Rust Control in Glyphosate Tolerant Wheat Following Application of the Herbicide Glyphosate

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ABSTRACT

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In greenhouse and field trials, transgenically modified wheat (*Triticum aestivum*) genotypes with tolerance to glyphosate had extremely low infection types to leaf rust caused by *Puccinia triticina* when treated with a labeled rate of the herbicide glyphosate prior to inoculation with leaf rust. A surfactant solution and a nonglyphosate herbicide had no effect on leaf rust development on the glyphosate tolerant wheat. Glyphosate had a systemic effect in reducing leaf rust development. The leaf rust control by glyphosate decreased with reduced application rates and longer periods of time between glyphosate application and leaf rust infections. The field and greenhouse tests indicated that control of leaf rust in wheat conditioned by glyphosate is transitory and is effective for at least 21, but not more than 35, days after application. Application of glyphosate also reduced infection types on wheat caused by the stem rust fungus, *Puccinia graminis* f. sp. *tritici*. Given these results and evidence from the literature that glyphosate can have adverse effects on other pathogens, including other rust fungi, additional investigation of the fungicidal properties of glyphosate are warranted, with particular attention to the timing of glyphosate application relative to fungal infection. The effects of glyphosate on the soybean rust fungus, *Phakopsora pachyrhizi*, an emerging pathogen in North America, merit immediate investigation.

Additional keywords: induced resistance, *Puccinia recondita* f. sp. *tritici*, transient resistance

Several crops, including canola, cotton, maize, and soybean, have been transgenically modified to tolerate application of the broad-spectrum herbicide glyphosate (10). Glyphosate can be applied to these crops to kill emerged weeds without injuring the crop. Transgenic wheat (*Triticum aestivum* L.) tolerant to the herbicide glyphosate was recently developed (37), but commercial deployment has been deferred indefinitely pending acceptance of genetically modified wheat in world markets.

While investigating the agronomic merits of advanced breeding lines containing a glyphosate tolerance gene, we discovered that such lines in field plots showed almost no leaf rust (caused by *Puccinia triticina* Eriks.) infections for a period of at least 2 weeks following glyphosate application at the early boot stage. Other plots that had glyphosate treatments at the three- to five-leaf stage (21 days prior to the boot stage) had high leaf rust infection, similar to control plants that were not treated with glyphosate. This finding and the results of other research showing that glyphosate may be inhibitory to other fungal species (11,13,30,32,33,35), including other rust fungi (36), suggest that this may be an unforeseen benefit of glyphosate application to crops transgenically modified to tolerate this herbicide. An immediate impact may be realized in helping to control the soybean rust fungus, *Phakopsora pachyrhizi* Sydow, an emerging pathogen of soybean in North America.

The objectives of these experiments were to (i) confirm the control of leaf rust conditioned by application of glyphosate on glyphosate tolerant wheat; (ii) determine the duration of the control and if it was systemic; and (iii) determine if glyphosate application also controlled stem rust of wheat caused by *Puccinia graminis* f. sp. *tritici*.

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MATERIALS AND METHODS

Plant materials. Personnel at Monsanto Co. (St. Louis, MO) transformed the wheat cultivar Bobwhite with the EPSPS (5-enolypyruvyl-shikimate-3-phosphate synthase) gene *aroA*:CP4 that provides tolerance to glyphosate (37). In 1998, two selections of Bobwhite, each containing a different transformation event (designated 33391 and 33512), were crossed with three wheat genotypes developed at the University of Minnesota: cultivars HJ98 (7), McVey (6), and Oklee (1). The F_1 hybrids were backcrossed for four generations as the female parent to the Minnesota wheat cultivars with selection for glyphosate tolerance at each generation. A single plant selection from each of the Minnesota lines was used for each backcross generation. These were designated HJ98-R, McVey-R, and Oklee-R. A glyphosate tolerant line, with the pedigree Ingot*5/33391, which originated from South Dakota State University, was included in one field experiment. Checks used in various experiments included the cultivars Thatcher, Max, and Ingot, which are susceptible to leaf rust; Ivan, which is leaf rust resistant; and Little Club, which is stem rust susceptible (Table 1).

Greenhouse tests: leaf rust. *Fall 2000.* Seeds of the recurrent parents HJ98-R, McVey-R, and Oklee-R, and the glyphosate tolerant lines HJ98*5/33512, Oklee*5/33512, McVey*5/33512, HJ98*5/33391, Oklee*5/33391, and McVey*5/33391 were planted as three seeds per pot in 15-cm-diameter pots filled with Metromix 2000 (Scotts-Sierra Horticultural Products, Marysville, OH) in fall 2000. Three pots per treatment were placed in the greenhouse at 15 to 25°C with supplemental metal halide lighting 8 to 12 h per day. At the boot stage (Feekes growth stage 9, [15]), plants were sprayed with either glyphosate (formulated as Roundup Ultra [Monsanto]) at the concentration of 4.5 mg acid equivalent (a.e.) ml^{-1} or water plus the nonionic surfactant Class Act (5% vol/vol [Agriliance, St. Paul, MN]) as a control. Treatment was delivered with a spray bottle until leaf wetness (in a volume of approximately 160 liters ha^{-1}). At 22 to 25 days after the spray treatment, plants were inoculated by spraying 1 to 2 mg of leaf rust urediniospores suspended in 350 µl of Soltrol 170 oil (Phillips Petroleum, Bartlesville OK). Three *P. triticina* virulence phenotypes (races), MBRJ, THBJ, and

