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MOLECULAR BIOLOGY & PHYSIOLOGY

Gossypium Lines Resistant to *Rotylenchulus reniformis* Vary in Sensitivity to the Herbicide Fluometuron

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ABSTRACT

Reniform nematode (*Rotylenchulus reniformis*) resistance is being transferred to *Gossypium hirsutum* from its distant relatives. Reports of fluometuron damage to LONREN lines with nematode resistance from *G. longicalyx* raised concerns about introducing herbicide sensitivity from other nematode resistance sources. The research objective was to evaluate 15 sources of reniform nematode resistance for their reaction to fluometuron three weeks after planting in a replicated greenhouse trial: two *G. herbaceum* accessions, four *G. arboreum* accessions, three *G. barbadense* accessions, three *G. hirsutum* accessions, and three *G. hirsutum* lines with resistance introgressed from *G. barbadense* (FR-05) or *G. longicalyx* (LONREN-1 and LONREN-2). The control genotype was *G. hirsutum* cultivar Deltapine 161 B2RF. Across all herbicide rates tested, mean herbicide injury ratings for *G. arboreum* accessions were greater than the control, whereas *G. barbadense* GB 713 and TX 110 were less. Regression analysis of herbicide rates indicated that injury increased linearly with increasing herbicide rate for all accessions, although *G. arboreum* A_2-083 had more injury than the control. Regression analysis of herbicide rates indicated that biomass decreased linearly with increasing herbicide rate for all accessions, although *G. barbadense* GB 713 and Pima PHY 800 exhibited greater biomass reduction than the control. Across all herbicide rates tested, mean electron transport rates of all *G. herbaceum* and *G. arboreum* accessions and *G. barbadense* Pima PHY 800 were lower than the control. The relationship between herbicide rate and electron transport rate was curvilinear, with similar decreases in electron transport rate in response to increasing herbicide concentration for all lines. Increased sensitivity to fluometuron could be introduced into *G. hirsutum* through crosses with distantly related species, but with the exception of *G. arboreum* A_2-083, the lines did not respond to the herbicide differently from the control.

Reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) causes significant economic losses to cotton (*Gossypium hirsutum* L.) in the southeastern U.S. (Koenning et al., 2004; Robinson, 2007). Symptoms of infection by reniform nematode include stunted plants, nutrient deficiencies, fewer bolls, smaller bolls, and reduced lint percentage (Jones et al., 1959; Koenning et al., 2004; Robinson, 2007). The greatest yield losses occur in the Mid-South states of Louisiana, Mississippi, and Alabama, where losses from 4 to 6% have been reported in recent years; Blasingame and Patel (2011, 2012, 2013) estimated combined yield losses of 92,456; 137,679; and 105,402 bales in 2010, 2011, and 2012, respectively, in these states.

Nematicides and crop rotation have been used to manage reniform nematode. Nematicides can be effective (Faske and Starr, 2006; Koenning et al., 2007; Rich and Kinloch, 2000), but concerns about expense and negative effects on human health and the environment have contributed to a reduction in the number of products available (Starr et al., 2007). Rotation to nonhost crops reduces reniform nematode populations temporarily, but the population recovers rapidly in the subsequent cotton crop (Davis et al., 2003; Koenning et al., 2004; Stetina et al., 2007). Unfortunately, no upland cotton cultivars with resistance to reniform nematode are currently available (Robinson, 2007; Robinson et al., 1999). Resistance has been identified in related *Gossypium* species: *G. longicalyx* Hutch. & Lee (Dighe et al., 2009), *G. aridum* (Rose & Standl.) Skov. (Romano et al., 2009; Sacks and Robinson, 2009), *G. barbadense* L. (Gutiérrez et al., 2011), and *G. arboreum* L. (Erpelding and Stetina, 2013; Sacks and Robinson, 2009). However, introgression of the resistance from these sources is not simple and can include several risks. Moving undesirable traits along with the nematode resistance (Nichols et al., 2010; Wallace et al., 2013) is possible, and the

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insertion of the foreign chromosome segment could potentially disrupt the expression of desirable genes in the plant (Chapala et al., 2012; Nichols et al., 2010).

