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Atrazine Tolerance in Warm-Season Grass Seedlings

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Atrazine Tolerance in Warm-Season Grass Seedlings[1]

C. C. Bahler, K. P. Vogel, and L. E. Moser[2]

ABSTRACT

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] effectively controls many annual weeds in established warm-season range grasses but can damage some of these grasses in the early seedling stage. This study determined the relative atrazine tolerance of seedlings of selected warm-season grasses and evaluated the effectiveness of a greenhouse bioassay for seedling atrazine tolerance in range grasses. Seed of 'Pathfinder' switchgrass [*Panicum virgatum* L.], 'Nebraska 54' indiangrass [*Sorghastrum nutans* (L.) Nash], 'Butte' side-oats grama [*Bouteloua curtipedula* (Michx.) Torr.], an experimental blue grama 'PMK 1483' [*B. gracilis* (H.B.K.) Lag ex Steud], 'Plains' bluestem (*Bothriochloa ischaemum* var *ischaemum* (L.) Keng), 'Caucasian' bluestem [*B. caucasica* (Trin.) C.E. Hubbard] 'Blaze' little bluestem [*Schizachyrium scoparium* (Michx.) Nash], and a Nebraska experimental prairie sandreed [*Calamovilfa longifolia* (Hook) Scribn.] were planted in the greenhouse in a 1:1 soil-sand mixture that contained 0, 1, 2, or 3 mg kg^{-1} atrazine. Seed lots from one cycle of field selection for atrazine tolerance of indiangrass and side-oats grama were included. A field study was also conducted on a silty clay loam (Typic Argiudoll) and on a loamy sand (Udic Haplustoll) site using little bluestem, blue grama, prairie sandreed, Caucasian bluestem, and Plains bluestem. Survival ranking at 3 mg kg^{-1} atrazine, in the greenhouse, was: Caucasian bluestem = switchgrass > Plains bluestem > prairie sandreed > indiangrass (check) = indiangrass (cycle 1) > side-oats grama (cycle 1) = side-oats grama (check) = blue grama. Atrazine also reduced height in the surviving seedlings. Height reduction in did not appear to be related to seedling survival. Greenhouse data correlated closely with the loamy sand site because the bioassay used a soil-sand mixture. Excellent stands of Plains and Caucasian bluestem were obtained on the silty clay loam site at all atrazine rates. The bioassay was an effective screen to separate grasses with differing atrazine tolerances.

Additional index words: Switchgrass, Side-oats grama, Indiangrass, Blue grama, Little bluestem, Caucasian bluestem, Plains bluestem, Prairie sandreed, Herbicide tolerance.

ATRAZINE [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s* triazine] controls a wide variety of annual weeds in certain warm-season range grasses. However, many grasses that are tolerant to atrazine when established are susceptible as seedlings. Martin et al. (1982) demonstrated a wide range of tolerance among warm-season range grasses. Switchgrass [*Panicum virgatum* L.] and big bluestem (*Andropogon gerardi* Vitman) seedlings were atrazine tolerant. However, indiangrass [*Sorghastrum nutan* (L.) Nash], side-oats grama [*Bouteloua curtipedula* (Michx.) Torr.], and sand lovegrass [*Eragrostis trichodes* (Nutt.) Wood] were not sufficiently tolerant to use atrazine at establishment.

Tolerance to the *s*-triazines is probably due to metabolic degradation. Detoxification of atrazine has been documented in corn [*Zea mays* (L.)] and sorghum (*Sorghum vulgare* Pers.). Detoxification can occur through modification of the atrazine molecule by N-dealkylation, 2-hydroxylation, or glutathione conjugation or any combination of the above pathways (Shimbukuro, 1967, Lamoureux et al., 1970; Lamoureux et al., 1973). Giant foxtail (*Setaria faberi* Herrm.), fall panicum (*Panicum dichotomiflorum* Michx.), and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] metabolize atrazine via N-dealkylation and 2-hydroxylation pathways (Thompson, 1972). Their tolerance to atrazine is directly related to the rate of atrazine detoxification.

