

AN ABSTRACT OF THE THESIS OF

J. Hugh B. Butler for the degree of Doctor of Philosophy
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Title: Sethoxydim Herbicide in Red Fescue and Bentgrass:
Levels and Mechanisms of Selectivity.

Abstract approved: Redacted for Privacy
Arnold P. Appleby

The herbicide rate required to reduce growth by 50% (GR_{50}) of 11 cultivars of red fescue (Festuca rubra L. and F. rubra ssp. commutata) and for four cultivars of bentgrass (Agrostis tenuis Sibth. and A. stolonifera L. var. palustris) to sethoxydim (2-[1-(ethoxyimino) butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) was determined in greenhouse and laboratory experiments. Field experiments compared only red fescue cv. 'Pennlawn' and bentgrass cv. 'Penncross'. The GR_{50} values for Pennlawn red fescue in the field, the greenhouse, and the laboratory were 30 kg/ha, 15 kg/ha, and 5.3 mM, respectively. Penncross bentgrass was 400, 1400, and 12,900 times more susceptible than red fescue in the field, in the greenhouse, and in laboratory experiments, respectively. Part of the difference in tolerance was due to the different stage of growth of the plants. The milli-molar concentration of xylene and surfactant used as a solvent for the technical grade sethoxydim was as toxic to red fescue without sethoxydim as when sethoxydim was included. Cultivars of red fescue differed in their response to sethoxydim, but bentgrass cultivars did not. The GR_{50} 's of other species tested in

the greenhouse were > 4 kg/ha for annual bluegrass (Poa annua L.), 0.22 kg/ha for tall fescue (F. arundinacea Schreb.), 0.37 kg/ha for downy brome (Bromus tectorum L.), and 0.017 kg/ha for Italian ryegrass (Lolium multiflorum Lam.).

Translocation and metabolism of ^{14}C sethoxydim was studied in whole plants of Pennlawn red fescue and Penncross bentgrass and contrasted with that of soybeans (Glycine max L.) cv. 'Amsoy'. More ^{14}C translocated from the treated leaf in soybeans than in red fescue or bentgrass. Little movement of ^{14}C occurred in bentgrass during 7 days after application of herbicide. The four major metabolites measured in these experiments constituted over 80% of the total activity applied. The principal solvent system for differentiating metabolites was a 1:4 (v/v) benzene:acetone mixture. The metabolite with an Rf value 0.5 appeared more in red fescue than in bentgrass, but the quantity of the metabolite was insufficient to ascribe all the tolerance of red fescue to the appearance of metabolites. Not all the ^{14}C radioactivity applied to red fescue could be accounted for in the plants and in the nutrient solution, whereas all the radioactivity could be accounted for in bentgrass and in soybean. Some of the radioactivity in red fescue was given off as a gas and was trapped in monoethanolamine, but not enough was captured to account for all the ^{14}C applied to the plants. The loss of ^{14}C suggests that red fescue tolerance is physiological, either a lack of a site of activity or an effective method of detoxifying sethoxydim.

SETHOXYDIM HERBICIDE IN RED FESCUE AND BENTGRASS:
LEVELS AND MECHANISMS OF SELECTIVITY

by

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Sethoxydim Herbicide in red Fescue and Bentgrass:
Levels and Mechanisms of Selectivity.

INTRODUCTION

Sethoxydim is a broad-spectrum grass herbicide selective in broadleaf crops. Sethoxydim controls a wide variety of grasses including cool- and warm-season annual and perennial species. Research on soil persistence and on aspects of plant response to sethoxydim has been reported. Conspicuously tolerant are several fine-leaf fescues, including red fescue and rattail fescue [Vulpia myuros (L.)K.C. Gmel.].

Research presented in Chapter 1 included a wider range of rates than has been used in other work. The objectives were to compare the tolerance of red fescue with that of bentgrass, to observe the effect of growing conditions on the sensitivity of the test species, and to study the difference in tolerance of several cultivars of these two species. Italian ryegrass cv. 'Marshall', tall fescue cv. 'Dawn', downy brome, and annual bluegrass were included in laboratory and greenhouse experiments for comparison with red fescue and bentgrass.

The objective of the research presented in Chapter 2 was to determine the mechanism of selectivity of sethoxydim between red fescue and bentgrass. Results from initial experiments suggested comparison with previously published information on soybeans. Hence, soybeans were included in the later experiments for comparison. The two hypotheses were that red fescue translocated less herbicide than bentgrass, and that metabolites extracted would account for the difference in tolerance between the two species.

Chapter 1. Tolerance and Selectivity of Sethoxydim in Red Fescue and Bentgrass.

Abstract. The herbicide rate required to reduce growth by 50% (GR_{50}) of 11 cultivars of red fescue (Festuca rubra L. #FESRU and F. rubra ssp. commutata) and for four cultivars of bentgrass (Agrostis tenuis Sibth. #AGRTE and A. stolonifera L. var. palustris #AGRST) to sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) was determined in greenhouse and laboratory experiments. Field experiments compared only red fescue cv. 'Pennlawn' and bentgrass cv. 'Pencross'. The GR_{50} values for Pennlawn red fescue in the the field, the greenhouse, and the laboratory were 30 kg/ha, 15 kg/ha, and 5.3 mM, respectively. Pencross bentgrass was 400, 1400, and 12,900 times more susceptible than Pennlawn red fescue in the field, in the greenhouse, and in laboratory experiments, respectively. Plants were at different growth stages in the different experiments. The milli-molar concentration of xylene and surfactant used as a solvent for the technical grade sethoxydim was as toxic to red fescue without sethoxydim as when sethoxydim was included. Cultivars of red fescue differed in their response to sethoxydim, but bentgrass cultivars did not. The GR_{50} 's of other species tested in the greenhouse were > 4 kg/ha for annual bluegrass (Poa annua L. #POAAN), 0.22 kg/ha for tall fescue (F. arundinacea Schreb. #FESAR), 0.37 kg/ha for downy brome (Bromus tectorum L. #BROTE), and 0.017 kg/ha for Italian ryegrass (Lolium multiflorum Lam. #LOLMU).

INTRODUCTION

Sethoxydim, a broad-spectrum grass killer that is selective in broadleaf crops (4, 6, 10, 11, 12), controls a wide variety of grasses including cool- and warm-season annual and perennial species. Research on soil persistence (9, 13) and aspects of plant response to sethoxydim (3, 5) has been reported. Several fine-leaf fescues, including red fescue and rattail fescue [Vulpia myuros (L.)K.C. Gmel. #VLPMY] (1, 8), were highly tolerant to sethoxydim in several experiments at Oregon State University (2)¹.

This research included a wider range of rates than has been used in other work. The objectives were to compare the tolerance of red fescue with that of bentgrass, to observe the effect of growing conditions on the sensitivity of the test species, and to study the difference in tolerance of several cultivars of these two species. Italian ryegrass cv. 'Marshall', tall fescue cv. 'Dawn', downy brome, and annual bluegrass were included in laboratory and greenhouse experiments for comparison with red fescue and bentgrass.

¹ Crop Science Dept. 1984. Weed Control Annual Report, Corvallis, OR.

MATERIALS AND METHODS

Laboratory experiments. Seeds of the 19 grass cultivars and species were surface sterilized with a 0.02% sodium hypochlorite solution for 5 min and rinsed for an additional 10 min in distilled water. Twenty seeds of each were counted onto a germinating blotter in a 50-mm square petri dish. Several molar concentrations were prepared by successive dilutions of technical sethoxydim (50% sethoxydim in xylene). Two distilled water checks and a treatment with the concentration of xylene equivalent to the amount contained in the highest rate of sethoxydim were included in each replicate.

Oxtoxynol² was added to the stock solution at 1% v/v. Seven ml of the appropriate concentrations were added to each petri dish. The petri dishes were sealed by wrapping in laboratory film and were placed on edge in a germinating cabinet. Germination temperatures were 15/25 C with a 12-h photoperiod. Germination, shoot length, and root length were measured after 7 days. Each treatment was replicated twice, and the experiment was conducted three times. Pennlawn red fescue and Penncross bentgrass were used in two other experiments to determine the response of the cultivars to a full range of concentrations of xylene and surfactant.

Greenhouse experiments. Eleven cultivars of red fescue, and eight other grass cultivars and species were thinned to 1/cm in 300-by 500-mm flats. Plants were grown for 4 weeks at approximately 20/15 C with a 14-h photoperiod with an average light intensity

²Triton X-100, Rohm and Haas, Spring House, PA.

of $450 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ (PAR). Plants were at the 3-leaf stage at spraying. Herbicides were applied with a compressed-air greenhouse sprayer, using TeeJet 8002 flat fan nozzle tips. Crop Oil concentrate³ was included in all treatments at an equivalent rate of 2.5 L/ha including an Oil check treatment. Herbicides were applied in 200 L/ha of solution except the 50 and 100 kg/ha rates for which multiple passes were made. Plants were harvested at the soil level and weighed fresh 4 weeks after spraying and results expressed as a percentage of the two check treatments. Each treatment was replicated twice, and experiments were conducted three times. The experiment also was conducted twice with plants grown in 200-mm square plastic pots. Experimental procedures were the same except that plants were grown in washed sand and fertilized with Long Ashton nutrient solution (7).

Field experiments. Two field trials were conducted at Hyslop Research Farm near Corvallis in 1983 and in 1984 on a Woodburn silt loam, a fine silty, mixed, mesic family of Aquultic Argixerolls. Pennlawn red fescue and Penncross bentgrass were sown with a four-row planter at 300-mm row spacings into a fine seedbed. Sub-plot size was 5 by 2 m arranged in a split-plot design with time of application as main plots and rate of herbicide as the sub-plot treatments. The 1983 experiment was sown in mid-September, and the

³ BASF Wyandotte Corp., Parsippany, N.J.

1984 experiment was sown in mid-April. Treatments were applied 2 months and 6 months after planting in each experiment. The plants had 3 to 5 leaves, 1 to 2 tillers, and were approximately 10 cm tall at the first spraying date and were 15 to 20 cm tall and had 10 to 20 tillers per plant at the later spraying date. All treatments except the 50 kg/ha rate were applied in 200 L/ha of solution with a unicycle or back-pack mounted compressed-air precision sprayer equipped with 8002 TeeJet nozzles at 210 kPa. The 50-kg/ha treatment was applied as a double application of the 25 kg/ha rate. Crop Oil was added at an equivalent rate of 2.5 L/ha to all treatments. Two untreated plots and one plot treated only with Crop Oil were included in each main plot. Plots were scored visually for growth reduction every month for 4 months after treatment.