MCDS (18), were inoculated separately onto different adult plants. The inoculated plants were placed overnight in a dew chamber at 18°C, and then placed on a greenhouse bench with the temperature and lighting conditions described earlier. Infection types were evaluated 12 days after inoculation using the scale of 0 to 4 (18): 0 = immune, with no visible necrosis or uredinia; ; = hypersensitive fleck with no sporulation; 1 = small uredinia surrounded by necrosis; 2 = small uredinia surrounded by chlorosis; 3 = moderate size uredinia without chlorosis or necrosis; 4 = large uredinia without chlorosis or necrosis. Methods for increasing the leaf rust urediniospores, storage of inoculum, inoculation procedures, and evaluation of leaf rust infection types were as described in (26).

Spring 2001. Seeds of HJ98-R and glyphosate tolerant line HJ98*5/33391 were planted as two seeds per pot in 15-cm-diameter pots filled with Metromix 2000 and grown to adult plant stage in the greenhouse in spring 2001 using the temperature and lighting regimes described for the fall 2000 experiment. Two pots per treatment were used, and plants were sprayed with one of two formulations of glyphosate, Roundup Ultra (4.5 mg a.e. ml^{-1}) or Rodeo, a glyphosate-containing formulation with no additives or surfactants (4.5 mg a.e. ml^{-1} [Monsanto]); the nonglyphosate broadleaf herbicide Discover (Clodinafop, 11 mg a.i. ml^{-1} [Syngenta Crop Protection, Greensboro, NC]); or the surfactant Class Act (5% vol/vol) at various growth stages from the four-leaf stage (Feekes 3) to flag leaf emergence (Feekes 8-9). At the boot stage (Feekes 9), plants were sprayed with either glyphosate (Roundup Ultra or Rodeo, 4.5 mg a.e. ml^{-1}) or water plus the nonionic surfactant Class Act (5% vol/vol) as a control. At the boot stage treatments, some of the flag leaves, the targets for subsequent leaf rust inoculations, were temporarily covered with a plastic bag during the herbicide/surfactant treatment. Treatments were delivered as described earlier. Following complete emergence of the spike, plants were inoculated with leaf rust virulence phenotype THBJ. Rust severity was rated 14 days later using the modified Cobb scale (23), and resistance response was rated using the response scales in (26).

Spring 2002. Seeds of Ivan (resistant check), Thatcher (susceptible check), wheat lines HJ98-R, and glyphosate tolerant line HJ98*5/33391 were planted as three seeds per pot in 15-cm-diameter pots filled with Metromix 2000 and grown in the greenhouse in spring 2002. Two pots per treatment were used, and temperature and lighting were as previously described for the fall 2000 experiment. At the three-leaf stage, plants were sprayed with one of two formulations of glyphosate: MON0139 (an experimental formulation with no additives [Monsanto]) or Roundup Ultra at active ingredient rates equivalent to the labeled rate (1.0× = 4.5 mg a.e. ml^{-1}), 0.5×, or 2.0×. One day after the spray treatment, plants were inoculated with leaf rust virulence phenotype MBRJ. The recurrent parent was not sprayed with glyphosate, and a second set of HJ98*5/33391 plants was sprayed with the same glyphosate treatments at the five-leaf stage, 11 days after leaf rust inoculation. The three- and five-leaf stage treatments were evaluated for leaf rust infection types at 14 and 21 days after inoculation, respectively, with the 0 to 4 infection type scale (18).

Greenhouse tests: stem rust. Seeds of the wheat genotypes McVey (resistant check), Little Club (susceptible check), HJ98, and glyphosate tolerant line HJ98*5/33391 were planted as three seeds per pot in 15-cm-diameter pots filled with Metromix 2000 and grown in the greenhouse in spring 2002. Plant growth conditions were as described for the fall 2000 experiment. At the three-leaf stage, two pots each containing HJ98*5/33391 plants were sprayed with Roundup Ultra (4.5 mg a.e. ml^{-1}) as described earlier or were not treated. One day after the glyphosate treatment, all plants were inoculated with virulence phenotype TPMK (25) of the wheat stem rust fungus *P. graminis* f. sp. *tritici*. The inoculated plants were placed in a mist chamber at 18°C for 16 h, with 4 h of incandescent lighting at the end of the incubation period. Stem rust infection types were evaluated 12 days after inoculation with a 0 to 4 scale similar to that used for leaf rust (25).

