Inhibition of *Drechslera teres* Sclerotioid Formation in Barley Straw by Application of Glyphosate or Paraquat

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ABSTRACT

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Field-grown barley straw was inoculated with *Drechslera teres* f. sp. *teres* or *D. t. maculata*, treated with two herbicides used in no-tillage barley production, and then incubated in controlled conditions to induce sclerotioid structure morphogenesis (resting form). Formulated herbicides containing glyphosate or paraquat were applied at three different concentrations. Applied at the recommended field rate, these herbicides significantly reduced sclerotioid structure production by *D. teres*. In addition, their morphology and myceliogenesis were modified in the presence of both herbicides at the recommended field rate. Glyphosate was more inhibitory than paraquat; no sclerotioid structures were produced in straw when glyphosate was applied before colonization by *D. teres*. The effect of the herbicides at 10^{-8} and 10^{-4} M varied depending on the herbicide, concentration of the active ingredient, method of application, and forma specialis of the pathogen.

Net blotch caused by *Drechslera teres* (Sacc.) Shoemaker, the anamorph of *Pyrenophora teres* Drechs., is an important foliar fungal disease of barley (*Hordeum vulgare* L.). The two recognized forms of this pathogen, *D. t. teres* and *D. t. maculata*, are present in France (2,3). *D. t. teres*, which produces distinct, well-defined, netlike symptoms, is responsible for the reticulated facies (net blotch) of the disease, while *D. t. maculata* causes polymorphic symptoms that can be confused with atypical lesions induced by other barley pathogens (2,3).

An important feature of the biology of *D. teres* is the diversity and longevity of its resting forms on straw. In this regard, crop residues can play a decisive role in the epidemiology of the disease (2,10). In both formae speciales of the pathogen, the black subspherical fructifications observed in vitro and in vivo on straw, which have an elastic texture and are covered with dark setae, are the sclerotioid structures that give rise to perithecia (teleomorph: *P. teres*) if fertilized by spermatia or, if fertilization is unsuccessful, to sclerotia (resting form) (2).

The spread of barley net blotch in France has reached epidemic proportions over the last 10 yr and has become more prevalent than major fungal diseases of barley such as powdery mildew and scald. Interaction of a number of components of the barley agroecosystem may promote the development of the disease. For example, modifications of crop management (surface tillage and/or direct drilling) may result in the persistence of straw on the surface of the soil, i.e., of a natural substrate for the survival and production of initial inoculum. The farm chemical environment (fertilizers, pesticides, growth regulators) may directly or indirectly modify the behavior of barley pathogens, particularly *D. teres*.

Previous studies utilizing agar culture showed that herbicides had either stimulatory or inhibitory effects on the development of *D. teres*, depending on the growth stage of the fungus and the concentration of the active ingredient (14). Glyphosate has been shown to inhibit or stimulate the growth of various saprophytic straw-inhabiting fungi (6).

Infested straw residues in minimum and no-tillage systems are likely to be exposed to herbicides. Glyphosate and paraquat are the most commonly used herbicides in reduced cultivation and direct drilling of barley and might possibly interfere with the ability of *D. teres* to develop and to persist on crop residues.

Our objective was to determine the effect of glyphosate and paraquat on sclerotioid structure production by *D. teres* on autoclaved barley straw.

MATERIALS AND METHODS

Pathogen isolation and storage. *D. t. maculata* (the spot form) and *D. t. teres* (the net form) were isolated from surface-sterilized barley leaves collected from the Agricultural Experimental Station of Monlon near Toulouse, France. A pure culture was obtained upon transfer onto a 5% V8 juice agar medium. The isolates were stored as sclerotia in the dark at 10 C, according to the technique described by Barrault (2). Isolates of *D. t. teres* and *D. t. maculata* were transferred onto an agar medium low in carbohydrates (modified Czapek medium containing 0.1% saccharose), which favors sclerotioid structure formation. Following incubation for 10 days in the dark at 23 C, barley straw was cut to 4-cm lengths (stem fragments were split lengthwise and autoclaved for 25 min at 115 C) and applied to the surface of the cultures. Fructifications appeared after approximately 15 days following incubation at 20 C in the dark.

Herbicides. Two commercial herbicides, glyphosate (Roundup) and paraquat (Gramoxone 2000), were used at concentrations of 10^{-8} and 10^{-4} M and at the recommended field rates, corresponding to 64×10^{-3} M for glyphosate and 2×10^{-2} M for paraquat.

