Weed Technology. 2002. Volume 16:464–466

Note

Giant Foxtail (*Seteria faberi*) Seedling Assay for Resistance to Sethoxydim[1]

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Abstract: Repeated application of acetyl-coA carboxylase–inhibiting herbicides, such as sethoxydim, may select for resistant (R) weed populations, making a rapid and reliable seedling bioassay a useful tool. Such a bioassay was developed to determine shoot and root responses of giant foxtail seedlings to sethoxydim. Root and shoot elongation of susceptible (S) and R giant foxtail seedlings was measured at 3 and 6 d after exposure to 0.1 to 100 mg/L sethoxydim. A bioassay concentration of 10 mg/L sethoxydim easily discriminated between S and R biotypes of giant foxtail at 6 d after exposure, with R:S shoot and root growth ratios of 3 and 10, respectively.
Nomenclature: Sethoxydim; giant foxtail, *Seteria faberi* Herrm. #[3] SETFA.
Additional index words: ACCase inhibitor, bioassay.
Abbreviations: ACCase, acetyl-coA carboxylase; DAT, days after treatment; R, resistant; S, susceptible.

INTRODUCTION

Application of herbicides is the dominant method of controlling weeds in many agricultural fields. Several annual grass species are evolving resistant (R) biotypes after repeated applications of acetyl coAcarboxylase (ACCase)-inhibiting herbicides, such as sethoxydim (Heap et al. 2001). Sethoxydim-R giant foxtail populations were first identified in Wisconsin in 1991 (Stoltenberg and Wiederholt 1995). Populations of giant foxtail resistant to ACCase inhibitors are also known in Iowa, and analogous green foxtail [*Setaria viridis* (L.) Beauv.] populations occur in Alberta, Manitoba, and Saskatchewan, Canada (Heap et al. 2001).

Determination of herbicide resistance may be time consuming and costly. However, rapid bioassays can be used to distinguish between differences in responses of weed biotypes to herbicides. For example, a pollen test discriminates between biotypes of blackgrass (*Alopecurus myosuroides* Huds.) resistant and susceptible to ACCase inhibitors (Letouze and Gasquez 2000), as does a quick test based on severed tillers of this same species (Boutsalis 2001). Seedling bioassays used to detect resistance to ACCase inhibitors have also been developed for blackgrass (Letouze and Gasquez 1999), wild oat (*Avena fatua* L.) (Murray et al. 1996), and rigid ryegrass (*Lolium rigidum* Gaud.) (Heap and Knight 1986), but no such tests exist for any of the foxtails (*Setaria* spp.). The objective of this study was to devise a rapid and reliable seedling bioassay for sethoxydim-R and -S seedlings of giant foxtail.

MATERIALS AND METHODS

All seeds used in these experiments originated from giant foxtail populations in Wisconsin.[4] Seeds from the R biotype were harvested from greenhouse-reared plants whose seedlings had been sprayed with sethoxydim plus crop oil at rates equivalent to 0.3 kg ai/ha and 1 L/ha, respectively. Seeds from S plants were harvested directly from field-grown plants. All seeds were immersed for 3 d in cold water (0 C) to break seed dormancy and then soaked for 1 min in 1% sodium hypochlorite, followed by distilled water to inhibit fungal growth. Rinsed seeds were placed into petri dishes with blotter paper saturated with 1 g/L gibberellic acid to promote germination and to enhance subsequent seedling growth. Petri dishes were incubated at 21 C in the dark, and seeds were allowed to germinate.

After germination, 10 seedlings were chosen, each with 3 to 6 mm rootlets, and transferred into new petri

[1] Received for publication August 20, 2001, and in revised form January 4, 2002.

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[3] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

[4] Seeds were provided by David Stoltenberg and the late R. Gordon Harvey, Department of Agronomy, University of Wisconsin, Madison, WI.

dishes with blotter paper saturated with sethoxydim at 0, 0.1, 1, 10, and 100 mg/L. The 10 seedlings were placed in a line across the middle of the petri dish, with all rootlets facing toward the bottoms of the inclined petri dishes. Petri dishes were incubated at 21 C in the dark. Dishes were positioned vertically to allow upward shoot and downward root growth. A randomized complete block experimental design was used during incubation. Each of the four replications was placed in a separate plastic bag to minimize variance in conditions within a replication.

Both root and shoot lengths were measured from the crown to the tip of the root or shoot and recorded 0, 3, and 6 d after sethoxydim treatment (DAT). Seedling growth increments were determined for 3 and 6 DAT by subtracting measurements on these days from the initial measurements.

The entire experiment was conducted three times. Data for shoot growth from the three trials were combined after ANOVA revealed no effects of the experiment or the experiment by treatment interaction ($P > 0.1$). However, differences among experiments did occur for root growth, therefore; results for roots are presented for each experiment separately. To determine if the S and R biotypes varied in their response to individual sethoxydim treatments, a two-sample *t* test for treatment and mean was used assuming unequal variance. Lastly, growth increments for all seedlings were grouped into 5-mm intervals, and frequency histograms were constructed to compare the distributions of growth increments of both biotypes.