Field plot tests 2002. Plant materials included the glyphosate tolerant lines HJ98*5/33391 and Oklee*5/33391, HJ98, Oklee, and the leaf rust susceptible cultivar Max. Wheat lines were planted in 1.2-m rows, 0.3 m apart, with 4 g of seed. Two replications were planted on 2 May 2002 at St. Paul, MN. The border areas of the plots were seeded with Max as a rust spreader. The spreader rows were inoculated with a mixture of five leaf rust virulence phenotypes at the end of May (Feekes 7-9). Ten different herbicide treatments were used on the glyphosate tolerant lines: untreated control, sprayed at the three-leaf stage 28 days after planting, and eight treatments that were sprayed at the three-leaf stage plus a single additional application 7 to 54 days later in 7-day intervals. Glyphosate was applied as the Roundup Ultra formulation at 4.5 mg a.e. ml^{-1} in a volume of 160 liters ha^{-1} using a plot sprayer. Plots were rated for leaf rust severity at 76 and 82 days after planting using the modified Cobb scale (23), and for resistance response with the scale in (26).

Field plot tests 2003. Plant materials included the leaf rust susceptible cultivar Ingot, a glyphosate tolerant line in an Ingot genetic background (Ingot*5/33391), and the leaf rust susceptible cultivar Max. The wheat lines were planted in plots consisting of four 1.2-m rows, 0.3 m apart, with 4 g seed per row. Adjacent plots were 0.4 m apart. Two replications were planted on 1 May 2003 at St. Paul, MN. The border area of the plots was seeded with Max as a rust spreader. The spreader rows were inoculated with a mixture of five leaf rust virulence phenotypes at the end of May (Feekes 7-9). Seven different herbicide treatments were used on Ingot*5/33391: untreated control, sprayed at the five-leaf stage (31 days after planting) or 7 days after the five-leaf stage (38 days after planting) with Roundup UltraMax at the labeled rate (1.0× = 5.4 mg a.e. ml^{-1}), 0.5×, or 2.0×. Plots were rated for leaf rust severity and resistance response as previously described at 74, 77, and 81 days after planting.

RESULTS

In our first experiment conducted in the fall of 2000, glyphosate tolerant wheat lines were inoculated with each of four leaf rust virulence phenotypes 22 to 25 days after treatment with glyphosate or a control containing only a nonionic surfactant at the boot stage. The glyphosate tolerant lines all had extremely low infection types of 0 (immune) to ; (fleck) when treated with a glyphosate solution prior to

Table 1. Leaf rust and stem rust reactions of plant materials

Genotype	Leaf rust reaction[a]	Stem rust reaction
Recurrent parents[b]		
HJ98-R	MR-MS	R[c]
McVey-R	MR-MS	R
Oklee-R	MR-MS	R
Glyphosate tolerant lines[d]		
HJ98*5/33391	MR-MS	R[c]
HJ98*5/33512	MR-MS	R
McVey*5/33391	MR-MS	R
McVey*5/33512	MR-MS	R
Oklee*5/33391	MR-MS	R
Oklee*5/33512	MR-MS	R
Checks		
HJ98	MR-MS	R
Oklee	MR-MS	R
Ingot	S	R
Max	S	S
Ivan	R	R
Thatcher	S	R
Little Club	N/A	S

[a] The recurrent parents and checks were inoculated with races of leaf rust virulent against them.

[b] The recurrent parents were single plant selections made from the parental cultivar.

[c] The stem rust virulence phenotype used in this study, TPMK, was virulent against HJ98-R and HJ98*5/33391. These lines are resistant to prevalent stem rust virulence phenotypes.

[d] 33391 and 33512 are different transformation events of the gene *aroA*:CP4 that provides tolerance to glyphosate into the cultivar Bobwhite. Both selections were crossed to the recurrent parent HJ98, McVey, or Oklee and backcrossed four times with selection for glyphosate tolerance at each generation.

Table 2. Leaf rust infection types of adult plants of glyphosate tolerant wheat lines treated with glyphosate prior to inoculation with leaf rust in the greenhouse during fall 2000

Genotype	Virulence phenotype	Leaf rust infection type[a]		
		Unsprayed	Water + surfactant[b]	Glyphosate[b]
Recurrent parents				
HJ98-R	MBRJ	23	23^{+}	
HJ98-R	THBJ	23^{+}	23^{+}	
McVey-R	MBRJ	;2^{-}	;2^{-}	
McVey-R	MCDS	23	22^{+}	
McVey-R	THBJ	23	23	
Oklee-R	MCDS	23;	23	
Oklee-R	THBJ	22^{+}	23;	
Oklee-R	MBRK	23	23	
Glyphosate tolerant lines[c]				
HJ98*5/33391	THBJ		23	0
HJ98*5/33391	MCDS		23	0
HJ98*5/33512	MBRJ		23	0
HJ98*5/33512	MCDS		0; 2	0;
HJ98*5/33512	THBJ		23	0
McVey*5/33391	THBJ		23	0;
McVey*5/33391	MCDS		23	;
McVey*5/33512	MCDS		23	0;
McVey*5/33512	THBJ		23	0;
McVey*5/33512	MBRK		;2^{-}	;
Oklee*5/33391	MCDS		0; 2^{+}	0
Oklee*5/33391	THBJ		23	0
Oklee*5/33512	MCDS		;	0
Oklee*5/33512	MBRK		;2^{-}	0
Oklee*5/33512	THBJ		22^{-}	0

