Suppression of Brown Patch Disease of Creeping Bentgrass by Isolates of Nonpathogenic *Rhizoctonia* spp.

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Research was supported in part by grant A0449 from the Natural Sciences and Engineering Research Council of Canada.
Accepted for publication 13 February 1984 (submitted for electronic processing).

ABSTRACT

Burpee, L. L., and Goulty, L. G. 1984. Suppression of brown patch disease of creeping bentgrass by isolates of nonpathogenic *Rhizoctonia* spp. Phytopathology 74:692-694.

Turfgrass isolates of *Rhizoctonia* spp., with binucleate hyphal cells (BnR), were studied as potential antagonists of *Rhizoctonia solani*. In each of three field experiments, creeping bentgrass developed significantly less disease when inoculated with BnR 24 hr before inoculation with *R. solani* than when inoculated with *R. solani* alone. Disease was not observed in plots inoculated with BnR alone. Significant differences in suppressive ability were observed among BnR isolates. BnR and *R. solani* were isolated from bentgrass leaves and grain inoculum that were removed from infested plots at the termination of each experiment. Disease was not suppressed in plots where an isolate of *Epicoccum* from turfgrass was substituted for BnR.

Additional key words: *Agrostis palustris*, *Ceratobasidium* spp.

Fungi that are similar to *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk), but have binucleate rather than multinucleate (>2) hyphal cells, have been isolated from aerial and subterranean parts of several plant species (1,6,20), including turfgrasses (5,13,14,18). Isolates of these binucleate *Rhizoctonia* spp. (BnR) from turfgrasses may be placed into two broad taxonomic groups: BnR that fit the species concept of *R. cerealis* van der Hoeven (teleomorph *Ceratobasidium cereale* Mur. et Burp. [15]) and BnR that do not fit this concept (5). *R. cerealis* causes yellow patch disease of several turfgrass species (5). Other BnR have been isolated from turfgrasses showing symptoms similar to those caused by *R. solani* (13). In a few instances, BnR and *R. solani* have been isolated from diseased leaves and crowns of creeping bentgrass (*Agrostis palustris* Huds.) and Kentucky bluegrass (*Poa pratensis* L.) collected from the same site (L. L. Burpee, *unpublished*). Further evidence indicating that a close association exists between BnR and *R. solani* was supplied by Martin et al (14), who isolated more propagules of BnR than propagules of *R. solani* from a stand of tall fescue (*Festuca arundinacea* Schreb.) with a history of *R. solani* infection.

Pathogenicity tests have revealed that most BnR tested, other than *R. cerealis*, are nonpathogenic or weakly virulent on turfgrasses (13,18). The inability of most BnR isolates to cause disease indicates that the apparent close association between BnR and *R. solani* does not enhance disease. However, it is possible that isolates of BnR may suppress disease caused by *R. solani*. This study was designed to test this disease suppression hypothesis.

MATERIALS AND METHODS

Three experiments were conducted consecutively. Methods used in experiments 2 and 3 were similar to those used in experiment 1, but modifications were made on the basis of the results of the previous experiment(s).

Experiment 1. Isolates of BnR and *R. solani* were collected from turfgrasses grown in various geographic regions (Table 1). The fungi were stored as mycelium and sclerotia on autoclaved rye grain at −5 C. Isolates were recovered by placing three to five infested grains on acidified potato-dextrose agar (APDA) in petri dishes held at 23 C for 4–5 days. Inoculum was prepared by transferring mycelial plugs from colonies on APDA to 250-ml Erlenmeyer flasks containing moist autoclaved rye grain (100 cm^3 grain, 20 ml H_2O). Flasks were incubated for 2 wk at 23 C. Infested grain was then removed and macerated in a food blender for about 30 sec on the chop cycle, then taken to the field.

