Effect of Glyphosate Herbicide on Pseudothecia Formation by *Pyrenophora tritici-repentis* in Infested Wheat Straw

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ABSTRACT

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Field-grown wheat straw infested with *Pyrenophora tritici-repentis* was treated with several herbicides used in no-tillage wheat production and then incubated under moist conditions in the greenhouse. Formulated herbicides containing bromoxynil, dicamba, glyphosate, 2,4-D, or paraquat, applied at labeled rates, significantly reduced ascocarp production by *P. tritici-repentis*. The glyphosate-containing herbicide Roundup (Monsanto Agricultural Co., St. Louis, MO) was the most effective; no ascocarps were produced in straw treated with this material. In further experiments with this herbicide, greenhouse-grown straw infested with *P. tritici-repentis* was treated with it either before incubation under conditions conducive to ascocarp development or at one of several times after incubation had started. The herbicide completely inhibited ascocarp development if applied before the conducive environment was imposed, but this inhibition diminished as the time delay increased between inoculation and treatment. Experiments conducted on autoclaved, inoculated straw indicated that the herbicide does not greatly reduce the mycelial growth rate of *P. tritici-repentis*. It was not determined whether the effect of the formulated material is due to glyphosate or to the nonherbicidal components of the material.

Pyrenophora tritici-repentis (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoem.) is a crop-residue-borne pathogen that causes tan spot, a foliage disease of wheat (*Triticum aestivum* L. emend. Thell.). The pathogen produces pseudothecia on infested straw during periods of moderately high moisture (6). Because primary inoculum for tan spot epidemics consists of ascospores produced in these straw-borne pseudothecia, the disease is most common and most severe in conservation tillage.

Infested straw residues in continuous, minimum-tillage wheat are likely to be exposed to herbicides. Roundup (Monsanto Agricultural Co., St. Louis, MO), containing the active ingredient glyphosate, is one of the most commonly used herbicides in reduced cultivation and direct drilling. Roundup has been shown to inhibit vegetative growth, spore formation, and spore germination of *Septoria nodorum* (Berk.) Berk. (5). It has also been reported to inhibit or stimulate the growth of various saprophytic straw-inhabiting fungi (2). In most studies, it is not possible to distinguish between the activity of the herbicidal ingredient (glyphosate) and the nonherbicidal components of the formulation. In the rare cases when these nonherbicidal materials were available, the antifungal activity appeared to be due largely to them (2).

The effect of formulated herbicides on *P. tritici-repentis* has not been examined previously. Our objective was to determine the effect of herbicides, particularly the glyphosate-containing herbicide Roundup, on ascocarp production by this pathogen. Although most previous studies of fungus response to glyphosate-containing herbicide have been conducted in agar culture, we performed our experiments with nonsterile wheat straw, because the effect of the herbicide on *P. tritici-repentis* might be influenced by nutrient availability and the activity of other microorganisms; the tests were performed with straw exposed to prolonged continuous moisture. We also determined the toxicity of the formulated herbicide to mycelium of *P. tritici-repentis* in autoclaved wheat straw.

MATERIALS AND METHODS

Infested straw. Initial experiments to test ascocarp inhibition by five herbicides were conducted with field-grown straw infested with *P. tritici-repentis*. This straw was collected after harvest in 1986 from a wheat field infected with tan spot, near Manhattan, Kansas. It was stored indoors in burlap bags and was used about 6 mo after harvest.

In further experiments with the glyphosate-containing herbicide, greenhouse-grown straw infested with the pathogen was used, in order to reduce variability in the experiments. Mature greenhouse-grown plants were inoculated with conidial suspensions of *D. tritici-repentis* isolate 6R180 (2,500 conidia per milliliter), by the method of Raymond et al (8). Straws were harvested from diseased plants approximately 6 wk after inoculation and stored dry at room temperature (22–24 C) until used. This straw typically had become colonized by such saprophytic fungi as *Alternaria, Cladosporium*, and *Epicoccum*, in addition to *P. tritici-repentis* (authors, *unpublished*). Uninfested straw, also colonized by saprophytic fungi, was obtained from uninoculated greenhouse-grown plants for use in some experiments. The straw pieces used in all experiments were taken from the top two internodes and were cut to a length of 5 cm.

