RESISTANCE TO BEACON IN DOWNY BROME

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Introduction

Downy brome (*Bromus tectorum*) is widely distributed throughout the western United States and is a major weed of Kentucky bluegrass grown for seed in the Pacific Northwest. Prior to the recent registration of primisulfuron (Beacon), growers relied on terbacil (Sinbar), metribuzin (Sencor or Lexone), and high rates (2 lb/a) of dicamba (Banvel) for control of downy brome. While terbacil is relatively effective if applied at maximum labeled rates preemergence to downy brome and moved into the soil with irrigation or timely rainfall, Kentucky bluegrass tolerance is quite marginal. Growers often damage their stands with treatments that achieve only partial weed control. While metribuzin has better crop safety than terbacil, downy brome control is extremely variable from year to year. Efficacy of dicamba is strongly dependent on downy brome size in late fall and severity of winter weather. Dicamba treatments often result in total weed control failures. Neither terbacil, metribuzin, nor high rates dicamba can be used in seedling stands of Kentucky bluegrass. Because of problems with all three of these older herbicides, growers were eager to adopt use of primisulfuron on Kentucky bluegrass grown for seed. We included this herbicide in many of the treatment combinations in long-term residue management by herbicide treatment studies conducted in Kentucky bluegrass grown for seed at Madras and La Grande, OR. Because resistance to ALS inhibitors has sometimes developed within a few years of use, we saved samples of downy brome seed from several treatments with and without primisulfuron over the duration of these studies.

Materials and Methods

Kentucky bluegrass was seeded in 1992 at Madras and La Grande, and no grass control herbicides were applied before the first grass seed harvest in 1993. Post-harvest residue management treatments (field burn, bale/flail chop/rake, and 'vacuum sweep') were applied annually to the 60 by 135 ft main plots from 1993 through 1996, and herbicide treatments were generally reapplied to the same 9 by 60 ft. subplots from fall 1993 through fall 1995. However, a few herbicide treatments were altered during that period because of excessive crop damage or excessive downy brome density. Herbicides were applied with a pull-type plot sprayer supplying 26 gal/a at 30 psi through 8003 flat fan nozzles. All primisulfuron treatments included 0.25% R-11 surfactant. Average time between the two applications was 8 weeks. In the 1996-97 growing season, 3 out of 15 plots in each residue management block were untreated checks. The other 12 plots received an early fall treatment of primisulfuron plus terbacil followed by a late fall treatment of either primisulfuron plus terbacil or primisulfuron alone where Kentucky bluegrass stands were thin. Downy brome growth at the early fall applications ranged from the 1-leaf to 4-leaf stage, while it ranged from the 4-leaf to the 12-tiller stage at the late fall applications. Weed density was measured by counting all downy brome plants present in a 7-ft-wide strip centered each plot. In cases where downy brome density exceeded 1000 plants/plot, sub-sampling was used. A swather was used to cut 5-ft-wide strips out of the centers of the plots, after which the adjacent edges of the plots (2 ft + 2 ft) were swathed together. Windrows were picked up and threshed with a plot combine when dry. Seed from each plot was bagged.

Samples of downy brome seed were collected during the cleaning of Kentucky bluegrass seed harvested from 1994 to 1997, and a 55-plot subset of these samples was screened in the greenhouse for resistance to 0.0352 lb/a (0.75 oz product/a) primisulfuron plus 0.25% surfactant applied at the 2-leaf growth stage. Greenhouse-grown downy brome was treated using a compressed-air spray chamber supplying 27 gal/a at mid-canopy height at 30 psi. In the rate response study, primisulfuron was applied at 0.0312, 0.0625, 0.125, 0.25, 0.375, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 16, and 32 times the standard 0.0352 lb/a rate. The greenhouse screening trial was conducted twice. Because quantities of seed from the 1994 and 1995 harvests were extremely limited, only seed from the 1996 harvest was used in the first study. The second study was conducted with seed from all three years, exhausting the seed supply for many of the plots. Results from the two studies were similar, and only data from the second study are shown.

Field Observations

Primisulfuron provided 81 to 94% control of downy brome when split-applied in early and late fall (Table 1). A split-application of primisulfuron plus terbacil tank-mixes provided 98 to 100% control for the first two years, dropping to 97% control at Madras in the third year. By 1996, downy brome control in several plots treated with various combinations of primisulfuron and terbacil for three consecutive years was noticeably lower. Whether downy brome survival in those plots was due to sprayer skips, different weather patterns, or increased herbicide resistance was not clear. The replication to replication variability in downy brome density in primisulfuron plus terbacil treatments in the 1995-96 growing season was high, with counts per plot ranging from 0 to 572 for one treatment. Downy brome density in 11 of 36 plots at Madras treated with the most effective combinations of primisulfuron plus terbacil was at least 10 times higher than in the other 25 plots, strongly suggesting that herbicide resistance was developing. Since successful reproduction is the most critical step in evolution, we classified downy brome in individual field plots as resistant if its density increased over the previous year. This definition says that a weed is resistant if it can increase in abundance from year to year when treated with the same herbicide. Because our pattern of swathing and combining (centers of plots and pooled edges of plots) redistributed weed seed passing through the combine, effective downy brome density for the previous year in the neighborhood of each plot was calculated as one-half of the density observed in that plot plus one-quarter of the density observed in each of the two neighboring plots. This calculation ignores the effects of seed shattering within a plot before combining and those of seed blowing between plots at any time.