One example of the risk associated with genetic modification of an adapted upland cotton background during the introgression process is illustrated in the response of LONREN and NEMSTACK breeding lines, which have reniform nematode resistance from *G. longicalyx* (Bell et al., 2009; Dighe et al., 2009), a species that is nearly immune to the reniform nematode. When researchers planted the lines and their progeny in fields across the southeastern U.S., early-season stunting that translated to poor yields at harvest was observed at many locations (Bell et al., 2009; Nichols et al., 2010). Examination of crop management practices at each site where stunting was observed suggested an association between plant damage and the use of the preemergence herbicides fluometuron or prometryn, both of which inhibit photosystem II (Bell et al., 2009, Nichols et al., 2010). Further tests under controlled conditions confirmed that plants homozygous for the reniform nematode resistance were more sensitive to these herbicides than heterozygous breeding lines or their homozygous susceptible siblings (Bell et al., 2009). However, further evaluation of these materials in fields with high reniform nematode pressure but where photosystem II inhibiting herbicides were not used also showed stunting of plants, suggesting that responses to herbicides and nematodes are indistinguishable and might be confounded (Nichols et al., 2010).

Fluometuron (*N,N*-dimethyl-*N'*-[3-(trifluoromethyl)phenyl]urea) was introduced for commercial use in cotton in 1965 (Melnikov, 1971) and remains one of the most commonly used soil-applied herbicides in cotton for control of annual grasses and small-seeded broadleaf weeds. It acts on susceptible plants by inhibiting photosynthesis and has prolonged residual action of two to five months (Porterfield et al., 2002). Fluometuron can 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. Although cotton is able to break down fluometuron (Eshel, 1969; Rogers and Funderburk, 1968), injury has been noted under cool wet conditions. Fluometuron is more readily absorbed by roots from soil application, than by leaves from foliar application (Eshel, 1969; Rubin and Eshel, 1977). The amount and rate at which it is absorbed, translocated, and subsequently metabolized, can vary among plant species and also between plant growth stages (Rubin and Eshel, 1977, 1978). Whereas fluometuron use in *G. hirsutum* and *G. barbadense* varieties is well established, the relative susceptibility of other cotton species is not documented.

Damage to *G. longicalyx*-derived lines that had been exposed to photosystem II inhibiting herbicides raised concerns about introducing herbicide sensitivity from other potential resistance sources. Therefore, the objective of this research was to evaluate 15 sources of reniform nematode resistance currently being used in germplasm improvement programs for their reaction to fluometuron in a replicated greenhouse trial.

MATERIALS AND METHODS

Fifteen lines with reniform nematode resistance were evaluated for their reaction to fluometuron. The lines tested were: *G. herbaceum* accessions A_1-017 and A_1-024; *G. arboreum* accessions A_2-083, A_2-100, A_2-190, and A_2-194; *G. barbadense* accessions Pima PHY 800, GB 713, and TX 110; *G. hirsutum* accessions T19, T1347, and T1348; and three *G. hirsutum* lines with resistance introgressed from *G. barbadense* (FR-05) or *G. longicalyx* (LONREN-1 and LONREN-2). The control genotype was *G. hirsutum* cultivar Deltapine 161 B2RF. Six seeds of each line were planted on top of 450 cm^3 of a mixture of sandy loam soil and sand (3:1 by volume) in 10-cm square pots. Fluometuron (Flo-Met 4L, Arysta Life Science North America Corporation, Cary, NC) added to 100 cm^3 additional soil mix (1:1 sandy loam soil:sand by volume) at rates of 0, 0.34, 0.67, 1.01, 1.34, and 1.68 kg a.i./ha was used to cover the seeds, and care was taken during watering to avoid disturbing soil. The field rate for fluometuron varies with soil type, but generally is in the range of 0.89 to 1.12 kg a.i./ha.

The experiment was conducted twice; trial 1 was planted on 18 November 2010 and harvested on 9 December 2010, and trial 2 was planted on 12 January 2011 and harvested on 3 February 2011. Pots containing all combinations of genotype and herbicide were arranged in a completely randomized design in each greenhouse trial and were treated as the experimental units. Natural light was supplemented between 0400 and 1900 h with three 1,000-watt high pressure sodium lamps spaced 2 m apart at 1.4 m above the plants. To maximize the number of observations in the herbicide concentration range of greatest interest (based on preliminary tests, data not shown), two pots of each

genotype were included in each trial for herbicides concentrations ranging from 0.34 to 1.34 kg a.i./ha, whereas only one pot of each genotype represented the lowest and highest herbicide concentrations.