Analysis of results. Results were analyzed using standard analysis of variance and linear regression methods. GR_{50} 's were calculated from the regression models.

RESULTS AND DISCUSSION

Laboratory experiments. Red fescue was tolerant to high concentrations of sethoxydim treatments (Table 1.1), whereas bentgrass was remarkably sensitive (Table 1.2). Rates as low as 0.3 μM reduced bentgrass root and shoot length by 50%. Red fescue root length was influenced at lower concentrations of sethoxydim than was shoot length, whereas the converse was true for bentgrass (Figures 1.1 and 1.2). Root length was less variable than shoot length (mean R^2 for red fescue and bentgrass was 0.91 and 0.70, respectively). Although red fescue was 15,900 times more tolerant than bentgrass to the sethoxydim-xylene formulation (mean GR_{50} for all cultivars = 5.88 mM and 370 nM for red fescue and bentgrass, respectively), the tolerance of red fescue was masked by the presence of the xylene. The tolerance of red fescue to high concentrations of sethoxydim suggested the inclusion of xylene as a check treatment because technical grade sethoxydim was supplied as a 50% xylene mixture. Xylene was more toxic than the sethoxydim-xylene solutions to Pennlawn red fescue (Figure 1.1). The sethoxydim possibly had a stimulatory effect on the fescue, while the xylene was detrimental. Xylene was neither stimulatory nor phytotoxic to bentgrasses at the low concentrations used to dissolve the sethoxydim.

Some red fescue cultivars were more tolerant to sethoxydim than others ($P = 0.005$) (Table 1.1), but cultivars of bentgrass did not differ ($P = 0.23$) (Table 1.2). GR_{50} 's of the red fescue cultivars varied by 50%, although some of this difference may have resulted from the differences in tolerance of the cultivars to xylene rather than to sethoxydim. Greater variability among elongating roots and

shoots prevents clear differentiation of the cultivars, although Cascade tended to be the most tolerant of the red fescue cultivars to sethoxydim-xylene solutions.

Greenhouse experiments. Red fescue was not only tolerant to sethoxydim over a range of rates from 0.5 to 10 kg/ha (Figure 1.3), but was stimulated by sethoxydim at the lower rates. High rates of sethoxydim caused leaf tip burn and stunted growth. Rates of 50 and 100 kg/ha damaged all plants, although not all plants were killed even at 100 kg/ha. Susceptibility of bentgrass was in striking contrast to that of red fescue, with an overall GR_{50} of 15 g/ha (Figure 1.4). Growth of bentgrass was not stimulated at very low rates of sethoxydim in most experiments. Data from stimulatory rates were deleted in calculating the GR_{50} 's of cultivars of red fescue.

Red fescue cultivars differed in response to sethoxydim ($P = 0.01$) (Table 1.1), but bentgrass cultivars did not ($P = 0.25$) (Table 1.2). 'Koket' red fescue, the most tolerant cultivar, was 50% more tolerant than Pennlawn. Cultivars responded similarly to increasing rates of sethoxydim. Because the experiment did not test density, differences between cultivars may be due to their varying ability to withstand the effects of intraspecific competition rather than their actual tolerance. The average increase in fresh weight compared with untreated plants depended on the cultivar, ranging from 0 to 60%. Variability of yield with the cultivar 'Futura' was high because of poor germination in all experiments. Application of Crop Oil stimulated plant growth by an average of 12% over the check plants.

Field experiments. Sethoxydim injured red fescue more in the fall planting than in the spring planting ($P = 0.04$) (Figure 1.5). Red fescue was damaged by rates of sethoxydim above 1 kg/ha at the first application date in the fall-planted experiment, but rates of 5 to 10 kg/ha caused little damage in the spring planting at the corresponding application time. Damage at the early time of spraying in the fall-planted experiment may have been accentuated by freezing weather 2 weeks after application. Less damage occurred in plots with areas covered by snow than in plots without snow. Red fescue was injured more from early application of sethoxydim than from later applications in both experiments ($P = 0.005$). Only the fall-planted, early-sprayed plot treated with 50 kg/ha, showed visual damage 6 months after application.

Bentgrass sensitivity to sethoxydim was not different between the two planting dates (Figure 1.6). Bentgrass was affected at rates greater than 20 g/ha and higher rates caused substantial growth reductions. Sethoxydim applied at 250 g/ha reduced bentgrass growth 75 and 95% in the fall and the spring experiments, respectively, by the fourth week after application. All plants in plots treated with 50 g/ha or more were dead 6 months after application.

General discussion. Red fescue tolerated 4 times normal field use rate without obvious injury symptoms. Although early damage to Pennlawn red fescue in the fall-planted, early-sprayed treatments could be seen at 1 kg/ha of sethoxydim, after 6 months, 5 kg/ha of sethoxydim was the lowest rate at which damage was seen. Pennlawn red fescue was one of the more sensitive red fescue cultivars so other cultivars would not likely be damaged in field use. Treatment

with sethoxydim is not recommended when application is to small plants, and prior to freezing temperatures. Once the fescue plant has developed, it is remarkably tolerant. The considerable tolerance of red fescue to sethoxydim in petri dishes and the toxicity of the xylene suggest that damage was caused by the solvent rather than by the sethoxydim itself. The seeds and subsequent seedlings were in direct contact with the sethoxydim-xylene solution for 7 days which suggests differences in uptake were not likely a cause of the tolerance.

Hosaka et al. (8) reported a number of fescue species tolerant to sethoxydim, but did not report on the tolerance of the species to their solvent system. Results of our study suggest that phytotoxicity of sethoxydim to these Festuca species is caused to the xylene. Tall fescue was much less tolerant to sethoxydim than red fescue in germination, greenhouse studies, and field studies¹. The physiological basis for the difference between these species should be investigated. Annual bluegrass and downy brome were tolerant to field-use rates of sethoxydim as has been reported previously (1, 8).

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We thank BASF Wyandotte Corp. for supply of sethoxydim and financial assistance. The senior author gratefully acknowledges the financial support of the National Research Advisory Council of New Zealand during the tenure of his study at Oregon State University while on leave from the Ministry of Agriculture and Fisheries.

Table 1.1. GR₅₀ values for red fescue estimated from laboratory and greenhouse studies^a.

Cultivar or Species	GR ₅₀	
	Laboratory	Greenhouse
	(mM)	(kg/ha)
Pennlawn	5.3 ^b	15.2 ^c
Polar	5.4	16.4
Futura	3.5	19.3
Hawk	6.4	18.3
Wintergreen	7.2	17.9
Ruby	5.6	17.6
Cascade	8.1	19.0
Koket	5.8	23.2
Banner	6.6	17.4
Waldorf	5.3	16.3
Scarlet	5.4	18.1

^aAverage of two replicates per experiment and three experiments.

^bPredicted from linear regression of reduction in elongation of fescue roots grown in a range of 0.01 mM to 10 mM concentrations in the laboratory.

^cPredicted from linear regression of reduction of fresh weight of leaves as percentage of check plants. Rates of sethoxydim applied were 1.0 to 20 kg/ha.

Table 1.2. GR₅₀ values for bentgrass and sensitive species estimated from laboratory and greenhouse studies^a.

Cultivar or Species	Laboratory	Greenhouse
	GR ₅₀ (nM)	GR ₅₀ (g/ha)
Highland bentgrass	429 ^b	14.2 ^c
Seaside bentgrass	316	11.6
Astoria bentgrass	319	13.2
Penncross bentgrass	413	10.9
Fawn tall fescue	942	216.0
annual ryegrass	631	16.7
downy brome	10850	372.0

^aAverage of two replicates per experiment and three experiments.

^bPrediction from linear regression of reduction in elongation of roots grown in a range of 0.1 to 500 nM concentrations in the laboratory.

^cPredicted from linear regression of reduction of fresh weight of leaves as percentage of check plants. Rates of sethoxydim used in prediction were from 0.62 to 20 g/ha for species with GR₅₀'s of less than 50 g/ha, and from 0.62 to 500 g/ha for species with GR₅₀'s over 100 g/ha.

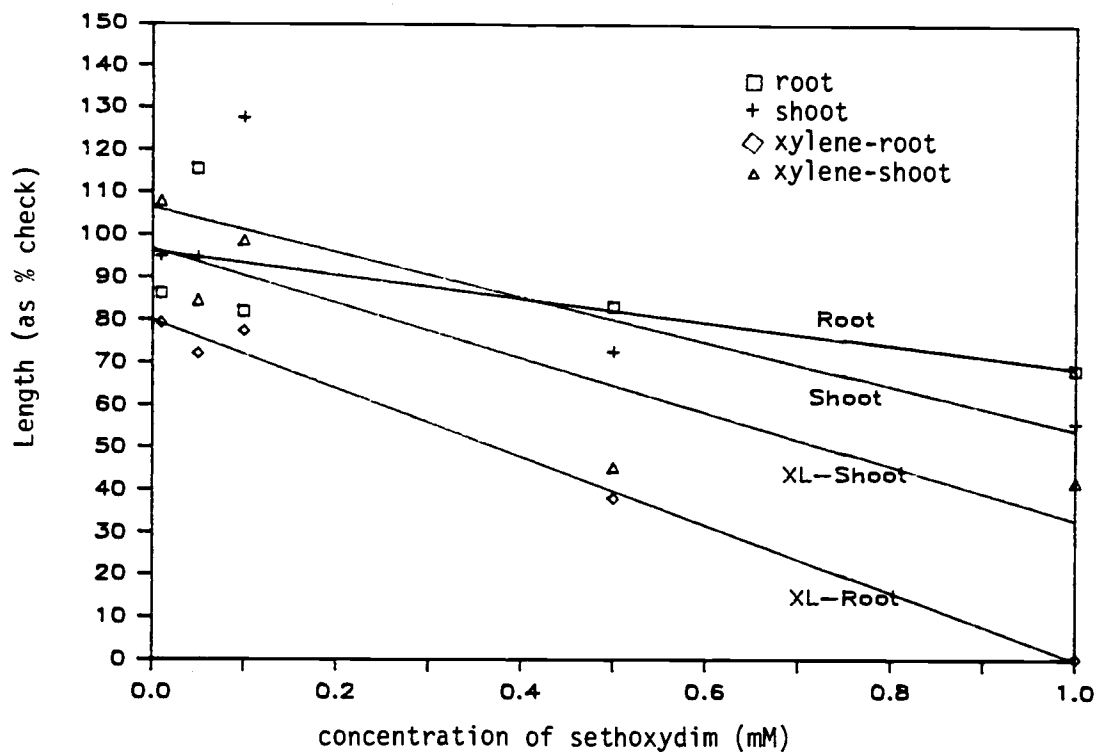


Figure 1.1. Length of Pennlawn red fescue seedlings after exposure to mM concentrations of sethoxydim. Seedlings were grown for 7 days in a germinating cabinet. Regressions equations are for concentrations from 0.01 to 1.0 mM. Length reduction(y)= $96.3 - 27.8 * \text{sethoxydim rate}$, $R^2=0.83$ for root length, $y=106.7 - 52.8 * \text{rate}$, $R^2=0.67$ for shoot length, $y=80.2 - 80.5 * \text{rate}$, $R^2=0.99^{**}$ for xylene root length, and $y=97.1 - 64.4 * \text{rate}$, $R^2=0.8^*$ for xylene shoot length.

