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Tolerance of Cotton (*Gossypium hirsutum*) Seedlings to Preemergence and Postemergence Herbicides with Four Modes of Action

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Abstract
The current dominant weed control program in cotton relies heavily on glyphosate. Typical glyphosate-based weed control programs require repeat applications. A residual herbicide might reduce the number of herbicide applications needed, and potentially reduce costs. Residual herbicides that can be used postemergence in cotton are limited in number, and there are few studies evaluating the response of cotton to over-the-top application of herbicides. A greenhouse study evaluated response of cotton to fluometuron, propazine, metolachlor, pyrithiobac, and glyphosate. Most of these herbicides caused minimal crop injury; however, fluometuron and propazine caused significant visual injury when applied over-the-top at the cotyledon and two-leaf stages, but less injury when applied preemergence.

Introduction
Cotton weed control is difficult for numerous reasons, including a lack of available herbicides, a relatively uncompetitive crop canopy, and slow inherent growth. Cotton growth is further slowed by soil-borne diseases, insect damage, and herbicides (10,22).

Cotton can require upwards of eight weeks of weed-free maintenance to achieve maximum yields, which is a longer period than is required in corn or soybean (4). Cotton also requires higher temperatures for rapid growth than does soybean (*Glycine max*) or corn (*Zea mays*). Most cotton growth models use a base temperature of 15.5°C (28) versus base temperatures of 10°C for most other summer-annual row crops and weeds (2,16,25). Cotton is also planted at relatively low densities, further reducing the competitive ability of cotton as compared to other agronomic row crops.

Fewer herbicides are available for use in cotton than for corn or soybean. For example, the first selective, postemergence broadleaf herbicide for cotton, pyrithiobac (2-chloro-6-[(4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid, sodium salt), was not registered until 1996, despite a long-standing need for such a product. Before the advent of transgenic, herbicide-resistant cotton cultivars, five or more conventional herbicides and three or more between-row tillage operations were often used in cotton (20). In 1997, weed control in cotton was improved significantly by the registration of transgenic, glyphosate-resistant cotton cultivars (7).

Glyphosate (N-(phosphonomethyl) glycine) is a very broad-spectrum, postemergence herbicide (17). Its efficacy and reliability have made it the dominant herbicide used for cotton weed control programs, as evidenced by the wide use of transgenic, glyphosate-resistant cultivars (27) However, cotton's slow early-season growth still makes glyphosate-based programs somewhat less effective in cotton than are similar programs in corn or soybean. Weed control in corn or soybean can typically be accomplished with one to two glyphosate applications, whereas in cotton, three or more herbicide applications plus supplemental tillage are sometimes needed to control weeds. Additionally, before 2006, the commercially-available glyphosate-resistant cultivars lacked reproductive tolerance to glyphosate applied over-the-top after the four-leaf stage (24). Later applications often disrupt boll formation and sometimes reduce yield (12). On these cultivars, glyphosate should only be applied as a carefully-directed spray, targeted beneath cotton foliage to minimize contact with the plant (32). Taller weeds growing in proximity to the cotton may not be controlled because the spray will be directed away from these weeds as well. Glyphosate has essentially no soil residual activity (26), so additional weeds may germinate immediately after treatment. Recently, glyphosate-resistant Palmer amaranth has been discovered (6). This particular weed species has always been troublesome, and glyphosate has been an excellent control option. However, if glyphosate-resistant Palmer amaranth becomes widespread, residual herbicides will become more important.

One or more soil residual herbicides added to a glyphosate-based weed management program in cotton could reduce the number of herbicide applications and the associated fuel, labor, and equipment costs (30). Additionally, there is significant interest in using residual herbicides to prevent the development of glyphosate-resistant weeds. Although many herbicides with soil-residual activity may injure cotton, data are limited on cotton response to such herbicides when they are applied postemergence to the crop. The herbicide fluometuron (*N*,*N*-dimethyl-*N*'-[3-(trifluoromethyl)phenyl]urea) has both soil and foliar activity. Fluometuron may be used in cotton either preemergence, post emergence, or as a directed spray. Until the advent of glyphosate-resistant cotton, fluometuron was sometimes applied over the top of cotton up to the two to three leaf stage, despite some injury to the crop, because other options were not available.

