# Response of Rhizoctonia Blights of Tall Fescue to Selected Fungicides in the Greenhouse

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## ABSTRACT

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Benomyl, carboxin, PCNB, iprodione, chlorothalonil, and triadimefon were sprayed on tall fescue (*Festuca arundinacea*) plants in greenhouse experiments to determine their effect on foliar blight in plants inoculated with isolates of *Rhizoctonia solani*, binucleate *Rhizoctonia*-like fungi, and *R. zeae* after fungicide treatment. Benomyl treatments did not prevent increase of disease caused by binucleate *Rhizoctonia*-like fungi in one experiment, and on some benomyl-treated plants, disease was more severe than on unsprayed, inoculated plants. Isolates of *R. zeae* caused as much or more blight on benomyl-treated plants than on untreated plants inoculated with *R. zeae*. PCNB was ineffective against *R. zeae* and one of the *R. solani* isolates tested. Carboxin, triadimefon, iprodione, and chlorothalonil were effective in preventing infection by all *Rhizoctonia* spp. and *Rhizoctonia*-like fungi. Results indicated that all *Rhizoctonia* spp. induced disease on tall fescue and that the effectiveness of fungicide treatments in reducing disease varied among the *Rhizoctonia* spp.-fungicide combinations.

Several species of *Rhizoctonia* may be pathogenic on turfgrasses (1–6). These species include *Rhizoctonia solani* Kühn, the causal agent of brown patch of turfgrasses, certain binucleate *Rhizoctonia*-like fungi (RLF) (3–6), and *R. zeae* Voorhees (9). One of the binucleate RLF, *R. cerealis* van der Hoeven, induces "yellow patch" of turfgrasses (3) as well as sharp eyespot of wheat (2). These fungi (*R. solani, R. cerealis,* certain other binucleate RLF, and *R. zeae*) have been reported as pathogens of tall fescue (*Festuca arundinacea* Schreb.) (4,5,9).

Turfgrass diseases such as brown patch on golf course greens and fairways and, to a more limited extent, on home lawns are managed by use of prophylactic and therapeutic sprays. Adequate control of these diseases requires correct identification of specific pathogens involved. Research on sensitivity of *Rhizoctonia* spp. to fungicides in vitro demonstrated that there may be differential isolate and species responses to fungicides (10). Schatla and Sinclair (15) reported that field isolates of *R. solani* from cotton differed in sensitivity to pentachloronitrobenzene (PCNB).

The purpose of this research was to evaluate foliar disease in tall fescue induced by *R. solani, R. zeae,* and binucleate RLF as affected by preinoculation fungicide treatments. This evaluation should give information not only on comparative pathogenic abilities of the *Rhizoctonia* spp. and binucleate RLF but also on the effects of these fungicides on the different *Rhizoctonia* spp. and binucleate RLF in vivo compared with in vitro effects reported in a previous study (10).

## MATERIALS AND METHODS

Six isolates representing two *Rhizoctonia* spp. and an unknown binucleate RLF (5) were chosen from 16 isolates used in previous experiments on in vitro response to fungicides (10). These included two isolates of *R. solani* (RS 96 and RS 44) from diseased bermudagrass (*Cynodon dactylon* L.) and tall fescue, respectively; two isolates of binucleate RLF (Bn 15 and Bn 109) obtained from ladino clover (*Trifolium repens* L.) roots and pathogenic on tall fescue (5); and two isolates of *R. zeae* (RZ 42 and RZ 197) obtained from diseased tall fescue foliage. Inoculum was prepared by growing these isolates on sterile tall fescue seed for 14 days at 28 C in the dark. Fescue seed and water (1:1, w/v) were autoclaved (121 C) for 30 min, allowed to cool, and a 1-cm agar disk from a potato-dextrose agar (PDA) culture of the appropriate test isolate was introducted in the sterile medium. During the incubation period, flasks containing the inoculum were shaken periodically to allow homogeneous growth of the fungal mycelia throughout the fescue seed medium.