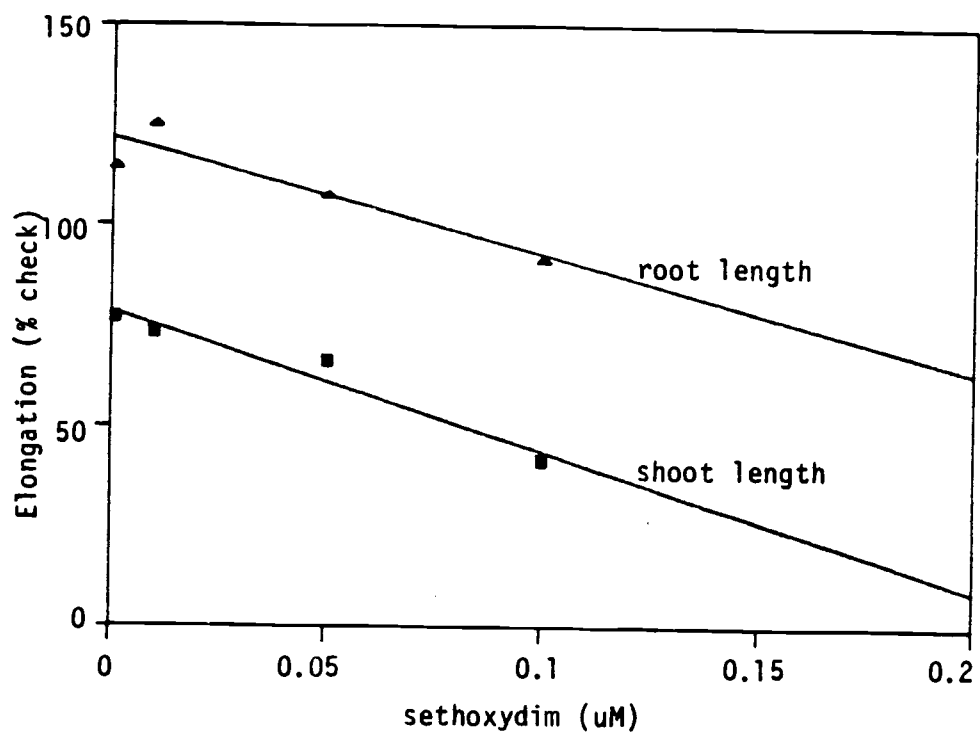


Figure 1.2. Length of Penncross bentgrass seedlings after exposure to nM concentrations of sethoxydim measured after 7 days growth in a germinating cabinet.

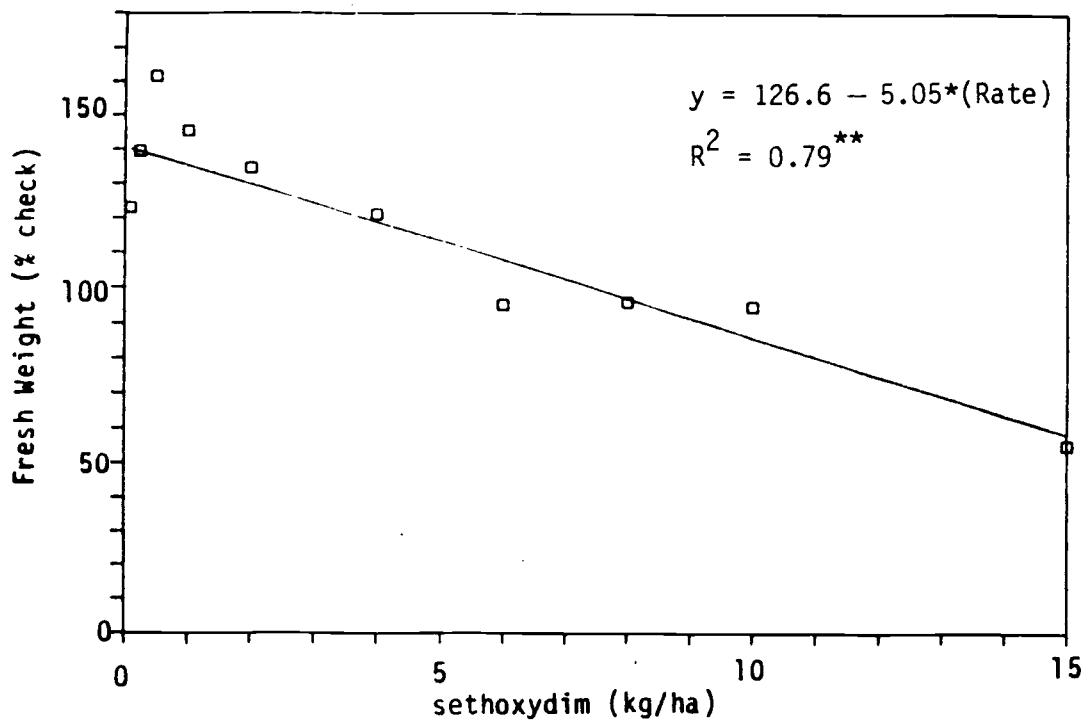


Figure 1.3. Shoot fresh weight of Pennlawn red fescue plants grown in the greenhouse and sprayed with sethoxydim. Fresh weight measured 4 weeks after treatment.

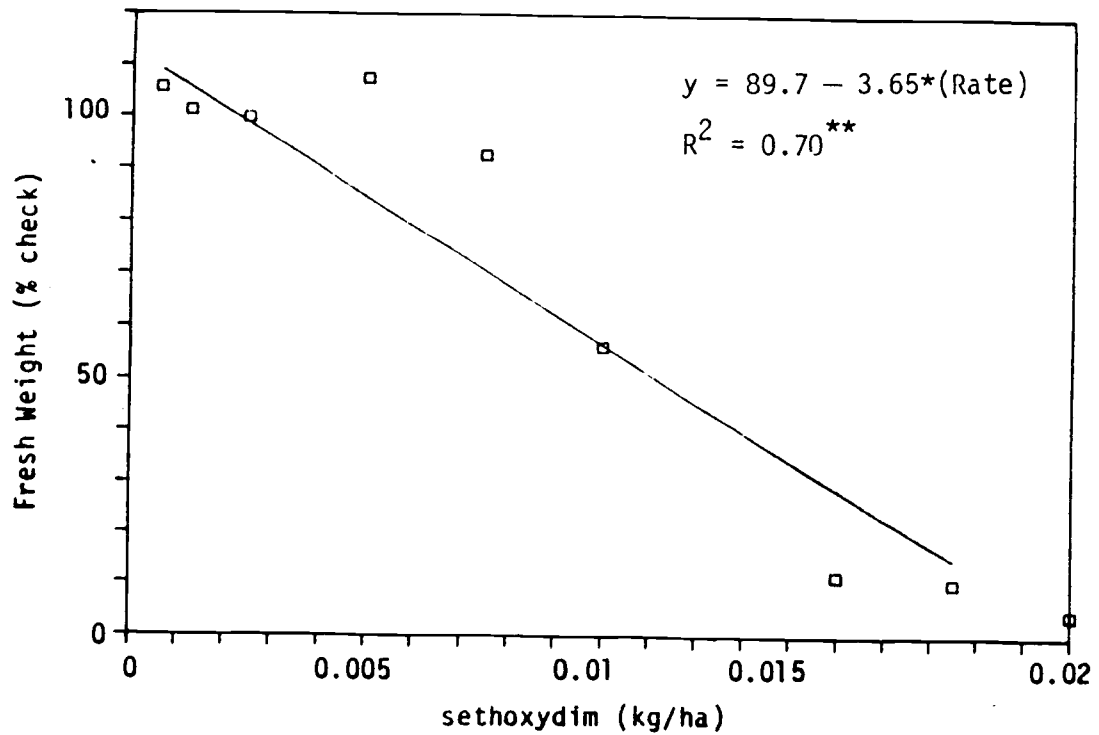


Figure 1.4. Shoot fresh weight of Penncross bentgrass plants grown in the greenhouse and sprayed with sethoxydim. Fresh weight measured 4 weeks after treatment.

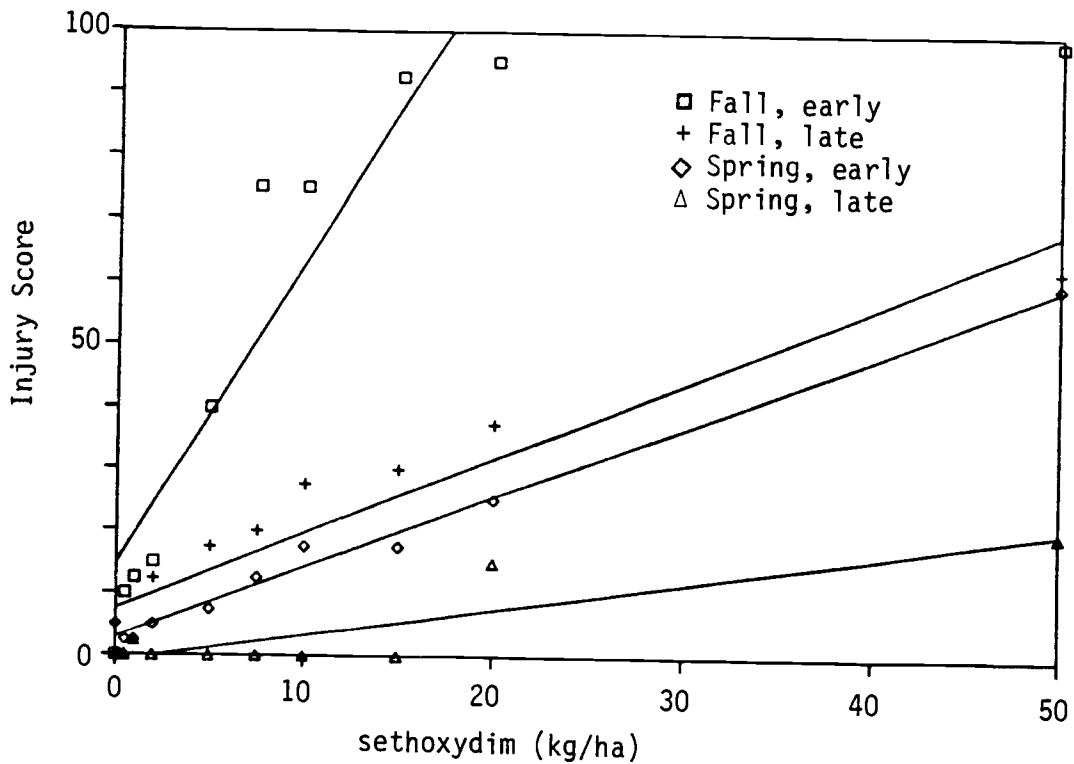


Figure 1.5. Visual evaluation of field experiment with Pennlawn red fescue 8 weeks after application of sethoxydim. Regression equations are in the form of

injury score = constant + slope * sethoxydim rate.

