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Using Leaf Fluorescence for Evaluating Atrazine Tolerance of Three Perennial Warm-season Grasses

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Abstract

Atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] blocks photosynthetic electron transport in susceptible plants. The energy from the interrupted electron transport is fluoresced from the leaves of atrazine-treated plants. The purpose of this study was to evaluate leaf fluorescence as a nondestructive bioassay of the relative atrazine tolerance of 3 perennial, warm-season grasses. Leaf section of switchgrass [*Panicum virgatum* L.] (high tolerance), indiangrass [*Sorghastrum nutans* (L.) Nash] (intermediate tolerance), and sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.] (lower tolerance) were placed in distilled water for 20 min and then in atrazine solutions. Fluorescence readings were taken prior to and after the atrazine treatment with a portable fluorometer. The difference between the 2 readings provided a reliable measure with low variability of the relative atrazine tolerance of the grasses studied and was effective on greenhouse-and field-grown plants. Optimum atrazine concentrations and incubation periods were 10^{-3} M (atrazine in distilled H_2O) and 30 min, respectively.

Key Words: *Bouteloua curtipendula* (Michx.) Torr., *Panicum virgatum* L., *Sorghastrum nutans* (L.) Nash., indiangrass, sideoats grama, switchgrass, photosynthesis, atrazine

Atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] is a widely used herbicide in corn (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench]. It has been used to shift species composition on rangelands and to aid establishment of grass seedings (Samson and Moser 1982, Waller and Schmidt 1983, Martin et al. 1982). A nondestructive bioassay for quantifying the relative atrazine tolerance of rangeland plants could be used in both ecological and management studies to predict the effects of atrazine application on the indigenous vegetation of specific sites.

Atrazine blocks electron flow in Photosystem II of photosynthesis, from Q to the plastoquinone pool (Ashton and Crafts 1981, Pfister and Arntzen 1979). The energy built up at Q by the inhibition process is either released as heat or as fluoresced light. The amount of fluorescence increases as atrazine binding to the thylakoid membrane increases and results in fluorescence that can be spectrophotometrically detected.

Ahrens et al. (1981) evaluated the effects of atrazine on leaf fluorescence of resistant and susceptible biotypes of 6 weed species. Fluorescence of leaf sections from susceptible biotypes increased when exposed to atrazine but fluorescence of resistant biotypes did not increase. They also evaluated leaf fluorescence of strains of wheat (*Triticum aestivum* L.), sorghum and soybeans [*Glycine max* (L.) Merr.] reported to differ in atrazine tolerance. They detected relatively small fluorescence differences among strains of these plants following treatment with atrazine, but these fluorescence values were not well correlated with atrazine tolerance as measured in field and greenhouse studies. Leaf fluorescence detected difference in atrazine tolerance among biotypes of broadleaved weeds and between atrazine-resistant wild turnip rape (*Brassica campestris* L.) and atrazine-susceptible, cultivated rapeseed (*B. campestris*) and between corn and quackgrass [*Agropyron repens* (L.) Beauv.] (Ali and Machado 1981).

Warm-season, perennial forage grasses differ in atrazine susceptibility, particularly as seedlings when they have the lowest threshold of atrazine tolerance (Martin et al. 1982, Bahler et al. 1984). Seedlings of indiangrass [*Sorghastrum nutans* (L.) Nash] are susceptible and seedlings of sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.] are extremely susceptible to atrazine, while switchgrass [*Panicum virgatum* L.] seedlings are tolerant (Bahler et al. 1984). Established plants of all 3 species tolerate atrazine applied at rates used for annual weed control.

The purpose of this study was to evaluate leaf fluorescence as a nondestructive bioassay of the relative atrazine tolerance of perennial, warm-season grasses using the grasses with known atrazine tolerance as indicator species. Factors investigated included concentration of atrazine test solutions and time periods required for the assay.