Aqueous solutions of fungicides were prepared at concentrations of 100 and 1,000 mg a.i./L and sprayed on 4-wk-old tall fescue plants grown in 10-cm-diameter clay pots with 20–25 plants per pot. The soil mix was a steam-pasteurized mix consisting of loamy soil, sand, and peat moss (4:1:1, v/v) amended with 1 g of 8-8-8 (NPK) granular fertilizer per kilogram of soil mix. Plant foliage was trimmed to a height of 12 cm and sprayed 24 hr later for 2 sec (until runoff), resulting in final fungicide rate treatments of 0.8 and 0.9 mg a.i./pot. Control plants were sprayed similarly with tap water. The fungicides used were wettable powder formulations of benomyl, carboxin, PCNB, triadimefon, iprodione, and chlorothalonil.

Sprayed plants were allowed to dry for 18–20 hr before they were inoculated by placing 0.5 g (fresh weight) of the fescue seed inoculum on the soil surface. Foliage in each pot was covered with a clear plastic bag to maintain high atmospheric moisture, and the pots were placed in clay dishes and kept moist by subirrigation during the experiments. The greenhouse temperature was 26–32 C and the temperature within the plastic bags covering the pots did not exceed 33 C (mean 28 C). Foliar disease severity was evaluated 3, 7, and 10 days after inoculation by visual estimation of foliar blight severity based on the Horsfall-Barratt rating system (8).

Responses of the foliar blights to fungicide treatments were evaluated in two experiments (each repeated once). Benomyl, carboxin, and PCNB were tested togther (experiments 1 and 2) and iprodione, triadimefon, and chlorothalonil were tested together (experiments 3 and 4) in factorial combinations of the six isolates, three fungicides, and two fungicide concentrations plus no-fungicide controls. All plants sprayed with fungicides or water controls were inoculated with each isolate, using four replicates

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(pots) for each treatment combination and each isolate control. The pots were arranged in a completely random design on a greenhouse bench for each experiment after inoculations. The data reported were based on experiments 1 and 2 for benomyl, carboxin, and PCNB and experiment 3 for triadimefon, iprodione, and chlorothalonil. Results of experiment 4 were similar to experiment 3. Only data from the 10-day rating for each experiment are reported.

Data were analyzed by analysis of variance for the incomplete factorial using a model for completely random treatments (17). Single degree-of-freedom contrasts were made to compare certain *Rhizoctonia* spp. and binucleate RLF or certain individual isolates.

## RESULTS

Foliar lesions characteristic of infection by *Rhizoctonia* were apparent on untreated plants after 3 days of incubation in all experiments, and disease severity continued to increase according to ratings made 7 and 10 days after inoculation. The final (10-day) ratings were analyzed to allow maximum resolution of isolate and fungicide treatment differences.

Isolate, fungicide, and fungicide concentration treatments were in factorial combination for each experiment. Because analysis of the factorial portions of each experiment indicated significant first-order interactions involving fungicides, data in all experiments were analyzed further for each fungicide to determine which isolates responded differently to different fungicides or fungicide concentrations. Linear contrasts were performed on the factorial portion of each experiment to determine whether different types of fungicides (systemic or nonsystemic) had different effects on disease severity for all isolates. Results of these analyses indicated no significant difference between inoculated plants sprayed with systemic fungicides versus nonsystemic fungicides in any of the experiments.

Results of experiments 1 and 2 differed. In the first experiment, there was no significant difference in disease severity in untreated plants induced by specific isolate types; on the average, isolates of binucleate RLF or *R. zeae* induced disease as severe as that induced by isolates of *R. solani* (Table 1). In experiment 2, however, isolates of *R. solani* and *R. zeae* induced significantly more disease on untreated plants than isolates of binucleate RLF. Disease severity on untreated plants induced by binucleate RLF was much greater in experiment 1 (Table 1) than in experiments 2 (Table 2) or 3 (Table 3). *R. solani* isolate RS 96 also induced more severe disease in experiment 1 than in experiments 2 and 3. Data were analyzed further to determine disease severity induced by each isolate on specific fungicide-treated plants according to fungicide concentrations (Tables 1–3). *R. zeae* isolates induced more severe