$y=29.6+1.96(\text{rate})$, $R^2=0.56^{**}$ for the fall-planted, early-sprayed treatments, $y=7.47+1.22(\text{rate})$, $R^2=0.90^{**}$ for the fall-planted, late-sprayed treatments, $y=2.89+1.14(\text{rate})$, $R^2=0.99^{**}$ for the spring-planted, early-sprayed treatments, and $y=0.98+1.19(\text{rate})$, $R^2=0.77^{**}$ for the spring-planted, late-sprayed treatments.

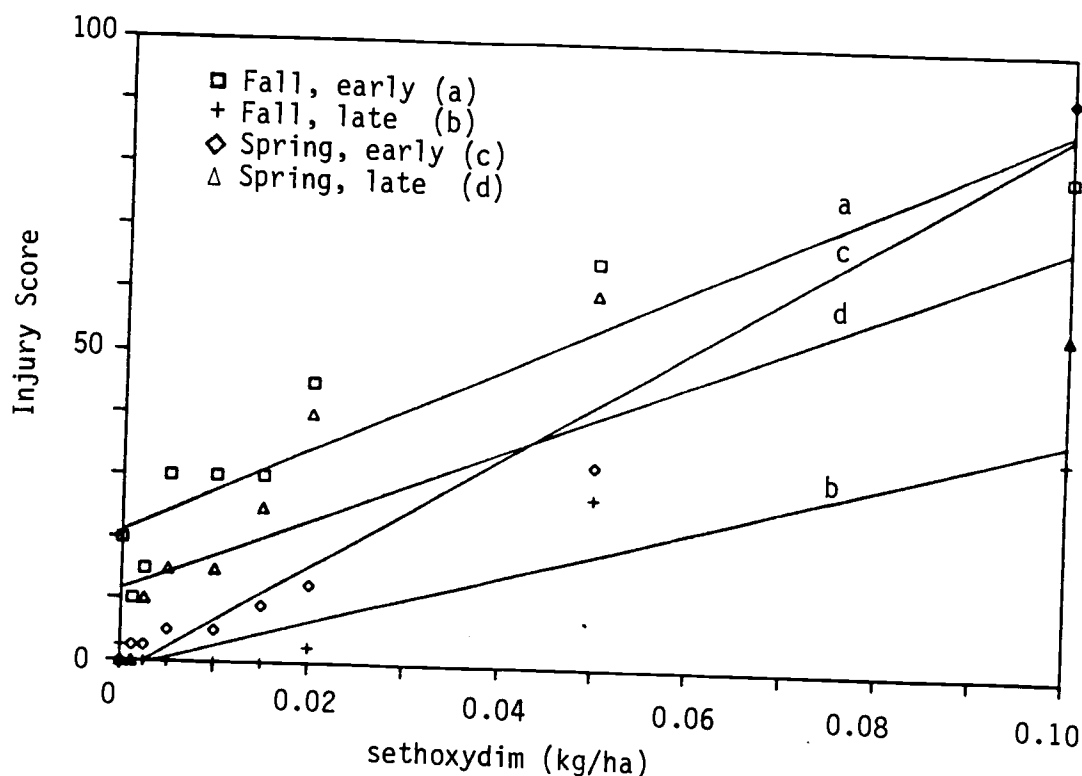


Figure 1.6. Visual evaluation of field experiment with Penncross bentgrass 8 weeks after application of sethoxydim. Regression equations are in the form of

injury score = $b_0 + b_1 \cdot \text{rate of sethoxydim (rate)}$.

$y=21.07+0.66(\text{rate})$, $R^2=0.87^{**}$ for the fall-planted, early-sprayed treatments, $y=-1.39+0.39(\text{rate})$, $R^2=0.90^{**}$ for the fall-planted, late-sprayed treatments, $y=-2.19+0.39(\text{rate})$, $R^2=0.97^{**}$ for the spring-planted, early-sprayed treatments, and $y=11.5+0.57(\text{rate})$, $R^2=0.71^{**}$ for the spring-planted, late-sprayed treatments.

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Chapter 2. Translocation and Metabolism of ^{14}C Sethoxydim in Red Fescue, Bentgrass, and Soybean.

Abstract. Translocation and metabolism of ^{14}C sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)-propyl]-3-hydroxy-2-cyclohexen-1-one) were studied in whole plants of red fescue (Festuca rubra L. 'Pennlawn' #FESRU) and colonial bentgrass (Agrostis tenuis Sibth. 'Penncross' #AGRTE) and contrasted with that of soybeans (Glycine max L. 'Amsoy'). More ^{14}C translocated from the treated leaf in soybeans than in red fescue or bentgrass. Little movement of ^{14}C occurred in bentgrass 7 days after application of herbicide. The four major metabolites measured in these experiments constituted over 80% of the activity applied. The principal solvent system for differentiating metabolites was a 1:4 (v/v) benzene:acetone mixture. A metabolite (R_f value 0.5) was found in greater amounts in red fescue than in bentgrass, but the quantity of metabolites was insufficient to explain all the tolerance of red fescue. Only 70% of the ^{14}C radioactivity applied to red fescue could be accounted for in the plants and in the nutrient solution, whereas 90 to 100% of the radioactivity could be accounted for in bentgrass and in soybeans. Some of the radioactivity (<1%) in red fescue was given off as a gas and was trapped in monoethanolamine, but not enough was captured to account for all the ^{14}C applied to the plants. The loss of ^{14}C suggests that red fescue tolerance is physiological, either a lack of a site of activity or an effective method of detoxifying sethoxydim.

INTRODUCTION

Sethoxydim is a recently developed broad-spectrum grass killer that is registered for use on several crops in the United States and in other parts of the world. Sethoxydim controls many grass plants at rates as low as 0.1 kg/ha. In contrast, fine-leaf fescues, including red fescue and rattail fescue [Vulpia myuros (L.)K.C. Gmel. #VULMY], tolerate rates of 30 kg/ha or higher¹. Red fescue was at least 400 times more tolerant to sethoxydim than bentgrass in field experiments, and it was up to 12,900 times more tolerant in studies measuring elongating roots². The cause of this tolerance in red fescue has not been published, although various cytological, histological, and physical selectivity studies have been reported (1, 5, 7, 9, 10, 15). Swisher and Corbin (13) reported that the basis of selectivity between soybeans and johnsongrass [Sorghum halepense (L.) Pers. #SORHA] was not due to uptake and movement of sethoxydim, but may be because of differential metabolism. They were unable to show sufficient difference in appearance of metabolites to explain the margin of selectivity reported. Campbell and Penner (4) reported that sethoxydim was translocated in alfalfa (Medicago sativa L.), navy bean (Phaseolus vulgaris L.), barnyardgrass (Echinochloa crus-

¹Crop Science Dept. 1984. Weed Control Annual Report, Corvallis, OR.

²J.H.B. Butler, Ph.D. Thesis, 1985. Oregon State Univ., Corvallis, OR.

galli (L.) Beauv. #ECHCA), and quackgrass (Agropyron repens (L.) Beauv. #AGRRE) to metabolic sinks, and few differences were evident between species. Inhibition of photosynthesis is not the primary mechanism of action of sethoxydim (6, 11, 12). A clear mechanism of selectivity between tolerant and sensitive species has not been established. Lipid metabolism or RNA processes may be involved in selectivity (11, 12) but there are no supporting data. Tolerance in red fescue is probably not a result of difference in uptake, because the greatest margin between red fescue and bentgrass was found when seedlings were grown in milli-molar concentrations of sethoxydim (2).

The objective of these studies was to determine the mechanism of selectivity of sethoxydim between red fescue and bentgrass. Results from initial experiments suggested comparison with previously published information on soybeans (6, 13). Hence, soybeans were included in the later experiments. The hypotheses tested were that red fescue translocated less herbicide than bentgrass, and that metabolites extracted would account for the difference in tolerance between the two species.

MATERIALS AND METHODS

Seeds were germinated in moist paper towels and transferred to 75-mm square plastic pots filled with washed sand (16 mesh), or they were germinated in the pots. Pots were placed in a growth chamber maintained at 30C during the day and 25C at night. Light intensity was maintained at $800 \text{ uE}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ for the 14-h day photoperiod. Long Ashton nutrient solution (8) was supplied every fourth day in addition to daily watering. Three-week-old plants were used in the experiments.

^{14}C translocation experiments. Plants were rinsed from the sand culture and transferred to 25-ml erlenmeyer flasks filled with 15 ml of half-strength Long Ashton nutrient solution. Plants were supported in the nutrient solution by sealing the neck of the flask with laboratory plastic film. Plants were then placed back in the growth chamber for 24 h before treatment with 2.5 nmoles of ^{14}C sethoxydim (70,000 dpm) applied in 1 ul hexane. Specific activity of the ^{14}C sethoxydim was 10.3 mCi/mmole and was labelled in the number 4 position on the ring. The droplet was placed on the upper surface 1 cm from the tip of the first leaf for the grasses and on the center leaflet of the first trifoliate leaf of the soybean. Plants were returned to the growth chamber until sampling. At sampling time, nutrient solution for all treatments was changed and sampled. The plants not sampled were returned to the growth chamber. Grass plants were separated into treated leaf, other leaves, and roots; the same divisions were made for soybeans and also included stems. Samples were dried, and either combusted in a sample oxidizer or solubilized

with 1 ml of Protosol³ for 24 h and then bleached with 0.1 ml of hydrogen peroxide for 3 h before scintillation cocktail was added to the vial. Samples were assayed using standard liquid scintillation (LSC) procedures.