In a study of 53 grass species of the subfamilies Festucoideae, Panicoideae, and Eragrostoideae, N-dealkylation, 2-hydroxylation, and glutathione conjugate catabolism pathways were found to occur in all species (Jensen et al., 1977). The major difference found between the species was the rate at which atrazine was metabolized. Species that showed atrazine tolerance all formed the atrazine-glutathione conjugate, which is a completely detoxified form of atrazine. Big bluestem and sand bluestem (*Andropogon hallii* Hack), in the seedling stage, appear to detoxify atrazine through glutathione conjugation (Jensen et al., 1977).

Reaction to atrazine also depends on soil properties. Lavy (1968) reported that the adsorption of the *s*-triazines increased and plant uptake of C^{14} atrazine decreased as soil acidity, organic matter, and clay content increased. LeBaron (1970) also showed that atrazine activity in soil was reduced by increased soil acidity and organic matter.

Tests for atrazine resistance in higher plants have involved bioassays using soil-incorporated atrazine (Winkle et al., 1981); floating leaf disks in broadleaf weeds (Hensley, 1981); or etiolated growth in cool-season grasses (Young and Evans, 1978). The purpose of this study was to determine the relative atrazine tolerance of common warm-season prairie grass seedlings used in the mid- and north central USA and to evaluate a bioassay as a technique for detecting atrazine-tolerant plants.

MATERIALS AND METHODS

Greenhouse Study

Seed lots of 'Pathfinder' switchgrass, 'Nebraska 54' indiangrass, 'Butte' side-oats grama, an experimental USDA/SCS blue grama 'PMK 1483' [*Bouteloua gracilis* (H.B.K.) Lag ex Steud], 'Plains' bluestem [*Bothriochloa ischaemum* var. *ischaemum* (L.) Keng], 'Caucasian' bluestem [*B. caucasica* (Trin.) C.E. Hubbard], 'Blaze' little bluestem [*Schizachyrium scoparium* (Michx.) Nash], and a Nebraska experimental prairie sandreed [*Calamovilfa longifolia* (Hook.) Scribn.] were used. Also used were seed from a Nebraska 54 indiangrass and a Butte side-oats grama population produced by one cycle of selection for seedling atrazine tolerance. Selection was accomplished by planting Nebraska 54 indiangrass and Butte side-oats grama in rows and then broadcasting 2.24 kg ha^{-1} atrazine after planting. Seed was harvested from the surviving plants.

The seed used in the greenhouse study was cold stratified for 14 days at 5°C in a 24 g L^{-1} captan {*N*-(trichloromethyl) thio]-4-cyclohexene-1,2-dicarboximide)} solution. A 1:1 soil-sand mix (Table 1) was weighed (17.7 kg per flat) and placed in paper bags. Zero, 1, 2, or 3 mg kg^{-1} atrazine concentrations in the soil-sand mix were obtained by pipetting 18 mL of 1000, 2000, or 3000 mg L^{-1} atrazine-methanol solution,

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respectively, evenly over the soil surface in each bag. The paper bags were left open to allow the methanol to evaporate (approximately 3 h), leaving the atrazine. Then the bags were closed and inverted 20 times to thoroughly mix the atrazine throughout the soil-sand mix. After shaking, the soil-sand mix was placed in greenhouse flats (0.6 × 0.3 m) and moistened by subirrigation. After moistening (approximately 1 h) 100 seeds per strain were planted in single rows of 0.3 m in length in each flat. Further watering of the flats was done by an alternating of top watering and subirrigation to prevent leaching the atrazine out of the flats.

The experimental design was a split-plot randomized complete block, with atrazine level as whole plots and grass species as subplots. One flat was a whole plot. Four replications (blocks) were used and the study was repeated once. Plant counts were taken after complete emergence (14 days after planting) and after 26 days to calculate the percent survival for each species at each atrazine rate. Susceptible grass seedlings did not begin to die from atrazine until about 10 days after emergence. Data are presented as percent survival. Percent survival was calculated by:

$$\frac{\text{no. of plants surviving 26 days after planting}}{\text{no. of plants 14 days after planting (emergence)}} \times 100$$

Plant height was measured every 3 days from Day 14 to 26 to determine the degree of stunting. However, only the heights from Day 26 were used in the analysis, because of serial correlation between the heights. Height for each species was determined by an average of three plants selected randomly in the row. Height was measured by holding the leaves upright and measuring from the soil surface. Measurements were not taken in any row that had less than 10 plants.