TABLE 1. Mean disease severity[a] of foliar blights of tall fescue untreated and treated with benomyl, carboxin, and PCNB and inoculated with *Rhizoctonia solani*, binucleate *Rhizoctonia*-like fungi (RLF), or *R. zeae* (experiment 1)

| | | Fungicide concentration (mg a.i./L) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Benomyl | | Carboxin | | PCNB | |
| *Rhizoctonia* isolate[b] | Untreated | 100 | 1,000 | 100 | 1,000 | 100 | 1,000 |
| RZ 197 | 5.00 | 5.50 | 7.00 | 4.25 | 0.75 | 4.50 | 5.50 |
| RZ 42 | 6.25 | 5.75 | 6.75 | 5.00 | 2.75 | 4.00 | 5.00 |
| RS 44 | 6.25 | 5.75 | 5.00 | 4.75 | 2.75 | 5.50 | 5.75 |
| RS 96 | 7.00 | 5.50 | 3.75 | 1.75 | 0.50 | 0.75 | 2.25 |
| BN 109 | 5.00 | 4.75 | 6.00 | 1.75 | 1.75 | 1.00 | 4.50 |
| BN 15 | 4.75 | 4.75 | 4.25 | 4.75 | 1.75 | 0.75 | 1.00 |
| Isolate contrast: | | | | | | | |
| 1. BN vs. RS | ns[c] | ns | ns | ns | ns | ** | ns |
| 2. RZ vs. RS | ns | ns | ** | ns | ns | ns | ns |
| 3. BN vs. RZ | ns | ns | * | ns | ns | ** | * |
| 4. RS 44 vs. RS 96 | ns | ns | ns | * | ns | ** | * |
| 5. BN 109 vs. BN 15 | ns | ns | ns | * | ns | ns | * |

[a] Disease severity was evaluated by visual estimation based on the Horsfall-Barratt rating system, where 0 = 0%, 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–93%, 9 = 94–97%, 10 = 98–99%, and 11 = 100% blight.

[b] RS, RZ, and BN refer to the *R. solani, R. zeae*, and binucleate RLF isolates, respectively.

[c] ns, *, and ** denote no significant difference, or significant differences at $P = 0.05$ and $P = 0.01$, respectively.

TABLE 2. Mean disease severity[a] of foliar blights of tall fescue untreated and treated with benomyl, carboxin, and PCNB and inoculated with *Rhizoctonia solani*, binucleate *Rhizoctonia*-like fungi, or *R. zeae* (experiment 2)

| | | Fungicide concentration (mg a.i./L) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Benomyl | | Carboxin | | PCNB | |
| *Rhizoctonia* isolate[b] | Untreated | 100 | 1,000 | 100 | 1,000 | 100 | 1,000 |
| RZ 197 | 4.75 | 6.75 | 7.25 | 3.75 | 1.25 | 4.25 | 3.75 |
| RZ 42 | 7.25 | 7.25 | 6.75 | 4.75 | 0.50 | 7.00 | 6.00 |
| RS 44 | 7.25 | 1.00 | 0.50 | 7.00 | 1.25 | 7.75 | 6.00 |
| RS 96 | 2.50 | 1.00 | 0.25 | 1.50 | 1.50 | 3.00 | 1.50 |
| BN 109 | 2.50 | 0.75 | 0.25 | 1.25 | 1.25 | 1.75 | 0.75 |
| BN 15 | 2.50 | 0.75 | 0.25 | 0.75 | 0.50 | 2.50 | 1.00 |
| Isolate contrast: | | | | | | | |
| 1. BN vs. RS | **[c] | ns | ns | ** | ns | ** | ** |
| 2. RZ vs. RS | * | ** | ** | ns | ns | ns | ns |
| 3. BN vs. RZ | ** | ** | ** | ** | ns | ** | ** |
| 4. RS 44 vs. RS 96 | ** | ns | ns | ** | ns | ** | ** |
| 5. BN 109 vs. BN 15 | ns | ns | ns | ns | ns | ns | * |

[a] Disease severity was evaluated by visual estimation based on the Horsfall-Barratt rating system, where 0 = 0%, 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–93%, 9 = 94–97%, 10 = 98–99%, and 11 = 100% blight.

[b] RS, RZ, and BN refer to the *R. solani, R. zeae*, and binucleate RLF isolates, respectively.

[c] ns, *, and ** denote no significant difference, or significant differences at $P = 0.05$ and $P = 0.01$, respectively.