Effect of herbicides on ascocarp production. Five formulated commercial herbicides, containing the active ingredients dicamba, 2,4-D, glyphosate, paraquat, and bromoxynil, were tested for their effect on ascocarp production in field-grown straw. These herbicides were chosen after preliminary tests in which nine herbicides commonly used in minimum-tillage wheat farming were compared for their effects on ascocarp production by *P. tritici-repentis*. Of the nine herbicides, the five listed above all produced some degree of inhibition (1). The herbicide solutions were prepared at their highest labeled concentrations; these concentrations, and the trade names of the formulated herbicides, are shown in Table 1.

Straw pieces were dipped individually in one of the five herbicide solutions for about 5 sec and then transferred to glass canning jars (1,000 ml) laid on their sides. Each jar contained 250 cm^3 of vermiculite, which was spread evenly over the bottom side of the jar and moistened with 135 ml of water. The lid of each jar had a hole (10 mm in diameter) in the center to allow ventilation and heat exchange. A total of 16 straw pieces treated with the same herbicide were placed in each jar, and there were two replicate jars per herbicide treatment. Two check treatments were included; straw was treated with water in one and with a surfactant solution (spreader-sticker 497-30-B, Cargill, Minneapolis, MN) in the other. The jars were placed on a bench in a greenhouse at 25 ± 5 C and were watered when the

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vermiculite looked dry (about every 7 days). After 1 mo ascocarps per straw were counted. Because previous work (7) had shown that ascocarps less than 200 μm in diameter rarely produce ascospores, the ascocarps were classified as small (less than 200 μm in diameter) and large (200–800 μm in diameter), and the number in each size class was recorded. The experiment was repeated once.

Because the glyphosate-containing herbicide showed the greatest inhibition of ascocarp production in the above-described experiment, further tests were conducted with this herbicide to determine the effect of the dose and the timing of application on the inhibition. Formulated herbicide concentrations of 32 ml/L (1.3% a.i.), 41.6 ml/L (1.7% a.i.), and 62.5 ml/L (2.6% a.i.) were used. The second is the most concentrated labeled rate (1 pt/3 gal) for wheat fields. Because preliminary tests (Sharma, *unpublished*) had shown that the effect of the herbicide solution on *P. tritici-repentis* is not altered by filter-sterilization (i.e., the effect is not due to a microbial contaminant), all further tests were conducted with unsterilized herbicide. Greenhouse-grown infested straw was used instead of field-grown straw, in order to reduce variability in the experiment. In these tests, straw pieces were covered with wet vermiculite for 2 hr, to moisten them, and then were randomly divided into five groups. In one group (the 0-day treatment) the moistened straw pieces were dipped in the herbicide solution at one of the three concentrations and placed in jars as described above, but with six straws per jar. The straws of the remaining four groups were placed in jars without further treatment, six pieces per jar. On days 5, 7, and 10 the straws in three of these groups, respectively, were dipped in the herbicide. Thus the infested straws were incubated under conditions favorable for ascocarp development (in jars with moist vermiculite) for various time periods before being exposed to the herbicide. Straw pieces in the check treatment were not treated with the herbicide. There were two replicate jars per treatment. This experiment was repeated (once) with a separate, replicated check treatment for each moisture period (0, 5, 7, and 10 days); the check straws were dipped in water when the respective herbicide-treated straws were dipped in herbicide. Ascocarps were counted on day 21.