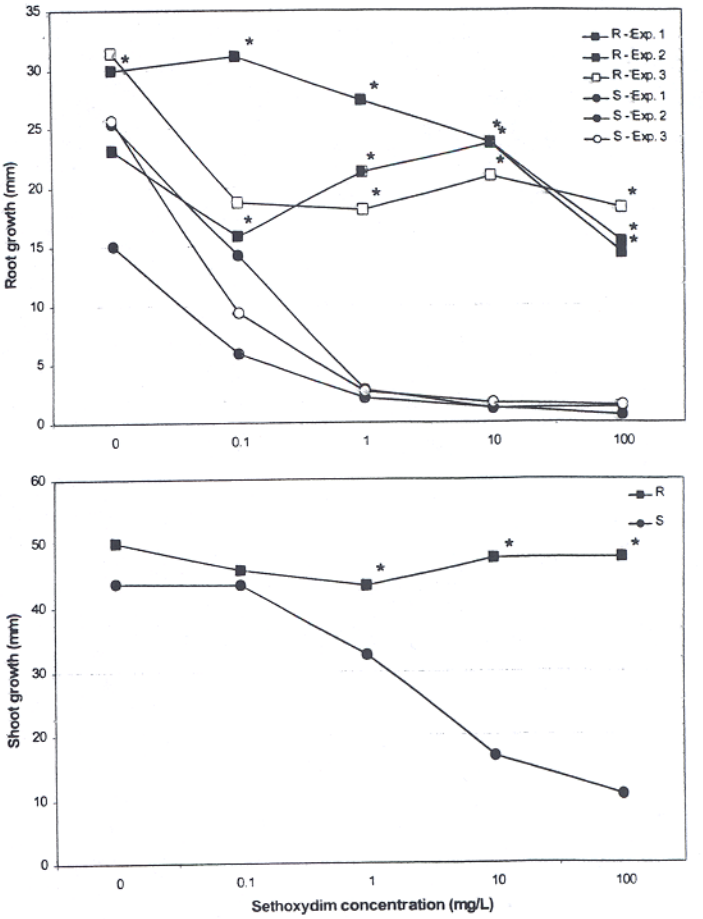

Figure 1. Root and shoot growth increments of giant foxtail seedlings 6 d after exposure to increasing sethoxydim solutions. Data from three experiments were combined for shoot growth but kept separate for root growth because of the significant experiment by treatment interactions. Asterisks (*) indicate differences between resistant (R) and susceptible (S) biotypes, according to *t* tests performed within each experiment for roots and across experiments for shoots.

RESULTS AND DISCUSSION

At both 3 and 6 DAT the S biotype showed a decline in root and shoot growth increments with increasing sethoxydim concentration, but only the more definitive data for 6 DAT are presented (Figure 1). The R biotype showed only a slight decline in root growth at 100 mg/L. The *t* tests indicated differences in growth ($P < 0.05$) between the S and R biotypes at 1, 10, and 100 mg/L sethoxydim concentrations for roots and shoots. Although root growth of S seedlings tended to be lower than that of the R seedlings even in the absence of sethoxydim, this difference was significant in only one of the three experiments. At ≥ 1 mg/L sethoxydim, root growth increments of S seedlings were negligible and easily distinguishable from the much higher values of the R seedlings.

The sethoxydim concentration chosen for bioassays of giant foxtail seedlings was 10 mg/L. A clear statistical distinction could be made at this concentration between S and R giant foxtail seedlings in shoot and root growth, even with the variability in root growth among experiments (Figure 1). Furthermore, this concentration did not affect root growth of the R biotype, as did the 100 mg/L sethoxydim treatment. The average R:S ratio at 6 DAT for shoot growth increments at 10 mg/L of sethoxydim was about 3, whereas it was about 10 for root growth increments.

Frequency distributions of shoot growth for S and R biotypes at 10 mg/L sethoxydim at 6 DAT are shown in Figure 2. Both biotypes have distinct distributions of growth, which means that the two biotypes can be distinguished relatively easily and with little error. Basically, at 6 DAT shoots < 29 mm are susceptible and those > 29 mm are resistant to sethoxydim. With this distinction, only about 8% of the S seedlings would be mis-

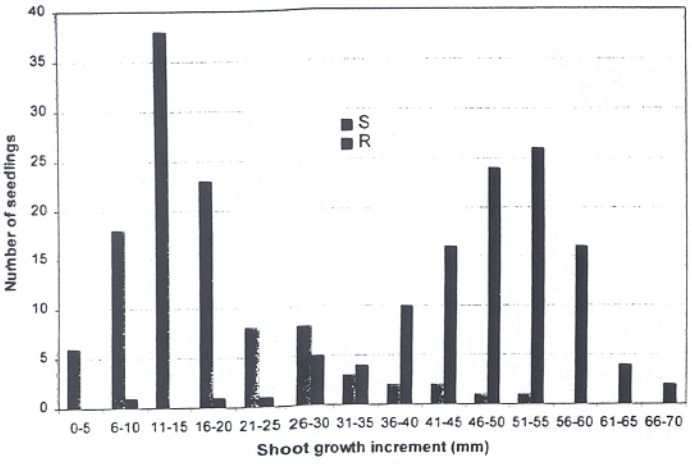

Figure 2. Distributions of shoot growth increments of resistant (R) and susceptible (S) biotypes of giant foxtail seedlings 6 d after exposure to a 10 mg/L sethoxydim solution.

classified as R, and only 6% of R seedlings would be misclassified as S. Similarly, roots < 3 mm are S and those > 3 mm are R (data not shown), with 0% and 10% misclassification, respectively.

The results of this study indicate that seedling bioassay provided a rapid test for the determination of sethoxydim resistance in giant foxtail. The significant differences between the S and R giant foxtail biotypes in root and shoot growth at 10 mg/L of sethoxydim allow quick and reliable distinction of biotypes, with misclassification rates ≤ 10%.

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