Effect of herbicides. *Sclerotioid structure morphogenesis.* Two methods were used to apply treatments. In the preinoculation treatment, sterile straw fragments were dipped in one of the herbicide solutions for 1 min, dried on sterile filter paper, transferred onto the cultures, and incubated at 20 C in the dark until sclerotioid structures formed. In the postinoculation treatment, after fructifications developed on untreated straw fragments (15 days at 20 C in the dark), 1 ml of each herbicide solution was applied by atomized spray to each fragment. The petri dishes were then incubated at 20 C in the dark for another 15 days. The number of sclerotioid structures forming on the treated straw was counted and expressed as the number unit of surface area (square centimeter). The effect of each herbicide was estimated with respect to controls, which consisted of sterile straw fragments dipped into sterile water or atomized with 1 ml of distilled water per straw. For each application method, a randomized block design with five replications was used. Each replication consisted of a petri dish with four straw pieces. The experiment was conducted twice.

Sclerotioid structure morphology and myceliogenesis. Morphology was assessed from measurements made with a micrometer (magnification 10 × 10, average diameter of fructifications with three replications and 10 observations selected randomly per replication) and presence

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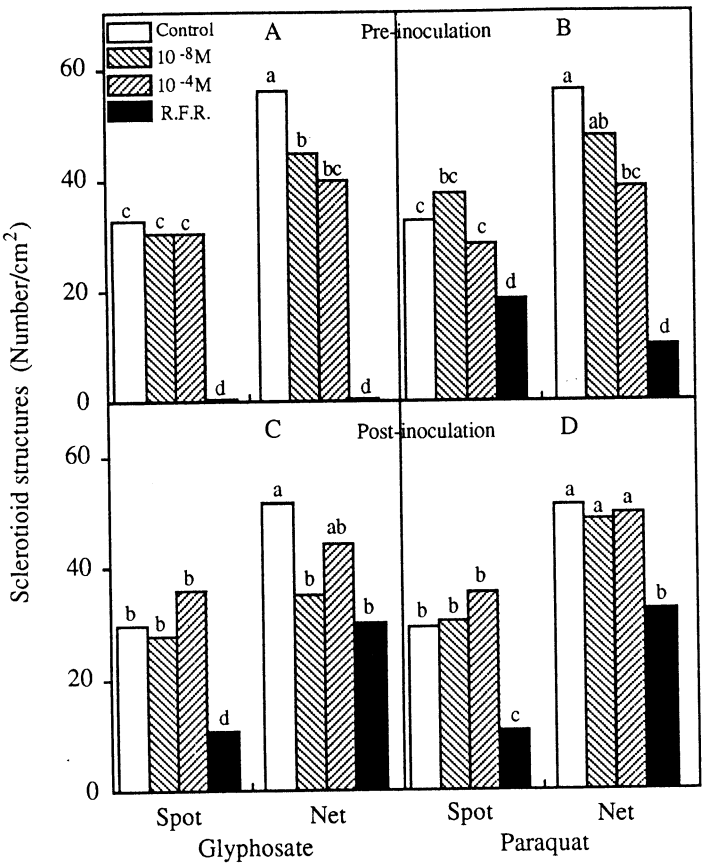

Fig. 1. Effect of glyphosate and paraquat on sclerotioid structure morphogenesis of *Drechslera teres* f. sp. *maculata* (spot) and *D. t. teres* (net) on barley straw. R.F.R. = recommended field rate. Histograms with the same letter are not significantly different according to the Newman-Keuls test ($P = 0.05$).