Materials and Methods

Incubation Time and Atrazine Concentration

An initial trial was conducted in June 1983 to determine the atrazine concentration and incubation period which would produce clearly differentiated fluorescence levels between untreated and atrazine-treated leaf sections. Established (1-year-old) and immature (4-month-old) indiangrass and established sideoats grama grown in a greenhouse were used. Preliminary trials showed that there were no differences in fluorescence among leaves on a tiller or among tillers on a plant. Four leaf sections were taken from the middle of fully expanded leaves of established and immature plants of indiangrass and sideoats grama. The leaf sections were 2 cm long and the margins of the leaf section were removed. The prepared leaf sections were placed in a petri dish containing distilled water and allowed to equilibrate under full sunlight (approximately 900 μ mol photon m^{-2} s^{-1}) on a greenhouse bench for 20 min, then the leaf sections were removed from the distilled water and initial fluorescence readings were taken.

The amount of fluorescence from the leaf sections was determined with a Model SF-20 plant productivity fluorometer (Richard Brancker Research Ltd.)[1]. Fluorescence is measured through a probe illuminating the surface of the leaf with monochromatic light centered around the 670 nm wavelength from a light-emitting diode (LED) lens. The fluorescence from the leaf is then collected through another collector lens sensitive to the fluorescing wavelengths (>710 nm). Before fluorescence measurements were taken, light output of the probe was calibrated at 4 J m^{-2} s^{-1}. The probe aperture was then reduced from 5 mm to 3 mm by a glass cover slip using black electrical tape because the standard aperture was too large for the leaf sections used.

After excess moisture was blotted, a leaf section was placed on the probe with the lower surface of the leaf section facing the aperture and lenses. A 10-sec adaption period was used and then the fluorescence of the leaf section was read for 50 sec. The terminal fluorescence value (F^t) was recorded (Richard et al. 1983).

The leaf sections were then placed into petri dishes containing 0, 10^{-2} (saturated), 10^{-3}, or 10^{-4} M atrazine-distilled water solutions containing 0.1% X-77 surfactant (contains alkylarylpolyoxyethyleneglycol, fatty acids, and isopropanol) The surfactant was used in all

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experiments and permitted complete suspension of atrazine in the saturated, 10^{-2} M solution. The leaf sections were incubated in the atrazine-distilled water solution for the assigned time period under full sunlight on a greenhouse bench. Fluorescence readings were taken at 1-hr intervals and the change in relative fluorescence (CRF) was calculated; CRF = (RF at time, t) –(RF at ti); where CRF = change in relative fluorescence, RF = relative fluorescence, and ti = initial time. The change in relative fluorescence was used rather than the fluorescence readings to reduce the variability among experimental units.

A second experiment was conducted in July 1983 to further investigate the incubation period which would result in the maximum difference in fluorescence between the control and the treated leaf sections. An incubation period of 120 min was used and fluorescence readings taken every 30 min. This interval period was chosen because fluorescence readings peaked after 1 hr in the first experiment. After the equilibration period, leaf sections were placed in distilled water, 10^{-2} M (sideoats grama), or 10^{-3} M (indiangrass) atrazine solution. The 10^{-2} M solution was used for sideoats grama because it gave the optimal fluorescence response after 1 hr of incubation in the initial trial. The 10^{-3} M atrazine concentration was chosen for the indiangrass because it was the middle concentration used in the initial trial and no differences among the 3 concentrations of atrazine were detected. Leaf sections were prepared and fluorescence readings were taken as described previously.

The experimental design for both experiments was a completely random design with a factorial treatment arrangement. Four leaf sections (subsamples) were sampled for each treatment per individual plant (the experimental unit) and averaged. Treatments were replicated 3 times and the second experiment was repeated.