The herbicide propazine was once registered for grain sorghum; however, cotton producers in West Texas occasionally used this herbicide on cotton for residual weed control and preliminary research also showed this potential (18).

This study was conducted to document seedling cotton response to postemergence glyphosate, fluometuron; s-metolachlor (2-chloro-*N*(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide); propazine (6-chloro-*N*,*N*'-bis(1-methylethyl), 1,3,5-2,4-diamine); and pyrithiobac. The four residual herbicides were selected because experience suggested that they could be applied postemergence to cotton with minimal crop injury. The four herbicides also represent four additional chemical classes of herbicides commonly used in agronomic crops and three different modes of herbicide action.

Herbicide Evaluation

Six cotton seed were planted in 10-cm pots containing a Boskett fine sandy loam soil (fine-loamy, mixed, thermic, Mollic Hapludalf) with a pH of 6.1 and 0.5% organic matter. The cotton was the glyphosate-resistant cultivar, 'Delta & Pineland 5415RR,' and all cotton was from the same seed lot. Treatments were a factorial arrangement of five herbicides, five herbicide rates, and four application timings (except for glyphosate). Fluometuron, metolachlor, propazine, and pyrithiobac were applied preemergence, at the cotyledon stage, and at the two-leaf stage and four-leaf stage. Because glyphosate has no preemergence activity, it was only applied at the postemergence timings.

The herbicides were applied at zero, one quarter, one half, one, and two times their label-recommended rates (or rates that were recommended in other crops in the case of propazine) as shown in Table 1. Treatments were applied using a CO_2-pressurized backpack sprayer at approximately 165 kPa of pressure and an application volume of 187 liter/ha. Nozzles were Tee-Jet (Spraying Systems Co., Carol Stream, IL) XR11002VS tips with a spacing of 38 cm. Treatments were replicated two times per trial, and the entire experiment was repeated three times. The repetitions of experiment were considered a random effect and a factorial analysis of variance was done for each herbicide. Greenhouse temperatures were 10 to 20°C at night and 20 to 30°C daytime with ambient sunlight of approximately 1,000 to 1,500 umol/m²/sec.

Table 1. Herbicides, label rates, chemical families, modes of action, and descriptions of uses that were applied to cotton at early growth stages.

Herbicide Rate used Chemical family	**Mode of action** Description
Fluometuron 1.12 kg ai/ha Urea	**Photosystem II electron transport inhibitor** A preemergence herbicide commonly used in cotton. This herbicide has also occasionally been used postemergence, but with a high risk of crop injury.
Glyphosate 0.84 kg ai/ha Amino acid	**EPSP Synthase Inhibitor** A postemergence, non-selective herbicide often used on transgenically modified, herbicide-resistant crops.
Propazine 1.12 kg ai/ha Triazine	**Photosystem II electron transport inhibitor** A preemergence and postemergence herbicide that was formerly registered on grain sorghum; however, experiments have shown potential to use propazine in cotton (18).
Pyrithiobac 0.07 kg ai/ha Pyrimidinyl thio-benzoates	**ALS Inhibitor** A selective postemergence broadleaf herbicide for cotton.
s-metolachlor 1.34 kg ai/ha Chloroacetamide	**Exact mode of action not known, inhibits shoot growth** Primarily a preemergence herbicide used in corn, cotton and soybean for control of grass and small-seeded broadleaf weeds. Some use as a residual tank-mix partner with postemergence herbicides.

Two weeks after application (± 2 days), cotton plants were visually rated for injury using the standard weed science percentage scale where 0% indicates healthy plants and 100% indicates plant death (13). At the time the ratings were done, a Minolta 502 SPAD (Specialty Products Agricultural Division, Plainfield, IL) meter was used to measure relative chlorophyll content of the plant. These meters measure the differential light attenuation of the leaves at 640 nm (red) and 940 nm (near-IR), and the readings have been shown to represent a relative chlorophyll measurement (23).