[a] Infection types: 0 = no flecks or uredinia; ; = hypersensitive chlorotic or necrotic flecks; 1 = small uredinia surrounded by necrosis; 2 = small uredinia surrounded by chlorosis; 3 = moderate size uredinia without necrosis or chlorosis; + = larger uredinia than normal; – = uredinia smaller than normal. A range of infection types is indicated by more than one infection type, with the predominant type listed first. Ratings are an average from nine plants per treatment.

[b] Glyphosate (4.5 mg a.e. ml^{-1}) or control (water plus nonionic surfactant) was applied at the boot stage (Feekes 9), and plants were inoculated with leaf rust 22 to 25 days after treatment.

[c] Genotypes 33391 and 33512 indicate different glyphosate tolerant Bobwhite lines.

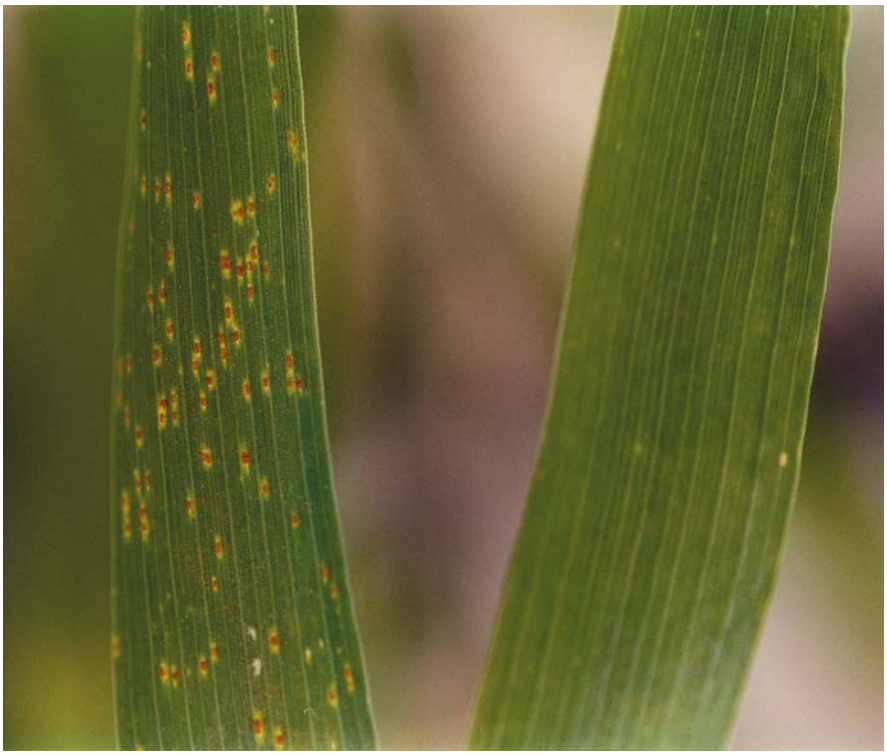

Fig. 1. Flag leaves of the glyphosate tolerant wheat line McVey*5/33512 20 days after inoculation with leaf rust race THB. Leaves on right and left were treated with Roundup Ultra herbicide or water (control), respectively, 22 days prior to leaf rust inoculation.

leaf rust inoculation (Table 2, Fig. 1). Adult plants of the recurrent parents HJ98-R, McVey-R, and Oklee-R had infection types of ;2– to 23+ to virulence phenotypes MBRJ, MBRK, MCDS, and THBJ of *P. triticina*. The recurrent parents had similar or identical infection types when sprayed with a control treatment of an aqueous solution of a nonionic surfactant 21 to 22 days prior to inoculation with the leaf rust isolates. The intermediate infection types of the isolates on the recurrent parents indicated that HJ98-R, McVey-R, and Oklee-R have some adult plant resistance to leaf rust. The glyphosate tolerant lines with HJ98, McVey, and Oklee backgrounds had low to intermediate infection types of ; (fleck) to 23 when treated with the nonionic surfactant solution.

