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Ecological fitness of acetolactate synthase inhibitor–resistant and –susceptible downy brome (*Bromus tectorum*) biotypes

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Studies were conducted to determine the relative fitness and competitive ability of an acetolactate synthase (ALS) inhibitor–resistant (R) downy brome biotype compared with a susceptible (S) biotype. In previous research, the mechanism of resistance was determined to be an altered ALS enzyme. Seed germination of the R biotype was compared with that of the S biotype at 5, 15, and 25 C. There were no different germination characteristics between R and S biotypes at 15 and 25 C. However, the R biotype germinated 27 h earlier than the S biotype and had reached over 60% germination when the S biotype initially germinated at 5 C. Under noncompetitive greenhouse conditions, growth of the R biotype was similar to that of the S biotype on the basis of shoot dry weight, leaf area, and plant height. Seed production of the R biotype was 83%, when compared with the S biotype, but seeds of the R biotype were larger than those of the S biotype. Replacement series experiments were conducted in the greenhouse to determine the relative competitive ability of R and S biotypes. No difference in competitive ability was observed between R and S biotypes on the basis of shoot dry weight, leaf area, or plant height. Thus, it appears that ALS-resistance trait is not associated with growth penalty in either noncompetitive or competitive conditions. In the absence of ALS inhibitors, these results suggest that the R biotype would remain at a similar frequency in a population of R and S biotypes.

Nomenclature: Downy brome, *Bromus tectorum* L. BROTE.

Key words: Herbicide resistance, ALS inhibitor, fitness, germination, competition.

Since the first triazine-resistant weed common groundsel (*Senecio vulgaris* L.) was discovered, herbicide resistance has been reported in most herbicide classes (18 classes) and in 171 weed species (Heap 2004; Ryan 1970). Such weed populations are rapidly increasing in number. Acetolactate synthase (ALS) inhibitors are one of the most important herbicide classes used in many cropping systems. The first resistance selected with ALS inhibitors was reported in prickly lettuce (*Lactuca serriola* L.) in 1987, only 5 yr after introduction of the sulfonylurea (SU) herbicides (Mallory-Smith et al. 1990). Since then, 83 weed species have developed resistance to ALS inhibitors (Heap 2004).

Models for the evolution of herbicide resistance include fitness as an important component (Gressel and Segel 1978, 1982; Maxwell et al. 1990). Evolutionarily, fitness can be defined as survival and reproductive success. Relative fitness between herbicide-resistant (R) and -susceptible (S) biotypes is important in population dynamics when the herbicide has been removed from the system (Maxwell et al. 1990). The population structure will be determined by the relative fitness of each biotype in the absence of the herbicide. The relative fitness of R and S biotypes is determined by survivorship of seeds and plants, and reproductive success is determined by seed production and competitive ability.

Considerable research on the relative fitness of herbicide-R and -S biotypes has been reported. Triazine-R biotypes have been shown to be less fit and competitive than S biotypes in many weed species. However, no consistent differences have been measured in the relative fitness of ALS inhibitor–R biotypes (Saari et al. 1994). No difference in growth rates was observed in SU herbicide–R and –S kochia [*Kochia scoparia* (L.) Schrad.] biotypes (Christoffoleti et al. 1997; Thompson et al. 1994b). However, it has been reported that an SU herbicide–S prickly lettuce biotype produced 31% more shoot dry weight and accumulated biomass 52% faster than the R biotype (Alcocer-Ruthling et al. 1992a). The relative competitiveness of an SU herbicide–R biotype was compared with the S biotype in prickly lettuce and kochia (Alcocer-Ruthling et al. 1992a; Thompson et al. 1994b). The R biotype had similar competitiveness when compared with the S biotype in both plant species.

An ALS inhibitor–R downy brome biotype was identified in Kentucky bluegrass (*Poa pratensis* L.) seed fields at Athena, OR, in 1998 (Ball and Mallory-Smith 2000). Previous research showed that the R biotype was resistant to the ALS inhibitors, primisulfuron, sulfosulfuron, and propoxycarbazone-sodium (Park and Mallory-Smith 2004). The resistance mechanism of this biotype was an altered target site.

The objective of this research was to determine the relative fitness of the R biotype, compared with the S biotype, regarding seed germination, growth rate, seed production, and competitiveness. This information will add insight into potential fitness differences between the R and S biotypes. Differential fitness, if it exists, could be helpful in developing resistance management strategies.