Uniform application of split-applied primisulfuron plus terbacil at the Madras and La Grande test sites in the fall of 1996 helped to clarify the situation. Downy brome density in the untreated checks increased by 33-fold at Madras and 47-fold at La Grande (Table 2). In 100 plots at each site, the primisulfuron plus terbacil treatment performed as it had in the first two years of the study, controlling of 99% of the downy brome. In the other 44 treated plots at each site, downy brome density increased an average of 9-fold at Madras and >3-fold at La Grande, or control relative to the untreated checks of 73% at Madras and 93% at La Grande. When the same data were grouped by previous year treatment rather than by plot, 20 treatments at Madras and 25 treatments at La Grande showed no signs of resistance, and achieved 99% control of downy brome. However, 16 treatments at Madras and 11 treatments at La Grande did show resistance, with downy brome densities increasing an average of 6-fold and 3-fold, respectively. Interestingly, only one of the 16 treatments showing resistance at Madras came from the field burn residue management treatment, while the other 15 treatments were nearly equally divided between ‘vacuum-sweep’ and bale/flail chop/rake residue management.

Greenhouse Screening

A 55-plot subset of samples of downy brome seed collected from Kentucky bluegrass seed harvested from 1994 to 1996 was screened in the greenhouse for resistance to 0.0352 lb/a primisulfuron applied at the 2-leaf stage. Progression of symptoms on downy brome treated with primisulfuron included a rapid cessation of growth and a gradual discoloration of the treated leaves, followed by the appearance of healthy, new tillers at the base of the plant within two to three weeks after treatment for the resistant biotypes. Plants that did not begin to regrow within three to four weeks after treatment eventually died. Because all downy brome plants from Madras and La Grande were initially injured by primisulfuron, resistance was simply measured as the ability to survive and regrow. Average downy brome survival increased from 5.9% for seed harvested in 1994, to 7.7% for 1995, and to 19.7% for 1996 (Table 3). Of particular concern was the increase over time in plots where primisulfuron failed to achieve 88% or better control. There were 2 such plots out of 51 tested for the 1994 seed, 8 out of 50 for the 1995 seed, and 19 out of 55 for the 1996 seed.

Dose response curves were generated for seed harvested from three plots (410, 301, and 344) at Madras in 1996, and the $LD_{98\%}$ for the most resistant type (plot 344) was 7-fold greater than for the most susceptible type (Fig. 1). $LD_{98\%}$ for plot 301 was 2-fold greater than plot 410. Plot 344, the most resistant type, had a 6-fold increase in downy brome density in the field from 1995 to 1996 (data not shown). The two resistant biotypes in seed from the 1994 harvest came from plots 236 and 238, which were centered only 18 ft. apart, suggesting a possible common origin for the resistance sometime prior to 1994.

Figure 1. Response to primisulfuron rates by downy brome seed archived from the 1996 harvest of three plots at Madras, OR. Logistic regressions of mortality versus rate (solid lines). Note logarithmic rate axis. Rates ranged from 32X to 1/32X the standard 0.75 oz product/a treatment.

Resistance Mechanism

ALS activity in the three biotypes used in the dose response trial plus another known susceptible was similarly inhibited by imazapyr (Arsenal) and chlorsulfuron (Glean) (Dale Shaner, pers. comm.), implying that resistance was not target-site based. Other greenhouse studies showed that primisulfuron-resistant biotypes were cross resistant to sulfosulfuron (Maverick). Phytotoxicity of sulfosulfuron on the resistant types was dramatically increased by tank-mixing with an organophosphate insecticide, implying that resistance was based on metabolic degradation of sulfonylurea herbicides.

Conclusions

Primisulfuron use in Kentucky bluegrass grown for seed rapidly selected downy brome biotypes with resistance to herbicide. The speed with which populations of this weed evolved resistance suggests that grass seed growers must be vigilant if they hope to continue using this herbicide. Cross resistance to sulfosulfuron suggests that wheat growers also will need to worry about resistant downy brome. Because this resistance evolved within a controlled test plot, we know more about the factors leading to this case of herbicide resistance than is usually known.