The experiment was conducted on a 5-yr-old stand of creeping bentgrass cultivar Penncross maintained at the University of Guelph Horticultural Research Station, Cambridge, Ontario. Mowing, fertilization, and irrigation schemes were similar to those prescribed for bentgrass golf putting greens (2). Bentgrass was inoculated with BnR isolates Bn 154, Bn 165, or Bn 190 24 hr before inoculation with *R. solani* isolate Rh 40. About 25 cm^3 of chopped grain inoculum of each BnR isolate was distributed by hand over circular plots 16 cm in diameter surrounded by plastic cylinders 10 cm tall. Plots infested with isolate Rh 40 and/or a BnR isolate served as individual treatments. Plots of uninoculated bentgrass and bentgrass treated with uninfested autoclaved rye grain served as controls. Immediately after inoculum was dispersed, plots were sprayed with 20 ml of water and covered with wooden frames 300 × 100 × 15 cm overlain with 4-mil transparent plastic sheeting (8). The frames were removed after 24 hr and about 20 cm^3 of Rh 40 inoculum was placed in the center of each plot. Plots were then sprayed with 20 ml of water and recovered with the wooden incubation frames. Temperatures were monitored by placing thermograph sensors under two of the incubation frames. Treatments were arranged in a randomized complete block design with four replicates.

TABLE 1. Origin of *Rhizoctonia* isolates used in the study of brown patch suppression on creeping bentgrass

Isolate	Species	Host or source	Geographic origin	Date isolated
Bn 4	BnR[a]	*Agrostis*	Ohio	1974
Bn 154	BnR	*Stenotaphrum*	Texas	1979
Bn 165	BnR	*Poa*	Pennsylvania	1980
Bn 190	BnR	*Agrostis*	Ontario, Canada	1982
Rh 40	*R. solani* (AG4)[b]	*Lolium*	Pennsylvania	1977
Rh 41	*R. solani* (AG5)	*Agrostis*	Pennsylvania	1977

[a] BnR = *Rhizoctonia* sp. with binucleate hyphal cells.
[b] AG = anastomosis group.

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Disease severity (percent necrotic leaf tissue per plot) was estimated 6 and 8 days after inoculation with Rh 40, using the Horsfall-Barratt rating system (12). Percent disease values were subjected to analysis of variance and means were statistically separated using Duncan's modified (Bayesian) least significant difference test (19). Leaf tissue samples and fragments of grain inoculum were collected when the experiment was terminated. The material was washed for 1 hr in running tap water, blotted dry, and placed on APDA in petri dishes stored at 23 C. Fungi growing from the samples were identified after 5–10 days of incubation.

Experiment 2. Experimental design and inoculation techniques were the same as those described in experiment 1, except an additional control treatment consisting of plots treated with uninfested autoclaved rye grain plus Rh 40 inoculum was included. This treatment was evaluated to determine if rye grain not infested with BnR influenced disease caused by Rh 40. Disease severity was estimated at 24-hr intervals after inoculation with Rh 40. Fungi infesting leaf tissue samples and fragments of grain were isolated as described in experiment 1.

Experiment 3. To determine the influence of different isolates of *Rhizoctonia* on disease suppression, experiment 2 was repeated using BnR isolates Bn 4 and Bn 165 and *R. solani* isolates Rh 40 and Rh 41 (Table 1). The effect of a common turfgrass epiphyte on disease caused by *R. solani* was evaluated by substituting an isolate of *Epicoccum* sp. for a BnR isolate in one treatment.