Metabolism studies. Plants (as previously described) were harvested at 3 days or at 6 days. Plant parts were separated as in the previous experiment. Soybean samples were ground with a mortar and pestle, while the bentgrass or red fescue samples were ground in a 5-ml glass tissue-grinder with 2 ml of absolute methanol. The mixture was then filtered through filter paper with six 2-ml aliquots of methanol. The filtrate was dried at room temperature with a stream of pre-pure N₂ gas (O₂ < 10 ppm) for approximately 1 h to a volume of 1 ml. A 0.1-ml sample was used for assaying with liquid scintillation, and a 0.1-ml aliquot was spotted onto a thin-layer chromatography (TLC) plate⁴. TLC plates were developed in an acetone:benzene (1:4 v/v) solvent to 150 mm. After drying, regions of R_f 0.0 to 0.1, 0.2 to 0.3, 0.45 to 0.55, and 0.9 to 1.0 were scraped off into a scintillation vial and assayed using LSC. Remaining silica also was scraped from the TLC plates and assayed using LSC procedures. Samples were checked against standards and checks at various stages, including assessment of losses at filtering, at drying, at TLC stages, and at counting. Full ¹⁴C activity balance was done in all experiments.

³ Protosol 0.5M tissue and gel solubilizer. New England Nuclear, Boston, MA.

⁴ Baker Si250(4C). J.T. Baker Chemical Co., Philipsburg, NJ 08865.

RESULTS AND DISCUSSION

^{14}C translocation experiments. More ^{14}C sethoxydim moved in soybean than in red fescue or in bentgrass. ^{14}C moved from the treated leaf into the other leaves in soybean (about 15% per day) (Figure 2.1). In red fescue, although ^{14}C moved out of the treated leaf (5% per day) (Figure 2.2), the ^{14}C in the other leaves did not increase. Very little ^{14}C moved from the treated leaf into other leaves in bentgrass (Figure 2.3). The greater movement of ^{14}C in soybean can be attributed partly to the absence of leaf burn. Hexane caused more leaf burn on leaves of red fescue and bentgrass than on the trifoliolate leaf of the soybeans, thus allowing more ^{14}C movement. In addition, soybeans had greater leaf surface, so the non-treated leaves provided a more substantial sink to move sethoxydim out of the treated leaf into the non-treated leaves (4). More ^{14}C moved from bentgrass into the nutrient solution than in red fescue, resulting from disruption of cell membrane integrity of the bentgrass allowing leakage of the ^{14}C into the nutrient solution. The nutrient solution was changed every 2 days, creating a concentration gradient from the plant roots to the nutrient solution, thus enhancing movement of the ^{14}C into the solution.

Recovery of ^{14}C in checks was higher when samples were combusted than when solubilized (91 to 98% versus 80 to 83%, respectively). An explanation for the less efficient recovery of the ^{14}C is that some of the ^{14}C was still bound in the structure of the plants even after 24 h of solubilization. Consequently, it was not counted in LSC. Corrections for color quenching during LSC were unable to account for the difference.

Metabolism studies. Sethoxydim undergoes a number of non-biological changes including degradation into other metabolites with exposure to light, warm temperatures (3), oxidation conditions, aqueous solutions, and development on TLC plates⁵. For these reasons, samples were not dried in a heated vacuum oven.

Complex changes in the amount of metabolites were evident 3 days after treatment with labeled sethoxydim in all species. Although Campbell and Penner (3) measured 10 metabolites of sethoxydim, only four major metabolite regions on the TLC's had sufficient counts to identify positively either by scraping or by using a radiographic TLC plate scanner. Rf regions were 0.0 to 0.1, 0.20 to 0.25, 0.45 to 0.60, and 0.9 to 1.0. These bands constituted 90 to 95% of the activity on the plate. Stock solution ¹⁴C sethoxydim corresponded to Rf 1.0. The Rf 0.0 metabolite was a non-biological degradation product. That accounted for more than 30% of the ¹⁴C activity after 6 to 24 h in aqueous solutions (Figure 2.4). The Rf 0.25 and Rf 0.5 bands constituted between 10 to 15% of the activity (Figure 2.4). These two bands only become important when ¹⁴C sethoxydim was applied to, and extracted from, plant tissue. The metabolites did not form if ¹⁴C sethoxydim stock was added to plant extract in methanol.

⁵ BASF Wyandotte Corp. Notes for Experimenters with BAS 9052 H-4-¹⁴C
May 1981.

The major difference between species was in the sum of the two metabolites Rf 0.25 and Rf 0.50 values at 3 days after treatment. The sum of ^{14}C activity of these two metabolites was similar in red fescue and soybean, but bentgrass had less ^{14}C at Rf 0.25 and Rf 0.50 than red fescue or soybean. In red fescue, the ^{14}C activity at Rf 0.50 in the extract of non-treated leaves accounted for 30% of the activity compared to less than 15% with bentgrass (Table 2.1). The proportion of activity at Rf 0.50 in the red fescue and soybean was similar to that reported by Swisher and Corbin in soybeans (13). The ^{14}C activity in the other Rf bands was reduced or increased, depending on the level of activity at Rf 0.50. At 6 days, no Rf 0.25 or Rf 0.50 metabolites were detected in bentgrass. Movement into the other leaves from the treated leaf during the 3 days may have masked any small changes. Data from metabolism experiments confirmed the results from the translocation experiments. Movement of ^{14}C sethoxydim in red fescue and bentgrass was not very different (Table 2.2), although more ^{14}C moved into bentgrass roots.

Recovery of ^{14}C sethoxydim. Recovery of ^{14}C was lower in red fescue than in bentgrass or soybean in most translocation and metabolism studies with whole plants. All of the ^{14}C was recovered in experiments when excised coleoptiles were treated with ^{14}C sethoxydim in aqueous solutions (data not shown). Recovery of ^{14}C from red fescue declined over the 7 to 8 days of the studies in both combustion and solubilization experiments (Figures 2.1, 2.2, and 2.3).

In experiments to trap ^{14}C , treated plants were enclosed in a clear-vented chamber. Air was extracted from the chambers and

bubbled through two separate testtubes filled with 5 ml of a trapping agent. Radioactivity was not successfully trapped with sodium hydroxide, and monoethanolamine was only slightly more successful (Figure 2.5). ^{14}C was found in the trap, but activity was insufficient (1% of total applied activity) to account for the low recoveries. Two explanations are possible: (a) insufficient air may have been drawn out through the system, although the flow rate was sufficient to change the air in the container 8 to 10 times each sampling; or (b) the ^{14}C may not have been present as CO_2 and so was not trapped in the monoethanolamine. Most of the ^{14}C loss in most of the experiments occurred in the first day. ^{14}C was not lost when stock ^{14}C sethoxydim was applied to dry plant material rather than to intact living plants and kept for 7 days at room temperature.

Other losses in the experiments could be accounted for. Drying samples in the pre-pure N_2 stream accounted for a 15 to 21% loss. Loss of ^{14}C stock solution applied directly to the TLC plate was 2%.

General discussion. Sethoxydim was a difficult herbicide to work with because of its instability in solution, in light, and on chromatography media (3). Sethoxydim could not be detected by gas liquid chromatography (data not shown), so TLC procedures were used. But, because sethoxydim degrades on TLC plates⁵, the data presented must be qualified by the assumption that the metabolites so presented are valid biological metabolites. Campbell and Penner (3) reported that some metabolites are phytotoxic.

Definitive conclusions from this study are as follows. Tolerance of red fescue to sethoxydim cannot be attributed to lack of

translocation. Translocation of ^{14}C sethoxydim followed the same pattern in red fescue as in bentgrass. Soybean, with its greater leaf area, translocated more ^{14}C sethoxydim. The loss of ^{14}C in red fescue cannot be explained; losses occurred in all experiments and may not have not been in the form of CO_2 . Unexplained loss of ^{14}C was reported by Veerasekaran and Catchpole (14) with a similar herbicide, alloxydim sodium. Over 40% of the ^{14}C applied was eliminated from the treated plants within 7 days (14). Our results show similar losses with intact plants.

The technique of using cell cultures as a possible biological method (13) may not be appropriate if metabolites are volatile. The volatile metabolites may remain in the cell culture instead of escaping into the air. Red fescue did metabolize sethoxydim, and the amount of metabolite appearing after 3 days was similar in proportion to those in soybeans. The variability of our results concerns us because in four experiments that included soybean, none produced similar results. Replicate variation was not high, but variation among experiments was high. We cannot explain the subtle changes in experimental procedures that may have been present.

The immediate change of sethoxydim applied to a plant is from a non-polar into a polar compound. The metabolite at Rf 0.0 was in similar quantities in most of the plants, suggesting that it could be phytotoxic. Transformation into metabolites Rf 0.25 and Rf 0.50 steadily increased over the next 6 days. Because the treated leaf still had intact sethoxydim, sethoxydim continued to move into the rest of the plant, replenishing the Rf 1.0 and encouraging its subsequent non-biological transformation into the Rf 0.0 metabolite.

The steady rise of the two metabolites, Rf 0.25 and Rf 0.50, in both red fescue and soybeans suggests inactivation of the sethoxydim into non-phytotoxic compounds.

Red fescue may be different than soybean in that it seems to further convert the ^{14}C sethoxydim into a compound that escapes from the system. CO_2 is the likely escape compound. We were unable to verify CO_2 loss with our techniques and suggest future study using different CO_2 capturing techniques.

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Table 2.1 Proportions of ^{14}C sethoxydim in various Rf regions on TLC plates extracted from treated leaves or from the balance of the plants (excluding roots)^a.

Day of Sampling	Rf Value ^b	Untreated Leaves			Treated Leaves		
		Bentgrass	Red Fescue	Soybean	Bentgrass	Red Fescue	Soybean
----- % of Activity as metabolite at Rf value -----							
3 days	0.00	32.5	7.6	28.9	37.9	27.5	32.6
	0.25	22.7	23.9	19.0	9.8	8.6	16.0
	0.50	12.2	30.4	40.7	23.9	40.0	40.1
	1.00	32.6	38.1	11.4	28.4	24.0	11.3
6 days	0.00	54.0	39.1	40.3	53.9	55.4	46.5
	0.25	0.0	6.9	18.4	14.0	10.4	27.5
	0.50	0.0	41.9	30.6	14.6	16.4	18.3
	1.00	42.3	12.2	10.6	17.6	17.7	7.7

^aAverage of two experiments with four plants in each experiment.