Quantification of Atrazine Tolerance among Grasses

Established plants of indiangrass, sideoats grama, and switchgrass were sampled from the greenhouse and a field nursery to determine if CRF values were related to atrazine tolerance of seedlings of these grasses. The greenhouse plants and field plants were in late-boot to early-head phenological stages at time of sampling in late July and early August 1983. The top leaf of a vegetative tiller from each species in the nursery was removed and taken to the greenhouse in a erlenmeyer flask containing distilled water. The time spent in transport from the nursery to the greenhouse was less than 10 min. Greenhouse and nursery samples were handled as described previously and 4 leaf sections were used per leaf. After equilibration in distilled water, leaf sections were incubated in 10^{-3} M atrazine-distilled water solution for 90 min. Relative fluorescence was determined at 30-min intervals.

The experiment was a factorial with a randomized complete block design with 3 replications. Four leaf sections were sampled per plant and were treated as subsamples. In all studies, least significant differences ($LSD_{.05}$) values were calculated for detecting statistical differences among treatments.

Results and Discussion

Incubation Time and Atrazine Concentration

Changes of relative fluorescence of established indiangrass plants were similar among the 10^{-2}, 10^{-3}, and 10^{-4} M atrazine levels, but indiangrass at all 3 atrazine concentrations had significantly higher CRF when compared to the control after 1 hr of incubation (Fig. 1B). The CRF for atrazine-treated leaves peaked at 1 hr of incubation and then decreased (Fig. 1B). For untreated leaves, the CRF values peaked after 2 hr and then decreased during the remaining incubation period. Immature indiangrass had similar CRF patterns as those of established plants except that peak CRF values occurred after 2 hr for the atrazine-treated leaf sections and after 3 hr in the untreated leaves (Fig. 1A). CRF values for the control and the 10^{-3} M atrazine treatment on immature indiangrass had not changed after 1 hr (Fig. 1A). This anomaly is unexplained and did not occur in subsequent experiments.

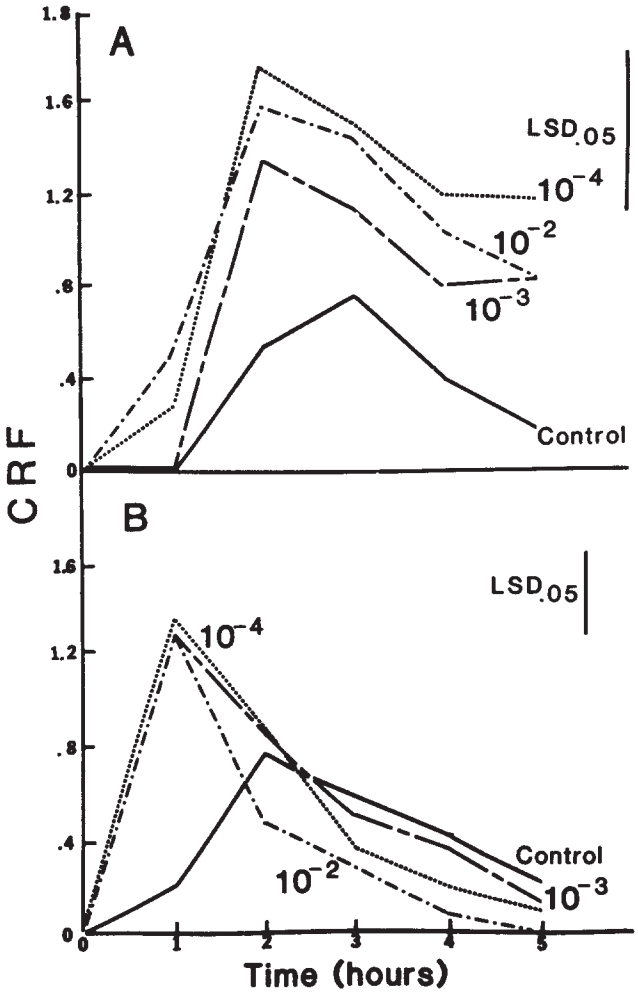

Fig. 1. *The change in relative fluorescence (CRF) of leaf sections of immature (A) and established (B) indiangrass incubated in 0, 10^{-2}, 10^{-3}, or 10^{-4} M atrazine for a 5-hr period.*