RESULTS

Experiment 1. Over the 8-day experimental period, mean day and night temperatures within the incubation chambers were 29.5 and 22.5 C, respectively. Disease severity was significantly ($P = 0.05$) lower in plots infested with BnR isolates plus Rh 40 than in plots infested with Rh 40 alone (Fig. 1) 6 and 8 days after inoculation. Isolates Bn 154 and Bn 165 provided a significantly ($P = 0.05$) greater degree of disease suppression than isolate Bn 190. Disease did not develop in plots infested with isolates of BnR alone. BnR and *R. solani* were isolated from washed leaf and grain

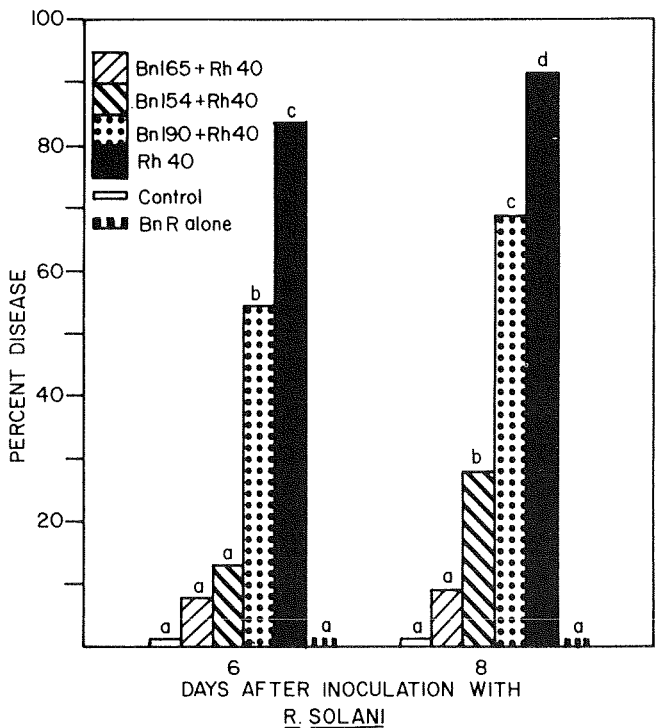

Fig. 1. Disease severity on creeping bentgrass cultivar Penncross inoculated with isolates of binucleate *Rhizoctonia* spp. (Bn 154, Bn 165, and Bn 190) and *Rhizoctonia solani* (Rh 40). Bentgrass was inoculated with binucleate isolates 24 hr before inoculation with *R. solani*. Bars with the same letter do not represent significantly different ($P = 0.05$) results according to Duncan's modified (Bayesian) least significant difference test.

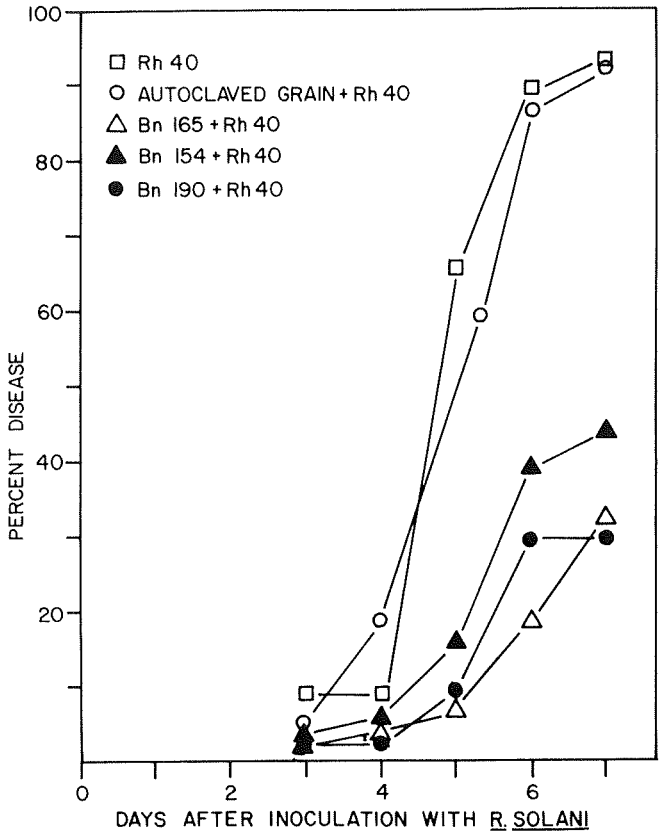

Fig. 2. Disease progress on creeping bentgrass cultivar Penncross inoculated with nonpathogenic isolates of binucleate *Rhizoctonia* spp. (Bn 154, Bn 165, and Bn 190) and *Rhizoctonia solani* (Rh 40). Bentgrass was inoculated with binucleate isolates 24 hr before inoculation with *R. solani*. DLSD values at $P = 0.05$ for days 3–7 were 2.18, 14.05, 20.37, 24.94, and 15.62, respectively.