Adult plants of HJ98-R and glyphosate tolerant lines with the HJ98 background also were tested with different formulations of glyphosate and adjuvants (Table 3). The objective of this experiment was to determine if the leaf rust control observed was due to glyphosate per se or could be produced by other adjuvants or herbicides and if the effect was systemic. The glyphosate tolerant lines showed no signs of leaf rust when treated with the glyphosate formulations Roundup and Rodeo 17 days prior to inoculation with leaf rust, and 10S-20S leaf rust rating when treated with the surfactant Class Act or the nonglyphosate herbicide Discover. For the treatments at 49 days after planting, flag leaves of the glyphosate tolerant lines were protected to prevent coverage by the Roundup Ultra and Rodeo glyphosate formulations or the surfactant Class Act. The protected flag leaves showed no sign of leaf rust infection when inoculated 16 days after the Roundup Ultra treatment, had only a trace level of infection with the Rodeo treatment, and had infection types and severity comparable to the controls when treated with the surfactant Class Act. The recurrent parent had leaf rust ratings ranging up to 20S with no treatment, or when treated with the surfactant Class Act or Discover 25 days before inoculation with leaf rust. The glyphosate tolerant lines had rust ratings up to 20S in the unsprayed treatment. The same glyphosate tolerant lines had visible signs of leaf rust infection when glyphosate formulations of Roundup Ultra and Rodeo were applied 23 to 38 days prior to leaf rust inoculation, but ratings were lower as the time between glyphosate application and leaf rust inoculation was reduced.

The transitory effect of glyphosate application on leaf rust infection was examined in a field trial in 2002. Glyphosate tolerant lines in HJ98 and Oklee backgrounds were treated with Roundup Ultra at weekly intervals starting at 28 days after planting up to 84 days after planting (Table 4). The susceptible cultivar Max had leaf rust ratings of 60S and 80S at 82 days after

planting. The glyphosate tolerant lines in HJ98 and Oklee backgrounds that were not treated with glyphosate had leaf rust ratings similar or identical to the respective recurrent parents. Both the HJ98 and Oklee glyphosate tolerant lines had lower leaf rust ratings compared with the recurrent parent and untreated lines when the glyphosate treatment was between 6 and 27 days prior to leaf rust rating. Glyphosate treatments more than 34 days before, or treatments after the initial leaf rust ratings had little or no effect on leaf rust ratings. The recurrent parents HJ98 and Oklee had final leaf rust ratings of 20MR-MS and 40MS-S, respectively, at 82 days after planting. Based on leaf rust rating in Max, leaf rust inoculum was probably present and increasing in the plots at 50 days after planting.

The effect of glyphosate application rate, timing, and formulation type on leaf rust infection was tested in a greenhouse study on seedling plants in spring 2002. The glyphosate formulations Roundup Ultra, which contains proprietary adjuvants to increase plant uptake and optimize weed control, and MON0139, which is free of any adjuvants, were applied at different rates at two different times (Table 5). The labeled rate (1.0×) of Roundup Ultra applied at the three-leaf stage, followed by leaf rust inoculation the following day, resulted in the glyphosate tolerant lines of HJ98 having a 0 infection type (immune response) compared with a 3+ infection type for the HJ98 recurrent parent that was not treated with glyphosate. The 0.5× and 2.0× rates of Roundup Ultra also resulted in 0 infection types when applied to the glyphosate tolerant lines at the three-leaf stage. The glyphosate tolerant wheat plants showed no adverse effects at the higher rates of glyphosate. The 0.5× and 1.0× rates of MON0139 applied to the glyphosate tolerant lines at the three-leaf stage resulted in leaf rust infection types of 23 and 0 – ;23, respectively, with relatively

Table 3. Leaf rust ratings of glyphosate tolerant wheat with herbicides and surfactant applied at various growth stages prior to inoculation with leaf rust in the greenhouse during spring 2001

Genotype	Treatment date (days after planting)	Days from glyphosate trt. to leaf rust inoc.	Leaf rust rating[a] following treatment				
			Unsprayed	Roundup[b]	Rodeo[b]	Class Act	Discover
Recurrent parent							
HJ98-R			10S-20S			20S	5MS-20S
Glyphosate tolerant lines							
HJ98*5/33391	No treatment	…	5S-20S				
HJ98*5/33391	27	38		5S-10MS			
HJ98*5/33391	33	32		Trace S-5S			
HJ98*5/33391	42	23		Trace S	5S		
HJ98*5/33391	48	17		0	0	10S-20S	10S
HJ98*5/33391	49 (flag covered)[c]	16		0	0-trace S	10S-40S	

[a] Leaf rust inoculation of flag leaves with virulent phenotype THBJ was done at 65 days after planting. Rust severity was estimated with the modified Cobb scale (23). MS = moderate size uredinia surrounded by chlorosis, S = large uredinia without chlorosis. Each rust rating represents four plants with a range of infection types and severities separated by a dash.
[b] Roundup Ultra and Rodeo both contain glyphosate and were applied at the concentration of 4.5 mg a.e. ml^{-1}.
[c] (flag covered) indicates that the flag leaf, the target for leaf rust inoculation, was protected during the herbicide/surfactant treatment.