The CRF patterns for sideoats grama were similar to those of established indiangrass with the CRF peaking after 1 hr of incubation in atrazine and then decreasing but at a slower rate than the indiangrass (Fig. 2). Leaf sections incubated with 10^{-2} M atrazine solution had a significantly higher CRF than the 10^{-3} or the 10^{-4} M atrazine-treated leaf sections (Fig. 2). CRF values for untreated sideoats grama leaf sections continued to increase with incubation whereas those of indiangrass declined after 2 or 3 hr (Fig. 1). The electron transport system may stay functional longer in the sideoats grama leaf sections than in those of indiangrass.

The initial study demonstrated that the greatest differences between control and treated leaf section for CRF occurred in the initial 2 hr of treatment. In the subsequent study, fluorescence readings were recorded every 30 min for 2 hr. CRF values of treated and untreated leaf sections were significantly different ($P<0.05$) after 30 min of incubation (Fig. 3). The CRF of treated leaf sections stabilized after 30 min and then started to decline after 60 min of incubation. This trend occurred for both sideoats grama and established and immature indiangrass plants. The CRF values for untreated sideoats grama increased slowly over the 120-min incubation period whereas those for untreated indiangrass increased more rapidly up to 60 or 90 min and then declined (Fig. 3).

Quantification of Atrazine Tolerance among Grasses

There were no significant differences between field- and greenhouse-grown plants in the CRF responses after treatment with atrazine, and a Bartlett's test showed homogeniety of variances so the data were combined for presentation. The CRF values of

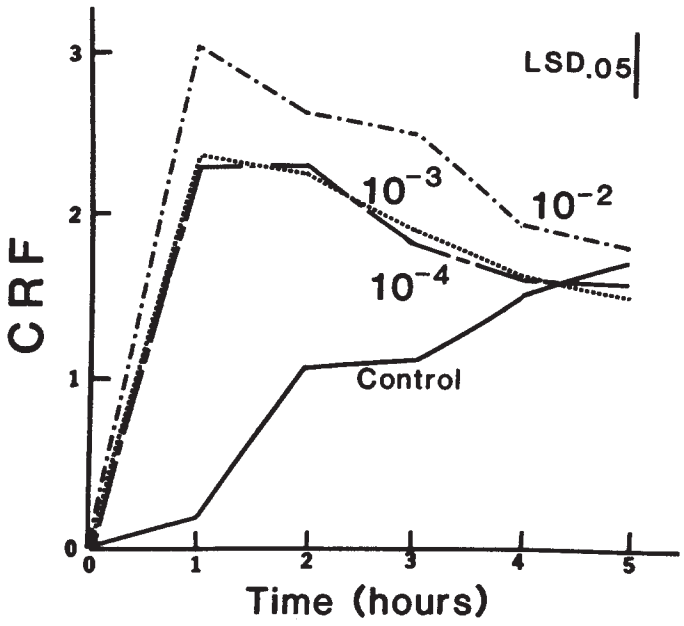

Fig. 2. *The change in relative fluorescence (CRF) of leaf sections of sideoats grama incubated in 0, 10^{-2}, 10^{-3}, or 10^{-4} M atrazine for a 5-hr period.*

