

Anti-senescence Activity of Chemicals Applied to Kentucky Bluegrass

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Abstract. This study was conducted to determine the potential anti-senescence activity of certain chemicals by monitoring changes in gross CO₂ exchange with senescence of excised leaves of Kentucky bluegrass (*Poa pratensis* L.). One day following foliar applications of benzyladenine (BA), triadimefon, and propiconazole, with and without chelated Fe (8% Fe phosphate citrate), Kentucky bluegrass leaves were excised, floated on distilled water in petri dishes, and placed in a darkened growth chamber. Gross CO₂ exchange rates (CER) were recorded 1, 4, 7, and 10 days after excision (DAE). Foliar applications of Fe, BA, triadimefon, or propiconazole applied alone induced an anti-senescence response. Combinations of Fe with the chemicals delayed excision-induced leaf senescence, but no significant increase in anti-senescence activity was obtained from the Fe and chemical combinations as compared to the materials applied alone. Chemical names used: N-(phenylmethyl)-1H-purin-6-amine (benzyladenine, BA); 1-(4-Chlorophenoxy)-3,3-dimethyl-1H-1,2,4-triazol-1-yl)-2-butanone (triadimefon); 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole (propiconazole).

Foliar treatments with BA, a synthetic cytokinin, have delayed or prevented leaf senescence in many plants (Thimann, 1980; Kane and Smiley, 1983; Tao et al., 1983). Triadimefon and propiconazole, systemic triazole fungicides frequently used for plant disease control, have delayed senescence of small grain crops in the field (Kettlewell et al., 1982; Baker et al., 1984). Kane and Smiley (1983) reported that triadimefon increased chlorophyll retention of Kentucky bluegrass leaves. Foliar applications of chelated Fe have also been reported to delay senescence of french bean (*Phaseolus* spp.) (Garg and Hemantaranjan, 1988). The greening effect of foliar applications of Fe on Kentucky bluegrass has been documented (Yust et al., 1984) and has been attributed to the requirement of Fe as a precursor in chlorophyll biosynthesis (Miller et al., 1982).

The objective of this research was to determine the potential anti-senescence activity of triadimefon, propiconazole, or BA applied alone and with chelated Fe on excised Kentucky bluegrass leaves.

Materials and Methods

Experiments were performed in October and repeated in December 1987. Kentucky bluegrass 'Georgetown' plugs (5 cm in diameter and 7.5 cm deep) were taken from the field 4 weeks before the initiation of the October study. The plugs were planted in 7.6-cm (215 cm³) diameter styrofoam cups filled with a Groseclose silt loam soil (a clayey, kaolinitic mesic Typic Hapludult) with a pH of 6.2. Holes were punched in the bottom of each cup and the plugs were subirrigated by placing the cups on a plastic-lined greenhouse bench containing 2.5 cm of water.

Triadimefon, propiconazole, and BA were applied to the Kentucky bluegrass at levels of 150, 42, or 6 mg a.i./m² growing area, respectively. Chelated Fe phosphate citrate (RGB Laboratories, Kansas City, Mo.) was applied alone or in combination with the three organic materials at 112 mg Fe/m² area. All

materials were applied with a compressed-air boom sprayer that delivered 123 liter-ha⁻¹ at a pressure of 276 kPa. Plugs were sprayed twice to ensure sufficient leaf coverage.

The youngest fully expanded leaves were excised from individual plants 1 day after spraying. Leaf tips were removed and 3 cm² of leaf tissue were harvested. Excised leaf segments were floated on distilled water in uncovered petri dishes and placed in a dark growth chamber maintained at 22C to accelerate senescence (Thimann and Satler, 1979). The experiment was designed as a completely randomized 4 × 2 factorial (4 chemical treatments × 2 Fe levels) observing the effects of BA or the triazoles applied with and without Fe across the four measurement dates. Each treatment was replicated five times.

Measurements of gross CER of detached leaves were made on the same leaf 1, 4, 7, and 10 DAE. Leaves were removed from the petri dishes in the darkened growth chamber just before measurement, blotted dry on tissue paper, and immediately placed in a Decagon LD-2 (Decagon, Pullman, Wash.) leaf disk electrode unit with an effective chamber volume of 5 cm³ that enclosed the 3 cm² of leaf tissue. The opened air flow rate into the sample chamber and reference line was 0.05 liter-min⁻¹. Differential CO₂ concentrations were monitored by passing standard and sample air lines through an infrared gas analyzer (model AR-600, Anarad, Santa Barbara, Calif.). Dark respiration CO₂ exchange was allowed to reach a steady-state level and this value was recorded.

Measurement of CO₂ exchange in the light was initiated by turning on a light source mounted 30.5 cm above the LD-2 chamber that was focused through a 150-mm lens and delivered 750 μmol-s⁻¹-m². The temperature of the leaf segments was maintained at 20C by pumping cooled water through the water-jacketed chamber. Steady-state CER values attained in light and darkness were recorded and gross CER was calculated by subtracting CO₂ exchange in the dark from CO₂ exchange in the light (Edmisten and Wolf, 1988).

Repeated time analyses using Wilke's criterion for the hypotheses of time effects and time × treatment interactions were performed, since repeated measurements were made on the same leaf material (Morrison, 1976). Data for gross CER measure-

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Abbreviations: CER, CO₂ exchange rates; DAE, days after leaf excision.

ments were analyzed by contrasts and Dunnett's procedure for each date when time effects were significant.