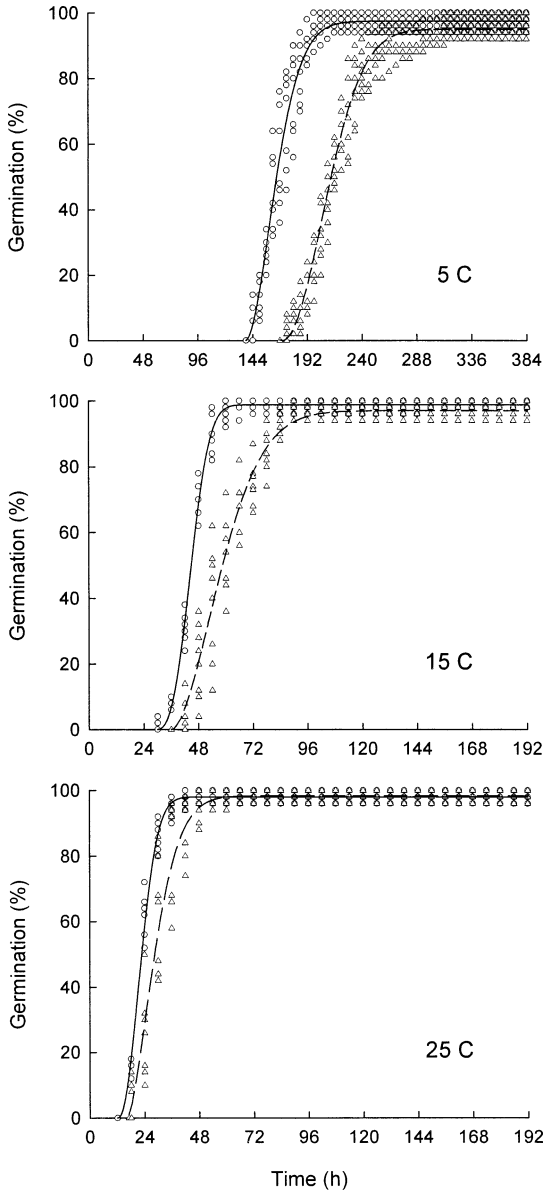

FIGURE 1. Cumulative germination percentage for acetolactate synthase inhibitor–resistant (R) ○ and –susceptible (S) △ downy brome biotypes at 5, 15, and 25 C. Solid lines are regression curves for the R biotype, and dashed lines are regression curves for the S biotype.

Materials and Methods

Plant Material

Seeds of R and S biotypes were collected from Kentucky bluegrass seed fields near Athena, OR, in 1998. Seed samples were screened to confirm resistance or susceptibility to primisulfuron in the greenhouse with rates equivalent to 40 g ai ha^{-1} primisulfuron (data not shown). The seeds were vernalized at 5 C for 6 wk, and then 10 plants from each biotype were grown in the greenhouse with a 25/20 C day/night temperature and a 16-h photoperiod. After the plants senesced, seeds from 10 plants were collected and combined by biotype. The seeds were cleaned and stored at room temperature until experiments were initiated.

Germination Test

Seed germination of R and S biotypes was compared at constant 5, 15, and 25 C in germination chambers[1] with 24-h light. Fifty seeds of each biotype were placed on two Whatman No. 2 filter papers in 10-cm-diam plastic petri dishes. Three milliliters of distilled water was placed in each petri dish and was subsequently added when required. At 6-h intervals, germinated seeds were counted and removed from the petri dishes. A seed was counted as germinated when the length of radicle was greater than the seed diameter. After 3 wk, the viability of ungerminated seeds was tested with tetrazolium chloride (Peters 2000).

Experiments were conducted in a completely randomized design with four replications and were repeated. The same seed sources and germination chambers[1] were used for both experiments. The cumulative germination was calculated with the Weibull equation (Brown and Mayer 1988; Shafii et al. 1991):

$$y = M\langle 1 - \exp\{-[K(t - L)]^C\}\rangle \quad [1]$$

where y is the cumulative germination percentage at time t and M, K, L, and C are empirically derived constants. M is the maximum cumulative germination, K the rate of increase, L the lag in germination, and C the shape parameter. Contrast procedures for M, K, and L parameters and for the joint hypotheses of M, K, L, and C parameters were conducted between R and S biotype models at all temperature regimes using PROC IML within SAS (SAS 1987).