TABLE 3. Mean disease severity[a] of foliar blights of tall fescue untreated and treated with triadimefon, iprodione, and chlorothalonil and inoculated with *Rhizoctonia solani*, binucleate *Rhizoctonia*-like fungi, or *R. zeae* (experiment 3)

| | | Fungicide concentration (mg a.i./L) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Triadimefon | | Iprodione | | Chlorothalonil | |
| *Rhizoctonia* isolate[b] | Untreated | 100 | 1,000 | 100 | 1,000 | 100 | 1,000 |
| RZ 197 | 5.75 | 2.00 | 1.25 | 4.25 | 0.75 | 4.75 | 0.25 |
| RZ 42 | 5.50 | 1.50 | 1.50 | 4.00 | 1.25 | 4.25 | 1.00 |
| RS 44 | 8.00 | 2.25 | 1.25 | 1.75 | 0.50 | 7.00 | 0.75 |
| RS 96 | 3.25 | 2.50 | 2.50 | 2.25 | 0.75 | 3.00 | 0.75 |
| BN 109 | 3.25 | 1.25 | 1.50 | 2.75 | 0.25 | 2.25 | 0.75 |
| BN 15 | 2.25 | 1.00 | 1.00 | 2.00 | 0.00 | 0.75 | 0.25 |
| Isolate contrast: | | | | | | | |
| 1. BN vs. RS | **[c] | * | ns | ns | ns | ** | ns |
| 2. RZ vs. RS | ns | ns | ns | * | ns | ns | ns |
| 3. BN vs. RZ | ** | ns | ns | * | * | ** | ns |
| 4. RS 44 vs. RS 96 | ** | ns | * | ns | ns | ** | ns |
| 5. BN 109 vs. BN 15 | ns | ns | ns | ns | ns | * | ns |

[a] Disease severity was evaluated by visual estimation based on the Horsfall-Barratt rating system, where 0 = 0%, 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–93%, 9 = 94–97%, 10 = 98–99%, and 11 = 100% blight.

[b] RS, RZ, and BN refer to the *R. solani, R. zeae*, and binucleate RLF isolates, respectively.

[c] ns, *, and ** denote no significant difference, or significant differences at $P = 0.05$ and $P = 0.01$, respectively.

disease on benomyl-treated plants than those of *R. solani* or binucleate RLF (Tables 1 and 2). Disease severity induced by *R. zeae* was usually greater on benomyl-treated plants than on untreated plants (Tables 1 and 2). Binucleate RLF also induced considerable disease on benomyl-treated plants in one experiment (Table 1).

In general, carboxin at 1,000 mg/L was effective in inhibiting disease development associated with most isolates (Tables 1 and 2). Both isolates RS 96 and BN 109 were inhibited also at 100 mg/L in experiment 1 but both induced fairly low disease severity in experiment 2, even in the absence of fungicide (Table 2). Isolates of binucleate RLF were significantly more sensitive than isolates of *R. solani* or *R. zeae* to carboxin at 100 mg/L in experiment 2 but not in experiment 1.

Isolates of binucleate RLF induced significantly less disease than isolates of *R. solani* and *R. zeae* on PCNB-treated plants in both experiments (Tables 1 and 2). Isolate RS 44 of *R. solani* was unaffected by PCNB treatment, but disease induced by RS 96 was dramatically reduced by PCNB in experiment 1.

For the overall factorial portion of experiment 3, there was a significant interaction with fungicides and fungicide concentration as before, so effects were analyzed separately for each fungicide. Binucleate RLF induced less severe blight on triadimefon-treated plants than did *R. solani* isolates as a group, but this difference was small (Table 3). Generally, plants treated with triadimefon did not develop severe foliar blight when inoculated with any isolate of *Rhizoctonia*.

Disease severity of plants treated with iprodione varied with the concentration of iprodione, but the concentration effect did not vary according to isolate (ie, there was no isolate × concentration interaction). Isolates of *R. zeae* induced more disease on iprodione-treated plants than isolates of *R. solani* or binucleate RLF (Table 3). Similar results were apparent on iprodione-treated plants inoculated with binucleate RLF compared with those inoculated with *R. zeae* isolates. On plants treated with 1,000 mg of iprodione per liter, disease did not develop appreciably after inoculation with any isolates of *Rhizoctonia* regardless of species or type (Table 3).

