37.7	30.0	27.9	39.3	38.3	39.6	58.3	35.2	37.5	42.7
Leaf above treated leaf	17.6	14.2	17.7	11.3	30.8	15.2	7.1	9.4	13.0	8.8	20.0	27.1
Leaf below treated leaf	10.0	7.0	9.2	7.6	6.1	8.7	8.2	9.4	11.7	10.0	11.4	12.4
Pseudostems (Leaf sheaths + meristem)	25.1	37.8	41.9	30.0	35.0	46.2	32.6	36.9	44.3	36.7	44.9	43.1
Roots	19.2	12.3	10.0	11.1	12.6	15.6	11.5	10.0	10.9	8.4	12.3	17.8
Experiment 3 (96 HAT):												
Treated leaf	43.1	35.8	52.5	34.0	41.5	38.6	42.2	57.2	34.3	48.0	28.5	52.5
Leaf above treated leaf	16.6	5.6	13.2	10.0	8.1	6.3	12.9	7.7	11.1	6.7	5.8	21.0
Leaf below treated leaf	10.0	9.1	14.6	8.0	10.0	7.7	8.6	11.4	7.4	10.0	6.4	10.3
Pseudostem (Leaf sheaths + meristem)	45.7	30.6	42.6	45.5	40.0	27.6	39.2	47.2	37.2	37.4	31.8	52.6
Roots	19.3	8.0	25.6	18.0	21.8	14.2	15.7	20.0	15.4	20.5	13.3	26.9

[a]Treatment 1 = plants dipped in a 18 μM haloxyfop-methyl solution before adding ^{14}C-haloxyfop-methyl on top of second leaf.
[b]Treatment 2 = plants dipped in a 18 μM haloxyfop-methyl + 1085 μM DMA-dicamba solution before adding ^{14}C-haloxyfop-methyl on top of second leaf.
[c]HAT = hours after treatment.
Note: Plants for 96 HAT were taken from a batch 2 days younger than those for 48 HAT.

Appendix table 51. ^{14}C-haloxyfop-methyl absorption and movement in tall fescue as influenced by dicamba. Experiments 4 and 5.

Plant part	Treatment 1[a]							Treatment 2[b]						
	I	II	III	IV	V	VI	AVG	I	II	III	IV	V	VI	AVG

-- (CPM) ------------------------------------

Experiment 4 (48 HAT[c])

Plant part	I	II	III	IV	V	VI	AVG	I	II	III	IV	V	VI	AVG
Outside treated leaf	3963	3471	10145	6260	5899	5013	5792	---	6333	4900	9246	9120	5652	8268
Treated leaf	62349	54978	30786	32400	47725	29623	42977	27584	33487	34036	33013	32918	50053	35182
Leaf above treated leaf	335	310	379	254	398	331	335	99	402	237	472	151	592	326
Leaf below treated leaf	62	84	88	64	51	52	67	54	2749*	58	167	48	72	80
Pseudostem (Leaf sheaths + meristem)	2647	4390	2778	2325	3284	2647	3012	1121	2497	2094	1843	1437	2629	1937
Roots	358	503	560	619	698	442	530	195	316	395	428	190	297	304
Total inside plant	65751	60265	34591	35662	52156	33095	46920	29053	36702	36820	35923	34744	53643	37814
Total counts	69714	63736	44736	41922	58055	38108	52712	43412	43035	41720	45169	43864	59295	46083
% recovery[d]	70	64	45	42	58	38	53	43	43	42	45	44	59	46

Appendix table 51 continued.

Plant part	Treatment 1[a]							Treatment 2[b]						
	I	II	III	IV	V	VI	AVG	I	II	III	IV	V	VI	AVG

-- (CPM) --

Experiment 5 (96 HAT):

Plant part	I	II	III	IV	V	VI	AVG	I	II	III	IV	V	VI	AVG
Outside treated leaf	3509	2778	3501	3089	3041	4276	3366	3981	3347	3487	3333	5527	4472	4025
Treated leaf	27720	43066	29071	49021	37697	39221	37633	47646	43644	42540	42426	39301	26795	40392
Leaf above treated leaf	679	332	326	186	414	405	390	300	165	466	144	318	223	270
Leaf below treated leaf	80	99	88	115	98	71	92	86	64	115	40	73	59	73
Pseudostem (Leaf sheaths + meristem)	3418	4138	3643	1972	3040	2318	3088	1612	820	2856	1546	1809	1261	1651
Roots	623	560	892	452	476	596	600	358	237	418	266	564	277	353
Total inside plant	32520	48195	34020	51796	41725	42611	41803	50002	44930	46395	44422	42065	28615	42739
Total counts	36029	50973	37521	54835	44766	46887	45169	53983	48277	49882	47755	47592	33087	46763
% recovery	36	51	38	55	45	47	45	54	48	50	48	48	33	47