Germination parameters and standard errors for all biotypes at each temperature were similar and so data were combined over experiments. All parameter estimates were significantly different from zero ($P < 0.001$) on the basis ofasymptotic t tests, suggesting that the model was reasonable and all parameters were required in the model (data not shown). The lack of fit tests showed that there was no significant lack of fit in any models with the lowest P value of 0.369, indicating that the regression functions fit the data (data not shown).

Comparative Growth and Seed Production

Seeds of each biotype were vernalized at 5 C for 6 wk. The seedlings were transplanted into 15-cm-diam by 20-cm-deep plastic pots containing commercial potting mix[2] and fertilized with Osmocote.[3] Plants were grown in the greenhouse under 25/20 C day/night temperature and a 16-h photoperiod. A plant of each biotype from each replication was harvested at 5-d intervals. The first harvest was at 15 d after transplanting (DAT), and the last harvest was at 80 DAT, resulting in 14 harvest dates. Plants were cut at the soil surface, and leaf area and plant height were measured. Leaf area was determined with a Li-COR stationary leaf area meter.[4] The plants were dried at 75 C for 72 h and weighed. Ten plants of each biotype were retained for seed production. After plants senesced, seeds of each biotype were harvested and cleaned. The number and weight of seeds per plant were measured.

The experiment was a randomized complete block design with four replications and was repeated. Experiments were begun in August 2000 and 2001, respectively. The plant height, leaf area, and shoot dry weight data collected from both experiments were combined.

TABLE 1. Parameter estimates for germination models of acetolactate synthase inhibitor–resistant (R) and –susceptible (S) downy brome biotypes at temperatures 5, 15, and 25 C calculated by the Weibull equation, $y = M\,[1 - \exp(-(K(t - L))^C)]$, and contrasts for *M, K,* and *L* parameters and for the joint hypotheses of *M, K, L,* and *C* parameters.

Temperature	Parameter[a]	Parameter estimates		
		R (SE[b])	S (SE[b])	$F\,(P_r > F)$
5 C	*M*	97.43 (0.335)	94.97 (0.412)	4.34 (0.038)
	K	0.03 (0.002)	0.02 (0.002)	0.72 (0.396)
	L	137.80 (2.287)	164.70 (4.577)	4.24 (0.040)
	C	1.76 (0.162)	2.30 (0.236)	30.73 (0.001)[c]
15 C	*M*	98.52 (0.289)	97.19 (1.053)	0.39 (0.536)
	K	0.06 (0.006)	0.04 (0.004)	0.52 (0.473)
	L	29.02 (1.845)	36.51 (2.818)	0.52 (0.473)
	C	2.79 (0.349)	1.71 (0.253)	17.41 (0.001)[c]
25 C	*M*	98.22 (0.215)	97.36 (0.311)	0.01 (0.979)
	K	0.11 (0.007)	0.07 (0.004)	0.24 (0.622)
	L	15.41 (0.434)	20.00 (0.813)	2.14 (0.145)
	C	1.41 (0.106)	1.56 (0.120)	1.49 (0.206)[c]

[a] Abbreviations: *M*, maximum cumulative germination; *K*, rate of increase; *L*, lag in germination; and *C*, shape parameter.
[b] Asymptotic standard errors for estimated parameters.
[c] Contrast for joint hypotheses of parameter estimates (*M, K, L,* and *C*).

Shoot dry weight (W) at time t was fit using the Richards function:

$$W = A[1 \pm \exp(b - kt)]^{-1/n} \quad [2]$$

where parameter A is the asymptotic maximum shoot dry weight, b the time when the curve rises above zero, k a rate constant, and n the shape of the curve (Hunt 1982; Richards 1959). Leaf area and plant height data also were fit to the Richards function. The variability of the data increased as the time increased. To overcome unequal variance, the data for the shoot dry weight were analyzed by weighted nonlinear squares with time^{-3}, and the data for the leaf area and plant height were analyzed by weighted nonlinear squares with time^{-2}. The seed number and seed weight in R and S biotypes were compared using the PROC TTEST procedure at a significance level of 0.05.