Three weeks after planting, plants were rated for herbicide injury, electron transport rates were measured, and plant tissue was harvested for biomass determination. Each plant was scored for herbicide injury on a scale of 0 to 4 where 0 = no injury and 4 = maximum injury observed on any plant in the trial. Indications of injury included bleaching of interveinal tissue, with progression to tissue necrosis and plant death in extreme cases. Electron transport rates in the cotyledons of two plants per pot were measured between 1000 and 1200 h. These measurements were based on chlorophyll fluorescence, which is associated predominantly with photosystem II (Briantais et al., 1986; Turley and Pettigrew, 2011), and were made using an OS5-FL Modulated Fluorometer (Opti-Sciences, Inc., Tyngsboro, MA). After herbicide injury ratings were taken, all green tissue above the cotyledonary node was harvested from all plants in each pot. Plant material was dried at 60 °C for 48 h and a combined dry weight determined.

All analyses were completed using the mixed models procedure in SAS (SAS PROC MIXED, SAS version 9.2, SAS Institute Inc., Cary, NC) with the Kenward-Roger denominator degrees of freedom option ($p \leq 0.05$). Data from both trials were combined. Initial analysis treated trial as a fixed effect and revealed no significant interactions with treatments. Therefore, in the final analysis trial and trial interactions were modeled as random effects. Treatments had a factorial structure with 16 cotton lines and six herbicide rates. Analysis of variance (ANOVA) was used to compare lines, with post-ANOVA means separation based on differences of least squares means ($p \leq 0.05$). Regression analysis was used to determine the nature of the response to the herbicide, with herbicide rate modeled as a linear or curvilinear (logarithmic) trend. Contrasts compared response trends for lines to the control genotype ($p \leq 0.05$).

RESULTS AND DISCUSSION

Results indicate differential sensitivity of cotton species to fluometuron, with *G. hirsutum* and *G. barbadense* lines being more tolerant than *G. herbaceum* and *G. arboreum* (Table 1). In general, the *G. arboreum* and *G. herbaceum* lines had the highest injury ratings, lowest plant dry weights, and lowest electron transport rates. *Gossypium barbadense* accessions TX 110 and GB 713 had the least injury; these lines also were in the group with the highest electron transport rates. In general, an inverse relationship was noted between injury and either plant dry weight or electron transport rate. A similar relationship was reported by Kendig et al. (2007) for *G. hirsutum* treated with postemergence applications of fluometuron, where greater plant injury was associated with lower chlorophyll levels. Individuals utilizing *G. arboreum* or *G. herbaceum* lines as sources of reniform nematode resistance or other traits should be aware of their greater sensitivity to fluometuron. We evaluated F_2 seedlings representing 12 different BC_2F_1 plants from a wide cross between *G. hirsutum* and *G. arboreum* at a single herbicide rate of 1.01 kg a.i./ha (data not shown). Plant responses were comparable to *G. hirsutum* with respect to injury rating, electron transport rates, and plant biomass, suggesting that even though *G. arboreum* is more susceptible to fluometuron, susceptibility might be reduced or eliminated through the plant breeding process.

Foliar injury increased in a linear manner in response to increased rates of fluometuron on all of the lines tested. With the exception of *G. arboreum* A_2-083, which showed significantly more injury based on a comparison of slopes, the lines responded similarly to the control genotype *G. hirsutum* Deltapine 161 B2RF (Fig. 1). Plant dry weight decreased in response to increased rates of fluometuron on all of the lines tested. The linear reductions in biomass of *G. barbadense* accessions GB 713 and Pima PHY 800 in response to increased fluometuron rates were significantly greater than that observed for the control genotype *G. hirsutum* Deltapine 161 B2RF (Fig. 2). Even though mean visual injury scores in the *G. barbadense* lines were not negatively impacted across the six fluometuron rates in comparison to the control, plant dry weight regression analysis did indicate that they were sensitive to increasing herbicide rates. These results differ from previous research examining the response of *G. hirsutum* and *G. barbadense* lines to prometryn, another photosystem II inhibitor. Shoot length of *G. barbadense* Pima S-7 was reduced 40% at 13.4 kg/ha prometryn, whereas *G. hirsutum* 'DP 5415' shoot length was reduced 60% at 1.3 kg/ha prometryn and plants died at 2.7 kg/ha (Molin and Khan, 1996). Differences in herbicide sensitivity among photosystem II inhibitors may be due to different rates of metabolism of the two herbicides between *Gossypium* species.