[a]Treatment 1 = plants dipped in X-77 (0.05% v/v) before adding ^{14}C-haloxyfop-methyl on second leaf.
[b]Treatment 2 = plants dipped in DMA-dicamba (1085 μM), containing 0.05% v/v X-77 before adding ^{14}C-haloxyfop-methyl on second leaf.
[c]HAT = hours after treatment.
[d] = 100,000 CPM/plant were added on top of second leaf.
*Declared outliers
--- = missing value (malfunction of sample oxidizer).

Appendix table 52. Fresh weight of plant parts used to assess ^{14}C-haloxyfop-methyl uptake and translocation. Experiments 4 and 5.

Plant part	Treatment 1[a]							Treatment 2[b]						
	I	II	III	IV	V	VI	AVG	I	II	III	IV	V	VI	AVG
							(mg)							
Experiment 4 (48 HAT[c]):														
Treated leaf	33.8	26	30.5	33.7	27.7	32.2	31	30.4	43.3	33.8	42.2	30	29.9	35
Leaf above treated leaf	9.3	7.1	11.0	20.7	13.4	11.3	12	17.1	7.6	17.5	16.1	12.7	8.5	13
Leaf below treated leaf	10.6	6.9	7.6	14.6	6.0	6.9	9	9.0	13.6	10	14.4	7.9	8.0	10
Pseudostem (Leaf sheaths + meristem)	27.5	27.3	24.2	35.4	32.0	30.0	29	34.2	38.9	28.1	31.7	25.3	27.2	31
Roots	27.6	22.0	24.6	31.4	26.5	24.4	26	25.7	29.3	26.2	26.3	17.8	33.1	26
Experiment 5 (96 HAT)														
Treated leaf	34.2	30.0	28.8	40.3	34.0	30.7	33	28.7	50.7	28.6	34.3	42.3	22.8	35
Leaf above treated leaf	14.7	10.0	11.6	6.4	11.0	22.7	13	8.4	8.5	15.3	6.8	20.7	14.4	12
Leaf below treated leaf	9.3	7.0	8.1	14.9	8.4	7.0	9	11.2	15.0	13.6	7.5	11.6	7.3	11
Pseudostem (Leaf sheaths + meristem)	38.3	27.8	25.8	32.7	25.9	33.4	31	27.6	40.0	30.0	27.4	43.1	25.5	32
Roots	36.6	35.2	41.0	34.2	30.0	29.0	34	46.4	42.5	34.8	31.8	43.5	36.8	39

[a]Treatment 1 = plants dipped in X-77 (0.5% v/v) before adding ^{14}C-haloxyfop-methyl on second leaf.
[b]Treatment 2 = plants dipped in DMA-dicamba 1085 μM (containing 0.05% v/v X-77) before adding ^{14}C-haloxyfop-methyl on second leaf.
[c]HAT = hours after treatment.

Appendix table 53. ^{14}C-haloxyfop-methyl absorption and movement in tall fescue as influenced by DMA-dicamba. Experiments 6 and 7.

Plant part	Treatment 1[a]						Treatment 2[b]					
	I	II	III	IV	V	AVG	I	II	III	IV	V	AVG

-- (CPM) --

Experiment 6 (72 HAT[c]):

Plant part	I	II	III	IV	V	AVG	I	II	III	IV	V	AVG
Outside treated leaf	4151	4310	4624	4700	4920	4541	700*	6090	4709	4471	4391	4915
Treated leaf[d]	75648	71656	48207	40027	43026	55713	51261	50142	55738	45970	60203	52663
Leaf above treated leaf	3451	3294	210	245	1729	1786	4017	288	913	215	361	1159
Leaf below treated leaf	61	68	56	51	55	58	45	42	45	57	70	52
Pseudostem (Leaf sheaths + meristem)	3741	5731	4675	5114	6056	5063	3359	2065	2676	2393	4559	3010
Roots	350	370	502	540	506	454	192	300	169	306	309	255
Total inside plants	83251	81119	53650	45977	51372	63074	58874	52837	59541	48941	65502	57139
Total counts	87402	85429	58274	50677	56292	67615	58874	58927	64250	53412	69893	61071
% recovery	90	88	60	52	58	70	61	61	66	55	72	63