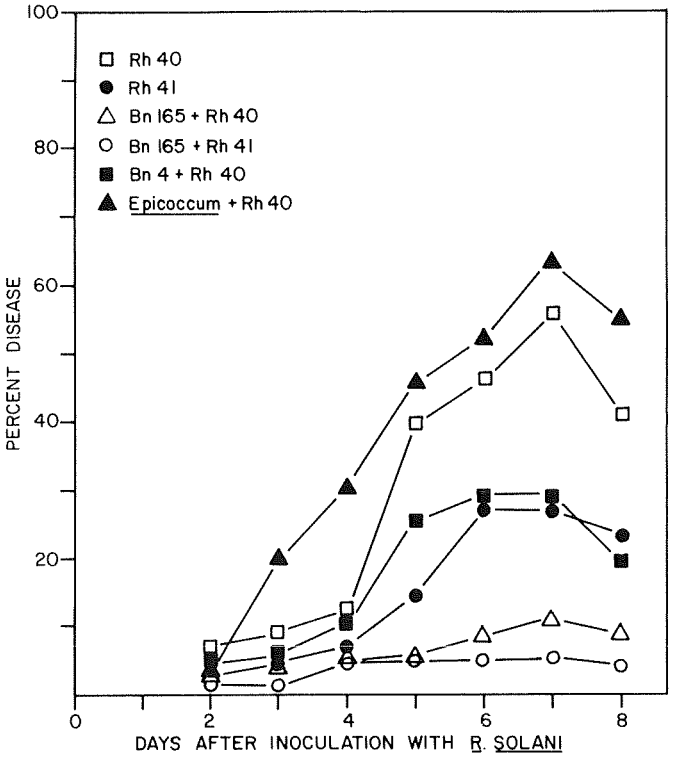

Fig. 3. Disease progress on creeping bentgrass cultivar Penncross inoculated with nonpathogenic isolates of binucleate *Rhizoctonia* spp. (Bn 4 and Bn 165), *R. solani* (Rh 40 and Rh 41), and an *Epicoccum* sp. Bentgrass was inoculated with binucleate isolates and *Epicoccum* 24 hr before inoculation with *R. solani*. DLSD values at $P = 0.05$ for days 2–8 were 11.73, 8.47, 19.27, 22.31, 25.25, 27.00, and 20.34, respectively.

samples collected from the plots 9 days after inoculation with Rh 40.

Experiment 2. Mean day and night temperatures within the incubation chambers were 34.0 and 25.5 C, respectively. Foliar necrosis was observed 3 days after inoculation with Rh 40. From days 3–7, disease severity was significantly ($P = 0.05$) lower in plots infested with BnR isolates plus Rh 40 than in plots infested with Rh 40 alone (Fig. 2). Disease severities were not significantly ($P = 0.05$) different among plots infested with the various BnR isolates plus Rh 40. Disease did not develop in plots infested with isolates of BnR alone. BnR and *R. solani* were isolated from washed leaf and grain samples collected from the plots 9 days after inoculation with Rh 40.