^bAverage Rf value for the band. Rf 0.0 is the band at 0.0 to 0.10, 0.25 is the band from 0.20 to 0.30, 0.50 is the band from 0.45 to 0.60, 1.0 is the band from 0.90 to 1.00.

Table 2.2 ^{14}C activity in various plant parts 3 days after treatment with ^{14}C sethoxydim.

Sampled Area	Bentgrass	Red Fescue	Soybean
	--- (% of Activity Applied) ^b -----		
Treated Leaf	78.9	55.4	46.6
Remainder of Leaves	6.5	11.3	37.8
Stem	--	--	6.5
Roots	1.3	0.7	1.5
Nutrient Solution	5.0	1.3	4.5
Total	92.1	68.7	96.9

^a ^{14}C applied as 1 μl drop of 2.5 μM ^{14}C sethoxydim.

^b Average of two experiments, four plants in each experiment.

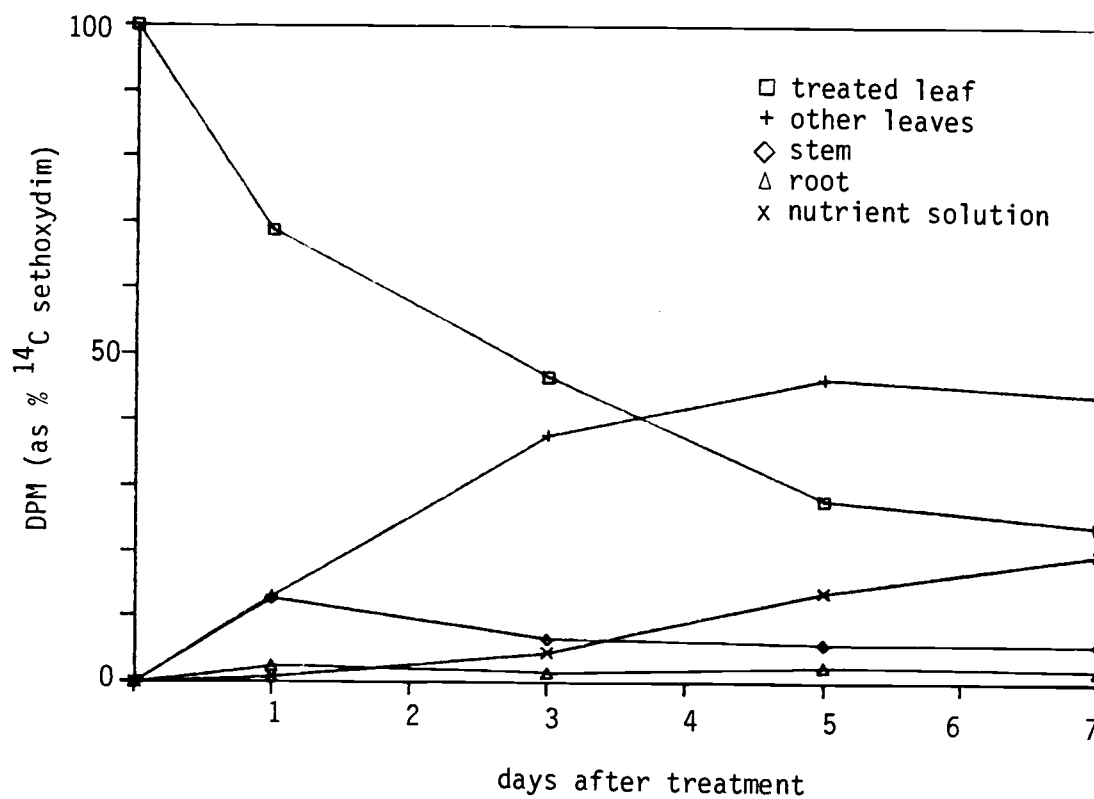


Figure 2.1 Movement of ^{14}C sethoxydim applied to soybean. 2.5 nM of ^{14}C sethoxydim applied to each plant. Average of 4 plants per experiment and two experiments.

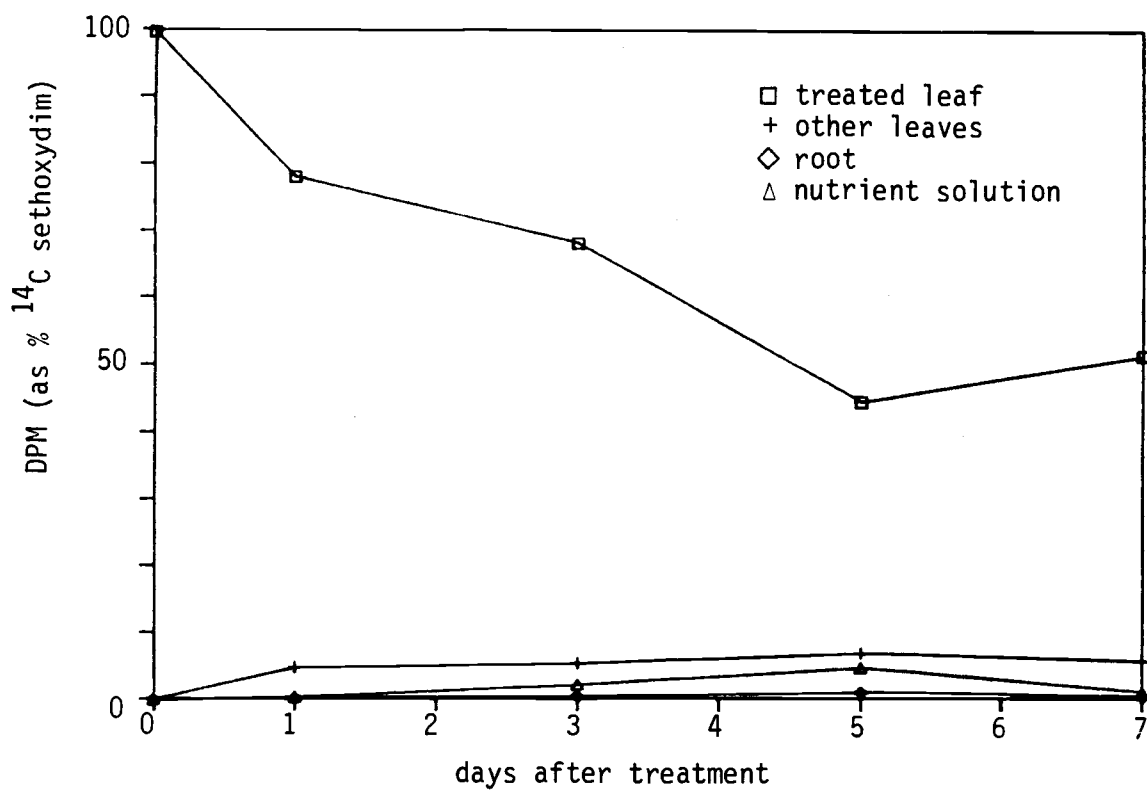


Figure 2.2 Movement of ^{14}C sethoxydim applied to red fescue. 2.5 nM of ^{14}C sethoxydim applied to each plant. Average of 4 plants per experiment and two experiments.

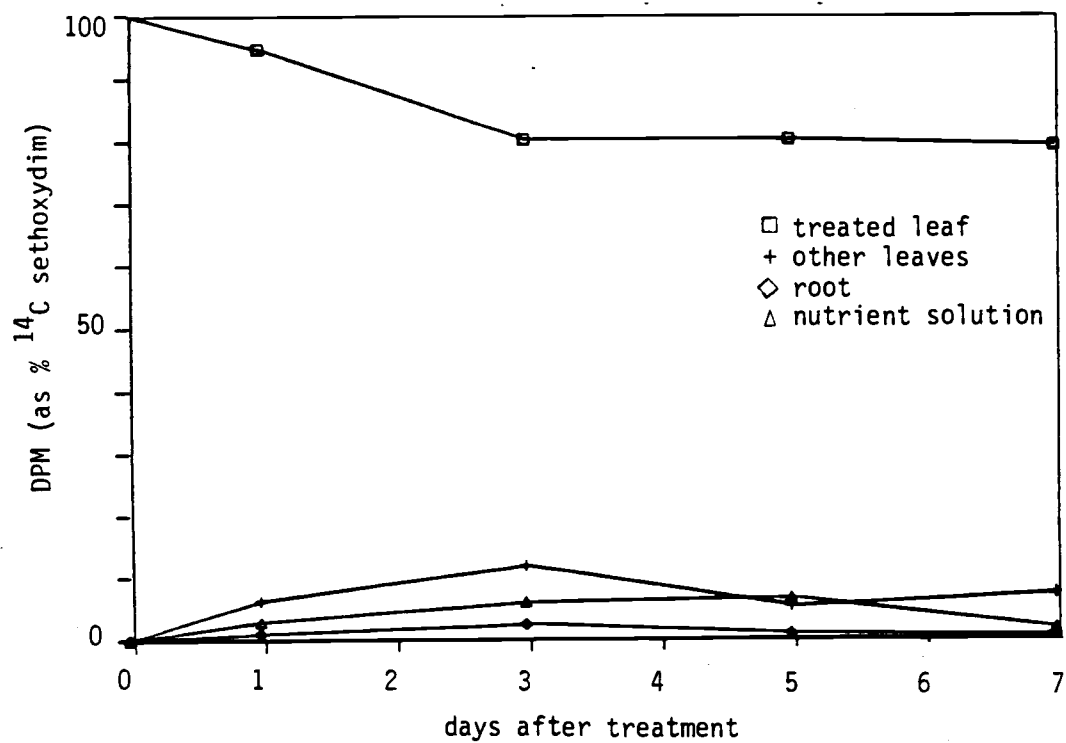


Figure 2.3 Movement of ^{14}C sethoxydim applied to bentgrass. 2.5 nM of ^{14}C sethoxydim applied to each plant. Average of 4 plants per experiment and two experiments.