The two greenhouse studies were found to be homogenous using Bartlett's test (Steel and Torrie, 1980) so data were combined. An analysis of variance (ANOVA) was performed on the combined data and a least significant difference (LSD) was used to compare height and percent survival means.

Field Study

The field study was conducted with Plains bluestem, Caucasian bluestem, little bluestem, blue grama, and prairie sandreed on two sites at the Univ. of Nebraska Field Station, Mead, NE. Site 1 was a silty clay loam (Typic Argiudoll) and Site 2 was a loamy sand (Udic Haplustoll) (Table 1). Site 1 was seeded on 2 June 1981 and Site 2 on 3 June 1981 with a grass plot drill (Vogel, 1978), on firm clean seedbeds. Each plot consisted of seven 4.6 m drill rows spaced 18 cm apart. Contiguous plots were separated on their ends by 3.0 (Site 1) and 1.5 m (Site 2) alleys of Pathfinder switchgrass. Pathfinder switchgrass was also seeded as a border around each of the experimental sites. All plots were seeded at a rate of 430 pure live seed (PLS) m^{-2} at a 1 cm depth.

Atrazine treatments were applied 3 June 1981 on Site 1 and 4 June on Site 2. Atrazine was applied with a small-plot sprayer at the rate of 0, 1.1, 2.2, or 3.4 kg (a.i.) ha^{-1}. These rates are approximately equivalent to 1, 2, and 3 mg kg^{-1} for the top 15 cm of soil. Pathfinder switchgrass alleys and borders were sprayed with 2.2 kg (a.i.) ha^{-1} atrazine. Site 1 was mowed on 30 July 1981 to a height of 10 cm for control of annual weeds. Both sites were sprayed with 2.5 kg (a.i.) ha^{-1} 2,4-D [2,4-dichlorophenoxy)acetic acid] on 12 Aug. 1981 for broadleaf weed control.

The experimental design was a split-plot randomized complete block design, with main plots being the grass species and subplots being atrazine rates. There were four replications on Site 1 and three replications on Site 2. Plant counts for Site 1 were taken 17 Sept. 1981 by visual estimation of stand population because of the heavy weed population. The visual estimations were based on the percent of the surface area of a plot that was occupied by the seeded species. On

Table 1. Soil characteristics of the field sites and greenhouse soil-sand mixture.

Soil characteristics	Greenhouse soil-sand mix	Silty clay loam (Typic Argiudoll)	Loamy sand (Udic Haplustoll)
Particle size (%)			
Sand	70.7	7.4	84.3
Silt	14.8	53.7	6.0
Clay	14.5	38.9	9.7
pH	6.4	5.8	6.1
Organic matter (%)	0.6	3.1	0.4
P mg Kg^{-1}	34.0	9.6	2.2
NO_3 mg Kg^{-1}	4.4	32.0	14.0

Site 2 all weeds were removed by hand on 17 Sept. 1981 and plant counts were determined by placing a metal grid containing 25 squares over the center five rows of each plot. Each square was 15 × 15 cm in size. The number of squares containing a grass plant were counted and the process was then repeated in another location on the same plot. Since each grid contained 25 squares and two grids were used per plot, the number of grids containing a seeded grass plant was multiplied by 2 to obtain percent stand.

RESULTS AND DISCUSSION

Greenhouse Study

The interaction between grass species and atrazine level was statistically significant ($P \leq 0.01$) for survival rate. Response of grass species reaction to atrazine was characterized as low, medium, or high degree of survival. Side-oats grama and blue grama had the lowest survival rate and the highest sensitivity in the atrazine treated flats (Fig. 1). For these grasses percent survival declined sharply at 1 mg kg^{-1} atrazine compared to the 0 atrazine rate and survival decreased further as atrazine concentrations increased. Both populations of side-oats grama and blue grama were the first grasses to show atrazine damage and to die. Evidently, little improvement has been made in atrazine tolerance with one cycle of field selection, since the percent survival mean of the side-oats grama check population was slightly higher than the Cycle 1 population at 1 and 2 mg kg^{-1} atrazine.