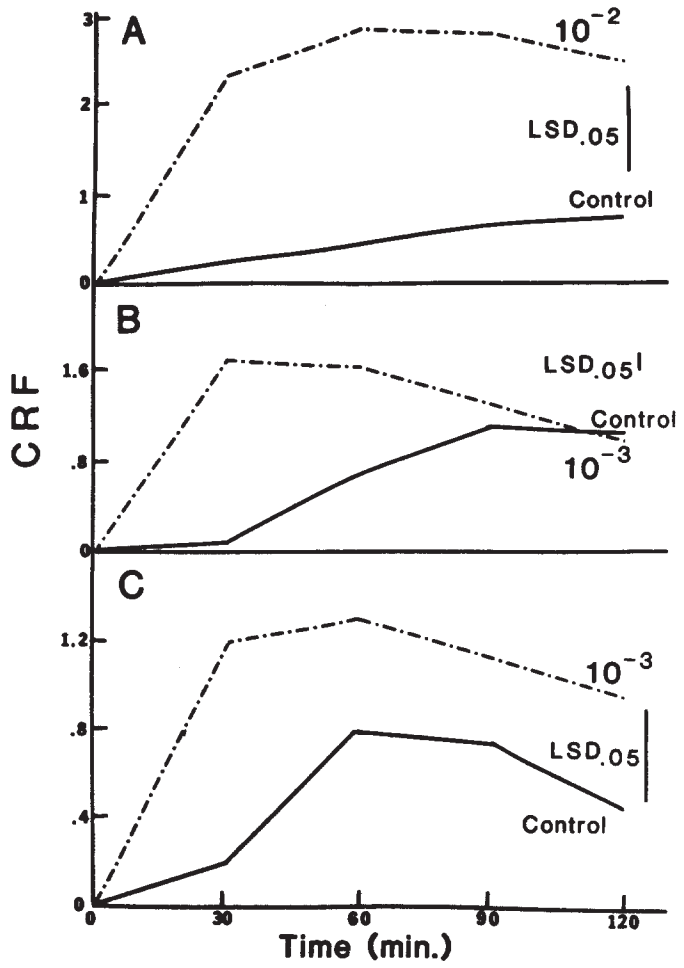

Fig. 3. *The change in relative fluorescence (CRF) of leaf sections of sideoats grama (A), immature indiangrass (B), or established indiangrass (C) incubated in 0 or 10^{-2} or 10^{-3} M atrazine for a 120-min period.*

switchgrass were significantly lower over the entire incubation period than those of sideoats grama and indiangrass ($P<0.03$) (Fig. 4). The reduced fluorescence of switchgrass, which indicates atrazine resistance, compares favorably to the atrazine resistance of seedling switchgrass observed by Martin et al. (1982) and Bahler et al. (1984). Higher levels of CRF of indiangrass and sideoats grama indicate low seedling atrazine tolerance which also was reported by Martin et al. (1982) and Bahler et al. (1984).

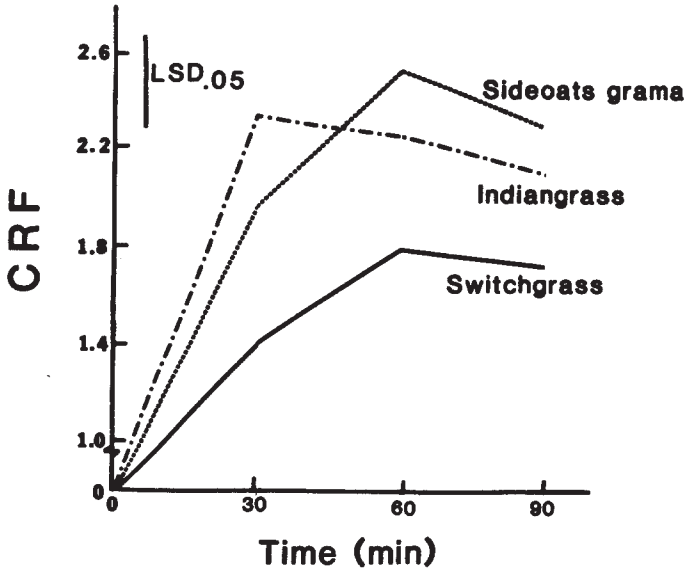

Fig. 4. *Change in relative fluorescence (CRF) of leaf sections of established indiangrass, sideoats grama, and switchgrass incubated in 10^{-3} M atrazine for a 90-min period (averages for greenhouse- and field-grown plants).*

Other members of the *Panicum* genus have been shown to detoxify atrazine by way of glutathione-peptide conjugation and 2-hydroxylation (Jensen et al. 1977). These same mechanisms may account for the tolerance of switchgrass to atrazine and its lower CRF values.