Table 1. Mean herbicide injury ratings, plant dry weights, and electron transport rates measured 3 wks after planting for 15 *Gossypium* lines and control genotype *Gossypium hirsutum* Deltapine 161 B2RF in greenhouse tests.

Line	Injury Rating (0 - 4)[z]		Plant Dry Weight (g)[y]		Electron Transport Rate (µmol electrons m^{-2} s^{-1})	
Gossypium arboreum A_2-194	2.19	a	0.008	f	36.2	e
Gossypium arboreum A_2-083	2.18	a	0.014	f	41.4	de
Gossypium arboreum A_2-100	1.79	ab	0.018	ef	38.8	e
Gossypium arboreum A_2-190	1.53	abc	0.015	f	55.1	cde
Gossypium herbaceum A_1-024	1.45	abcd	0.021	def	52.0	cde
Gossypium herbaceum A_1-017	1.39	bcd	0.017	f	59.2	cde
Gossypium hirsutum FR05-10	1.18	cd	0.039	bc	68.1	bcd
Gossypium barbadense Pima PHY 800	1.05	cd	0.040	bc	59.7	cde
Gossypium hirsutum Deltapine 161 B2RF	1.01	d	0.031	bcd	91.9	ab
Gossypium hirsutum T19	0.96	de	0.036	bcd	90.2	ab
Gossypium hirsutum LONREN-2	0.95	de	0.031	cde	86.1	ab
Gossypium hirsutum T1347	0.93	de	0.041	bc	99.1	a
Gossypium hirsutum LONREN-1	0.86	ef	0.042	bc	92.8	a
Gossypium hirsutum T1348	0.86	ef	0.044	b	78.1	abc
Gossypium barbadense TX 110	0.46	ef	0.039	bc	90.2	ab
Gossypium barbadense GB 713	0.34	f	0.067	a	96.3	a
F	*7.16*		*10.76*		*5.23*	
$p \leq$ F	*<0.0001*		*<0.0001*		*<0.0001*	

Means calculated across six herbicide rates ranging from 0 to 1.68 kg a.i./ha in two trials.

Within each column, means followed by the same letter are not significantly different based on differences of least squares means ($p \leq 0.05$).

[z] Visual rating on a 0 to 4 scale where 0 = no injury and 4 = maximum injury observed.

[y] Weight of all tissues above the cotyledons after drying for 48 h at 60 °C.

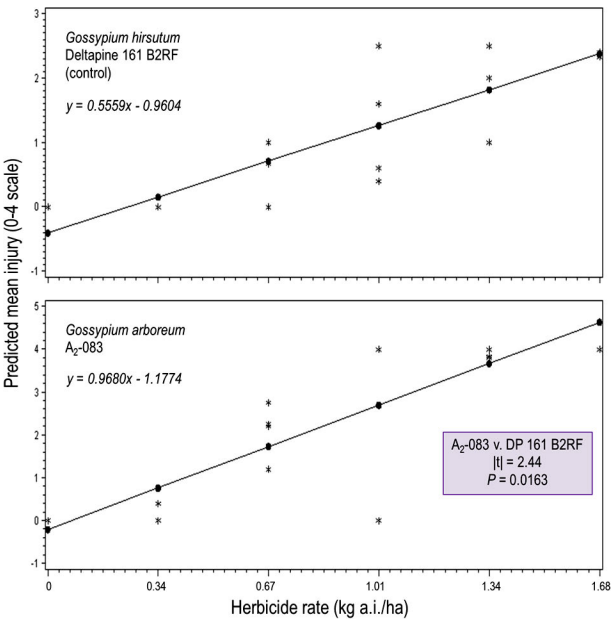

Figure 1. Herbicide injury response to increasing rates of fluometuron in control genotype *Gossypium hirsutum* Deltapine 161 B2RF and *G. arboreum* accession A_2-083; injury is a visual estimate on a 0 to 4 scale where 0 = no injury and 4 = maximum injury observed. Asterisks represent data points from two trials combined and means used to plot the regression line are indicated with solid circles.