Experiment 7 (72 HAT):

Plant part	I	II	III	IV	V	AVG	I	II	III	IV	V	AVG
Outside treated leaf	4496	3678	3288	4271	3976	3942	5725	6423	4541	6567	3349	5321
Treated leaf	39438	64591	79034	51531	58967	58712	50520	44409	60280	44418	60325	51990
Leaf above treated leaf	5569	2567	856	3820	983	2759	1737	364	153	2934	200	1078
Leaf below treated leaf	147	85	563*	67	97	99	43	60	100	76	139	84
Pseudostem (Leaf sheaths + meristem)	5655	5936	8322	7441	6710	6813	3952	4264	3385	4095	1281	3395

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Appendix table 53 continued.

Plant part	Treatment 1[a]						Treatment 2[b]					
	I	II	III	IV	V	AVG	I	II	III	IV	V	AVG
						(CPM)						
Roots	377	366	553	441	302	408	396	369	276	277	181	300
Total inside plants	51186	73545	89328	63300	67059	68884	56648	49466	64194	51800	62126	56847
Total counts	55682	77223	92616	67571	71035	72825	62373	55889	68735	58367	65475	62168
% recovery	55	76	91	66	70	72	61	55	67	57	64	61

[a]treatment 1 = 18 μM haloxyfop-methyl (0.05% v/v X-77); ^{14}C-haloxyfop-methyl.
[b]treatment 2 = 18 μM haloxyfop-methyl + 1085 μM DMA-dicamba (0.05% v/v X-77).
[c]HAT = hours after treatment.
[d]Treated leaf was washed twice in methanol (80% by vol) and once in pure chloroform before oxidizing.
97,000 CPM ^{14}C-haloxyfop-methyl added/plant for Experiment 6, 102,000 CPM added/plant for Experiment 7.
*Declared outliers.

Appendix table 54. Fresh weight of plant parts used to assess ^{14}C-haloxyfop-methyl uptake and translocation. Experiments 6 and 7.

Plant part	Treatment 1[a]						Treatment 2[b]					
	I	II	III	IV	V	AVG	I	II	III	IV	V	AVG
	--------------------------------------- (mg) ---------------------------------											
Experiment 6 (72 HAT[c]):												
Treated leaf[d]	32.8	38.7	43.0	39.9	35.1	37.9	26.4	34.3	37.1	37.3	51.3	37.3
Leaf above treated leaf	6.3	8.3	3.9	8.2	16.8	8.7	7.3	12.5	6.4	6.8	18.0	10.2
Leaf below treated leaf	8.9	8.7	8.0	8.5	7.9	8.4	6.1	10.2	9.0	8.4	10.0	8.7
Pseudostem (Leaf sheaths + meristem)	36.1	37.8	36.2	44.8	45.5	40.1	32.6	33.6	42.6	38.2	54.3	40.3
Roots	20.0	14.8	27.3	21.3	33.7	23.4	17.7	25.6	25.9	15.6	26.1	22.2
Experiment 7:												
Treated leaf	32.3	34.2	46.1	29.0	25.5	33.4	36.0	33.3	31.8	25.8	39.1	33.2
Leaf above treated leaf	11.0	8.9	4.4	7.2	8.7	8.0	11.0	20.0	3.8	6.0	6.4	9.4
Leaf below treated leaf	8.9	8.1	11.8	7.1	9.1	9.0	7.0	7.0	8.9	5.9	11.6	8.0
Pseudostem (Leaf sheaths + meristem)	37.0	43.2	45.3	39.1	38.4	40.6	40.0	48.1	33.7	34.0	31.8	37.5
Roots	15.0	16.1	27.9	25.7	29.0	22.7	18.7	24.8	12.3	15.0	36.4	21.4

[a]Treatment 1 = 18 μM haloxyfop-methyl (0.05% v/v X-77); 97,000 CPM ^{14}C-haloxyfop-methyl on adaxial surface of second leaf.
[b]Treatment 2 = 18 μM haloxyfop-methyl + 1085 μM DMA-dicamba (0.05% v/v X-77; 97,000 CPM ^{14}C-haloxyfop-methyl on adaxial surface of second leaf for experiment 6, 102,000 CPM/plant for experiment 7.
[c]HAT = hours after treatment.
[d] = treated leaf was washed twice in methanol (80% by vol) and once in pure chloroform before oxidizing.