Growth rate. The effect of the glyphosate-containing herbicide on the growth rate of *P. tritici-repentis* was tested with autoclaved straw under controlled environmental conditions. Vermiculite (60 cm^3) was spread in each of 27 culture plates (20 × 100 mm), 40 ml of water was added to each, and the plates were autoclaved for 20 min at 121 C. Uninfested greenhouse-grown straw pieces were moistened for 2 hr in wet vermiculite and then autoclaved. Three culture plates (replicates), each containing four straw pieces, were used for each treatment. For one set of treatments ("preinoculation"), straw pieces were dipped in the glyphosate herbicide at one of three different concentrations, placed in culture plates for 3 days, and then inoculated with conidia of *D. tritici-repentis*. In the other set of treatments ("postinoculation"), straws were treated with the herbicide 3 days after inoculation. Check straws were treated with water. The inoculum was a conidial suspension (2,000 propagules per milliliter) prepared from agar cultures of *D. tritici-repentis* as described previously (7). A 3-μl drop of the suspension was placed at one end of each straw. The plates were covered and incubated for 1 wk at 20 C. Two straws from each of the three replicate plates of each treatment were then removed, cut into 2-mm pieces, and plated sequentially on a medium consisting of water agar amended, after autoclaving, with 200 mg of chloramphenicol and 0.42 mg of triphenyltin hydroxide per liter. The plates were incubated at 18 C under a 12-hr daily photoperiod. They were examined microscopically, 1 wk after plating, for conidia or mycelium of *D. tritici-repentis*. From the number and location of straw segments supporting the fungus, a growth rate was calculated. After 1 mo the two straw pieces remaining in each culture plate with vermiculite were examined to determine the number of ascocarps per straw.

Data analysis. The Kruskal-Wallis test was used to analyze data from the experiment to compare various herbicides and also the experiment to compare growth rates of *P. tritici-repentis* on autoclaved straw in the presence or absence of the glyphosate-containing herbicide.

To analyze the effect of the herbicide dose and application timing on ascocarp production, regression analysis was used. A test for homogeneity of variances between the two repetitions of the experiment showed no heterogeneity, so the results were pooled for the analysis. A regression equation relating the number of ascocarps to the time of application was constructed for each of the three doses. The significance of the linear and quadratic coefficients for the time variables was determined for each dose, and the equations for the three doses were compared.

RESULTS

The formulated herbicides Buctril, 2,4-D, Paraquat, Banvel, and Roundup all significantly reduced ($P = 0.05$) the number of ascocarps produced by *P. tritici-repentis* on infested field straw (Table 1). No ascocarps were formed in the straw treated with the glyphosate-containing herbicide. The surfactant alone did not have any inhibitory effect.

At all three concentrations, the glyphosate-containing herbicide reduced the formation of ascocarps on infested greenhouse-grown straw (Table 2). Regression analysis of the effect of the herbicide on the formation of large ascocarps (more than 200 μm in diameter) indicated that the length of incubation time (i.e., the time during which the colonized straw remained moist before herbicide application) had a significant effect ($P = 0.05$) on the number of ascocarps produced (Fig. 1). In contrast, the three doses of herbicide were not significantly different in their effects. In regression equations for each of the three doses the linear and quadratic coefficients of time were significant ($P = 0.05$), but there was no significant difference in the intercepts of the three equations, and there was no significant interaction of dose with either the linear or the quadratic coefficient of time. The difference between the number of ascocarps on untreated straw and that on straw treated with the herbicide after 10 days of moist incubation was statistically significant, but small. There were no significant differences among the check treatments for days 0, 5, 7, and 10 in the repetition of the experiment. Analysis of numbers of total ascocarps

Table 1. Reduction in ascocarp production by *Pyrenophora tritici-repentis* on wheat straw treated with various herbicides[x]

	Common name	Trade name	Concentration[y] (%)	Ascocarps produced[z]
Herbicide treatment	Glyphosate	Roundup	1.7	0 c
	Paraquat	Paraquat	0.02	20 b
	2,4-D	2,4-D	6.0	18 b
	Dicamba	Banvel	4.8	13 b
	Bromoxynil	Buctril	0.6	15 b
Untreated check				83 a
Surfactant treatment				122 a

[x] Straw infested with *P. tritici-repentis* was collected from the field and then tested under laboratory conditions.
[y] Concentration of the active ingredient in the applied solution.
[z] Average number of ascocarps (more than 200 μm in diameter) per replicate group of 16 straws. Values followed by the same letter are not significantly different ($P = 0.05$) as determined by the Kruskal-Wallis test.