Table 4. Leaf rust ratings of glyphosate tolerant wheat lines in field plots in 2002 after glyphosate treatment at various growth stages[a]

Genotype	Glyphosate applic. (DAP)	Leaf rust rating (76 DAP)[b]			Leaf rust rating (82 DAP)		
		Days from trt. to LRR	Plot 1	Plot 2	Days from trt. to LRR	Plot 1	Plot 2
Susceptible check							
Max	None		10MS-S	30MS-S		60S	80S
Recurrent parents							
HJ98	None		Trace MS-S	Trace-5MS		20MR-MS	20MR-MS
Oklee	None		10MR-MS	20MR-MS			40MS-S
Glyphosate tolerant lines							
HJ98*5/33391	None		0-trace S	0-trace S	…	5MR-MS	5-10MS
HJ98*5/33391	28	48	Trace MS-S	5MS-S	54	5MR-10MS-MR	10MR-20MS
HJ98*5/33391	35	41	Trace-5MS	0-trace MS-S	47	5-10MR-MS	5MR-MS
HJ98*5/33391	42	34	Trace MS-S	0-5MS-S	40	5MR-MS	Trace MR-20MR-MS
HJ98*5/33391	49	27	0	0	33	Trace MR	Trace MS
HJ98*5/33391	56	20	0	0	26	Trace MR	0
HJ98*5/33391	63	13	0	0	19	0	0
HJ98*5/33391	70	6	0	0	12	0	0
HJ98*5/33391	77	–1	5MS-S	Trace MR-MS	5	5MR-MS	Trace MR
HJ98*5/33391	84	–8	0-5MS	5MR-MS	–2	Trace MR-20MR-MS	10MR-MS
Oklee*5/33391	None	…	20MS-S	30MS-S	…	40MR-MS	40MS-S
Oklee*5/33391	28	48	40MS-S	40MS-S	54	60MS-S	60MS-S
Oklee*5/33391	35	41	20MS-S	10MS-S	47	40MS-S	40MS-S
Oklee*5/33391	42	34	20MS-S	20MS-S	40	60MS-S	20-60MS-S
Oklee*5/33391	49	27	0-traceMS	0-5MR-MS	33	10MR-MS	10MR-MS
Oklee*5/33391	56	20	0	0	26	Trace MR-20-40MS	Trace-5MR-MS
Oklee*5/33391	63	13	0	0	19	0	0
Oklee*5/33391	70	6	Trace MR-MS	0	12	Trace MR	Trace MR
Oklee*5/33391	77	–1	20S-MS	30MS-S	5	20MS-S	30MS-S
Oklee*5/33391	84	–6	30MS-S	40MS-S	–2	40S	60MS-S

[a] Rust rating: 0 = no flecks or uredinia; R = small uredinia with necrosis; MR = mixture of small and large uredinia with necrosis; MS = moderate size uredinia with chlorosis; S = large uredinia. The preceding number indicates percent leaf area covered as estimated with the modified Cobb scale (23). Trace indicates less than 1% severity, … indicates missing data.
[b] DAP, days after planting; LRR, leaf rust rating.

few uredinia compared to the unsprayed recurrent parent HJ98-R for each treatment. The 2.0× treatment of MON0139 resulted in a 0 infection type when applied at the three-leaf stage. Treatments of Roundup Ultra and MON0139 had no effect on leaf rust infection type when applied at the five-leaf stage following leaf rust inoculation 11 days earlier at the three-leaf stage. This indicates that glyphosate application on wheat plants with previously established rust infections had no effect on rust development.

Different rates of glyphosate and timing of application were further tested in a field plot evaluation in 2003. For this test, a glyphosate tolerant line with an Ingot background was used because the recurrent parent Ingot is leaf rust susceptible. Leaf rust infections were visible on the susceptible cultivar Max and the recurrent parent Ingot at 74 days after planting, and progressed to reach ratings of 100S on Max and 40S on Ingot at 81 days after planting (Table 6). The treatments with 1.0× and 2.0× applications of Roundup UltraMax at 38 days after planting had much lower rust ratings at 74, 77, and 81 days after planting compared to the recurrent parent Ingot. The glyphosate tolerant line of Ingot that was not treated with Roundup UltraMax had the same leaf rust ratings as Ingot. The glyphosate tolerant lines that were treated with 0.5×, and 1.0× of Roundup UltraMax at 31 days after planting had the same leaf rust severities at 74 and 77 days after planting as the recurrent parent Ingot and the untreated glyphosate tolerant line of Ingot. At 81 days after planting, the glyphosate tolerant lines of Ingot that were treated with the 1.0× rate at 31 days after planting had rust ratings of 30S compared with 40S for the lines in the 0.5× treatment, the untreated glyphosate tolerant lines, and the recurrent parent Ingot. The 2.0× treatment at 31 days after planting resulted in a lower rust rating at 74, 77, and 81 days after planting compared with the recurrent parent Ingot. The 0.5× treatment at 38 days after planting did not result in a lower final rust rating compared with the recurrent parent Ingot.