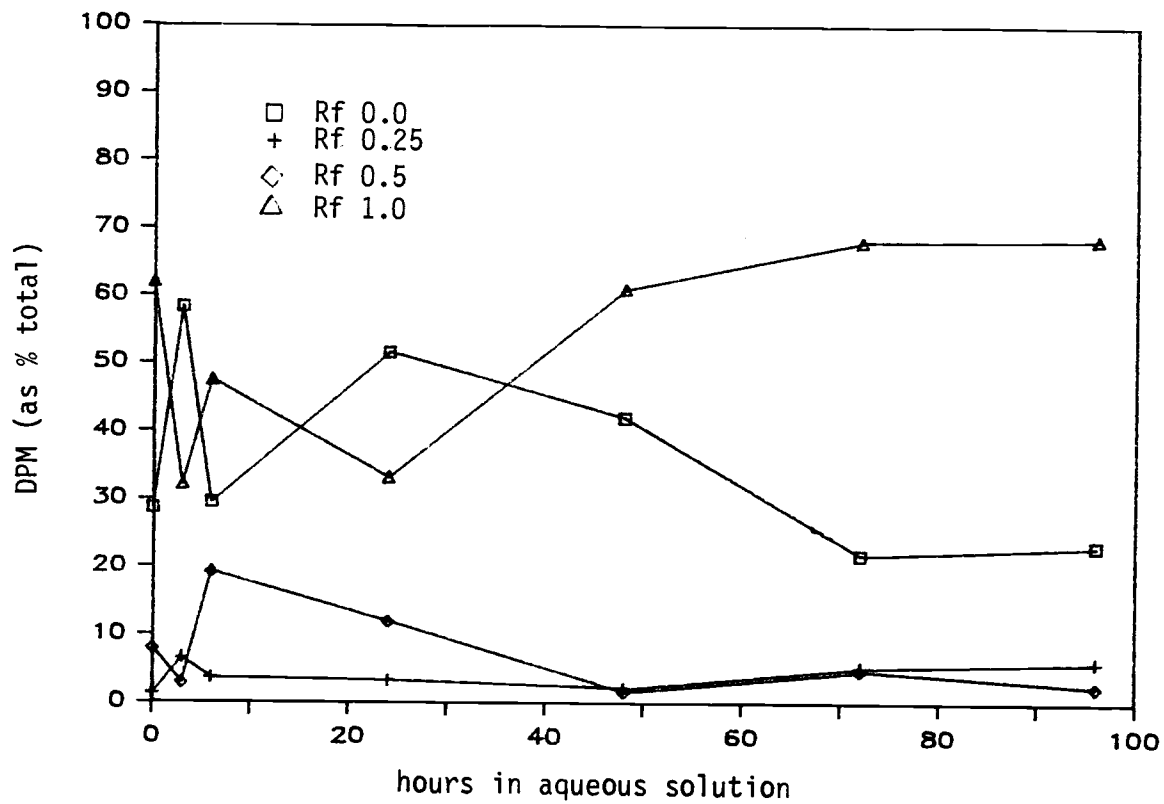


Figure 2.4 Non-biological transformations of ^{14}C sethoxydim in aqueous solutions.

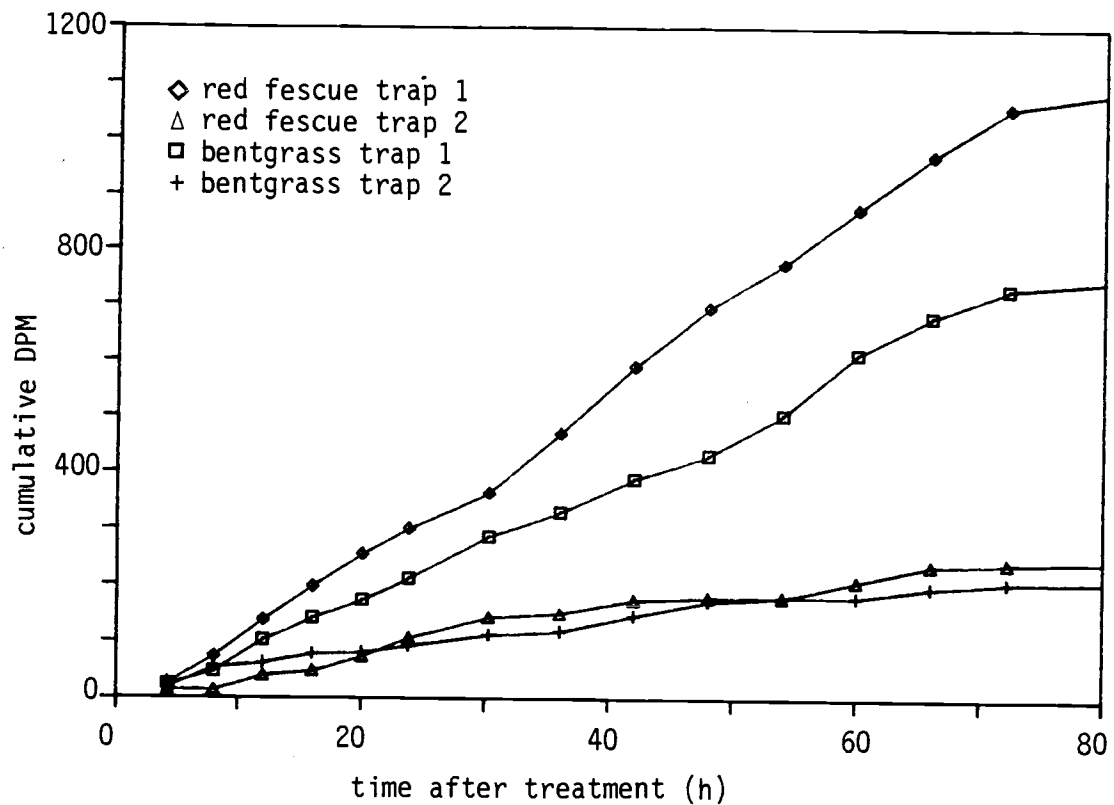


Figure 2.5 Cumulative loss of ^{14}C from bentgrass or from red fescue plants grown in a closed chamber. Air was extracted from the chamber and bubbled through two 5-ml monoethanolamine solutions. 7.5 nM ^{14}C sethoxydim was applied to each of six plants in the chamber.

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APPENDICES

Appendix Table 1. Red fescue fresh weight as % of the untreated plants in the greenhouse experiment.

Rate	Shoot Fresh Weight										
	Pennlawn	Polar	Futura	Hawk	Wintergreen	Ruby	Cascade	Koket	Banner	Waldorf	Scarlet
(kg/ha)	----- (% of check) ^b -----										
CO ^a	94	111	110	120	184	113	98	94	113	105	93
1	121	137	103	150	167	143	140	161	127	121	108
2	121	110	122	122	86	103	142	120	114	109	101
5	111	111	114	136	174	136	142	135	111	133	110
7.5	60	73	79	90	96	80	89	100	74	95	88
10	92	84	98	104	103	119	81	114	83	99	73
15	48	64	78	54	58	48	63	72	64	34	58
20	28	32	35	49	38	39	59	72	40	36	45
50	2	5	1	9	8	5	5	5	5	4	2

^aCO = Crop Oil check.

^bAverage of two replicates per experiment, and the experiment was conducted three times.

Appendix Table 2. Bentgrass and other sensitive species fresh weight as % of untreated^a plants in greenhouse experiments.

Rate	bentgrass				annual ryegrass	tall fescue	downy brome	annual bluegrass
	Highland	Seaside	Astoria	Penncross				
(g/ha)	-----(% of check) ^c -----							
CO ^b	96	96	99	104	91	92	96	98
2.5	76	62	111	83	106	103	127	115
5.0	71	73	90	88	96	103	115	145
10.0	84	94	47	59	110	67	128	78
20.0	42	37	26	32	58	36	121	85
50.0	40	10	12	11	29	17	107	75
100.0	5	5	8	3	53	10	90	70
500.0	4	3	4	2	10	7	28	108

^aGreenhouse experiments.

^bCrop Oil

^cAverage of two replicates per experiment, and the experiment was conducted three times.

Appendix Table 3. Tolerance of red fescue and bentgrass to application of sethoxydim in the field experiment.

Planting date	Time of Application	Constant ^a	Slope	GR ₅₀	R ²
Red fescue		----- (kg/ha) -----			
Fall	early	29.61	1.96	10.4	0.56
Fall	late	7.47	1.22	21.7	0.90
Spring	early	2.89	1.14	41.3	0.99
Spring	late	-0.98	1.13	45.1	0.77
bentgrass		----- (g/ha) -----			
Fall	early	21.07	0.66	43.8	0.87
Fall	late	-1.39	0.39	130.8	0.90
Spring	early	-2.19	0.89	58.7	0.97
Spring	late	11.50	0.57	67.3	0.71

^aRegression equations parameters, and GR₅₀ values.

Appendix Table 4. Effect of sethoxydim on elongation of red fescue roots germinated in petri dishes in the laboratory and grown in a range of molar concentrations^a.

Cultivar	b_0	b_1	se_{b_0}	se_{b_1}	R^2
	----- (mM) -----				
Pennlawn	90.05	-7.59	7.08	1.72	0.83
Polar	91.99	-7.72	4.20	1.02	0.93
Futura	75.17	-7.11	6.78	1.65	0.82
Hawk	116.45	-10.37	3.95	0.96	0.97
Wintergreen	100.33	-7.03	3.49	0.85	0.94
Ruby	94.99	-8.02	3.29	0.80	0.96
Cascade	93.43	-5.38	3.23	0.79	0.92
Koket	90.34	-6.93	3.59	0.87	0.94
Banner	107.14	-8.65	4.70	1.14	0.93
Waldorf	92.34	-8.01	4.20	1.02	0.94
Scarlet	101.55	-9.48	5.99	1.46	0.91
annual bluegrass	69.86	-79.91	10.20	20.3	0.84

^aAverage of two replicates per experiment and three experiments.

^bRegression equation parameters for linear regression over the range of concentrations of 10 to 0.01 mMolar. Regression equation is
Elongation(as % of check) = $b_0 + b_1(\text{concentration of sethoxydim})$

Appendix Table 5. Effect of sethoxydim on elongation of red fescue shoots germinated in petri dishes in the laboratory and grown in a range of molar concentrations^a.

Cultivar	b_0	b_1	se_{b_0}	se_{b_1}	R^2
	----- (mM) -----				
Pennlawn	92.98	-9.55	11.13	2.71	0.75
Polar	101.1	-10.28	12.44	3.03	0.74
Furtura	65.66	-6.37	4.37	1.06	0.90
Hawk	101.36	-10.44	11.78	2.85	0.77
Wintergreen	84.58	-7.97	12.9	3.14	0.62
Ruby	99.87	-9.92	16.5	4.03	0.60
Cascade	79.42	-5.48	9.48	2.30	0.58
Koket	79.80	-7.72	9.45	2.30	0.74
Banner	94.21	-8.92	11.30	2.75	0.72
Waldorf	73.35	-7.00	9.51	2.32	0.69
Scarlet	87.95	-8.96	11.92	2.90	0.70
annual bluegrass	49.90	-59.08	12.4	24.86	0.65

^aAverage of two replicates per experiment and three experiments.