(both sizes) yielded the same conclusions as those described for large ascocarps.

The glyphosate-containing herbicide not only inhibited ascocarp formation by *P. tritici-repentis* on wheat straw but also interfered with the structure and maturity of the few ascocarps that were produced. During normal ascocarp production by *Pyrenophora*, dark, spherical structures about 100 μm in diameter become visible in the straw tissue, enlarge to full size (350–800 μm in diameter), and then develop asci and ascospores under proper conditions of moisture and temperature. In some of the straw pieces we treated with the herbicide on day 0 or day 5, a number of structures consisting only of ring- or bowl-shaped stroma were formed (Figs. 2 and 3).

Although the glyphosate-containing herbicide suppressed the formation of ascocarps by *P. tritici-repentis*, it did not appear to be directly toxic to the fungus. In autoclaved straw treated with the herbicide 3 days after inoculation with *Pyrenophora*, the fungus grew at 80% of its rate in untreated straw. The growth rate was significantly reduced ($P = 0.05$), to 48% of that in untreated straw, when the herbicide was applied 3 days before inoculation with *Pyrenophora*. Within a treatment (i.e., pre- or postinoculation application of herbicide) growth rates were not significantly affected ($P = 0.05$) by the glyphosate concentration over the range tested (1.3, 1.7, and 2.6%). When the herbicide-treated straw was kept on moistened vermiculite for 1 mo, no ascocarps were formed in pre- or postinoculation treatments.

DISCUSSION

The herbicide Roundup, which contains glyphosate as the active ingredient, is effective in suppressing ascocarp development by *P. tritici-repentis* in field- and greenhouse-grown straw. It was not determined whether this effect is due to the glyphosate or the nonherbicidal components of the formulation. The herbicide is not toxic to mycelium of *P. tritici-repentis*. It appears to exert its effect on ascocarp production by interfering with the proper organization of the stromatic tissue. Consequently, its ability to disrupt ascocarp formation is highly dependent on the developmental stage at which it is applied to the fungus. If applied to straw after 10 days of conditions favorable for ascocarp development, the herbicide has little effect on subsequent ascocarp formation. However, we have determined, in a replicated study, that ascocarps formed following such late application of the herbicide failed to form ascospores, even after 6 wk of conditions that permitted abundant ascospore production in untreated straws (Sharma and Pfender, *unpublished*).

Other fungi have also been shown to be inhibited by glyphosate-containing herbicide. Mycelial growth on herbicide-amended agar is reduced in many fungi (2). The wheat pathogen *S. nodorum*, when studied in agar culture, showed inhibition of vegetative growth, spore formation, and spore germination in the presence of Roundup (5); however, in field experiments, the pathogen survived and sporulated on infested straw sprayed with the herbicide as well as on unsprayed straw (4). Some fungi are not greatly affected by the formulated herbicide. Of particular interest is the finding that the production of perithecia by *Chaetomium globosum*, a saprophytic cellulose decomposer, is not inhibited by the formulated herbicide, whether tested in pure culture or on straw buried in soil (3). In our study, the glyphosate-containing herbicide clearly affected the formation of ascocarps and thus the formation of ascospores by *P. tritici-repentis*, but it had a relatively small effect on mycelial growth at the concentrations we used. As indicated previously, the effect of Roundup on various fungi may be due either to the active ingredient (glyphosate) or to the nonherbicidal materials in the formulation. Since the nonherbicidal materials were not available to us, we cannot attribute the effect of the formulated

Table 2. Ascocarp production by *Pyrenophora tritici-repentis* on greenhouse-grown wheat straw treated with a glyphosate-containing herbicide at various times in a favorable environment

Time of application (days)[w]	Large ascocarps[x,y]				Total ascocarps[y]			
	Glyphosate concentration (%)[z]				Glyphosate concentration (%)[z]			
	0	1.3	1.7	2.6	0	1.3	1.7	2.6
0	54.5	0.2	0.0	1.0	63.0	0.5	0.0	3.0
5		14.5	0.5	0.0		15.0	3.2	4.5
7		4.2	2.0	1.5		9.5	12.2	7.2
10		26.8	25.8	28.2		35.2	37.2	34.0

[w] Number of days between the placing of infested straws in a moist environment at 22–24 C and the application of the herbicide.

