Glyphosate inhibits rust diseases in glyphosate-resistant wheat and soybean

Paul C. C. Feng*†, G. James Baley‡, William P. Clinton*, Greg J. Bunkers*, Murtaza F. Alibhai*, Timothy C. Paulitz§, and Kimberlee K. Kidwell‡

*Monsanto Biotechnology Research, St. Louis, MO 63017; ‡Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420; and §Root Disease and Biological Control Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA 99164-6430

Communicated by R. James Cook, Washington State University, Pullman, WA, October 11, 2005 (received for review July 19, 2005)

Glyphosate is a broad-spectrum herbicide used for the control of weeds in glyphosate-resistant crops. Glyphosate inhibits 5-enolpyruvyl shikimate 3-phosphate synthase, a key enzyme in the synthesis of aromatic amino acids in plants, fungi, and bacteria. Studies with glyphosate-resistant wheat have shown that glyphosate provided both preventive and curative activities against *Puccinia striiformis* f. sp. *tritici* and *Puccinia triticina*, which cause stripe and leaf rusts, respectively, in wheat. Growth-chamber studies demonstrated wheat rust control at multiple plant growth stages with a glyphosate spray dose typically recommended for weed control. Rust control was absent in formulation controls without glyphosate, dependent on systemic glyphosate concentrations in leaf tissues, and not mediated through induction of four common systemic acquired resistance genes. A field test with endemic stripe rust inoculum confirmed the activities of glyphosate pre- and postinfestation. Preliminary greenhouse studies also demonstrated that application of glyphosate in glyphosate-resistant soybeans suppressed Asian soybean rust, caused by *Phakopsora pachyrhizi*.

Phakopsora pachyrhizi | *Puccinia striiformis* f. sp. *tritici* | *Puccinia triticina* | disease control

Glyphosate is a broad-spectrum herbicide that inhibits 5-enolpyruvyl shikimate 3-phosphate synthase (EPSPS), a key enzyme in the synthesis of aromatic amino acids. Crops engineered with a glyphosate-insensitive EPSPS have been successfully commercialized, permitting in-crop application of glyphosate for postemergence control of weeds (1, 2). EPSPS is present in plants, fungi, and bacteria, but not in animals (3). Assuming the presence of a glyphosate-sensitive EPSPS, fungi and bacteria may be susceptible to the action of glyphosate. In pure culture, growth of many fungi was inhibited *in vitro* by glyphosate but only at very high concentrations (ED_{50} 100 to >1,000 mg/g) (4).

Crops are susceptible to many fungal and/or bacterial diseases. However, the fact that conventional crops are killed by glyphosate has complicated investigations on potential benefits of glyphosate in disease control. Glyphosate has been shown to interfere with the production of phenolic compounds from the shikimate pathway that contribute to plant defenses (5), thereby enhancing the rate of plant death caused by *Pythium* and other soil-borne fungal pathogens (6). However, studies of soil-borne fungal pathogens (*Fusarium*, *Rhizoctonia*, and *Sclerotinia*) in glyphosate-resistant (GR) soybeans have not detected increased disease with glyphosate application compared with other herbicide treatments (7, 8).

This article focuses on important rust pathogens of wheat and soybean. New races of the stripe rust fungus (*Puccinia striiformis* f. sp. *tritici*) caused multimillion dollar losses of wheat in the U.S. in 2002 (9). Asian soybean rust, caused by *Phakopsora pachyrhizi*, is endemic in Asia and was discovered in South America in 2001 (10) and in the U.S. in 2004 (11). This pathogen has a wide host range on other Leguminous species (12), and no resistant cultivars are currently available. Asian soybean rust is a potential threat to 70 million acres of soybean in the U.S., and fungicides are currently the only control option.

During the 2002 stripe rust epidemic in eastern Washington, we observed that a highly susceptible GR wheat cultivar had reduced stripe rust infection levels upon treatment with glyphosate. Our objective was to determine the effect of glyphosate on the incidence of leaf and stripe rusts in susceptible cultivars of GR wheat in a controlled laboratory environment. We measured preventive and curative activities and glyphosate as a potential inducer of systemic acquired resistance (SAR) in disease control. We determined the translation of our laboratory observations to a field environment with natural stripe rust inoculum. We also examined under greenhouse conditions the effect of glyphosate against the Asian soybean rust pathogen in GR soybeans.

Materials and Methods

Seeds of GR wheat were derived from the spring wheat cultivars Ingot (GR-Ingot, South Dakota State University) and Macon (GR-Macon) (13). Urediospores from *Puccinia triticina* and *Puccinia striiformis* f. sp. *tritici* (race PST-78) were provided by J. Kolmer (University of Minnesota, Saint Paul, MN) and X. Chen [U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), Washington State University], respectively. Glyphosate formulations (Roundup WeatherMAX, UltraMAX, and Ultra), as well as formulation controls without glyphosate, were provided by Monsanto. Phosphono[^{14}C]methyl-labeled glyphosate was obtained from NEN PerkinElmer. We obtained Soltrol 170 oil from Chevron Phillips Chemical and the flat fan spray nozzle (XR110015) from Spraying Systems (Wheaton, IL).

Glyphosate Spray on GR Wheat in the Growth Chamber. Seeds from GR-Ingot or GR-Macon were planted in Metromix 350 soil (Hummert International, St. Louis, MO) in 10-cm-square pots and germinated in the growth chamber (20°C day, 16°C night, 12-h photoperiod, 70% relative humidity, and 650 $\mu E \cdot m^{-2} \cdot s^{-1}$ light). Formulations were diluted with deionized water and applied over-the-top (OT) of plants from three- to seven-leaf stage by using a track-sprayer equipped with a commercially available flat-fan nozzle (XR110 015) at a height of 46 cm with a track speed and output volume equivalent to 187 liters/ha.