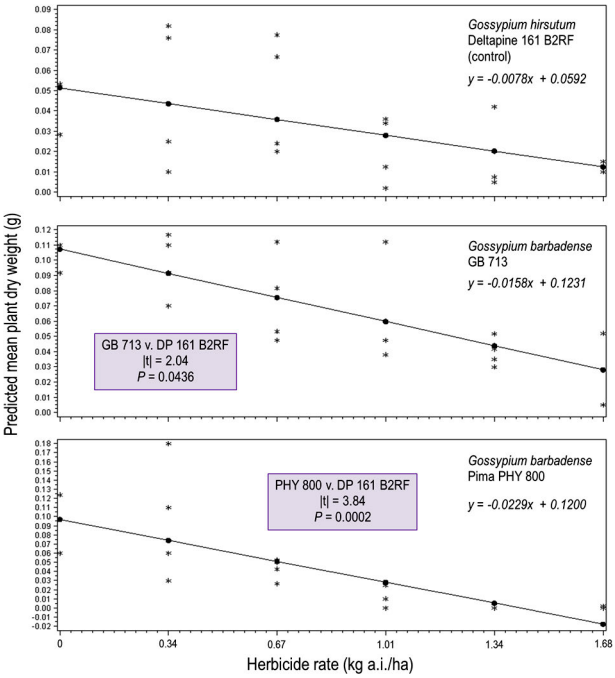

Figure 2. Plant biomass response to increasing rates of the herbicide fluometuron in control genotype *Gossypium hirsutum* Deltapine 161 B2RF and *G. barbadense* accessions GB 713 and Pima PHY 800. Asterisks represent data points from two trials combined and means used to plot the regression line are indicated with solid circles.

Electron transport rate decreased in response to increased fluometuron rates in a curvilinear manner (Fig. 3). None of the *Gossypium* lines responded differently from the control genotype *G. hirsutum* Deltapine 161 B2RF (data not shown). The increased foliar injury noted on *G. arboreum* A_2-083 and the increased biomass reductions on *G. barbadense* accessions GB 713 and Pima PHY 800 do not appear to be the direct result of differences in electron transport rate during peak daylight hours. Molin and Khan (1996) suggested that tolerance to prometryn could be related to less damage to the repair mechanisms in the plant, or to the existence of better repair mechanisms, and it is possible that these factors could contribute to the varying responses observed in this study. Because electron transport rates were not measured during periods of darkness, effects of the herbicide on the ability of the lines to repair damage to the reaction centers were not directly measured.

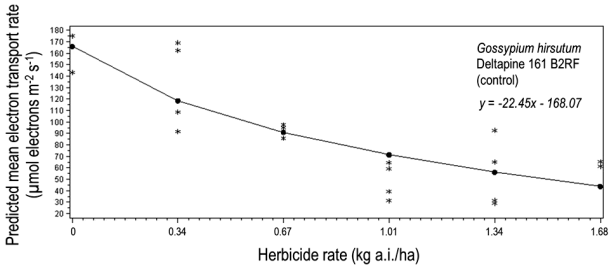

Figure 3. Electron transport rate response to increasing rates of the herbicide fluometuron in control genotype *Gossypium hirsutum* Deltapine 161 B2RF. Asterisks represent data points from two trials combined and means used to plot the regression line are indicated with solid circles.

In summary, the incorporation of *G. barbadense* sources of reniform nematode resistance into *G. hirsutum* would not appear to raise concern about inadvertently transferring increased sensitivity to fluometuron, at least across typical field application rates. This assumption is based on both limited injury to treated *G. barbadense* plants and previous reports of reduced sensitivity to other photosystem II inhibitor herbicides. However, there is indication that as a species group, that *G. barbadense* is more sensitive to fluometuron rate changes than *G. hirsutum* based on reduced plant dry weight. The diploid cotton species with reniform nematode resistance used here, i.e. *G. arboreum* and *G. herbaceum*, were notably affected by fluometuron application across a range of application levels. This was especially true with the *G. arboreum* accessions tested where the means were often significantly different from the *G. hirsutum* control. Consequently, fluometuron sensitivity could be an issue associated with reniform nematode resistance introgression efforts. Our experience, however, has not borne this out and may indicate that fluometuron sensitivity is not genetically linked with reniform nematode resistance.

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DISCLAIMER

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