Indiangrass (Fig. 1) was intermediate in survival rates at 2 mg kg^{-1} atrazine, when compared to the other species but still did not have an acceptable percent survival. Indiangrass (Cycle 1) at 1 mg kg^{-1} atrazine was significantly higher in tolerance in comparison to the check, however, the relationship was reversed at the 2 mg kg^{-1} atrazine rate. Little bluestem was also intermediate in survival rates at 1 and 3 mg kg^{-1} atrazine. The higher survival rate of little bluestem at 2 mg kg^{-1} atrazine than at 1 mg kg^{-1} atrazine is unexplained (Fig. 1).

Plains bluestem, Caucasian bluestem, switchgrass, and prairie sandreed were all relatively high in survival rate (Fig. 1). Switchgrass had the highest survival rate at 1 and 2 mg kg^{-1} atrazine for all species. At 2 mg kg^{-1} atrazine Plains bluestem, Caucasian bluestem, and prairie sandreed were similar in survival rate. At 3 mg kg^{-1} atrazine, switchgrass, and Caucasian bluestem were equivalent. Prairie sandreed had the lowest survival rate when compared to switchgrass, Caucasian bluestem, and Plains bluestem at 3 mg kg^{-1} atrazine.

The ranking of survival for all species at 3 mg kg^{-1} atrazine was ($P < 0.1$) Caucasian bluestem = switchgrass > Plains bluestem > prairie sandreed > little bluestem > indiangrass (Cycle 1) = indiangrass (check) > side-oats grama (check) = side-oats grama (Cycle 1) = blue grama. Indiangrass, side-oats grama, and switchgrass were studied by Martin et al. (1982) and our results were similar to theirs.

Plant heights decreased as atrazine rate increased (Fig. 2). Both populations of side-oats grama showed stunting, but so few plants remained that height measurements were not taken at 2 and 3 mg kg^{-1} atrazine rates. Blue grama survival was so severely reduced by atrazine that no height measurements were possible. In both populations of indiangrass height decreased significantly as atrazine increased from 0 to 3 mg kg^{-1}

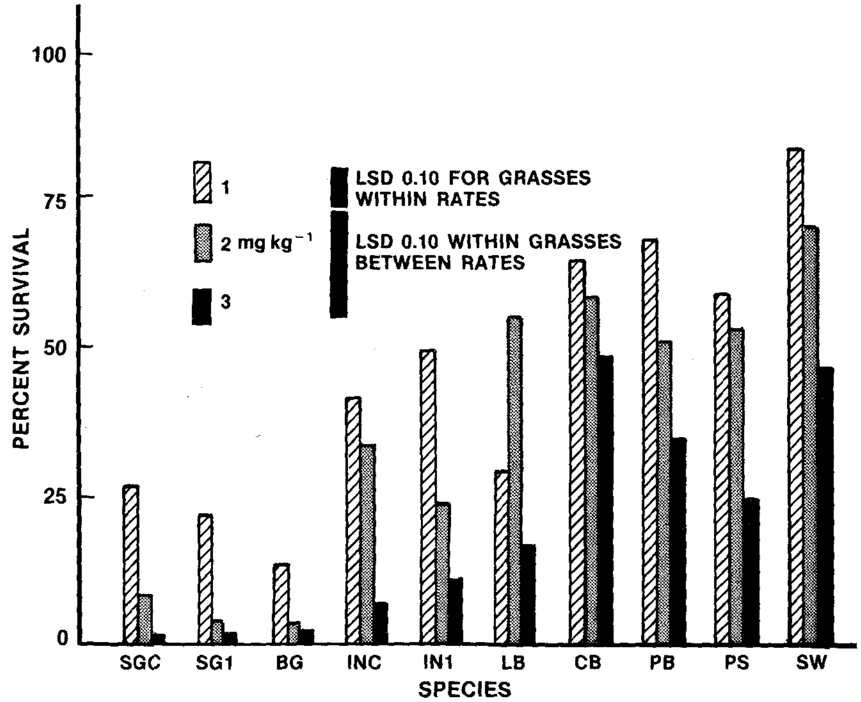

Fig. 1. The percent survival for each species at 1, 2, or 3 mg kg^{-1} atrazine, in the greenhouse study. SGC-side-oats grama (check); SGI-side-oats grama (Cycle 1); BG-blue grama; INC-indiangrass (check); IN1-indiangrass (Cycle 1); LB-little bluestem; CB-Caucasian bluestem; PB-Plains bluestem; PS-prairie sandreed; SW-switchgrass.