In some experiments, phosphono[^{14}C]methyl-labeled glyphosate (457 MBq/mmol specific activity, 98.2% purity) was added to the diluted glyphosate formulation at a concentration of ≈545

Conflict of interest statement: Glyphosate is manufactured by Monsanto Co., which employs P.C.C.F., G. J. Baley, W.P.C., G. J. Bunkers, and M.F.A. A patent has been applied for by the Washington State University Research Foundation, representing G. J. Baley, T.C.P., and K.K.K. G. J. Baley was hired by Monsanto after completion of his Ph.D. at Washington State University. His contribution to this article was made as a Ph.D. student at Washington State University.

Abbreviations: GR, glyphosate resistant; EPSPS, 5-enolpyruvyl shikimate 3-phosphate synthase; SAR, systemic acquired resistance; DBI, days before inoculation; DAI, days after inoculation; AUDPC, area under the disease progress curve; ae, acid equivalent; INA, 2,6-dichloro isonicotinic acid; WCI, wheat chemically induced; OT, over-the-top.

†To whom correspondence should be addressed. E-mail: paul.feng@monsanto.com.

www.pnas.org/cgi/doi/10.1073/pnas.0508873102

kBq of radioactivity per 40 ml of volume and sprayed as described above. Formulation controls of Roundup WeatherMAX or UltraMAX without glyphosate were diluted to a concentration of 0.1% and sprayed similarly. The surfactant concentration of 0.1% was similar to a spray solution of formulated glyphosate at a dose of 0.84 kg acid equivalent (ae)/ha (1× field use rate) at a volume of 187 liters/ha. In experiments for quantification of systemic glyphosate, the leaf targeted for spore inoculation was shielded from the [^{14}C]glyphosate OT spray by placing a disposable plastic straw over the leaf before the spray and removing the straw afterward.

Inoculation of Wheat Rust Spores and Disease Rating. The youngest, fully expanded leaf on the plant was marked for inoculation with leaf rust spores. Plants were laid down horizontally and covered with an absorbent towel except for the target leaf. Urediospores were suspended in Soltrol 170 oil at a concentration of 3 mg/ml and sprayed with an air brush at two passes per leaf.

After inoculation with leaf rust spores, GR-Ingot plants were placed under a mist tent within a growth chamber (20°C, no light, 100% relative humidity) for 24 h to allow for spore germination. To further ensure adequate humidity, the tent was equipped with a mister that was activated for 2 min at 8-min intervals. At the end of the 24-h incubation, the plants were returned to normal growing conditions described previously. Plants were rated from 11 to 15 days after inoculation (DAI) for disease severity based on percentage of leaf area covered with sporulating lesions (i.e., pustules) (14). In one experiment, the incidence of lesions without sporulation was also documented.

Stripe rust spores required slightly cooler conditions for growth and germination. After leaf inoculation as described above, GR-Macon plants were placed in the mist tent within the growth chamber at 12°C (no light and 100% relative humidity). After 24 h, the plants were returned to a growth chamber set at 14°C, 16-h photoperiod, 75% relative humidity, and 650 $\mu E \cdot m^{-2} \cdot s^{-1}$ light. Plants were rated for stripe rust severity 13–16 DAI.

Quantification of [^{14}C]Glyphosate in Tissues. Glyphosate concentration was quantified based on radioactivity. In most experiments, the tissue of primary interest was the inoculated leaf. At harvest, the leaf was excised and washed sequentially with water (30 ml) followed by methanol (5 ml), and the combined solutions were analyzed for leaf surface radioactivity. Radioactivity in the leaf was recovered by combustion analysis in a biological oxidizer (Packard 387 oxidizer) and translated to glyphosate mass based on specific activity and expressed as μg/g fresh weight. In some experiments, the glyphosate concentration in the remaining foliage and roots was also determined.

SAR Induction Experiments in GR Wheat. At 1 day before inoculation (DBI), glyphosate formulation (1× Roundup WeatherMAX), 0.1% formulation control (Roundup WeatherMAX without glyphosate), or 2,6-dichloro isonicotinic acid (INA) (200 μg/g in 0.1% formulation control) was applied OT to GR-Ingot plants at the five-leaf stage. The entire plant was inoculated the next day by application of stripe rust spores as described above. Two plants were harvested from each treatment from 0 to 144 h postinoculation, and leaf tissues were stored at −80°C.

Total RNA was isolated from frozen leaf tissue by using TRIzol reagent (GIBCO Invitrogen). RNA (15 μg) was denatured and separated on a 2% (wt/vol) agarose-6.6% (vol/vol) formaldehyde gel (15). Loading buffer containing ethidium bromide (Sigma) was used to confirm equal sample loading. Before RNA loading, the gel was photographed, rinsed in water for 10–15 min to remove formaldehyde and ethidium bromide, and then equilibrated with 20× SSC (3 M NaCl/0.3 M sodium citrate, pH 7.0). The RNA was blotted to a positively charged nylon membrane (Roche Diagnostics) by using 20× SSC and a Turboblot system (Schleicher & Schuell). After transfer, RNA was crosslinked to the membrane by UV irradiation. All prehybridizations and hybridizations were conducted at 42°C in a solution containing 5× SSC, 5× Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% BSA), 50% formamide, 1% SDS, and 100 μg/ml denatured salmon sperm DNA. ^{32}P-labeled probes were hybridized for 14–16 h, and the membrane was sequentially washed with 2× SSC in 0.1% SDS (twice for 10 min) at 22°C and 0.5× SSC in 0.1% SDS (twice for 5 min) at 50°C. The membrane was blotted, wrapped in plastic film, and exposed to x-ray film. Membranes were stripped of bound probe by agitating in boiling 0.005× SSC in 0.1% SDS for 30 min, air dried, and rehybridized to subsequent probes as described above.