Appendix table 55. Uptake and movement of ^{14}C-haloxyfop-methyl when the dimethylamine salt of dicamba was applied to separate leaves of the same plant. Experiment 1.

Plant part	Replication	Treatment[a]					
		1	2	3	4	5	6
		--- (CPM) ---					
Outside treated leaf	I	2379	8810	2022	9602	24290	21126
	II	1537	11820	2999	2173	8149	7301
	III	1934	5701	1845	2613	3173	6954
	AVG	1950	8777	2289	4796	11871	11794
Treated leaf	I	22002	6615	26105	10764	9972	9240
	II	19306	6912	21368	12936	22145	15309
	III	26069	7485	30577	9829	22098	7004
	AVG	22459	7004	26017	11176	18072	10518
Leaf above or below treated leaf	I	37	197	51	140	24	142
	II	33	93	32	195	28	117
	III	39	67	71	110	36	88
	AVG	36	119	51	148	29	116
Newer leaf (3rd leaf)	I	305	519	265	426	403	449
	II	261	411	314	404	215	455
	III	268	193	412	467	486	423
	AVG	278	374	330	432	368	442
Pseudostem (leaf sheaths + meristem)	I	1399	1474	2652	900	1229	698
	II	1645	690	1287	1062	1206	653
	III	1552	454	2863	1532	1497	729
	AVG	1532	873	2267	1165	1311	693
Roots	I	327	287	324	270	300	208
	II	324	165	270	240	220	147
	III	408	206	287	261	379	221
	AVG	353	219	294	257	300	192

[a]Treatment: 1 = 1st leaf dipped in X-77; ^{14}C-haloxyfop-methyl applied on 2nd leaf.
2 = 2nd leaf dipped in X-77; ^{14}C-haloxyfop-methyl applied on 1st leaf.
3 = 1st leaf dipped in 1085 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 2nd leaf.
4 = 2nd leaf dipped in 1085 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 1st leaf.
5 = 1st leaf dipped in 2170 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 2nd leaf.
6 = 2nd leaf dipped in 2170 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 1st leaf.
[b]Roots were allowed to air-dry for about 10 min before weighing. 59,000 CPM/plant were used.

Appendix table 56. Uptake and movement of ^{14}C-haloxyfop-methyl when the dimethylamine salt of dicamba was applied to separate leaves of the same plant. Experiment 2.

Plant part	Replication	Treatment[a]					
		1	2	3	4	5	6
		------------- (CPM) -------------					
Outside treated leaf	I	9214	6369	5423	6794	25555	16138
	II	7857	17311	3458	11862	11154	16283
	III	4934	19758	13302	21219	10734	23450
	AVG	7335	14429	7394	13292	15814	18624
Treated leaf	I	30207	19643	25725	15076	29763	9893
	II	31516	13606	43495	10793	30340	12171
	III	29626	12860	40550	8773	31914	7428
	AVG	30450	15370	36590	11547	30672	9831
Leaf above or below treated leaf	I	55	163	32	213	125	103
	II	60	368	87	115	81	171
	III	80	158	79	161	37	70
	AVG	65	230	66	163	81	115
Newer leaf (3rd leaf)	I	381	234	462	356	324	393
	II	553	555	205	272	406	610
	III	257	487	217	433	273	277
	AVG	397	425	295	354	334	427
Pseudostem (leaf sheaths + meristem)	I	2336	1512	2019	1462	2551	463
	II	2085	1725	2677	1071	1933	1267
	III	3030	1300	1731	931	1231	939
	AVG	2484	1512	2142	1155	1905	890
Roots	I	321	214	364	273	433	195
	II	367	445	528	190	301	147
	III	396	382	243	226	240	152
	AVG	361	347	378	230	325	165

[a]Treatment: 1 = 1st leaf dipped in X-77; ^{14}C-haloxyfop-methyl applied on 2nd leaf.

2 = 2nd leaf dipped in X-77; ^{14}C-haloxyfop-methyl applied on 1st leaf.

3 = 1st leaf dipped in 1085 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 2nd leaf.

4 = 2nd leaf dipped in 1085 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 1st leaf.

5 = 1st leaf dipped in 2170 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 2nd leaf.

6 = 2nd leaf dipped in 2170 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 1st leaf.

[b]Roots were allowed to air-dry for about 10 min before weighing. 95,500 CPM/plant were used.