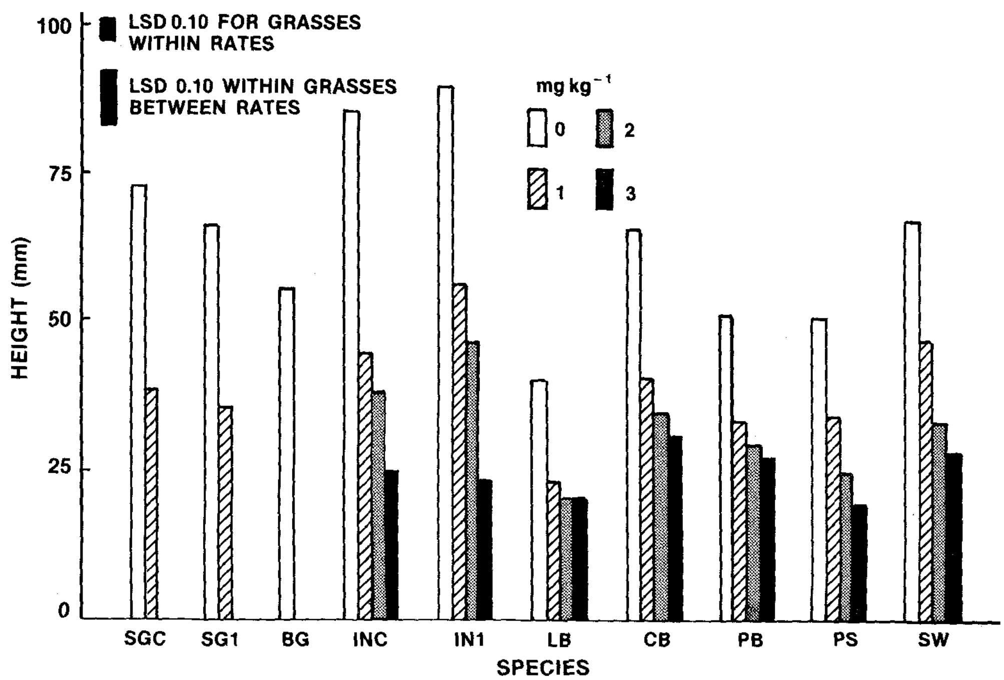

Fig. 2. Seedling height at 0, 1, 2, or 3 mg kg^{-1} atrazine, in the greenhouse study. SGC-side-oats grama (check); SGI-side-oats grama (Cycle 1); BG-blue grama; INC-indiangrass (check); IN1-indiangrass (Cycle 1); LB-little bluestem, CB-Caucasian bluestem; PB-Plains bluestem; PS-prairie sandreed; SW-switchgrass.

Table 2. The mean plant populations and stand for Caucasian bluestem, Plains bluestem, little bluestem, blue grama, and prairie sandreed of the greenhouse study and field study.

Species	Atrazine rate	Greenhouse study	Field study	
		soil-sand mixture	Silty clay loam (Typic Argiudoll)	Loamy sand (Udic Haplustoll)
	kg ha⁻¹	seedling row⁻¹	% stand	
Plains bluestem	0.0	31	68	58
	1.1	19	80	41
	2.2	21	69	29
	3.4	14	76	11
Caucasian bluestem	0.0	34	52	34
	1.1	29	70	23
	2.2	23	58	14
	3.4	12	85	7
Little bluestem	0.0	47	17	42
	1.1	14	20	3
	2.2	21	17	4
	3.4	5	7	3
Blue grama	0.0	25	24	47
	1.1	14	14	21
	2.2	1	11	6
	3.4	1	8	6
Prairie sandreed	0.0	45	8	21
	1.1	24	2	2
	2.2	19	10	1
	3.4	10	4	0
$LSD_{0.05}$ for atrazine rate within a grass†		21	19	18
$LSD_{0.05}$ for grasses		9	12	17

† LSD = Least significant difference.