Four weeks after the second postemergence application (± 2 days), all pots were destructively harvested to determine dry weights of above-ground plant tissue. Plants were dried on a greenhouse bench for 7 days, dry weights were measured, and converted to a percentage of the controls. Fresh weight and percentage moisture were also collected; however, moisture data showed no consistent trends and fresh weight data were similar to dry weight data and these data are not shown. Data were subjected to a preliminary factorial analysis of variance and numerous herbicide interactions were observed. Because the objective of this study was to determine if cotton growth stage affected cotton tolerance to herbicides, the analysis was repeated on a by-herbicide basis evaluating herbicide rate, timing, and the interaction of rate and timing. In this analysis we focused on rate by timing interactions because this interaction is an indication that cotton responded differently at different growth stages. The factorial structure of treatments resulted in numerous pots that were the "zero-rate" of each herbicide. These controls were combined and also used to convert weight data to percentage of the untreated to reduce variability between experimental trials.

Cotton Response to Herbicides

Glyphosate. While we observed a significant timing effect for all parameters, herbicide rate and the interaction of rate and timing was not a significant (Table 2). The lack of a rate effect indicated that glyphosate did not damage the cotton. There were differences in relative chlorophyll content and visual injury ratings with application time; however, these differences were due to cotton growth and visual appearance at the different sampling times. There was a main effect of timing as relative dry weight increased from 85%, 89%, and 100% of the untreated control with the cotyledon, two-leaf, and four-leaf application times, respectively. However, because there was no effect of glyphosate rate we believe that this difference (significant at the 0.03 level) indicates a very low level of phytoxicity that does not adversely affect the utility of the treatment. Glyphosate is a highly specific inhibitor of the shikimate pathway enzyme, 5-enolpyruvylshikimate-3 phosphate synthase (EPSPS), and has a very broad spectrum of activity against both mono- and dicotyledonous weeds (1,17). Glyphosate may be used on transgenic, glyphosate-resistant cotton cultivars, without visible crop injury, except for possible loss of fruiting forms, if applied after the four-leaf stage (12,32).

Table 2. Probability of effects of herbicide rates, stage of cotton growth at treatment, and their interaction on visual injury (%), chlorophyll meter measurements (SPAD), and relative dry weights (%).

		Rate	**Timing**	**Interaction**
Herbicide	**Parameter**	**(P-value)**		
Glyphosate	% Injury	0.5041	<0.0001	0.3741
	SPAD	0.7768	0.0007	0.7060
	Rel. dry weight	0.3913	0.0307	0.2384
Pyrithiobac	% Injury	0.4962	<0.0001	0.3839
	SPAD	0.1381	<0.0001	0.3049
	Rel. dry weight	0.5547	0.1914	0.5698
s-metolachlor	% Injury	0.0011	0.1228	0.0005
	SPAD	0.5454	<0.0001	0.8725
	Rel. dry weight	0.0596	<0.0001	0.0223
Fluometuron	% Injury	<0.0001	<0.0001	<0.0001
	SPAD	0.0027	<0.0001	0.2048
	Rel. dry weight	<0.0001	0.0031	0.2811
Propazine	% Injury	<0.0001	<0.0001	<0.0001
	SPAD	0.0006	<0.0001	0.1322
	Rel. dry weight	0.0007	0.0005	0.0886

Pyrithiobac. Similar to the glyphosate results, there were no significant effect of pyrithiobac rate or an interaction between rate and timing (Table 2). Pyrithiobac is a pyrimidinylthiobenzoates herbicide that inhibits acetolactate synthase (ALS), a key enzyme in the biosynthesis of branched chain amino acids (18). It was relatively recently (1996) registered for use in cotton as the first selective postemergence, over-the-top herbicide for broadleaf weed control, and also has a preemergence activity. Pyrithiobac is generally considered safe on cotton, although chlorosis and stunting may occasionally occur. Injury due to pyrithiobac treatment was not observed in our study.