Gene fragments from three wheat defense response genes and one wheat chemically induced (WCI) gene were used as probes. All gene fragments were generated by using PCR of genomic DNA from the wheat cv. Bobwhite. Primers were designed based on published sequences for wheat *PR-1* (accession no. AJ007349, 5′-AACCGGGGCGTCTTCATCA-3′ and 5′-TGCATACATTTACACGCTCCACAG-3′) and wheat thionin-like *WCI-3* genes (accession no. U32429, 5′-GCTCAGATGAAGATGGTTGCCG-3′ and 5′-TTGTGGACGCAGACCTCATAGC-3′) from cv. Kanzler (16), and for wheat peroxidase *Pxc-1a* (accession no. X56011, 5′-GGCAAACAGCGACCTGCCAGG-3′ and 5′-TCTATCAATCACGAGTTCACC-3′) and thaumatin-like *PR-5* genes (accession no. X58394, 5′-AGCACCCAGGACTTCTACGACATC-3′ and 5′-GTGCGACGTATAGAGGCTTCATG-3′) from cv. Cheyenne (17, 18).

Field Test for Stripe Rust Control in GR Wheat. A field trial with high endemic inoculum of stripe rust was established at the USDA-ARS Palouse Conservation Field Station, northeast of Pullman, WA in 2004. GR-Macon was planted in a block design in 1.5-m-wide by 3.6-m-long plots, and plants were sprayed with a glyphosate formulation (Roundup Ultra) by using a hand-held boom at 0.42–1.26 kg ae/ha (0.5× to 1.5× rates). Two no-spray controls flanked the sprayed plots. The first spray (i.e., preinfestation) was applied when the plants were at the four- to five-leaf stage (May 19, 2004) and did not have visible symptoms of rust. The second spray (i.e., postinfestation) was applied just before head emergence (June 30, 2004) at ≈7 days after the development of initial rust symptoms. GR-Macon is highly susceptible to local races of stripe rust, and heavy stripe rust inoculum levels resulted in adequate infection for recording disease severity in experimental plots. Stripe rust incidence (0–100%) and severity (1–9) (19) were recorded on July 2nd, 8th, and 12th, 2004. Area under the disease progress curve (AUDPC) was calculated for each treatment based on disease incidence from July 2–12. General contrasts were used to compare all of the glyphosate treatments to the no-spray control plots, and *P* values were calculated with Scheffé's *F* test (STATISTIX 7.0).

Greenhouse Evaluation of Asian Rust Control in GR Soybean. Two experimental genotypes (48 and 57, maturity Group 3) of GR soybean (*Glycine max*) were evaluated in the USDA-ARS, Foreign Disease Weed Science Research Unit, Biosafety Level 3, Plant Pathogen Containment Facility, Fort Detrick, MD (20). A single seed was planted into each 10-cm-diameter clay pot filled with planting mix containing soil, sand, vermiculite, perlite, and peat moss, with five replicate pots per treatment. Plants were grown for 21 days in greenhouse (29°C, 16-h supplemental light/day) and transferred inside the containment facility for subsequent inoculations. Glyphosate (Roundup Ultra) was applied at the 2nd trifoliate stage by using a Solo Spraystar 460 rechargeable spray bottle (Solo, Newport News, VA). The treatments were as follows: no spray; glyphosate 3 DBI at either 1.26 or 2.52 kg ae/ha (1.5× or 3.0× preinoculation); 1.5× 3 DBI

Table 1. Efficacy of glyphosate spray treatment before spore inoculation on % incidence of leaf rust in GR wheat cv. Ingot at varying growth stages

Spray 1 DBI, rust rating 13 DAI*	5-leaf $N = 11$	3-leaf $N = 8$	7-leaf $N = 4$
No spray control	100	NA	NA
Water	NA†	88	NA
Formulation control‡	NA	75	100
Glyphosate§	0	0	0

*Plants were sprayed OT 1 DBI and evaluated for the presence of sporulating lesions and pustules 13 DAI.
†NA, not analyzed.
‡Formulation control (0.1%) from Roundup UltraMAX or WeatherMAX without glyphosate.
§Roundup WeatherMAX at 0.84 kg ae/ha in 187 liters/ha volume (1×).

and at symptom appearance (1.5× pre- and postinoculation), and 1.5× at symptom appearance (1.5× postinoculation).

Three days after the initial glyphosate application, all plants were inoculated with the soybean rust pathogen, *P. pachyrhizi*, by using an equal urediospore mixture of four isolates from Brazil 01-1, Paraguay 01-2, Thailand 01-1, and Zimbabwe 01-1. The combined urediospores were suspended in distilled water containing 0.01% Tween 20 (vol/vol) at a concentration of 25,000 spores per ml, and ≈5 ml of inoculum was sprayed onto each plant by using an atomizer at 137 kPA pressure. Inoculated plants were placed into a dew chamber at 20°C for ≈18 h and moved to a greenhouse at 25°C until disease measurements were recorded at 14 and 21 DAI. The number of sporulating lesions was counted within two 1.2-cm-diameter circles on either side of the mid-rib of the center leaflet on the first trifoliate for each plant. The experiment was repeated, and data were analyzed at each sampling time by ANOVA, with treatment and soybean lines as main effects [least significant difference (LSD) test at $P \leq 0.05$, STATISTIX 7.0].