(Fig. 2). Caucasian bluestem and switchgrass showed similar stunting, which was greater than that seen with Plains bluestem. Switchgrass, although tolerant to atrazine, showed a marked decrease in height as atrazine concentration increased. The ranking for height reduction from 0 to 3 mg kg^{-1} atrazine was little bluestem < Plains bluestem < prairie sandreed < Caucasian bluestem = switchgrass < indiangrass (check) < indiangrass (Cycle 1). Under the conditions of this study, however, the amount of stunting does not appear to be related to plant survival.

Field Study

As expected atrazine damage was greater on the loamy sand site than on the silty clay loam site (Table 2). The silty clay loam site had higher organic matter content and smaller particle size than the loamy sand site (Table 1). According to Lavy's (1968) and LeBaron's (1970) research the high level of organic matter in the silty clay loam soil reduced the amount of atrazine available for uptake by the plants while in the loamy sand the atrazine activity was high because of the low soil organic matter content.

Plant survival on the loamy sand site followed the same trends as those in the greenhouse bioassay. Plant survival decreased as atrazine concentration increased for all species (Table 2). The similar pattern of plant survival in both the greenhouse soil-sand mixture and loamy sand site can be accounted for by the similarity in soil characteristics (Table 1).

Plains and Caucasian bluestems on the silty clay loam site tolerated atrazine at 1.1, 2.2, and 3.4 kg ha^{-1}. Stands of Plains and Caucasian bluestem on the silty clay loam site would have been suitable for forage harvest where atrazine had been applied. At the time stands were estimated these old world bluestems were 1 m tall and were headed. The switchgrass in the alleys and borders were also 1 m tall and headed with stands of 80 to 100% survival. Blue grama, little bluestem, and prairie sandreed, in contrast to Plains and Caucasian bluestem, all showed atrazine damage on both the silty clay loam site and loamy sand site (Table 2). They showed trends similiar to those from the greenhouse bioassay, which indicated that Plains and Caucasian bluestem were more tolerant to atrazine than the other species. Blue grama, little bluestem, and prairie sandreed did not produce enough forage for harvest the establishment year because of poor stands. None of the plots on the loamy sand site produced harvestable forage the establishment year except for a few of the Caucasian or Plains bluestem plots at the lower rate of atrazine. Some plants of Plains and Caucasian bluestems in these plots were headed when plants were counted. Stands of switchgrass in the alleys and borders on the loamy sand sites ranged from 30 to 60%.

In the previously reported research of Martin et al. (1982), preemergence applications of atrazine, in the field, resulted in sparse stands of indiangrass, side-oats grama, and sand lovegrass but switchgrass and big bluestem showed significantly little if any stand reductions. In the greenhouse bioassay described herein switchgrass had survival rates of 50% even at 3 mg kg^{-1} atrazine in a soil mix that had only 0.6% organic matter. Switchgrass can thus be used as a tolerance standard for evaluating atrazine sensitivity of warm-season range grasses.

Results of field studies by Martin et al. (1982) and our field trials both support the bioassay as an effective tool for separating warm-season grasses with differing atrazine sensitivities. The use of atrazine to establish these grasses is feasible for the high tolerance group, switchgrass and Plains and Caucasian bluestems, but not for medium or low tolerance groups, because a rate of 2.2 kg ha^{-1} atrazine is the minimum required for effective weed control. The use of the greenhouse bioassay to separate plants with differing tolerances also has potential for screening materials in a breeding program. Development of cultivars with atrazine tolerance in the seedling stage would permit the use of atrazine in their establishment.

Plant materials with atrazine tolerance equivalent to switchgrass in the greenhouse bioassay probably have adequate tolerance to atrazine to permit the use of atrazine as a preemergence herbicide for the establishment of their seedlings.

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