S-metolachlor. The two higher rates of s-metolachlor caused slight visual injury when applied preemergence, and at the cotyledon and two-leaf stages (Table 2 and Fig. 1) The highest rate of s-metolachlor had no effect on cotton dry weight; however, reductions were noted with lower rates applied at the cotyledonary stage. In agricultural use, s-metolachlor is known to cause occasional growth reductions. S-metolachlor did not affect SPAD readings; however, SPAD data are shown for consistency of data presentation with the other herbicides (Fig. 1). S-metolachlor, formerly the racemic metolachlor, is a chloroacetamide herbicide. Chloroacetamide herbides are chemically diverse in structure, but typically are used preemeergence and inhibit cell division (9). S-metolachlor is registered for preemergence or early postemergence use in cotton and as a pre-mix with glyphosate.

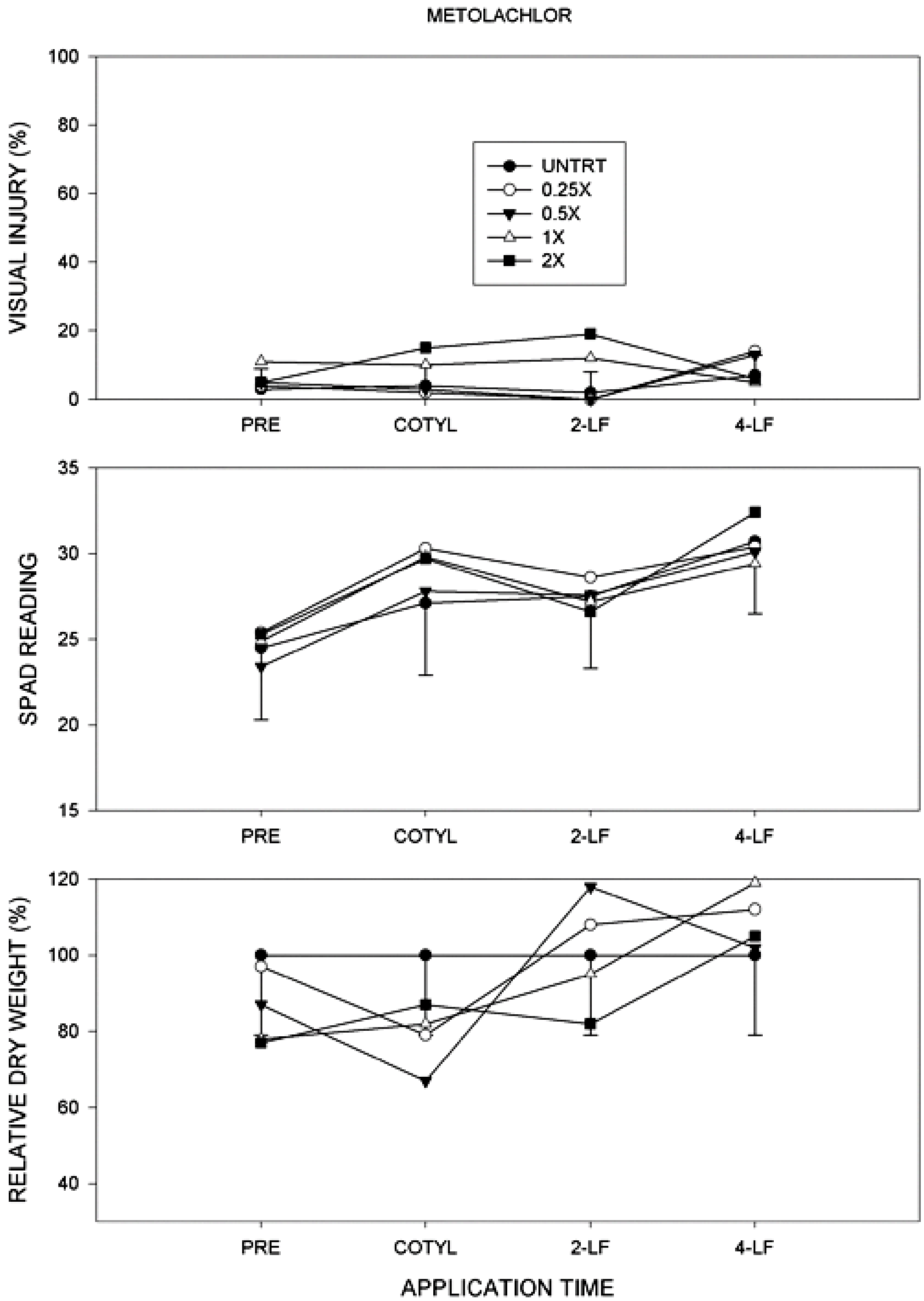

Fig. 1. Effect of application timing and s-metolachlor rate on visual injury, SPAD readings, and relative dry weight of cotton seedlings in a greenhouse herbicide response study. Herbicide treatments were applied preemergence (PRE), and at the cotyledon (COTY), two-leaf (2-LF), and four-leaf (4-LF) stage of cotton. LSD bars (5%) are located on the untreated data points. The 1X herbicide rate was 1.34 kg/ha.

The s-metolachlor label does not allow application to sand or loamy sand soils nor to areas where water is likely to pond over the soil. Significant injury can occur if these restrictions are not followed. Foliar phytotoxicity of s-metolachlor has been observed following the application of s-metolachlor at temperatures greater than 30°C (30). However the largest differences in dry weights between untreated and treated occurred at the cotyledon stage. Little adverse effect of metolachlor was found in this study, with effects being slight and frequently inconsistent among rates.

Fluometuron. At all application timings, injury tended to increase as the fluometuron rate increased (Fig. 2.). Although not all treatments differed statistically, there was a general rate response with several instances of differences occurring between higher and lower rates. The injury response was greatest with the cotyledon-stage applications, followed by the two-leaf applications. Injury response was least with preemergence timings which is the preferred way to use fluometuron. Chlorophyll readings followed an opposite trend, as expected because greater injury would lead to lower chlorophyll levels. The greatest response occurred following treatment at the two-leaf stage, with least response found following the preemergence application. With fresh and dry weights, the greatest reductions occurred at the cotyledon stage, and the least response occurred with the four-leaf application.

Before the advent of transgenic, glyphosate-resistant cotton, fluometuron was frequently used preemergence as well as for directed, early postemergence sprays. However, some applications of fluometuron were made post emergence, over-the-top (15). These data indicate that the cotyledon stage may be the most sensitive to fluometuron. Visual injury ratings and SPAD meter readings were affected less at preemergence timings while fresh and dry weights were affected more. It is possible that fluometuron causes a general reduction in growth, with relatively little yellowing or visual damage when used preemergence.