Results

Glyphosate Efficacy on Leaf Rust in GR-Ingot Wheat. Initial experiments in the growth chamber examined plants in the five-leaf stage that were either not sprayed or sprayed with a glyphosate formulation (Roundup WeatherMAX at 0.84 kg ae/ha, 1×), a typical field use rate. Although nonsprayed plants were 100% infected with rust pustules, none of the glyphosate-sprayed plants showed evidence of infection (Table 1).

The possibility that rust control might result from other reagents in the formulation besides glyphosate was also examined (Table 1). Plants at the three-leaf stage were sprayed 1 DBI with water, 0.1% formulation control (Roundup WeatherMAX without glyphosate), or 1× glyphosate formulation. When sprayed with water or formulation control, 88% or 75% of the plants were infected with rust, respectively; however, no rust was evident on plants sprayed with the glyphosate formulation. Similar results were obtained with plants at the seven-leaf stage.

Glyphosate Spray Timing Before Leaf Rust Inoculation in GR-Ingot Wheat. We measured the duration of rust control and the concentration of glyphosate in tissues. Plants at the three- or five-leaf stage were sprayed either 14 or 1 DBI with a 1× dose of glyphosate formulation augmented with [^{14}C]glyphosate. A single leaf was inoculated, and rust severity (0–100%) was rated 13 DAI. The leaf was then washed and combusted to recover glyphosate.

In all treatments (Table 2, treatments A–D), nonsprayed control plants produced consistent rust severity ranging from 24% to 29% 13 DAI. Plants in treatments A and B with glyphosate spray 14 DBI showed much reduced rust pustules (0.4% and 0.2%, respectively) but significant lesions (90% and 40%, respectively), indicating arrested sporulation that effectively prevented pathogen spread. Plants in treatments C and D with glyphosate spray 1 DBI showed no pustules and few lesions. Fig. 1 compares rust severity in leaves with no spray or with glyphosate spray 14 or 1 DBI. Our results showed that rust control was optimal right after glyphosate spray and persisted for at least 14 days.

On the day of inoculation (0 DAI), glyphosate concentration in the inoculated leaf was 9–10 μg/g from a 14 DBI spray (Table 2). In comparison, a 1 DBI spray generated much higher glyphosate concentrations (42–59 μg/g). Glyphosate concentrations 13 DAI declined slightly presumably from leaf export. Tissue concentrations of glyphosate were similar among 14 DBI treatments A and B, suggesting a fairly uniform pattern of

Table 2. Effect of [^{14}C] glyphosate spray before spore inoculation on leaf rust rating and correlation with glyphosate tissue concentration in GR wheat cv. Ingot

Leaf rust rating and glyphosate tissue concentrations	Treatment A	B	C	D
Plant leaf stage at glyphosate spray*	5	3	5	3
Plant leaf stage at spore inoculation†	7	5	5	3
Glyphosate spray timing, DBI‡	14	14	1	1
% Leaf rust rating, avg (SE)§				
% Pustules, no spray	28.8 (2.8)	26.0 (1.9)	27.0 (1.2)	24.0 (3.7)
% Pustules, 1× glyphosate spray	0.4 (0.2)	0.2 (0.1)	0	0
% Lesions, 1× glyphosate spray	90	40	10	10
Tissue glyphosate in μg/g, avg (SE)¶				
Inoculated leaf, 0 DAI	10.4 (2.1)	9.3 (3.5)	41.8 (4.4)	59.3 (13.5)
Inoculated leaf, 13 DAI	8.0 (1.3)	9.3 (1.5)	29.9 (6.5)	41.2 (7.0)
Plant foliage, 13 DAI	10.1 (2.0)	7.3 (2.0)	76.7 (13.4)	60.5 (10.6)
Roots, 13 DAI	8.6 (1.3)	5.7 (1.4)	23.6 (5.0)	33.9 (6.3)

*Roundup WeatherMAX (0.84 kg ae/ha in 187 liters/ha vol, 1×) containing [^{14}C]glyphosate was sprayed OT.
†Leaf rust spores were suspended in oil and applied onto a single mature leaf on each plant.
‡Roundup was sprayed before spore inoculation 14 or 1 DBI.
§The inoculated leaf was rated 13 DAI for percentage of leaf area with sporulating lesions (i.e. pustules) or percent incidence of lesions without sporulation. The results are the average of five plants and SE.
¶The inoculated leaf, after washing to remove surface residues, was combusted to recover radioactivity and translated to glyphosate concentration (μg/g fresh weight). The results are the average of five plants and SE.

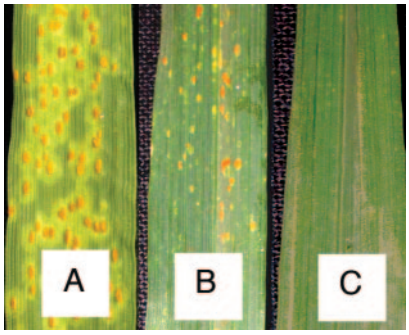

Fig. 1. The effect of glyphosate treatment on severity of leaf rust (*P. triticina*) in GR wheat cv. Ingot 13 DAI. Treatment A, no spray; treatment B, glyphosate formulation (Roundup WeatherMAX, 0.84 kg ae/ha, 1×) 14 DBI; treatment C, glyphosate formulation (1×) 1 DBI.