Appendix table 57. Fresh weight of plant parts used to assess uptake and translocation of ^{14}C-haloxyfop-methyl in tall fescue when placing DMA-dicamba in separate leaves. Experiment 1.

Plant part	Replication	Treatment[a]					
		1	2	3	4	5	6
		─────────────── (mg) ──────────────					
Treated leaf	I	44.4	11.7	33.2	9.0	39.4	9.0
	II	33.6	15.1	35.6	9.3	40.5	13.6
	III	43.4	12.1	33.3	13.4	54.4	9.2
	AVG						
Leaf above or below treated leaf	I	8.0	49.2	6.7	43.4	10.0	34.0
	II	7.0	48.7	7.5	62.0	8.4	50.2
	III	9.4	45.4	7.2	68.9	8.4	38.1
	AVG						
Newer leaf (3rd leaf)	I	30.5	82.4	17.9	82.2	78.6	69.2
	II	19.0	69.4	30.0	72.8	30.9	56.8
	III	21.8	85.7	26.9	49.2	55.0	63.8
	AVG						
Pseudostem (Leaf sheaths + meristem)	I	43.4	80.6	42.4	62.6	58.8	62.5
	II	38.0	70.0	41.3	74.6	47.7	67.3
	III	47.7	55.4	44.2	81.7	57.4	54.1
	AVG						
Roots[b]	I	50.0	35.6	14.3	23.8	56.6	54.4
	II	55.1	30.0	17.7	62.8	57.2	65.2
	III	68.0	64.0	30.0	60.0	79.5	60.2
	AVG						

[a]Treatment: 1 = 1st leaf dipped in X-77; ^{14}C-haloxyfop-methyl applied on 2nd leaf.
2 = 2nd leaf dipped in X-77; ^{14}C-haloxyfop-methyl applied on 1st leaf.
3 = 1st leaf dipped in 1085 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 2nd leaf.
4 = 2nd leaf dipped in 1085 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 1st leaf.
5 = 1st leaf dipped in 2170 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 2nd leaf.
6 = 2nd leaf dipped in 2170 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 1st leaf.

[b]Roots were allowed to air-dry for about 10 min before weighing.
59,000 CPM/plant were used.

Appendix table 58. Fresh weight of plant parts used to assess uptake and translocation of ^{14}C-haloxyfop-methyl in tall fescue when placing DMA-dicamba in separate leaves. Experiment 2.

Plant part	Replication	Treatment[a]					
		1	2	3	4	5	6
		--------------- (mg) --------------					
Treated leaf	I	50.0	10.0	27.0	8.7	43.2	8.2
	II	67.6	11.1	44.5	7.0	37.0	8.6
	III	36.7	10.8	35.1	10.0	46.6	8.3
	AVG						
Leaf above or below treated leaf	I	9.0	36.0	6.0	36.9	8.6	39.2
	II	14.1	54.8	10.6	33.9	10.0	35.0
	III	8.4	50.9	7.8	40.0	9.1	35.1
	AVG						
Newer leaf (3rd leaf)	I	38.2	8.8	41.0	38.0	22.8	75.0
	II	50.0	31.3	15.6	27.7	29.0	41.1
	III	16.6	69.0	8.0	55.5	21.0	65.3
	AVG						
Pseudostem (Leaf sheaths + meristem)	I	60.5	36.6	39.1	50.9	46.2	66.7*
	II	68.3	58.7	53.3	48.9	55.4	48.5
	III	41.7	57.4	32.4	46.4	48.2	74.9*
	AVG						
Roots[b]	I	32.1	30.0	19.1	24.7	25.3	16.1
	II	60.0	56.7	70.0	27.6	47.0	36.6
	III	40.0	76.0	33.3	30.0	22.7	23.7
	AVG						

*4th leaf starting to emerge.
[a]Treatment: 1 = 1st leaf dipped in X-77; ^{14}C-haloxyfop-methyl applied on 2nd leaf.
  2 = 2nd leaf dipped in X-77; ^{14}C-haloxyfop-methyl applied on 1st leaf.
  3 = 1st leaf dipped in 1085 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 2nd leaf.
  4 = 2nd leaf dipped in 1085 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 1st leaf.
  5 = 1st leaf dipped in 2170 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 2nd leaf.
  6 = 2nd leaf dipped in 2170 μM DMA-dicamba; ^{14}C-haloxyfop-methyl on 1st leaf.
[b]Roots were allowed to air-dry for about 15 min before weighing.
95,500 CPM/plant were used.