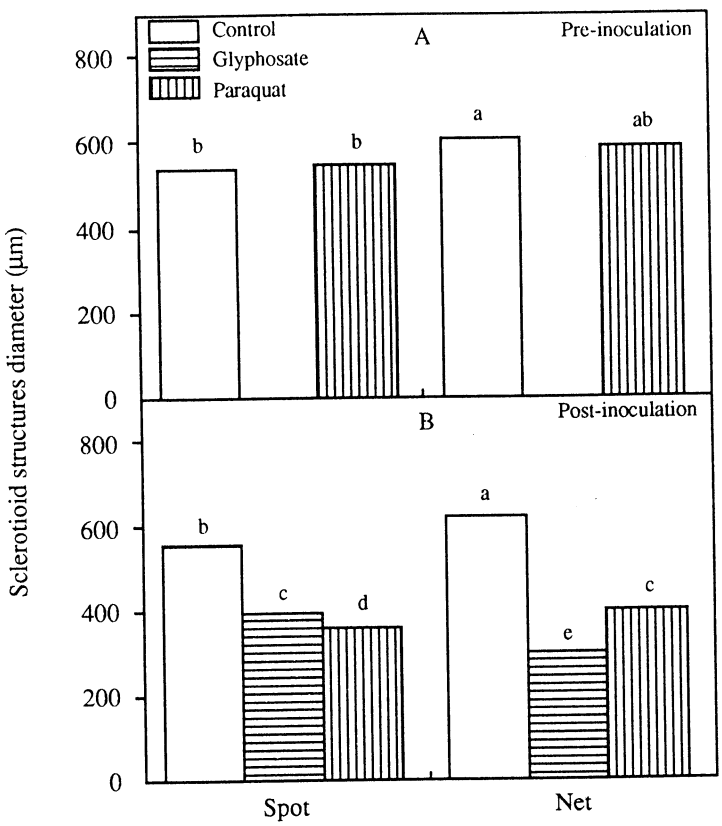

Fig. 2. Effect of glyphosate and paraquat on sclerotioid structure morphology of *Drechslera teres* f. sp. *maculata* (spot) and *D. t. teres* (net) on barley straw. Histograms with the same letter are not significantly different according to the Newman-Keuls test ($P = 0.05$).

of the setae at the periphery of the structure. After being counted, structures were isolated singly under sterile conditions and transferred onto a 5% V8 juice agar medium in petri dishes (100 mm in diameter). The dishes were incubated for 8 days under optimum conditions (darkness, 23 C), then the average diameter of each colony was measured (five replications).

Data analysis. Results of all experiments were subjected to statistical analysis (ANOVA). The Newman-Keuls test (homogeneous groups) was applied to treatment means ($P = 0.05$).

RESULTS

Sclerotioid structure morphogenesis. *Preinoculation treatment.* At the recommended field rate, glyphosate completely inhibited sclerotioid structure formation, irrespective of the forma specialis of the pathogen. At 10^{-4} and 10^{-8} M, partial inhibition was observed with *D. t. teres* but there was no significant effect on *D. t. maculata* (Fig. 1A).

Paraquat also had an inhibitory effect on both formae speciales of the pathogen at the recommended field rate, although the inhibitory effect was less than that of glyphosate. The inhibition observed at 10^{-4} M was not significant at 10^{-8} M on *D. t. teres* (Fig. 1B). At 10^{-8} and 10^{-4} M, paraquat had no significant effect on *D. t. maculata*, although a slight stimulation was observed at 10^{-8} M (Fig. 1B)

Postinoculation treatment. Glyphosate at the recommended field rate inhibited sclerotioid structure formation on both formae speciales of the pathogen. An inhibitory effect on *D. t. teres* was observed at 10^{-8} M (Fig. 1C). Paraquat had a significant inhibitory effect only at the recommended field rate (Fig. 1D). At 10^{-4} M, both glyphosate and paraquat stimulated *D. t. maculata*, but not significantly (Fig. 1C and D).

Sclerotioid structure morphology and myceliogenesis. The herbicides applied at the recommended field rate also affected sclerotioid morphology. Most of the structures were less developed and were covered by few setae. These observations led to the investigation of their morphology and myceliogenesis.

Preinoculation treatment. As glyphosate had precluded sclerotioid structure formation (Fig. 1), the morphology and myceliogenesis could not be investigated. Paraquat had no significant effect on morphology and myceliogenesis in both formae speciales of the pathogen (Figs. 2A and 3A).

Postinoculation treatment. The average diameter of the sclerotioid structures produced on barley straw treated with glyphosate or paraquat was significantly smaller than that of the control (Fig. 2B). As to myceliogenesis, the growth rate was significantly reduced (Fig. 3B) but the morphology of the mycelium was not modified.

DISCUSSION

The effect of both herbicides on *D. teres* varied according to the herbicide, concentration of the active ingredient, and forma specialis of the pathogen. *D. t. teres* generally was more sensitive to both herbicides than was *D. t. maculata*. The inhibitory effect on sclerotioid structure formation of *D. t. teres* was observed at herbicidal concentrations below the recommended field rate, whereas at the same concentrations, no effect or even a stimulatory effect was observed on *D. t. maculata*.

At the recommended field rate, glyphosate had a total inhibitory effect on sclerotioid structure morphogenesis when applied prior to the establishment of the pathogen. This result corroborated earlier observations on *Pyrenophora tritici-repentis* (12). Sharma et al (12) reported that glyphosate used at the recommended field rate suppressed ascocarp formation by *P. tritici-repentis* on wheat straw (preinoculation treatment). The weaker effect of paraquat on *D. teres* also had been observed on *P. tritici-repentis* (12).