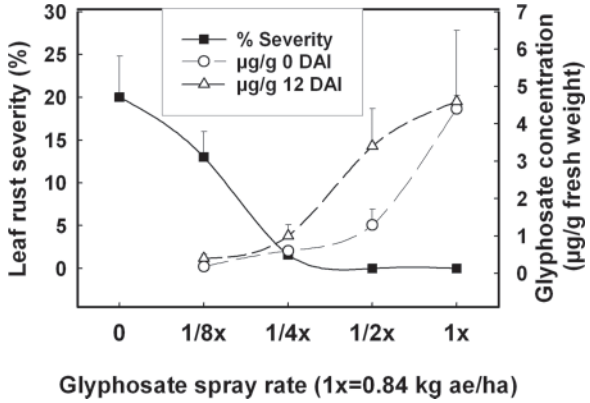

Fig. 2. Correlation of spray dose of glyphosate, severity of leaf rust (%), and systemic concentration of glyphosate in the inoculated leaf of GR wheat cv. Ingot. Roundup WeatherMAX (0.11–0.84 kg ae/ha, 1/8× to 1×) containing [^{14}C]glyphosate was sprayed 1 DBI onto plants with one mature leaf shielded from the spray. The shielded leaf was inoculated with leaf rust spores 1 day later, and leaf rust severity was rated 11 DAI. Systemic glyphosate concentration (µg/g fresh weight) in the inoculated leaf was determined by radioactivity 0 or 12 DAI.

glyphosate distribution in the plant. The major factor affecting tissue concentration was the timing of glyphosate spray. When the plants were sprayed at 14 DBI, tissue concentrations ranged from 6 to 10 µg/g, whereas a 1 DBI spray resulted in 24–77 µg/g. Because glyphosate is not metabolized in wheat, the decrease in tissue concentration likely resulted from distribution and dilution due to growth.

Our results showed that the level of disease control was proportional to tissue glyphosate concentration. One day after glyphosate spray, the tissue concentrations were high, resulting in complete rust control. At 14 days after spray, reduced tissue concentrations controlled rust sporulation but not lesion development. Analysis by autoradiography (data not shown) of the inoculated leaf demonstrated uniform distribution of glyphosate throughout the entire leaf and further implicated glyphosate in leaf rust control.

Correlation of Systemic Glyphosate with Leaf Rust Control in GR-Ingot Wheat. Analysis at 1 day after spray showed that ≈70% of the plant-intercepted glyphosate remained on the leaf surface, 21% localized within the foliage and 9% translocated to the roots (data not shown). Leaf tissues that intercept the spray contain both localized and translocated (i.e., systemic) glyphosate, whereas roots contain only systemic glyphosate. The following experiment was designed to determine the role of various glyphosate pools in rust control.

Examination by microscopy showed comparable spore germination on leaves that were sprayed or not sprayed with 1× glyphosate formulation, suggesting that leaf surface glyphosate does not prevent spore germination. Because the inoculated leaf was shielded from the glyphosate spray, it contained only systemic glyphosate imported from the rest of the plant. Nonsprayed plants showed 20% rust severity, which declined progressively with increasing glyphosate spray rate (Fig. 2). At 1/2× to 1× spray rates, rust severity was zero, indicating that systemic glyphosate is responsible for rust control. The leaf concentration of glyphosate at 0 DAI ranged from 0.2 to 4.4 µg/g from application of 1/8× to 1× dose and increased slightly by 12 DAI. These results indicated that the effective threshold of systemic glyphosate for leaf rust control is from 1 to 5 µg/g of leaf tissue, and rust control was proportional to glyphosate spray dose.

Glyphosate Spray Timing After Leaf Rust Inoculation in GR-Ingot Wheat. We examined potential curative activity of glyphosate. Plants at the five-leaf stage were first inoculated with rust spores followed by spray application of glyphosate formulation at various times. Nonsprayed plants showed 20% severity 12 DAI (Table 3). Plants sprayed with a formulation control without glyphosate just before (0.01 DBI) or after (0.01 DAI) spore inoculation showed reduced rust (10–11%), suggesting that surfactant alone may have some effect on spores. Rust was completely controlled when 1× glyphosate formulation was applied from 0.01 to 5 DAI. With the 5-DAI spray, leaf lesions were already visible, suggesting that infection was well underway but failed to progress to pustules.

Correlation of SAR Induction with Leaf Rust Control in GR-Ingot Wheat. We examined whether leaf rust control by glyphosate could be mediated through induction of SAR genes in GR-Ingot. The induction of four common SAR genes (*PR-5*, *WCI-3*, *Pxc-1a*, and *PR-1*) was measured after glyphosate application, and results correlated with leaf rust control. Northern analysis showed that *PR-5*, a thaumatin-like protein, was readily induced by application of 1× glyphosate formulation (Fig. 3). *PR-5* was maximally induced between 8 and 48 h after the glyphosate spray and could potentially mediate the activity of glyphosate against leaf rust. However, *PR-5* was also extensively induced by other treatments, including the formulation control, INA, or spore inoculation, and none of these treatment resulted in any rust control. These results indicated no relationship between induction of *PR-5* gene and leaf rust control.

For the remaining probes (*WCI-3*, *Pxc1*A, or *PR-1*), applica-

Table 3. Effect of application timing of glyphosate on % severity of leaf rust in GR wheat cv. Ingot

Spray bef/aft inoculation	% Leaf rust*	SE
No spray control	20.0	4.8
Formulation cont 0.01 DBI†	11.3	2.8
Formulation cont 0.01 DAI	10.0	2.7
Glyphosate 0.01 DAI‡	0.0	0.0
Glyphosate 1 DAI	0.0	0.0
Glyphosate 2 DAI	0.0	0.0
Glyphosate 5 DAI	0.0	0.0

*Leaf rust (%) was rated 12 DAI and reported as the average of five plants with SE.