[x] Average number of large ascocarps (more than 200 μm in diameter) per 5-cm-long straw after 21 days of incubation in a moist environment at 22–24 C.

[y] Each value is the average of two experiments, with two replicates per experiment and six straws per replicate.

[z] Concentration of the active ingredient (glyphosate) in the herbicide solution.

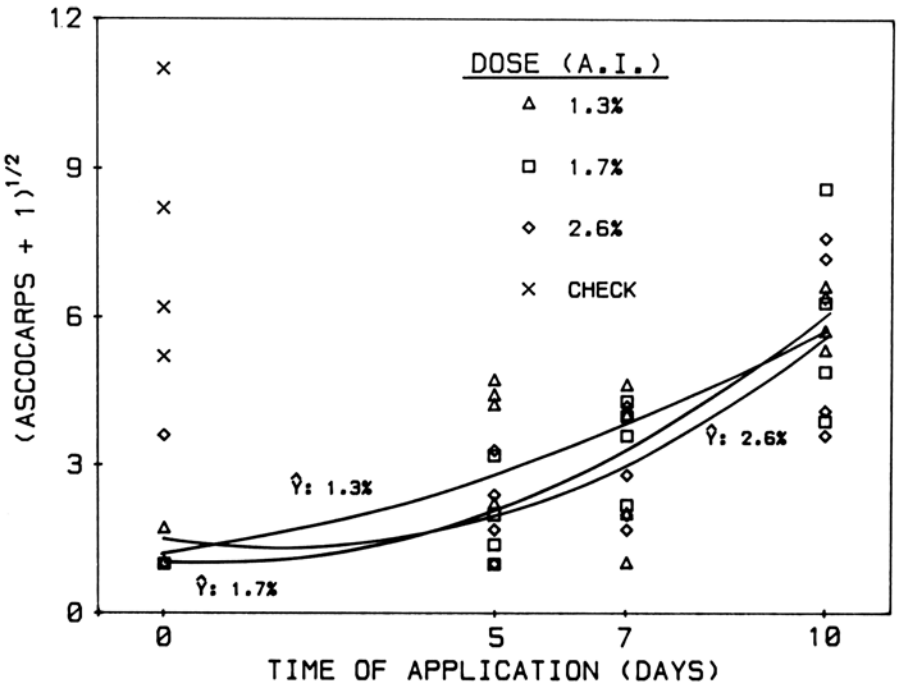

Fig. 1. Production of ascocarps by *Pyrenophora tritici-repentis* on nonsterile wheat straw treated with a glyphosate-containing herbicide at various times after the onset of environmental conditions favorable for ascocarp development. The herbicide was applied, at three different rates, by dipping straws in the solution. The straws were incubated on moist vermiculite in jars for a total of 21 days. The symbols indicate replicate data points (the average of six straws per replicate) transformed to reduce variance heterogeneity. Curves for predicted values ($\hat{Y}$) were fitted by nonlinear least squares regression. Regression equations for doses of 1.3, 1.7, and 2.6% a.i. are, respectively, $Y = 1.31 + 0.23t + 0.02t^2$, $Y = 0.97 - 0.05t + 0.05t^2$, and $Y = 1.68 - 0.30t + 0.69t^2$, where t is the number of days between the beginning of incubation and the application of the herbicide.

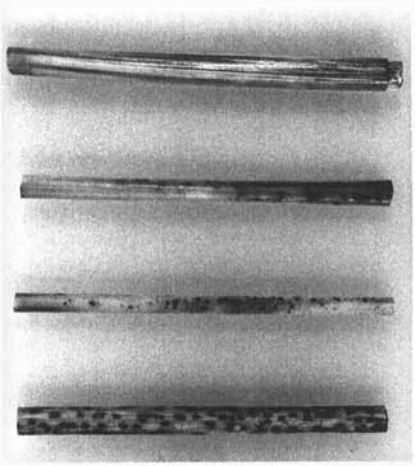

Fig. 2. Wheat straws infested with *Pyrenophora tritici-repentis* and treated with a glyphosate-containing herbicide on (top to bottom) day 0, day 5, and day 7 of a moist incubation period, and an untreated (check) straw (approximately 1×). The black structures are ascocarps of *P. tritici-repentis*.