Results and Discussion

Multivariate analyses using Wilke's criterion showed time to be highly significant and the time \times treatment interaction to be nonsignificant for CER data. Contrasts and Dunnett's procedure for treatment comparisons with a control were used as statistical analyses for each date of measurement for the CER data. Single degree of freedom contrasts indicated very few differences in any comparison of organic chemical treatments when applied alone or with chelated Fe. Therefore, these statistical analyses are not presented.

The gross CER decline for both experiments with time was anticipated as excision-induced senescence progressed (Table 1). The trend for decline in gross CER across time for the October experiment was linear, while gross CER values leveled off at 7 DAE in the December experiment (Table 1). Comparisons of CER values between chemical treatments and the untreated control indicated that the majority of differences were detected 10 DAE in the October experiment (Table 2). Although not significantly different from the control, all treated leaves, except for the triadimefon plus Fe treatment, tended to have larger CER values at 7 DAE in the October experiment (Table 2). BA, BA plus Fe, propiconazole, and Fe alone had significantly larger CER values 10 DAE in the October experiment.

Only propiconazole alone and BA plus Fe at 4 and 7 DAE, respectively, and BA plus Fe or propiconazole plus Fe at 10 DAE significantly increased gross CER over nontreated leaves in December (Table 2). Still, CER values for all treated leaves tended to be larger than the control leaves 10 DAE.

Foliar applications of chelated Fe applied alone resulted in CER values similar to those obtained with the combinations of BA or triazoles with Fe (Table 2). The CER values for the combination treatment of triadimefon plus Fe were unexplainably low 7 and 10 DAE in the October experiment. Contrasts comparing the grouping of organic materials plus Fe vs. Fe alone were not significantly different (statistical data not presented). This result indicated no additional or synergistic response in retarding senescence by combining chelated Fe with BA, triadimefon, or propiconazole.

The synthetic cytokinin BA and the combination of BA and chelated Fe tended to maintain the highest CER values across the 10-day measurement period in both experiments (Table 2). Previous reports have documented the anti-senescence activity of BA (Tao et al., 1983). Garg and Hemantaranjan (1988) indicated that chelated Fe applications retarded leaf senescence, stimulated chlorophyll production, and increased photosynthetic rates in beans (*Phaseolus* spp.). Responses to treatments of propiconazole or triadimefon alone were not significantly different

Table 1. Mean CER of Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles 1 day before excision.

Experiment	Gross CER ^z ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)			
	DAE			
	1	4	7	10
October	3.8	3.5	1.9	0.8
December	3.8	2.2	1.6	1.5

^zValues are means of five replications; none of the interactions were significant.

Table 2. CER of Kentucky bluegrass leaves 1, 4, 7, and 10 DAE following foliar applications of BA and triazoles alone or with Fe.

Chemical	Concentration ^z Chemical	Fe	Gross CER ^y ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)			
			DAE			
			1	4	7	10
<i>October</i>						
None	0	0	2.34	2.75	1.51	0.30
	0	112	3.26	3.88	2.34	1.01*
BA	6	0	4.31	3.99	2.30	1.10*
	6	112	3.90	3.01	2.50	1.15*
Propiconazole	42	0	4.29	4.29*	2.16	0.90*
	42	112	5.83*	3.81	2.27	0.71
Triadimefon	150	0	3.30	3.79	1.97	0.71
	150	112	2.89	2.71	0.57	0.16
LSD	---	---	2.25	1.39	1.03	0.57
<i>December</i>						
None	0	0	3.60	1.33	0.83	0.64
	0	112	4.27	2.11	1.70	1.33
BA	6	0	3.46	2.80	1.33	1.77
	6	112	4.40	2.13	2.59*	1.97*
Propiconazole	42	0	3.63	2.96*	1.54	1.38
	42	112	3.97	2.18	1.75	2.23*
Triadimefon	150	0	2.98	1.72	0.96	0.99
	150	112	4.27	2.39	1.68	1.49
LSD	---	---	2.17	1.58	0.92	1.29

^zLevels expressed in milligrams of active ingredient applied per square meter of growing area.

^yValues are means of five replications consisting of 3 cm² of leaf tissue per replication.

^xValues within the same column and same experiment are significantly different from the control at $P = 0.05$, according to Dunnett's test.

from each other at any time in either experiment (Table 2). The triazole-treated leaves tended to have CER values similar to BA-treated leaves, suggesting cytokinin-like activity of the triazoles.

As endogenous plant growth substances, cytokinins have acted as anti-senescence agents (Thimann, 1980). The triazoles have exhibited cytokinin-like growth effects on certain plants, but Fletcher and Arnold (1986) reported that the growth activity of triadimefon was a stimulation of root growth that indirectly could have led to increased plant cytokinin levels. Since the leaves in these experiments were excised 1 day after treatment, it is unlikely the anti-senescence response could have come from a promotion of plant cytokinin synthesis.

Prior studies with BA, the triazoles, and Fe indicated each of these chemicals generally stimulated both chlorophyll production and photosynthetic rates (Kane and Smiley, 1983; Kettlewell et al., 1982; Garg and Hemantaranjan, 1988). In our experiment, the treatments apparently maintained chlorophyll activity or at least retarded chlorophyll degradation. Frequent observations of leaf color as senescence progressed suggested that the rate of chlorophyll degradation in the treated leaves was reduced, since these leaves remained greener than the untreated leaves.

Foliar applications of triadimefon, propiconazole, and Fe are a part of many turfgrass maintenance programs. In addition to their respective uses as fungicides or fertilizers, the anti-senescence activity could theoretically promote an increase in photosynthetically active leaf area for the turf plant and further enhance plant growth and development. Further research to examine anti-senescence activity and growth regulator activities on intact plants is necessary to determine if these responses can be obtained in the field.

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