†Formulation control (cont) (0.1%), Roundup WeatherMAX without glyphosate, was sprayed OT at 15 min before spore inoculation (0.01 DBI).

‡Roundup WeatherMAX (0.84 kg ae/ha in 187 liters/ha vol, 1×) was sprayed at 15 min after spore inoculation (0.01 DAI).

AGRICULTURAL SCIENCES

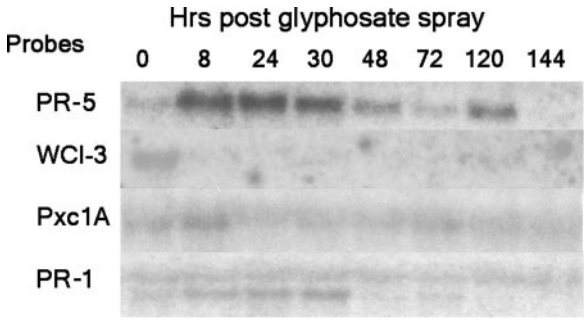

Fig. 3. Northern blot assay for induction of SAR genes (*PR-5*, *WCI-3*, *Pxc1A*, or *PR-1*) in leaf extracts from 0 to 144 h postspray of glyphosate in GR wheat cv. Ingot.

tion of 1× glyphosate resulted in little to no induction, suggesting no association with rust control activity of glyphosate (Fig. 3). As positive controls, we induced *WCI-3* by treatment with INA, and *PR-1* and *Pxc-1*a by rust spore inoculation; however, the induction of these genes resulted in no rust control. Based on these results, we conclude that rust control activity of glyphosate is not mediated through the induction of four common SAR genes that were examined. Our results do not exclude the possibility that the activity of glyphosate could be mediated through other SAR genes.

Field Efficacy of Glyphosate on Stripe Rust in GR-Macon Wheat. The effect of glyphosate on stripe rust was examined in the susceptible GR-Macon cultivar. Growth chamber results showed that glyphosate had both preventive and curative activities on stripe rust at levels similar to that of leaf rust (results not shown).

A field trial was conducted under ideal environmental conditions (warm days ≈25°C, cool nights ≈10°C with dew formation and sporadic rainfall) that resulted in heavy, natural infestation of stripe rust. Plants were sprayed with glyphosate formulation (Roundup Ultra) at 0.42–1.26 kg ae/ha (0.5× to 1.5× rates). Fig. 4 shows that the preinfestation spray of glyphosate (0.5× to 1.5×) effectively reduced the progression of stripe rust incidence as measured by AUDPC; however, rust severity remained high. Results indicated that preinfestation spray of

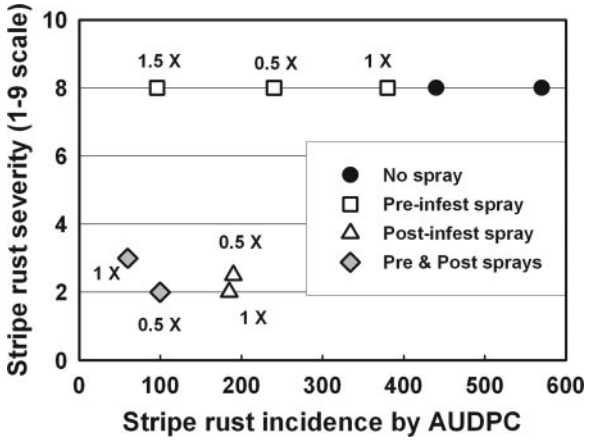

Fig. 4. Field evaluation of the effect of glyphosate treatment on stripe rust (*P. striiformis* f. sp. *tritici*) in GR wheat cv. Macon from natural inoculum near Pullman, WA. Roundup Ultra (0.42–1.26 kg ae/ha, 0.5× to 1.5×) was sprayed preinfestation on May 19, 2004 and/or postinfestation on June 30, 2004. Rust symptoms first appeared on June 25, 2004. Stripe rust incidence based on AUDPC was calculated from ratings on July 2nd, 8th, and 12th. The *y* axis describes rust severity (1–9) from July 12th.

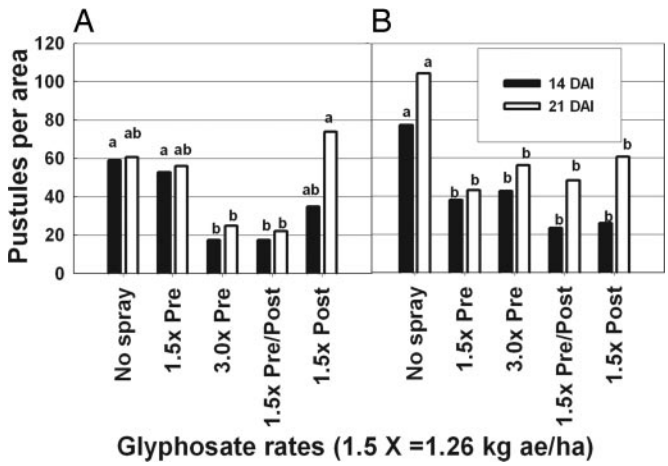

Fig. 5. Greenhouse evaluation of the effect of glyphosate on Asian soybean rust (*Phakopsora pachyrhizi*) in GR soybean genotypes 48 (*A*) and 57 (*B*). The glyphosate treatments were as follows: no spray; 1.5× preinoculation (1.26 kg ae/ha 3 DBI); 3.0× preinoculation (2.52 kg ae/ha 3 DBI); 1.5× pre- and postinoculation (1.26 kg ae/ha 3 DBI and at rust appearance); and 1.5× postinoculation (1.26 kg ae/ha at rust appearance). At 14 and 21 DAI, the number of sporulating lesions was counted within two 1.2-cm-diameter circles on either side of the mid-rib of the center leaflet on the first trifoliate for each plant ($n = 5$). Lesion numbers were analyzed by ANOVA with treatment and soybean lines as main effects. Within each genotype and sampling time, data points labeled with the same letter are not significantly different at $P \leq 0.05$.