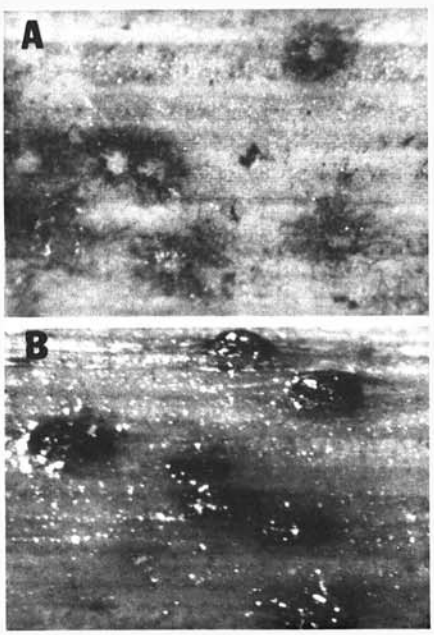

Fig. 3. Stromatic structures of *Pyrenophora tritici-repentis* produced on nonsterile wheat straw after a 3-wk incubation (16×) **(A)** Doughnut-shaped stroma occasionally produced on straws treated with glyphosate herbicide. **(B)** Mature ascocarps produced on untreated straws.

herbicide on *P. tritici-repentis* to the glyphosate or the nonherbicidal components specifically.

The usefulness of these findings for reducing primary inoculum of *P. tritici-repentis* in the field depends upon a number of considerations that have not yet been investigated. Most of these considerations are related to the moisture status of the infested straw. Absorption and retention of the herbicide by the straw is critical. Absorption into the straw, where the fungus is located, is likely to require that the straw be sufficiently wet for diffusion to occur. How well the material is retained against leaching is unknown, and how quickly the straw-inhabiting microorganisms degrade the herbicide (and how moisture status affects their metabolic activity) is also unknown. Relatively rapid degradation of glyphosate herbicide is accomplished in soil through microbial metabolism (9). The timing of herbicide application relative to periods of straw moisture favorable for ascocarp development must also be considered. Since there are currently no data concerning the cumulative effect of interrupted wet periods on ascocarp development, it is not possible to estimate the time of the developmental threshold beyond which glyphosate herbicide is no longer effective, i.e., a period comparable to the 10 days of continuous moisture in our study. There may, however, be a longer period of time during which the herbicide is effective, as indicated by our observation of ascospore inhibition even after ascocarps had formed. It is presently unknown whether the effect of the herbicide on ascocarp development would be reversible if the material were removed (e.g., by leaching) during the prolonged interval of interrupted wet periods that occur in the field.

All of this is complicated by the fact that *P. tritici-repentis* survives and produces its ascocarps predominantly in the upper layers of straw residue, where moisture fluctuates and the wet periods necessary for ascocarp production tend to be of short duration and unpredictable occurrence.

Another factor in applying these findings to field use is herbicide concentration. The concentrations we used represent the highest labeled concentrations, i.e., the highest amount of herbicide per acre applied in the lowest volume of carrier. Although this low-volume application could provide adequate coverage for a light amount of residue (e.g., in a field receiving some tillage but not complete tillage), a larger volume of carrier would be required to effectively treat the large amount of residue in a no-tillage field. At labeled amounts of herbicide per acre, lower concentrations would thus be required. Further tests (*unpublished data*) showed that straw dipped in the glyphosate-containing herbicide at concentrations as low as 0.25% a.i. produces significantly fewer ascocarps than untreated straw.

Effects of the herbicide on nontarget microorganisms, including some that may be antagonistic to *P. tritici-repentis*, could also be relevant to the field performance of this material.

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