Competitive Growth

The relative competitive ability of the R and S biotypes was compared under greenhouse conditions. Seeds of each biotype were vernalized at 5 C for 6 wk and then planted in 98 cell trays (26 ml cell^{-1}). Fourteen d after planting (DAP) at the three- to four-leaf stage, plants were transplanted into pots that were 40 by 40 cm with a volume of 48 L containing a commercial potting mix[2] fertilized with 50 g of Osmocote[3] per pot. Competition between biotypes was evaluated using a replacement series experiment at five biotype proportions (100:0, 75:25, 50:50, 25:75, and 0:100) at a constant planting density of 100 plants m^{-2} (16 plants pot^{-1}). Plants were grown in a greenhouse with 12-h supplemental lighting and 20/15 C day/night temperature. The experiment was conducted in a randomized complete block design with four replications and was repeated. Experiments were begun in November 2001 and February 2002, respectively. Pots were rearranged within each block every 5 d to reduce variation associated with differences in light regime. Plants were cut at the soil surface 60 DAT. Individual plant height and leaf area were measured.

Plants were dried at 75 C for 72 h and weighed for shoot dry weight. No edge effect was found between edge and nonedge plants on the basis of a Student's t test. Individual plant height, leaf area, and shoot dry weight data were subjected to analysis of variance (ANOVA). The experiment-by-treatment interaction was not significant, and data from the repeated experiments were combined. Total shoot dry weight and leaf area were compared with the theoretical yields for equal competitive ability using 95% confidence intervals. Statistical computations were carried out using PROC GLM within SAS (SAS 1987).

Results and Discussion

Germination Test

Total seed germination for R and S biotypes exceeded 95% at 5, 15, and 25 C (Figure 1). Ungerminated seeds from the 5 and 15 C treatments were viable (94 and 88% viable, respectively), on the basis of the tetrazolium test. No viable seeds remained from the 25 C treatment because seeds deteriorated. There was no difference in the number of ungerminated viable seeds between R and S biotypes on the basis of the tetrazolium test (data not shown).

The maximum cumulative germination between R and S was not different at 15 and 25 C (Table 1). Although a contrast procedure showed that the maximum cumulative germination was different between R and S biotypes at 5 C, the difference was only 2.5% (Table 1). Seeds of the R biotype germinated 27 h sooner than the S biotype at 5 C (Table 1). Germination onset between biotypes was not different at 15 and 25 C. The rate of germination for both R and S biotypes increased as temperature increased (Table 1). There were no differences in the rate of germination between R and S biotypes at any temperature regime. The *F* statistics comparing the joint hypothesis of *M, K, L,* and *C* parameters for germination models of R and S biotypes decreased as temperature increased and provided no significant difference at 25 C (Table 1). These results confirmed that differences in germination processes between R and S biotypes were greater as temperatures decreased.

Differences in germination between the R and S biotype might be important because downy brome germinates dur-