glyphosate, especially at higher use rates, provided early reduction in rust incidence, but the effect diminished over time. We estimate the duration of preventive activity for glyphosate to be ≈30 days. In comparison, application of glyphosate (0.5× or 1×) 1 week postinfestation, either alone or in combination with preinfestation treatment, significantly reduced both the incidence and severity ratings. Results from the field test verified laboratory results and demonstrated that glyphosate (0.5× to 1× rates) applied at appropriate timings can prevent and arrest stripe rust in GR wheat.

Greenhouse Evaluation of Glyphosate on Asian Rust (*P. pachyrhizi*) in GR Soybean. The effect of glyphosate on Asian soybean rust was evaluated in two germplasms of GR soybean. Application of glyphosate (3× or 2.56 kg ae/ha) 3 DBI significantly ($P \leq 0.05$) reduced sporulating lesions of rust by >70% 14 DAI in GR soybean 48 (Fig. 5*A*). Lower dose of glyphosate (1.5×) pre- and/or postinoculation was less efficacious. For GR soybean 57, all glyphosate treatments (1.5× or 3.0×), pre- and/or postinoculation, significantly ($P \leq 0.05$) reduced the number of lesions by 46–70% at 14 and 21 DAI (Fig. 5*B*). At high rates of glyphosate formulation, spot necrosis was observed on leaves from the high concentration of the surfactant. These preliminary results suggest that glyphosate is active against the Asian soybeans rust.

Discussion

After the initial 2002 field observation of reduced stripe rust disease in GR wheat, our laboratory results demonstrated that application of glyphosate provided both preventive and curative activities against leaf and stripe rusts in GR wheat. Leaf rust control was proportional to systemic glyphosate concentration, with complete control achieved at a tissue concentration between 1 and 5 μg/g that was attained at a spray dose typical for weed control. These results were confirmed quantitatively in the field under natural stripe rust pressure in 2004. Leaf rust control by glyphosate was not mediated through induction of four common SAR genes (21). Preliminary results from greenhouse

studies also showed activity of glyphosate against Asian rust in GR soybean.

The fact that glyphosate is active against wheat rust at low concentrations was unexpected in light of earlier reports showing the need for much higher glyphosate concentrations (100–1,000 mg/g) to inhibit fungal growth (4). We propose that the activity of glyphosate stems from inhibition of fungal EPSPS, the same mechanism ascribed to its herbicidal activity. We believe that as rust spores parasitize the plant host to acquire nutrients, they become exposed to a lethal dose of systemic glyphosate. This result was demonstrated during germination of rust aeciospores from *Puccinia lagenophora*, which were inhibited by glyphosate at 22 μg/ml glyphosate (22).

The sensitivity of fungal EPSPS to glyphosate was examined through bioinformatics analysis. Structural studies of EPSPS based on x-ray crystallography have identified key amino acids involved in catalysis (23, 24). These amino acids are highly conserved across species and have been used to characterize the interactions between glyphosate and EPSPS. In fact, the sensitivity of an EPSPs to glyphosate can be predicted by the presence of four unique amino acid motifs (4). A search of public databases showed genome sequences from 12 fungi. We deduced and aligned the amino acid sequences of fungal EPSPS and showed that all of them are predicted to be glyphosate-sensitive. Still, the presence of a glyphosate-sensitive EPSPS is no guarantee for activity because there could be alternative resistance mechanisms such as metabolism, uptake, or translocation (25, 26).

Our results showed that rust control depends on the systemic glyphosate concentration of a plant, which is determined by uptake and translocation efficiencies (27). Glyphosate is a water-soluble molecule that relies on surfactants to cross the foliar cuticle barrier (28), and over the years surfactant systems have been developed to facilitate this process (29). Preliminary studies comparing two commercial formulations showed that rust control in GR wheat was better with the formulation characterized for its higher efficiency of glyphosate uptake and translocation. Our results also imply that better rust control can be expected from GR crops engineered with a glyphosate-insensitive EPSPS gene, which conserves glyphosate, than with a glyphosate-deactivation gene.

Glyphosate is an efficacious, broad-spectrum herbicide. Our studies demonstrate that glyphosate may offer additional benefits of rust control in GR wheat and soybean. However, further research is needed to determine whether these findings can be translated into management recommendations for growers. Extensive field tests will be needed to determine the dose and timing of application under varying environmental conditions to establish the utility of glyphosate as a tool for rust management in GR wheat and soybean.

Note Added in Proof. A paper published in November 2005 by Anderson and Kolmer reported the control of leaf rust (*Puccinia triticina*) on glyphosate-tolerant wheat sprayed with glyphosate in greenhouse and field trials (30).

We are grateful for rust urediospores provided by Jim Kolmer and James Anderson (University of Minnesota, Saint Paul, MN) and Xianming Chen (Washington State University). Reid Frederick (USDA-ARS) is acknowledged for conducting the studies on Asian soybean rust at Ft. Detrick, MD. We thank Maolong Lu for bioinformatics analysis, Sophia Chen for microscopy studies, and Sally Metz, Claire CaJacob, and Danny Gigax for supporting this research. The initial field observations were obtained from research funded by a grant to K.K.K. from the USDA Biotechnology Risk Assessment Research Grants Program.