Appendix table 59. Percentages of polar unknown, haloxyfop acid, and haloxyfop-methyl recovered from tall fescue leaf macerates of plants dipped in formulated haloxyfop-methyl (FHM) or FHM + dimethylamine salt of dicamba (DMA-dicamba) 24 h after treatment.

Treatment	Polar unknown				Haloxyfop acid				Haloxyfop-methyl			
	I	II	III	IV[a]	I	II	III	IV[a]	I	II	III	IV[a]
	---------- % of total radioactivity scanned --------											
Experiment 1:												
FHM	43	72	49		53	26	48		4.4	1.9	2.8	
FHM + DMA-dicamba	73	65	72		23	33	25		3.0	1.8	2.9	
Experiment 2:												
FHM	32	47	32	41	61	49	62	54	7.6	3.9	6.0	4.4
FHM + DMA-dicamba	69	75	68	62	25	22	28	34	5.4	2.8	3.9	3.8

[a]For Experiment 1, only three replications were performed.

Appendix table 60. ^{3}H-IAA accumulation in tall fescue microsomal membrane vesicles.

Treatment	Experiment			AVG
	1	2	3	
	------------- ^{3}H-IAA CPM[a] -------------			
ethanol check	2662	1561	3707	2643
4 μM ionophore[b]	1390	1259	1521	1390
10^{-8} M IAA	2439	1491	3148	2359
10^{-6} M IAA	----	1453	3033	2243
10^{-4} M IAA	2351	1377	2496	2075

[a]Originally, 167,000 CPM/assay from ^{3}H-IAA (4 x 10^{-6} M IAA) were added.
[b]A mixture of valinomycin, nigericin, and carbonyl cyanide m-chlorophenylhydrazone.
--- = missing value.

Appendix table 61. ^{14}C-haloxyfop accumulation in tall fescue microsomal membrane vesicles as affected by dicamba. Experiments 1 and 2.

| | ^{14}C-haloxyfop accumulation | | | | |
| | Experiment 1 | | Experiment 2 | | |
Treatments	I	II	I	II	AVG
ethanol check	3753	3728	3103	3785	3592
4 μM ionophore[a]	1984	2232	1781	1730	1932
2 x 10^{-5} M dicamba	3357	4204	2580	2688	3207
10^{-3} M dicamba	2951	3031	1954	3038	2743

[a]A mixture of valinomycin, nigericin, and carbonyl cyanide m-chlorophenylhydrazone.

^{14}C-haloxyfop initially added:
    Experiment 1: 125,000 CPM/assay.
    Experiment 2: 130,000 CPM/assay.

Appendix table 62. ^{14}C-haloxyfop accumulation in tall fescue microsomal membrane vesicles as affected by dicamba. Experiments 2, 3, and 4.

| | ^{14}C-haloxyfop accumulation | | | | | | |
| | Exp. 2 | | Exp. 3 | | Exp. 4 | | |
Treatment	I	II	I	II	I	II	AVG
	----------------------- cpm --------------------						
ethanol check	1907	1685	1538	1564	1565	1495	1626
4 μM ionophore[a]	157	1109	903	980	1098	1149	900
10^{-4} M dicamba	1583	1434	1254	1223	1210	1382	1348
10^{-3} M dicamba	1383	1429	1195	1198	1308	1395	1318

[a]A mixture of valinomycin, nigericin, and carbonyl cyanide m-chlorophenylhydrazone.

^{14}C-haloxyfop initially added:
    Experiment 2 = 59400 CPM/assay
    Experiment 3 = 58500 CPM/assay
    Experiment 4 = 62500 CPM/assay

Appendix table 63. ^{14}C-haloxyfop accumulation in tall fescue microsomal membrane vesicles as affected by dicamba. Additional experiments.

| Treatment | ^{14}C-haloxyfop accumulation | | | | |
| | Exp. 1 | | Exp. 2 | | |
	I	II	I	II	AVG
			cpm		
ethanol check	1555	1494	1511	1415	1494
4 μM ionophore[a]	779	831	798	861	818
10^{-3} M dicamba	1029	1015	997	1097	1035
10^{-3} M dicamba + 4 μM ionophore	860	838	862	753	829

[a]A mixture of valinomycin, nigericin, and carbonyl cyanide m-chlorophenylhydrazone.

^{14}C-haloxyfop initially added:
    Experiment 1 = 33620 CPM/assay
    Experiment 2 = 33937 CPM/assay