^bRegression equation parameters for linear regression over the range of concentrations of 10 to 0.01 mMolar. Regression equation is Elongation(as % of check) = $b_0 + b_1$ (concentration of sethoxydim).

Appendix Table 6. Effect of sethoxydim on reduction of elongation of bentgrass and sensitive species roots to sethoxydim in the the laboratory.^a

Cultivar	b_0	b_1	se_{b_0}	se_{b_1}	R^2
	----- (nM) -----				
Highland bentgrass	110.76	-0.14	3.26	0.016	0.95
Seaside bentgrass	109.61	-0.19	7.47	0.036	0.87
Astoria bentgrass	96.35	-0.14	3.33	0.016	0.95
Penncross bentgrass	118.75	-0.17	3.84	0.018	0.95
Fawn tall fescue	95.32	-0.05	1.78	0.0085	0.89
annual ryegrass	102.31	-0.08	8.24	0.039	0.52
downy brome	108.61	-0.005	1.61	0.0076	0.11

^aAverage of two replicates per experiment and three experiments.

^bRegression equation parameters for linear regression over the range of concentrations of 0.1 to 500 nMolar. Regression equation is
Elongation(as % of check) = $b_0 + b_1(\text{concentration of sethoxydim})$

Appendix Table 7. Effect of sethoxydim on reduction elongation of bentgrass shoots and sensitive species shoots to sethoxydim in the the laboratory.^a

Cultivar	b_0	b_1	se_{b_0}	se_{b_1}	R^2
	----- (nM) -----				
Highland bentgrass	85.50	-0.18	17.35	0.083	0.53
Seaside bentgrass	56.51	-0.11	5.21	0.025	0.83
Astoria bentgrass	96.99	-0.19	8.31	0.040	0.85
Penncross bentgrass	78.31	-0.14	7.13	0.034	0.81
Fawn tall fescue	103.54	-0.18	4.50	0.021	0.91
annual ryegrass	88.12	-0.17	5.14	0.25	0.92
downy brome	95.06	-0.018	2.28	0.011	0.41

^aAverage of two replicates per experiment and three experiments.

^bRegression equation parameters for linear regression over the range of concentrations of 0.1 to 500 nMolar. Regression equation is
Elongation (as % of check) = $b_0 + b_1 * (\text{concentration of sethoxydim})$

Appendix Table 8. Effect of sethoxydim in reduction of fresh weight of red fescue grown in the greenhouse.^a

Cultivar	b_0	b_1	se_{b_0}	se_{b_1}	R^2
	----- (kg/ha) -----				
Pennlawn	126.6	-5.05	9.84	0.914	0.83
Polar	128.7	-4.79	7.75	0.72	0.88
Futura	122.1	-3.73	9.41	0.87	0.74
Hawk	146.0	-5.25	9.65	0.89	0.85
Wintergreen	152.0	-5.72	22.45	2.085	0.52
Ruby	139.0	-5.03	15.15	1.40	0.66
Cascade	145.8	-5.03	10.28	0.96	0.81
Koket	124.8	-4.17	10.24	0.951	0.75
Banner	134.0	-4.31	6.13	0.57	0.90
Waldorf	113.8	-5.14	11.71	1.09	0.78
Scarlet	113.8	-3.53	4.68	0.434	0.92

^aAverage of two replicates per experiment and three experiments.

^bRegression equation parameters for linear regression over the range of concentrations of 10 to 0.01 mMolar. Regression equation is
Elongation(as % of check) = $b_0 + b_1(\text{concentration of sethoxydim})$

Appendix Table 9. Effect of sethoxydim on reduction of fresh weight of bentgrass cultivars and sensitive species grown in the greenhouse^a.

Cultivar	b_0	b_1	se_{b0}	se_{b1}	R^2
	----- (g/ha) -----				
Highland bentgrass	92.5	-2.99	10.23	1.17	0.57
Seaside bentgrass	87.83	-3.27	10.37	1.89	0.60
Astoria bentgrass	111.1	-4.62	5.65	0.65	0.91
Penncross bentgrass	89.7	-3.65	9.25	1.06	0.70
Fawn tall fescue	86.7	-0.16	8.70	0.054	0.56
Marshall ryegrass	108.0	-3.46	5.80	0.67	0.84
downy brome	115.4	-0.18	4.17	0.026	0.85
annual bluegrass	No response for this range of rates.				

^aAverages of two replicates per experiment, and three experiments.

^bRegression equation parameters for a range of rates from 0.62 to 20 g/ha for species with GR_{50} 's of less than 50 g/ha, and from 0.62 to 500 g/ha for species with GR_{50} of over 100 g/ha.

Appendix Table 10. Soybean Metabolism: Combined data from Experiments EXP 84-012, 84-013 and 85-018

Experiment No.	85-018	84-012	84-013	85-018	84-012	84-013	84-006	Average
Treated Leaves								
	-----DPM-----			% of ¹⁴ C in band at corresponding Rf Value				
3 days								
0.00	210	470	480	20	26	51	57	32
0.25	90	250	240	9	13	26	8	16
0.56	640	1030	70	60	52	7	18	40
1.00	110	160	140	10	8	15	15	11
6 days								
0.00	70	790	710	34	40	64		46
0.25	90	340	190	50	13	18		27
0.56	20	1060	90	9	37	8		18
1.00	10	190	110	5	8	9		7
Untreated Leaves								
3 days								
0.00	60	50	12	39	16	31	24	28
0.25	50	20		33	7	15	10	19
0.56	10	200	170	9	69	43	42	40
1.00	30	20	40	17	7	9	22	11
6 days								
0.00	350	160	250	41	23	56		40
0.25	150	90	110	16	13	24		18
0.56	260	340	50	30	51	10		30
1.00	90	80	40	11	12	8		10

Appendix Table 11. Metabolites extracted from red fescue and bentgrass plants treated with 2.5nm ^{14}C and extracted with methanol.

Rf Value	Bentgrass	Red Fescue	Bentgrass	Red Fescue
	-----DPM-----		^{14}C (as % of total)	
Treated Leaf				
3 day				
0.00	1079	774	37	27
0.25	278	234	9	8
0.50	658	1143	23	40
1.00	807	702	28	24
6 day				
0.00	1751	1478	53	55
0.25	469	247	14	10
0.50	473	406	14	16
1.00	568	448	17	17
Remainder of Leaves				
3 day				
0.00	43	22	32	7
0.25	19	110	22	23
0.50	14	90	12	30
1.00	36	275	32	38
6 day				
0.00	68	30	52	38
0.25	2	15	0	8
0.50	5	49	0	41
1.00	80	19	42	12

^aMean of 4 plants from Experiment 84-009.

Appendix Table 12. Recovery of ^{14}C sethoxydim in metabolism experiments from red fescue and bentgrass^a.

Plant Part	Bentgrass	Red Fescue	Bentgrass	Red Fescue
	-----DPM-----		^{14}C (as % of total)	
3 day				
Treated Leaf	38497	40625	63.5	67.3
Other Leaves	3107	2107	5.1	3.5
Roots	350	210	0.6	0.3
Solution	2086	704	3.4	1.2
6 day				
Treated Leaf	42676	45426	70.4	75.3
Other Leaves	1851	1872	3.1	3.1
Roots	214	1011	0.4	1.7
Solution	1957	524	3.2	0.9

^aExperiments 84-009 and Experiments 84-106

Appendix Table 13. Loss of ^{14}C in bentgrass and red fescue after application of 2.5 nmol of sethoxydim applied as a 1 ul droplet.

Plant Part	Days	Bentgrass	Red Fescue	Bentgrass	Red Fescue
		----DPM----		^{14}C (as % applied)	
Treated	0	110000	98418	100.0	100.0
	1	89315	55909	81.2	56.8
	3	81723	42797	74.3	43.5
	5	77035	44034	70.0	44.7
	7	82167	38184	74.7	38.8
Leaves	0	0	0	0.0	0.0
	1	8536	4629	7.8	4.7
	3	8646	18696	7.9	19.0
	5	14255	11525	13.0	11.7
	7	13100	19197	11.9	19.5
Roots	0	0	0	0.0	0.0
	1	913	195	0.8	0.2
	3	2091	2412	1.9	2.5
	5	1600	1069	1.5	1.1
	7	594	1327	0.5	1.3
Solution	0	0	0	0.0	0.0
	1	840	7148	0.8	7.3
	3	15639	5561	14.2	5.7
	5	12566	6025	11.4	6.1
	7	9209	9196	8.4	9.3

^aData is average of 4 replicates and samples oxidized in sample combuster. Experiment 85-16B.

Appendix Table 14. Loss of ^{14}C in Red Fescue and Bentgrass in experiment to measure potential loss of CO_2^{a} .

Plant Part	Bentgrass	Red Fescue	Bentgrass	Red Fescue
	----DPM----		^{14}C (as % applied)	
Treated Leaf	192700	154520	66.5	53.3
Other Leaves	49440	30180	17.1	10.4
Roots	2441	1271	0.8	0.4
Solution	17610	1780	6.1	0.6

^aDPM and loss as % of total ^{14}C applied as three 1 ul droplet on 3 separate leaves and grown in a sealed container for up to 72 hours.

Exp No 85-017.

Appendix Table 15. Changes in analysis of sethoxydim in vitro^a.

Rf value	<u>bentgrass</u>		<u>fescue</u>		<u>standards</u>	
	time 1 ^b	time 2	time 1	time 2	std 1	std 2
0.0	0.8 ^c	0.8	41.6	0.1	2.4	0.8
0.1	0.0	1.2	6.1	.0	1.1	0.4
0.2	0.0	0.7	4.7	0.1	2.0	0.3
0.3	0.6	0.7	0.5	0.2	1.6	0.4
0.4	0.2	1.7	4.5	0.0	0.6	21.5
0.5	1.8	0.5	4.6	1.7	0.3	0.8
0.6	10.9	2.5	5.1	0.5	30.5	1.5
0.7	5.7	2.1	1.6	0.3	1.3	0.9
0.8	5.1	4.8	2.8	1.7	2.1	1.9
0.9	68.1	84.1	28.6	7.4	53.3	70.4
1.0	6.8	1.7	0.0	87.9	4.8	1.1

^aExpt85-015

^bTime 1 was before the samples were dried with pre-pure N₂, and time 2 was after drying the sample with pre-pure N₂.

^cProportion of ¹⁴C at that Rf value.