The effect of glyphosate application on stem rust (caused by *P. graminis* f. sp. *tritici*) infections in wheat also was examined. The recurrent parent HJ98-R, and a glyphosate tolerant line in an HJ98 background were treated with Roundup Ultra at the three-leaf stage and inoculated with stem rust virulence phenotype TPMK 2 days later. The glyphosate tolerant line had an infection type of 0 (immune) with the Roundup Ultra treatment. The recurrent parent HJ98 and the untreated glyphosate tolerant line had infection types of ;2 to 3+ to stem rust phenotype TPMK in two separate replications. The cultivars McVey and Little Club were included as resistant and susceptible checks, respectively. McVey had an infection type of 0, and Little Club had infection types of 3+ to 4. Therefore, glyphosate application also provides control of the stem rust fungus.

Table 5. Leaf rust infection types on seedlings of glyphosate tolerant HJ98 wheat following treatment with different formulations and rates of glyphosate in the greenhouse in spring 2002

	Glyphosate			
Genotype	**Formulation**	**Rate[a]**	**Timing[b]**	**Infection type[c]**
Resistant check				
Ivan	Unsprayed	Unsprayed	Unsprayed	;
Susceptible check				
Thatcher	Unsprayed	Unsprayed	Unsprayed	3+
Recurrent parent				
HJ98-R	Unsprayed	Unsprayed	Unsprayed	3+
Glyphosate tolerant line				
HJ98*5/33391	MON0139	0.5×	3 leaf	23 few
HJ98*5/33391	MON0139	1.0×	3 leaf	0–;23 few
HJ98*5/33391	MON0139	2.0×	3 leaf	0
HJ98*5/33391	Roundup Ultra	0.5×	3 leaf	0
HJ98*5/33391	Roundup Ultra	1.0×	3 leaf	0
HJ98*5/33391	Roundup Ultra	2.0×	3 leaf	0
HJ98*5/33391	MON0139	0.5×	5 leaf	3+
HJ98*5/33391	MON0139	1.0×	5 leaf	3+
HJ98*5/33391	MON0139	2.0×	5 leaf	3+
HJ98*5/33391	Roundup Ultra	0.5×	5 leaf	3+
HJ98*5/33391	Roundup Ultra	1.0×	5 leaf	3+
HJ98*5/33391	Roundup Ultra	2.0×	5 leaf	3+

[a] 1.0× rate = 4.5 mg a.e. ml^{-1}.
[b] Plants were inoculated with leaf rust virulence phenotype THBJ 1 day following the three-leaf glyphosate application.
[c] Infection types: 0 = no flecks or uredinia; ; = hypersensitive chlorotic or necrotic flecks; 1 = small uredinia surrounded by necrosis; 2 = small uredinia surrounded by chlorosis; 3 = moderate size uredinia without necrosis or chlorosis; + = uredinia larger than normal; – = uredinia smaller than normal. A range of infection types is indicated by more than one infection type, with the predominant type listed first. Ratings are an average from six plants per treatment.

Table 6. Leaf rust ratings of glyphosate tolerant Ingot wheat in field plots after treatment at different growth stages with three rates of glyphosate[a]

	Glyphosate		Leaf rust rating (DAP)[b]		
Genotype	**Rate[c]**	**Timing (DAP)[b]**	**74**	**77**	**81**
Susceptible check					
Max	Unsprayed	Unsprayed	5S	40S	Dead
Recurrent parent					
Ingot	Unsprayed	Unsprayed	5S-MS	20S-MS	40S
Glyphosate tolerant lines					
Ingot*5/33391	None	None	5MS-S	20MS-S	40S
Ingot*5/33391	0.5×	31	5MS-S	20MS-S	40S
Ingot*5/33391	1.0×	31	5MS-S	20MS-S	30S
Ingot*5/33391	2.0×	31	tMS	10MS-S	30S
Ingot*5/33391	0.5×	38	tMS	10MS-S	40S
Ingot*5/33391	1.0×	38	0	5MS-S	10MS-S
Ingot*5/33391	2.0×	38	0	0-tS	5MS-S

[a] Rust response: 0 = no flecks or uredinia; R = small uredinia with necrosis; MR = mixture of small and large uredinia with necrosis; MS = moderate size uredinia with chlorosis; S = large uredinia; t = trace level of infection. The preceding number indicates percent leaf area covered as estimated with the modified Cobb scale (23).
[b] Days after planting.
[c] 1.0× rate = 5.4 mg a.e. ml^{-1} as Roundup UltraMax.

DISCUSSION

The results of greenhouse and field testing confirmed our initial observation that application of glyphosate reduced or eliminated infection by *P. triticina* and also *P. graminis* f. sp. *tritici* in glyphosate tolerant wheat genotypes. How glyphosate inhibits rust infection is not known. The herbicide may induce a systemic resistance response in wheat, or may act directly as a systemic fungicide itself. We demonstrated the systemic effect of the treatments by protecting the flag leaf, the target for leaf rust inoculation, from the herbicide/surfactant. For both of the glyphosate-containing herbicides, Roundup Ultra and Rodeo, low leaf rust ratings were observed whether the flag leaf was protected or unprotected during the herbicide application. Also, in all but one experiment, one or more new leaves emerged during the time between glyphosate application and leaf rust inoculation. Since leaf rust infections were not observed on leaves that were exposed to leaf rust inoculum but not glyphosate, this also indicated that the

effect of glyphosate in reducing rust infections was systemic.