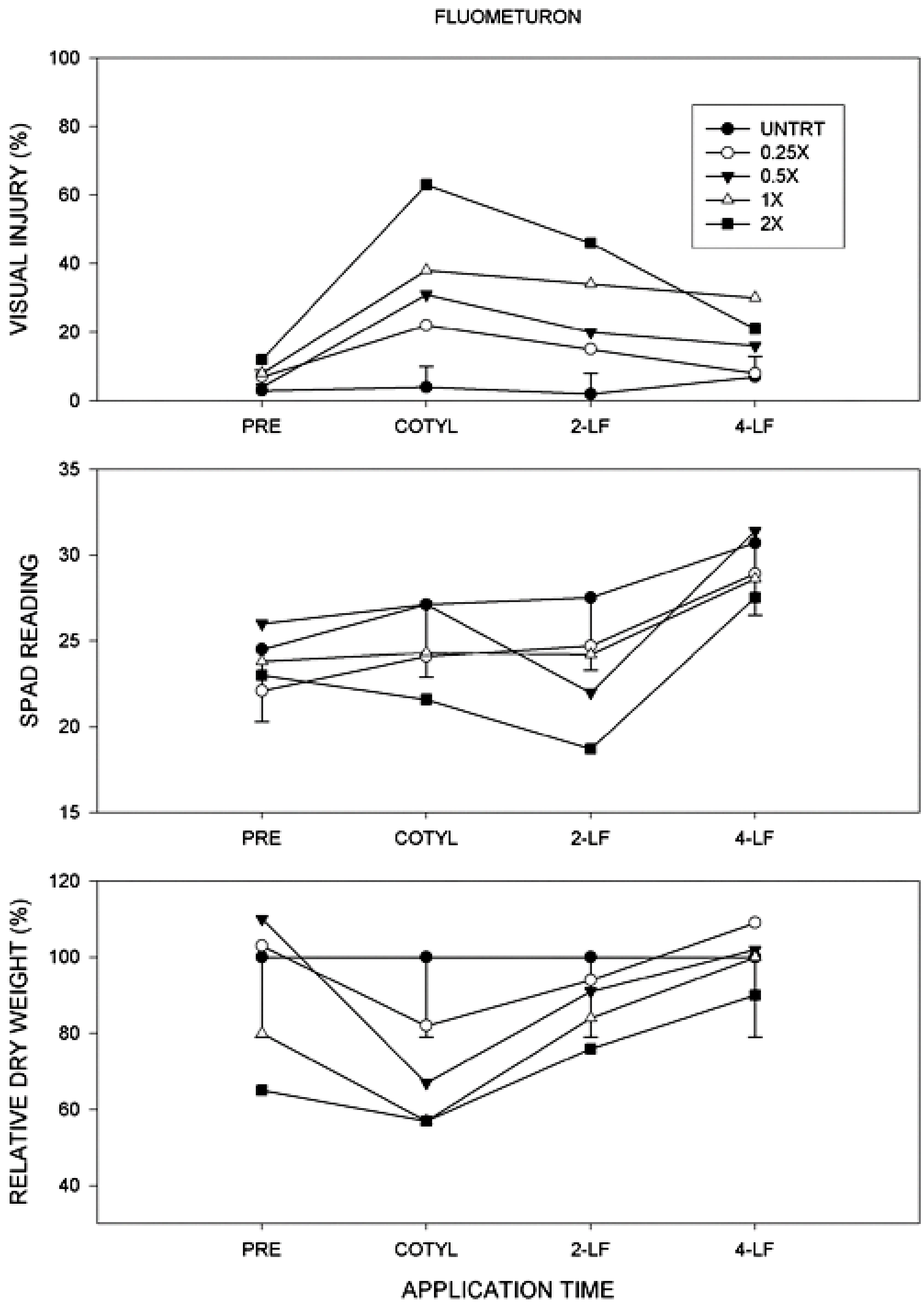

Fig. 2. Effect of application timing and fluometuron rate on visual injury, SPAD readings, and relative dry weight of cotton seedlings in a greenhouse herbicide response study. Herbicide treatments were applied preemergence (PRE), and at the cotyledon (COTY), two-leaf (2-LF), and four-leaf (4-LF) stage of cotton. LSD bars (5%) are located on the untreated data points. The 1X herbicide rate was 1.12 kg/ha.

Propazine. The same trends described with fluometuron were generally seen with propazine treatments (Fig. 3). Visual injury and weight reductions were greatest with cotyledon-stage applications. Although the trends with fluometuron and propazine were similar, crop response from the propazine was generally less. While these herbicides differ in chemical family, they share the same mode of action of inhibition of Photosystem II electron transport.