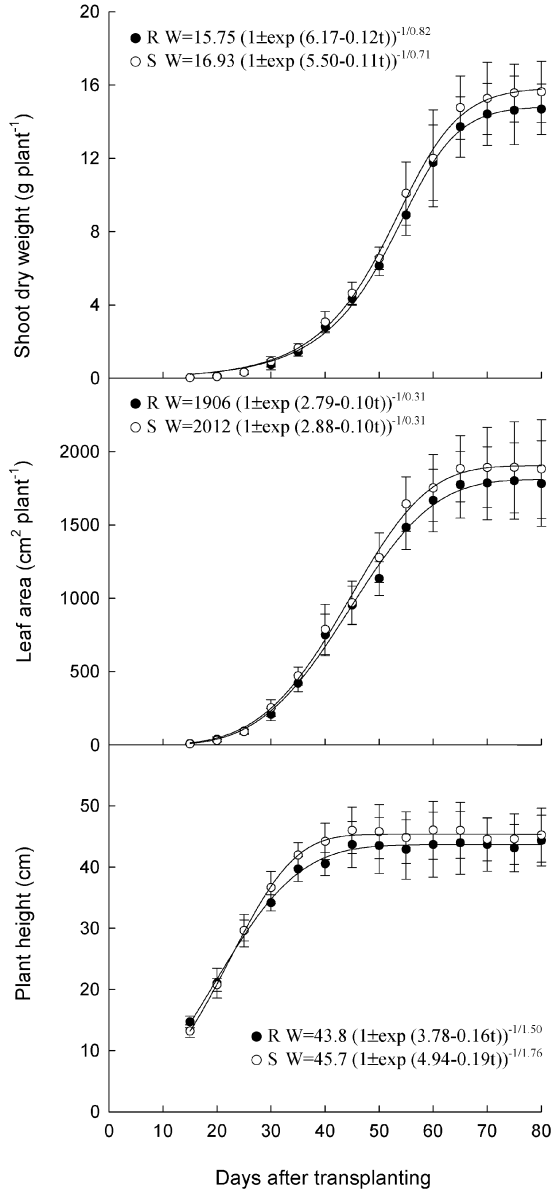

FIGURE 2. Regression curves and 95% confidence intervals for shoot dry weight, leaf area, and plant height of acetolactate synthase inhibitor–resistant (R) and –susceptible (S) downy brome biotypes under noncompetitive conditions. Nonlinear regression equation parameters, calculated using the Richards function: $W = A[1 \pm \exp(b - kt)]^{-1/n}$, where A is the asymptote, b the time when the curve rises above zero, k a rate constant, and n a shape parameter.

ing the late fall or winter when temperatures are cooler. Similar results were observed in SU-R Bibb lettuce (*Lactuca sativa* L.) isoline and kochia species (Mallory-Smith et al. 1992; Thompson et al. 1994a). The R near-isogenic line of Bibb lettuce germinated faster than the S line (Mallory-Smith et al. 1992). Dyer et al. (1993) showed that seeds of an SU-R kochia population germinated faster than seeds from an S population at 4.6 C but not at 10.5 C. They proposed that elevated levels of branched chain amino acids in the resistant seed could be responsible for faster germination. Thompson et al. (1994a) found that the difference in the rate of germination between R and S biotypes of kochia was temperature-dependent with the R biotype germinating faster at 8 and 18 C but not at 28 C.

TABLE 2. Seed production for acetolactate synthase inhibitor–resistant (R) and –susceptible (S) downy brome biotypes under noncompetitive conditions.

Biotype	Total seed	Seed weight	
	no. plant^{-1}	g 300 seeds^{-1}	g plant^{-1}
R	2,390 (88.3)[a]	0.89 (0.028)	6.54 (0.23)
S	2,883 (80.2)	0.73 (0.024)	6.61 (0.22)

[a] Standard errors are in parentheses.

Comparative Growth and Seed Production

Figure 2 shows the accumulation of shoot dry weight, leaf area, and plant height of R and S downy brome biotypes under noncompetitive conditions. The data showed a sigmoidal growth pattern. The maximum shoot dry weight and leaf area were reached at 70 DAT. The maximum plant height was reached at 40 DAT. Shoot dry weight, leaf area, and plant height of the R biotype were similar to those of the S biotype. Other parameter estimates for shoot dry

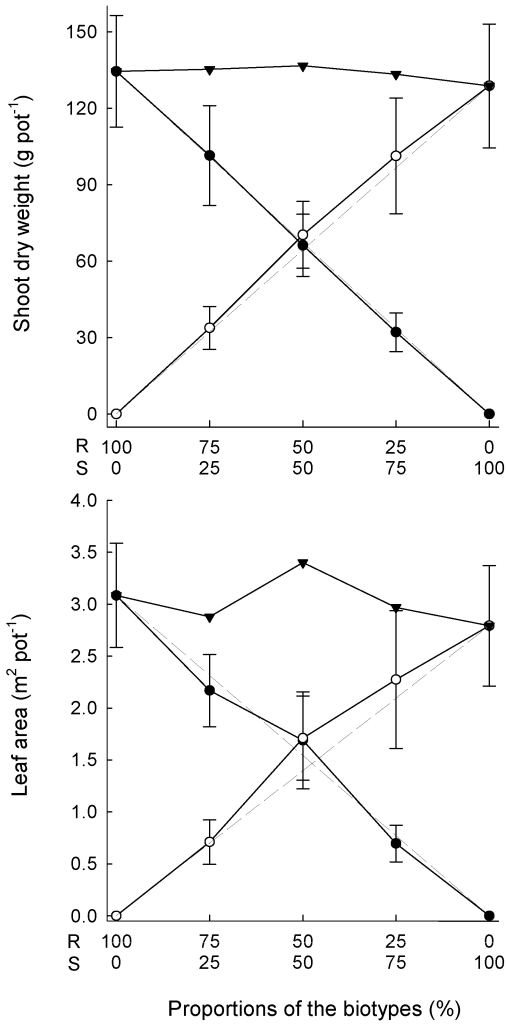

FIGURE 3. Shoot dry weight and leaf area per pot produced by acetolactate synthase inhibitor–resistant (R) or –susceptible (S) downy brome biotypes (R ●, S ○, and total ▼) grown at five proportions with a constant density (16 plants pot^{-1}). Dashed lines indicate theoretical shoot dry weight when the biotypes are equally competitive. Vertical bars represent 95% confidence intervals of the mean.