The higher CRF values of atrazine-treated indiangrass and sideoats grama possibly can be related to their mode of detoxification. When detoxification of atrazine was investigated in *Sorghastrum pellitus*, 64% of the absorbed atrazine was in an unaltered form (Jensen et al. 1977). If indiangrass also retains a high level of absorbed atrazine in an unaltered form then its detoxification mechanisms may not be as efficient as that of *Panicum*. Jensen et al. (1977) investigated members of the Chlorideae tribe which includes sideoats grama. All Chlorideae species investigated retained very large percentages of unaltered atrazine and N-dealkylated atrazine. N-dealkylation, while removing an alkyl group from atrazine, does not fully detoxify atrazine (Shimabukuro 1967). This characteristic appears to be present in the *Bouteloua* genus because seedlings of sideoats grama and blue grama [*Bouteloua gracilis* (H.B.K.) Lag ex Steud] are both extremely susceptible to atrazine injury (Martin et al. 1982, Bahler et al. 1984). This could account for the high CRF values in atrazine-treated sideoats grama.

Our data suggest that the leaf fluorescence technique may be useful for estimating the relative atrazine tolerance of warm-season, perennial grasses. It is effective for both field- or greenhouse-grown plants. Its use can possibly be expanded to other rangeland plants. Since leaf sections of different plants, both between and within species, can vary in leaf fluorescence after being incubated in distilled water, our method of using CRF values rather than the absolute relative fluorescence values used by Ahrens et al. (1981) and Ali and Machado (1981) to quantify relative differences in atrazine tolerance provides a means of equitably comparing spe-

cies. Experimental variation can also be reduced when using CRF values rather than the direct fluorescence reading. Our results indicate that incubation times of 30 min and atrazine concentrations of 10^{-3} N give CRF values that can be used to detect differences on the 3 species we studied.

Literature Cited

Ahrens, W.H., C.J. Arntzen, and W.E. Stoller. 1981. Chlorophyll fluorescence assay for the determination of triazine resistance. Weed Sci. 29:316-322.

Ali, A., and V.S. Machado. 1981. Rapid detection of triazine resistant weeds using chlorophyll fluorescence. Weed Res. 21:191-197.

Ashton, F.M., and A.S. Crafts. 1981. Mode of action of herbicides. 2nd ed. John Wiley and Sons, New York.

Bahler, C.C., K.P. Vogel, and L.E. Moser. 1984. Atrazine tolerance in warm-season grass seedlings. Agron. J. 76:891-895.

Jensen, K.I.N., G.R. Stephenson, and L.A. Hunt. 1977. Detoxification of atrazine in 3 Graminae subfamilies. Weed Sci. 25:212-220.

Martin, A.R., R.S. Moomaw, and K.P. Vogel. 1982. Warm-season grass establishment with atrazine. Agron. J. 74:916-920.

Pfister, K., and C.J. Arntzen. 1979. The mode of action of photsystem II -specific inhibitors in herbicide-resistant weed biotypes. Z. Naturforsch 34c:996-1009.

Richard, E.P., J.R. Goss, C.J. Arntzen, and F.W. Slife. 1983. Determination of herbicide inhibition of photosynthetic electron transport by fluorescence. Weed Sci. 32:361-367.

Samson, J.F., and L.E. Moser. 1982. Sod-seeding perennial grasses into eastern Nebraska pastures. Agron. J. 74:1055-1060.

Shimabukuro, R.H. 1967. Atrazine metabolism and herbicidal selectivity. Plant Physiol. 42:1269-1276.

Waller, S.S., and D.K. Schmidt. 1983. Improvement of eastern Nebraska tallgrass range using atrazine or glyphosate. J. Range Manage. 36:87-90.