Other investigations have shown that the effect of these herbicides depended on the herbicide, the concentration of the active ingredient, the experimental conditions, and the pathogen. Thus, at the recommended field rates, glyphosate and paraquat had no effect on growth and sporulation of *Stagonospora nodorum* on field-treated wheat residues (9). However, the number of pycnidia formed on wheat leaf fragments inoculated with *S. nodorum* spores issued from cultures that had been treated with 3×10^{-4} M of paraquat was markedly increased compared with the control (7). Perithecia of *Chaetomium globosum* (a saprophytic cellulose decomposer) developed on straw treated with glyphosate (3.8×10^{-3} M) but were inhibited on 10^{-4} M of paraquat (8). *Colletotrichum truncatum* acervuli and *Phomopsis* spp. pycnidia developed more rapidly on soybean stems treated with glyphosate or paraquat than on untreated stems (4); their number was increased in the presence of paraquat (5). Paraquat applied to wheat straw at 3.9×10^{-4} M inhibited the growth of *Gaeumannomyces graminis* var. *avenae*, *Eurotium* sp., *Fusarium culmorum*, and *Trichoderma viride*. The latter fungus also was inhibited by 3.9×10^{-5} M of paraquat (15).

The morphology of the sclerotioid structures, as well as their myceliogenesis, was affected by both herbicides, especially when they were applied after the appearence of the pathogen fructifications. These structures were less developed, less prominent, and covered by few setae; fertilization by spermatia may thus be assumed uncertain, to the extent that setae may act as trichogynae or receptive filament (3). As a result, teleomorphogenesis, and thus the ascosporal inoculum, would be inhibited. The mycelium growth rate would then be reduced.

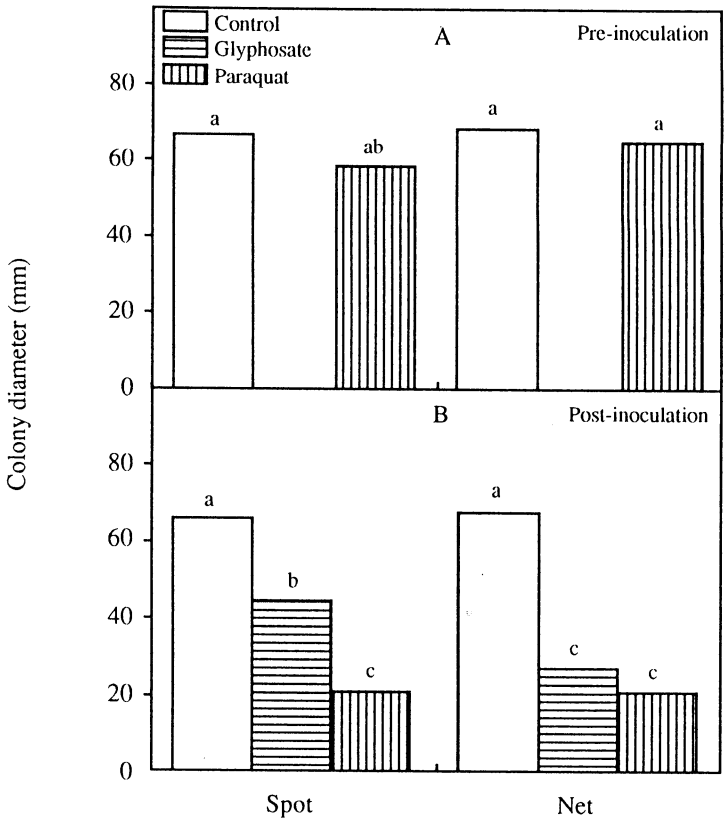

Fig. 3. Effect of glyphosate and paraquat on sclerotioid structure myceliogenesis of *Drechslera teres* f. sp. *maculata* (spot) and *D. t. teres* (net) on barley straw. Histograms with the same letter are not significantly different according to the Newman-Keuls test ($P = 0.05$).

These herbicides may not decrease the risk of an early outbreak of the disease as a result of a decrease in the amount of initial inoculum. The effect of the herbicides under controlled conditions would not be as likely to occur in the field. Several considerations should be investigated and are necessary to substantiate the present findings. The climatic conditions (temperature, light, humidity) might have an influence in the complex interactions among herbicide, pathogen, and plant. The amounts of herbicides on straw fragments in petri dishes were markedly higher than those present in the field. Herbicides in petri dishes are not degraded by a diverse heterotrophic microflora or by inorganic or photochemical processes. Relatively rapid degradation of glyphosate herbicide is accomplished in soil through microbial metabolism (13). Effect of the herbicide on nontarget microorganisms, including some that may be antagonistic to *D. teres*, could also be relevant to the field performance. Thus, paraquat impaired the growth of *T. viride* (15). This fungus was an efficient antagonist of *D. teres* (1,11), whether in vitro (with a nearly complete inhibition conidial germination) or in vivo (through decreased coleoptile and leaf attack and through decreased conidiogenesis on straw).

In the future, it would be relevant to consider field investigations to assess the effect of both herbicides on sclerotioid structure development by *D. teres*. Additionally, investigation of the effect of both active ingredients on spermatia morphogenesis and on fertilization of sclerotioid structures by the latter would deserve further consideration.

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