TABLE 3. Individual shoot dry weight, leaf area, and plant height for acetolactate synthase inhibitor–resistant (R) and susceptible (S) downy brome biotypes in a replacement series experiment at five proportions.

		Proportions of mixture (%)				
	Biotype	100 0	75 25	50 50	25 75	0 100
Dry weight (g)	R	8.4 (0.40)[a]	8.5 (0.56)	8.3 (0.77)	8.0 (1.20)	0
	S	0	8.5 (0.70)	8.8 (0.89)	8.4 (1.24)	8.0 (0.41)
Leaf area (cm^2)	R	1927 (163)	1,807 (194)	2,111 (439)	1,739 (444)	0
	S	0	1,774 (534)	2,139 (370)	1,894 (305)	1,745 (185)
Plant height (cm)	R	61.6 (0.85)	59.1 (1.26)	60.5 (1.78)	59.7 (2.15)	0
	S	0	60.2 (2.40)	61.9 (1.82)	59.5 (1.43)	60.3 (0.94)

[a] 95% confidence intervals are in parentheses.

weight, leaf area, and plant height models were similar between R and S biotypes, indicating similar rate and shape of the growth curves for both biotypes. Similar results had been reported in SU herbicide–R and –S kochia biotypes (Christoffoleti et al. 1997; Thompson et al. 1994b;).

The R biotype produced 17% fewer seed than the S biotype (Table 2). The total seed weight per plant was similar between R and S biotypes because seeds of the R biotype were heavier than those of the S biotype. The early germination of the R biotype at low temperature may be related to the larger seed of the R biotype. Purrington and Bergelson (1997) reported that SU herbicide–S *Arabidopsis thaliana* lines produced about 25% more seeds than the R lines, especially in nutrient-poor conditions. However, no difference in seed production was observed between R and S biotypes in prickly lettuce or kochia (Alcocer-Ruthling et al. 1992b; Thompson et al. 1994b).

Competitive Growth

When the R and S biotypes were grown in mixtures under competitive conditions, the shoot dry weight and leaf area per pot of the R biotype was similar tothat of the S biotype (Figure 3). The shoot dry weight per pot of the R and S biotypes were similar and corresponded to the theoretical response of two biotypes having equal competitive fitness. Individual shoot dry weight, leaf area, and plant height of R and S biotypes were similar in monoculture and were not different in any proportions of mixture (Table 3). ANOVA for individual shoot dry weight, leaf area, and plant height showed no proportion effect in R and S mixtures indicating no competition between the biotypes (data not shown).

Even though the R biotype produced 17% fewer seeds than the S biotype, the R biotype might dominate early in the season because of its earlier germination at low temperature and larger seed size. There were no differences in shoot dry weight, leaf area, and plant height between R and S biotypes under noncompetitive conditions. The R biotype was equally competitive to the S biotype. Thus, it appears that the altered target site trait conferring resistance to ALS inhibitors in the R biotype does not result in a growth penalty. In the absence of ALS inhibitors, these results suggest that the R biotype would remain at a similar frequency in a population of R and S biotypes.

Sources of Materials

[1] Controlled Environment Chamber, Hoffman Manufacturing Inc., P.O. Box 547, Albany, OR 97321.

[2] Sunshine Mix #1 potting mix, Sun Gro Horticulture Inc., 110 110th Avenue Northeast, Suite 490, Bellevue, WA 98004.

[3] OSMOCOTE® (14-14-14), Southern Agricultural Insecticides Inc., P.O. Box 218, Palmetto, FL 34220.

[4] LI-COR 3100 Leaf Area Meter, Li-COR Inc., 4421 Superior Street, Lincoln, NE 68504.