There was no significant isolate and fungicide concentration interaction associated with chlorothalonil-treated plants. Disease severity was low on chlorothalonil-treated plants across all isolates and isolate types for the high concentration, and all isolates were similarly inhibited. With 100 mg of chlorothalonil per liter, however, isolates of *R. solani* and *R. zeae* induced more severe disease than isolates of the binucleate RLF (Table 3). These responses mimicked the disease severities on untreated plants, with the exception of isolate BN 15.

## DISCUSSION

Variability between experiments 1 and 2 associated with the binucleate RLF isolates confounded disease response interpretations involving those isolates. The binucleate RLF used in these experiments were probably not *R. cerealis*, based on differences in hyphal cell diameter, temperature-grown characteristics (fungi used in these experiments had optima around 28 C [5], whereas *R. cerealis* grows optimally around 23 C [3]), and lack of anastomosis with *R. cerealis*. The decrease in disease severity between experiments 1 and 2 cannot be explained but could have been due to a change in the fungi or, more likely, physiological differences in the tall fescue plants used in different experiments resulting from unidentified differences in the experimental conditions.

In experiment 1, when disease induction by both binucleate RLF isolates was greater, benomyl treatment did not reduce disease severity induced by the binucleate RLF isolates as it appeared to in experiment 2. This lack of control in experiment 1 may have been due to a decrease in antagonistic fungi caused by the benomyl treatment, which was suggested as a possible explanation for an increase in the incidence of sharp eyespot of rye, caused by *R. cerealis*, after benomyl treatments (18).

Isolates of *R. zeae* also induced disease as severe or more severe on tall fescue plants treated with benomyl than on inoculated, untreated plants. This effect was consistent between experiments. Reduction in antagonistic microflora may also have been a contributing factor. The lack of control with benomyl against *R. zeae*-induced disease and the previous demonstration of benomyl tolerance of *R. zeae* in vitro (10) are significant because they demonstrate that a common turf fungicide may not be effective against all Rhizoctonia blights on turfgrasses. *R. zeae* has only recently been demonstrated to be a pathogen on tall fescue and other turfgrasses (9), however, and the frequency of its occurrence and relative importance on various turfgrasses is still to be determined. Carboxin, triadimefon, iprodione, and chlorothalonil inhibited *R. zeae* in vitro (10) and effectively prevented high disease severity on grass inoculated with *R. zeae*. PCNB was not effective in reducing disease on grass inoculated with *R. zeae*, although these isolates were moderately sensitive to PCNB in vitro (10).

Disease severities induced by *R. solani* isolates were generally lowered on plants treated with benomyl, triadimefon, iprodione, and chlorothalonil, which agrees with published accounts (13,14,16) and in vitro assays (10). PCNB and carboxin were less effective in reducing disease severity on *R. solani*-inoculated plants than the other fungicides used, but the effectiveness appeared to be isolate-related. These differences also corresponded to differences in isolate virulence; the isolate originally obtained from a lesion on tall fescue (RS 44) generally induced more severe disease than an isolate from diseased bermudagrass (RS 96).

These experiments, and experiments on direct fungicide effects (in vitro studies) on *Rhizoctonia* spp. and binucleate RLF (10), demonstrated that these fungi may respond differently to fungicides as a group and may also induce considerable disease on plants previously treated with some fungicides.

There are different fungi classified under the form-genus *Rhizoctonia*, some of which are ascomycetes as well as basidiomycetes (12). Presence of dolipore septa is evidence of basidiomycetous affinities (11), and all of the fungi used in these experiments had doliopore septa. Although fungal sensitivity to benomyl, for example, generally follows certain taxonomic boundaries, some basidiomycetes are insensitive (7). Therefore, it was not surprising that potentially related fungi in the *Rhizoctonia* complex responded differently to benomyl.

It is significant that all of these fungi may induce turf disease and that therapeutic or prophylactic sprays of certain fungicides used on tall fescue turf may give different degrees of control, no control, or even greater disease severity depending on the species of *Rhizoctonia* inducing the disease if environmental conditions remain conducive to disease development. Therefore, more research is needed on identification of *Rhizoctonia* spp. inducing turf disease with the goal of more firmly establishing their specific biology, pathology, and importance on different turfgrasses. Results of these and similar experiments should be useful in development and application of adequate control strategies.

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