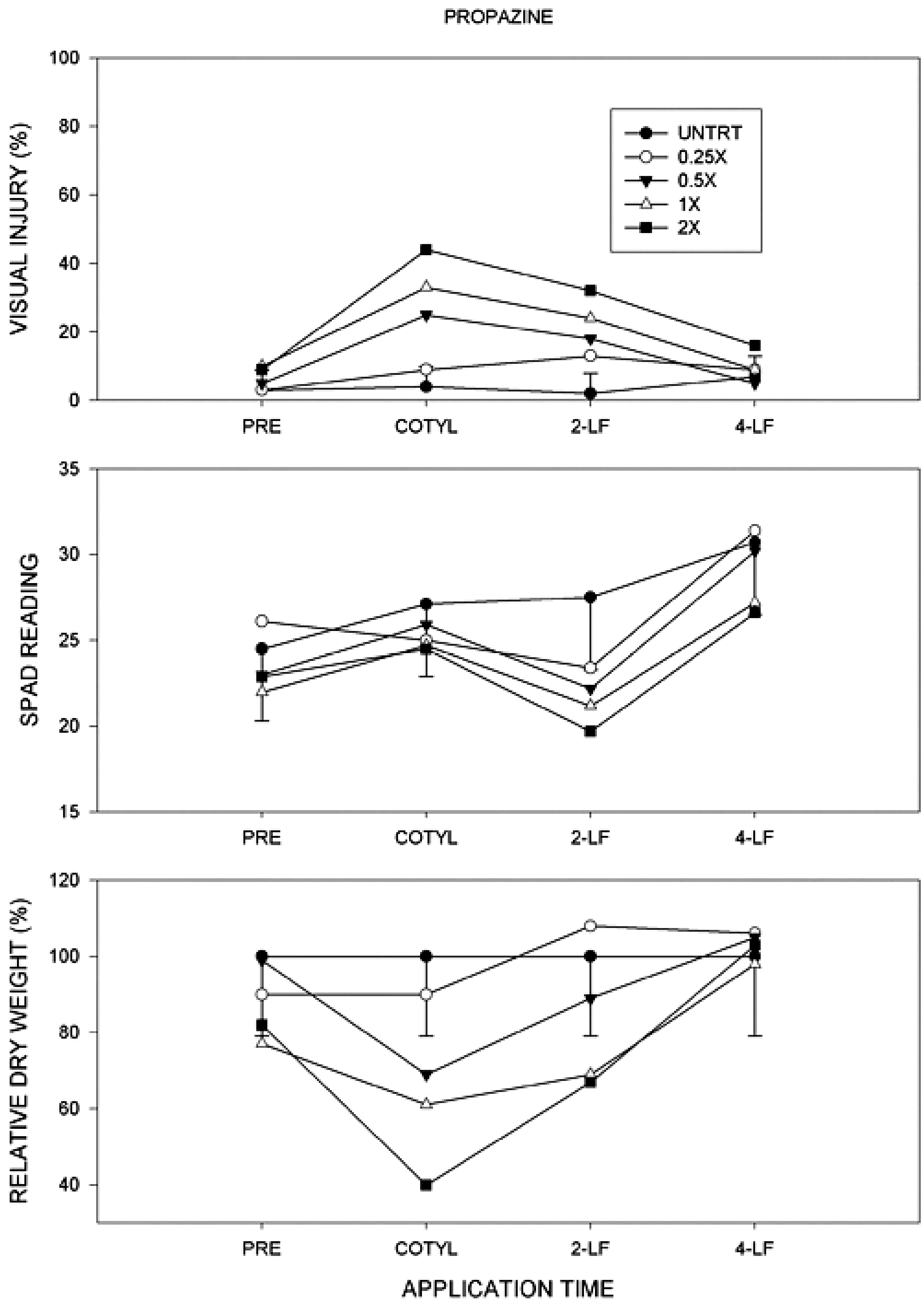

Fig. 3. Effect of application timing and propazine rate on visual injury, SPAD readings, and relative dry weight of cotton seedlings in a greenhouse herbicide response study. Herbicide treatments were applied preemergence (PRE), and at the cotyledon (COTY), two-leaf (2-LF), and four-leaf (4-LF) stage of cotton. LSD bars (5%) are located on the untreated data points. The 1X herbicide rate was 1.12 kg/ha.

Propazine is an s-triazine herbicide that inhibits Photosystem II (11). Propazine was primarily used for grain sorghum (*Sorghum bicolor* L.); however, some propazine was used on cotton in dry areas in Texas, where its use in sorghum was also common. In recent research, propazine has provided significant improvements in residual control when applied with glyphosate; however, minor crop injury was noted (18).

In summary, pyrithiobac showed essentially no potential for seedling injury. Metolachlor, showed few adverse effects, and is also registered for preemergence and postemergence use. Fluometuron has potential to injure small cotton, but could be used when better options for residual control are unavailable. Propazine is no longer registered for use in either sorghum or cotton, but its tolerance by cotton seedlings and young plants was similar to that found with fluometuron. Given that propazine has provided excellent residual pigweed control, a registration of propazine on cotton could be useful for management and prevention of glyphosate-resistant weeds.

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