Literature Cited

Alcocer-Ruthling, M., D. C. Thill, and B. Shafii. 1992a. Differential competitiveness of sulfonylurea resistant and susceptible prickly lettuce (*Lactuca serriola*). Weed Technol. 6:303–309.

Alcocer-Ruthling, M., D. C. Thill, and B. Shafii. 1992b. Seed biology of sulfonylurea-resistant and -susceptible biotypes of prickly lettuce (*Lactuca serriola*). Weed Technol. 6:858–864.

Ball, D. A. and C. A. Mallory-Smith. 2000. Sulfonylurea herbicide resistance in downy brome. Proc. West. Soc. Weed Sci. 53:41–42.

Brown, R. F. and D. G. Mayer. 1988. Representing cumulative germination. 2. The use of the Weibull function and other empirically derived curves. Ann. Bot. 61:127–138.

Christoffoleti, P. J., P. Westra, and F. Moore, III. 1997. Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 45:691–695.

Dyer, W. E., P. W. Chee, and P. K. Fay. 1993. Rapid germination of sulfonylurea-resistant *Kochia scoparia* accessions is associated with elevated seed levels of branched chain amino acids. Weed Sci. 41:18–22.

Gressel, J. and L. A. Segel. 1978. The paucity of plants evolving genetic resistance to herbicides: possible reasons and implications. J. Theor. Biol. 75:349–371.

Gressel, J. and L. A. Segel. 1982. Interrelating factors controlling the rate of appearance of resistance: the outlook for the future. Pages 325–347 *in* H. LeBaron and J. Gressel, eds. Herbicide Resistance in Plants. New York: J. Wiley.

Heap, I. 2004. International Survey of Herbicide Resistant Weeds. www.weedscience.com.

Hunt, R. 1982. Plant Growth Curves: The Functional Approach to Plant Growth Analysis. Baltimore, MD: Edward Arnold. Pp. 128–143.

Mallory-Smith, C. A., D. C. Thill, M. Alcocer-Ruthling, and C. Thompson. 1992. Growth comparisons of sulfonylurea resistant and susceptible biotypes. Pages 301–303 *in* Proceedings of the First International Weed Control Congress. Volume 2. Melbourne, Australia: Weed Science Society of Victoria.

Mallory-Smith, C. A., D. C. Thill, and M. J. Dial. 1990. Identification of herbicide resistant prickly lettuce (*Lactuca serriola*). Weed Technol. 4: 163–168.

Maxwell, B. D., M. L. Roush, and S. R. Radosevich. 1990. Predicting the evolution and dynamics of herbicide resistance in weed populations. Weed Technol. 4:2–13.

Park, K. W. and C. A. Mallory-Smith. 2004. Physiological and molecular basis for ALS inhibitor resistance in *Bromus tectorum* biotypes. Weed Res. 44:71–77.

Peters, J., ed. 2000. Tetrazolium Testing Handbook: Contribution No. 29 to the Handbook on Seed Testing. Lincoln, NE: Association of Official Seed Analysts.
Purrington, C. B. and J. Bergelson. 1997. Fitness consequences of genetically engineered herbicide and antibiotic resistance in *Arabidopsis thaliana*. Genetics 145:807–814.
Richards, F. J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10:290–300.
Ryan, G. F. 1970. Resistance of common groundsel to simazine and atrazine. Weed Sci. 18:614–616.
Saari, L. L., J. C. Cotterman, and D. C. Thill. 1994. Resistance to acetolactate synthase inhibiting herbicides. Pages 83–139 *in* S. B. Powles and J.A.M. Holtum, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Ann Arbor, MI: Lewis.
[SAS] Statistical Analysis Systems Institute. 1987. SAS/STAT™ Guide for Personal Computers. Version 6, Cary, NC: Statistical Analysis Systems Institute. 1028 p.
Shafii, B., W. J. Price, J. B. Swensen, and G. A. Murray. 1991. Nonlinear estimation of growth curve models for germination data analysis. Pages 19–42 *in* G. A. Milliken and J. R. Schwenke, eds. Proceedings of 1991 Kansas State University Conference on Applied Statistics in Agriculture. Manhattan, KS: Kansas State University.
Thompson, C. R., D. C. Thill, and B. Shafii. 1994a. Germination characteristics of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 42:50–56.
Thompson, C. R., D. C. Thill, and B. Shafii. 1994b. Growth and competitiveness of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 42:172–179.

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