1. Nida, D. L., Kolacz, K. H., Buehler, R. E., Deaton, W. R., Shuler, W. R., Armstrong T. A., Taylor, M. L., Ebert, C. C., Rogan, G. J., Padgette, S. R., *et al*. (1996) *J. Agric. Food Chem*. **44,** 1960–1966.
2. Padgette, S. R, Kolacz, K. H., Delannay, X., Re, D. B., Lavallee, B. J., Tinius, C. N., Rhodes, W. K., Otero, Y. I., Barry, G. F., Eichholtz, D.A., *et al*. (1995) *Crop Sci*. **35,** 1451–1461.
3. Kishore, G. M. & Shah, D. M. (1998) *Annu. Rev. Biochem*. **57,** 627–663.
4. Franz, J. E., Mao, M. K. & Sikorski, J. A. (1997) *Glyphosate: A Unique Global Herbicide* (Am. Chem. Soc., Washington, DC), monograph 189.
5. Johal, G. & Rahe, J. (1984) *Phytopathology* **74,** 950–955.
6. Lévesque, C. & Rahe, J. (1992) *Annu. Rev. Phytopathol*. **30,** 579–602.
7. Lee, C. D., Penner, D. & Hammerschmidt, R. (2000) *Weed Sci*. **48,** 710–715.
8. Sanogo, S., Yang, X. B. & Lundeen, P. (2001) *Plant Dis*. **85,** 773–779.
9. Chen, X.M., Moore, M., Milus, E. A., Long, D. L., Line, R. F., Marshall, D. & Jackson, L. (2002) *Plant Dis*. **86,** 39–46.
10. Yorinori, J. T., Paiva, W. M., Frederick, R. D., Costamilan, L. M., Bertagnolli, P. F., Hartman, G. E., Godoy, C. V. & Nunes, J., Jr. (2005) *Plant Dis*. **89,** 675–677.
11. Schneider, R. W., Hollier, C. A., Whitam, H. K., Palm, M. E., McKemy, J. M., Hernández, J. R., Levy, L. & DeVries-Paterson, R. (2005) *Plant Dis*. **89,** 774.
12. Bromfield, K. R. (1984). *Soybean Rust* (Am. Phytopathol. Soc., St. Paul, MN), monograph 11.
13. Kidwell, K. K., DeMacon, V. L., Shelton, G. B., Burns, J. W., Carter, B. P., Morris, C. F., Chen. X. & Bosque-Perez, N. A. (2003) *Crop Sci*. **43,** 1561–1563.
14. James, W. C. (1971) *A Manual of Assessment Keys for Plant Diseases* (Am. Phytopathol. Soc., St. Paul, MN), Key 1.2.
15. Sambrook, J., Fritsh, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Woodbury, NY), 2nd Ed., pp. 7.43–7.52.
16. Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K. H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., *et al*. (1996) *Plant Cell* **8,** 629–643.
17. Rebmann, G., Mauch, F. & Dudler, R. (1991) *Plant Mol. Biol*. **17,** 283–285.
18. Rebmann, G., Hertig, C., Bull, J., Mauch, F. & Dudler, R. (1991) *Plant Mol. Biol*. **16,** 329–331.
19. Line, R. F. & Qayoum, A. (1992) *Virulence, Aggressiveness, Evolution, and Distribution of Races of Puccinia striiformis (the Cause of Stripe Rust of Wheat) in North America, 1968–1987* (U.S. Dept. Agric., Washington, DC), bulletin 1788.
20. Melching, J. S., Bromfield, K. R. & Kingsolver, C. H. (1983) *Plant Dis*. **67,** 717–722.
21. Morris, S. W. Vernooij, B., Titatarn, S., Starrett, M., Thomas, S., Wiltse, C. C., Frederiksen, R. A., Bhandhufalck, A., Hulbert, S. & Uknes, S. (1998) *Mol. Plant–Microbe Interact*. **11,** 643–658.
22. Wyss, G. S. & Muller-Scharer, H. (2001) *Biol. Control* **20,** 160–166.
23. Stallings, W. C., Meguid-Abdel, S. S., Lim, L. W., Shieh, H. S., Dayringer, H. E., Leimgruber, N. K., Stegeman, R. A., Anderson, K. S., Sikorski, J. A., Padgette, S. R., *et al*. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5046–5050.
24. Padgette, S. R., Re, D. B., Gasser, C. S., Eichholtz, D. A., Grazier, R. B., Hironaka, C. M., Levine, E. B., Shah, D. M., Fraley, R. T. & Kishore, G. M. (1991) *J. Biol. Chem*. **266,** 22364–22369.
25. Feng, P. C. C., Tran, M., Chiu, T., Sammons, R. D., Heck, G. R. & CaJacob, C. A. (2004) *Weed Sci*. **52,** 498–505.
26. Lorraine-Colwill, D. F., Powles, S. B., Howkes, T. R., Hollinshead, P. H., Warner, S. A. J. & Preston, C. (2002) *Pestic. Biochem. Physiol*. **74,** 62–72.
27. Feng, P. C. C., Chiu, T., Sammons, R. D. & Ryerse, J. S. (2003) *Weed Sci*. **51,** 443–448.
28. Feng, P. C. C., Ryerse, J. S. & Sammons, R. D. (1998) *Weed Technol*. **12,** 300–307.
29. Feng, P. C. C., Sandbrink, J. J. & Sammons, R. D. (2000) *Weed Technol*. **14,** 127–132.
30. Anderson, J. A. & Kolmer, J. A. (2005) *Plant Dis.* **89,** 1136–1142.