Edge effects probably played a major role in the increase in resistance over time. Effective and ineffective treatments were often adjacent, with the spray pattern edge of an effective treatment providing a gradually decreasing rate of herbicide into the first 6 inches of an adjacent plot. Full rates of primisulfuron were needed to control even the most susceptible type, with survival rapidly increasing below the 0.5X rate. The harvest technique helped move weed seeds between adjacent plots. Tank-mixes of herbicides with alternate modes of action are often suggested as ways to slow the development of resistance. Such tank-mixes were present in these field trials, and were overcome by the resistance that downy brome developed. However, it is possible that the resistance evolved in the boundaries between neighboring treatments, and uniform applications of primisulfuron plus terbacil over an entire field might have remained effective much longer than they did as individual treatments within a larger test. The extremely high density of downy brome in the untreated checks and in those treatments that never were effective may have aided the rapid evolution of resistance by providing a large gene pool in which primisulfuron could select a resistant biotype.

Ongoing Studies and Future Plans

The dose response curves are being repeated for the three biotypes and for one additional plot from Madras, two plots from La Grande, and a putative target site mutant collected in the Columbia River Basin by Dr. Dan Ball. Preliminary results confirm the dose-response curves shown in Figure 1, and strongly suggest that the Columbia River basin biotype does possess target site resistance. Seed produced in the greenhouse from the first two greenhouse trials will be tested to determine whether the percent of resistant progeny has increased in the primisulfuron survivors. We hope to determine whether the seed from the field plots consists of a mixture of susceptible and resistant types. Seed from the 1997 harvest is available for nearly all of the 180 plots at each field site. We plan to screen this seed to produce a detailed map of the distribution of resistance across the 2.5 acre area of each site. These 9 ft by 60 ft grid maps will then be analyzed for spatial patterns, potentially locating the initial sources of resistance.

Table 1. Control of downy brome in Kentucky bluegrass grown for seed using primisulfuron alone or with split-applications of primisulfuron + terbacil.

Location	Timing	Rate	1993-94	1994-95	1995-96
		(lb a.i./a)	----------------- (% control)		-------------------
<u>Madras</u>					
Primisulfuron / Primisulfuron	Early fall / Late fall	0.0176 / 0.0176	82	94	91
Primisulfuron + terbacil* / Primisulfuron + terbacil*	Early fall / Late fall	0.0176 + 0.375 / 0.0176 + 0.375	98	99	97
<u>La Grande</u>					
Primisulfuron / Primisulfuron	Early fall / Late fall	0.0176 / 0.0176	81	92	83
Primisulfuron + terbacil* / Primisulfuron + terbacil*	Early fall / Late fall	0.0176 + 0.375 / 0.0176 + 0.375	100	98	99

*Terbacil rate in 1995-96 was reduced to 0.3 lb/a due to crop injury from terbacil carryover.

Table 2. Response of downy brome in Kentucky bluegrass grown for seed to split-applications of primisulfuron plus terbacil in 1996-97 following various herbicide treatments for previous three years.

Downy brome population increases over previous year's population (by category)	Madras	LaGrande
	-------------- (ratio 1997 to 1996)	----------------
Untreated checks	33.2X	47.1X
Treatments where resistance had developed	6.1X	3.1X
No. of treatments with apparent resistance	16	11
Control relative to untreated checks	82%	93%
Treatments without resistance	0.4X	0.5X
No. of treatments without resistance	20	25
Control relative to untreated checks	99%	99%
Plots where resistance had developed	8.9X	3.4X
No. of plots with apparent resistance	44	44
Control relative to untreated checks	73%	93%
Plots without resistance	0.3X	0.3X
No. of plots without resistance	100	100
Control relative to untreated checks	99%	99%

Treatments or plots were classified as having resistance if the ratio of downy brome density in 1997 to 1996 exceeded 1.

Table 3. Response of archived BROTE seed to primisulfuron.

Response of BROTE at 2-leaf stage to 0.0352 lb/a primisulfuron	Year of seed production		
	1993-94	1994-95	1995-96
	-----------------------(no. of plots in each category)-----------------------		
archived seed not viable	7	0	0
100% mortality	26	19	18
95-99% mortality	8	17	9
88-95% mortality	8	6	9
80-88% mortality	0	2	1
70-80% mortality	0	3	4
50-70% mortality	2	2	7
<50% mortality	0	1	7
Total no. of samples tested	51	50	55
Total no. of seedlings treated	1393	4303	3972
% of seedlings surviving	5.9	7.7	19.7

Primisulfuron was first applied in the 1993-94 growing season. BROTE seed was collected during cleaning of Kentucky bluegrass seed, and samples from selected treatments at Madras and LaGrande were archived in cold storage.