The leaf rust control conditioned by glyphosate was rate dependent and also transitory. In a greenhouse experiment, the formulation Roundup Ultra resulted in infection types of 0 (immune) when applied at the 0.5×, 1.0×, and 2.0× application rates. In contrast, MON0139, which is glyphosate without any additives, resulted in an infection type of 0 at only the 2.0× rate and was less effective in controlling leaf rust at the 0.5× and 1.0× rates compared with Roundup Ultra. A field test also showed that lower leaf rust ratings resulted from higher rates of glyphosate (Table 6). Both greenhouse and field tests indicated that glyphosate inhibits initial leaf rust infections in wheat for at least 21 days after treatment, but not more than 35 days after treatment. However, if infections during this time period only are suppressed this can result in substantial disease reduction throughout the growing season with a multicyclic pathogen such as leaf rust which produces infectious urediniospores within 7 to 9 days after the initial infection. Glyphosate application on wheat plants with previously established rust infections had no effect on rust development of those pustules, suggesting that the effect of glyphosate may be to inhibit only the early stages of infection.

The transitory effect could be due to dilution and breakdown over time of glyphosate in actively growing plant tissue, or a similar transitory effect of induced plant defense responses. Glyphosate and its metabolites are known to persist in glyphosate-tolerant soybeans, and in planta levels were correlated with the amount of glyphosate applied and timing of application (2). Glyphosate also is labeled for preharvest use in certain non-glyphosate-tolerant crops for late season weed control. Glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), and the plant-produced shikimic acid were found in the seeds of canola (8), pea, barley, flax (9), and wheat (5) following preharvest glyphosate applications. Therefore, our results are consistent with the theory that glyphosate in the plant tissue is either directly or indirectly responsible for inhibition of rust infections, and the transitory effect may be due to dilution or breakdown of glyphosate over time.

Glyphosate inhibits EPSPS (31), an enzyme in the shikimate pathway. This pathway is essential for synthesis of the amino acids phenylalanine, tyrosine, and tryptophan and is found in plants, fungi, and microorganisms (14,24,29). The effect of glyphosate on soil microbes has been investigated. Glyphosate application increased the population of several soil fungi in one study (17) and had no effect on soil microbes in another (3). Although transitory changes in microorganism populations were observed, Wardle (34) concluded that it is unlikely that glyphosate applications have any adverse effect on the soil microbial community.

Glyphosate has been shown to reduce sporulation, growth, and symptoms caused by other cereal fungal pathogens. Wheat pathogens suppressed by glyphosate applications include *Septoria nodorum* (the causal agent of leaf blotch in wheat) (13), tan spot (*Pyrenophora tritici-repentis*) (11,30), take-all (caused by *Gaeumannomyces graminis*), and Rhizoctonia root rot (caused by *Rhizoctonia solani*) (35). *Rhynchosporium secalis*, the barley scald pathogen, and *Pyrenophora teres,* the barley net blotch pathogen, were affected (33) as well as the fungus *Drechslera teres* (32). Therefore, we predict that the suppressive effect of glyphosate on leaf and stem rust may occur with other pathogens when glyphosate is applied to glyphosate tolerant wheat. Unfortunately, glyphosate tolerant wheat is no longer available for research because of Monsanto's decision to defer commercial development of this product.

Roundup Ready (glyphosate tolerant) soybeans (*Glycine max*) have been widely grown in the United States since 1998, and several studies have examined the interaction between glyphosate-treated Roundup Ready soybeans and soilborne pathogens. Results have shown no significant effect on *Sclerotinia sclerotium* (16,20,21); inhibitory effects (4) or no effect on *Rhizoctonia solani* (12); and effects ranging from inhibitory (28) to no effect (22) to stimulatory (27,28) on *Fusarium solani*.

The effects of glyphosate on aeciospore germination of the rust *Puccinia lagenophora* Cooke that is used for biological control of the weed *Senecio vulgaris* have been examined (36). Glyphosate concentrations as low as 0.025× and 0.0125× of the labeled rate resulted in significantly less spore germination compared with a water control and three other herbicides, linuron, 2,4-D, and MCPP, used at the same rates. All herbicides significantly reduced spore germination when used at their labeled rates.

Conclusions. We have shown that glyphosate acts either directly or indirectly to reduce or eliminate infection of leaf and stem rust in wheat. A growing body of literature suggests that glyphosate can have variable effects on other fungi as well. Because Roundup Ready crops have only become available in the last few years, few in vivo tests of the fungicidal properties of glyphosate have been conducted. Given the substantial acreage planted to Roundup Ready crops, especially soybean (85% of the more than 30 million hectares in the United States in 2004 [19]), investigation of the fungicidal properties of glyphosate are warranted, with particular attention to the timing of glyphosate application relative to fungal infection. The effects of glyphosate on the soybean rust fungus (*Phakopsora pachyrhizi*), an emerging pathogen in North America, merit immediate investigation.

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