Experiment 3. Day and night mean temperatures within the incubation chambers (over 8 days) were 30 and 21 C, respectively. Five to 8 days after inoculation, Rh 40 caused significantly ($P = 0.05$) more disease than Rh 41 (Fig. 3). Disease severity was significantly ($P = 0.05$) lower in plots infested with the *R. solani* isolates plus the BnR isolates than in plots infested with the *R. solani* isolates alone. The presence of *Epicoccum* in plots infested with Rh 40 had no significant effect on disease severity. Isolate Bn 165 had a significantly ($P = 0.05$) greater suppressive effect on disease caused by Rh 40 than did isolate Bn 4. Foliar necrosis was less evident in all plots because of growth of symptomless turfgrass foliage 8 days after inoculation with Rh 40 or Rh 41. Disease did not develop in plots infested with isolates of BnR alone or *Epicoccum* alone. BnR and *R. solani* were isolated from washed leaf and grain samples collected from plots 10 days after inoculation with Rh 40 or Rh 41. *Epicoccum* was isolated from washed grain but not from washed leaf samples.

DISCUSSION

Infestation of creeping bentgrass with nonpathogenic isolates of BnR resulted in significant ($P = 0.05$) suppression of brown patch disease in all three experiments. Among experiments, variation in the severity of disease caused by isolate Rh 40 and variation in the degree of suppression induced by isolates of BnR were probably attributable to uncontrolled differences in incubation temperatures. The relatively low disease severity ratings recorded during experiment 3 are attributed to temperatures increasing to 40 C on days 1, 2, 5, 6, and 7. However, disease suppression induced by isolates Bn 4 and Bn 165 was still observed.

The mechanism by which BnR isolates suppress brown patch can only be surmised. Observations of pairings among BnR and *R. solani* isolates in culture indicate that the BnR isolates used in this study are not hyperparasitic and they do not inhibit *R. solani* through antibiosis (L. L. Burpee, *unpublished*). However, other BnR isolates may be hyperparasites. Observations of hyphal interactions among more than 100 isolates of BnR and *R. solani*, recovered from turfgrasses and other plants, revealed that one BnR isolate, from soybean root, parasitized other isolates by producing hyphae that curled around host hyphae (L. L. Burpee, *unpublished*). This type of parasitism was similar to that reported by Butler (7) for an isolate of *R. solani*.

In the absence of hyperparasitic and antibiotic effects, nutrient competition and/or host-induced resistance (9) would appear to be plausible mechanisms of disease suppression caused by BnR isolates. The ability to isolate BnR from symptomless leaves of creeping bentgrass 8–10 days after inoculation indicates that hyphae of these fungi colonize leaf surfaces. Because growth and infection cushion formation by *R. solani* are influenced by host exudates (10), it is possible that competition between *R. solani* and BnR for leaf exudates would result in disease suppression. A nutrient-competition theory has been used to explain antagonism of *Botrytis cinerea* on leaf surfaces by bacteria and yeasts (4,11).

Nutrient competition between *R. solani* and epiphytic fungi, other than BnR, may also result in disease suppression. Frequent isolation of *Epicoccum* spp. from symptomless turfgrass leaves indicates that these fungi are common epiphytes (L. L. Burpee, *unpublished*). However, results from experiment 3 indicate that inoculation of creeping bentgrass with a turfgrass isolate of *Epicoccum* does not suppress brown patch. The isolate could not be recovered from leaf tissue 10 days after inoculation, indicating that it did not colonize the phyloplane well. These observations support the theory that spores of *Epicoccum* spp. seldom germinate and colonize nonsenescent tissue (3).

BnR occur widely in nature. Isolates have been recovered from such diverse sources as the rhizospheres of rye and soybean in Florida (16), peanut seed and roots of corn, snap bean, southern pea, cucumber, onion, and lima bean in Georgia (20), flax seedlings in Minnesota (1), turfgrasses in Pennsylvania (18), and hypocotyls of white beans in Ontario (W. McFadden, *personal communication*). To date, these fungi have been examined only as potential plant pathogens (1,6,17,18,20). Results presented here indicate that, under experimental conditions, nonpathogenic isolates of BnR can suppress turfgrass disease caused by *R. solani*. Given the apparent ubiquitous nature of BnR, it is quite possible that a similar but less intense suppression may occur under natural conditions over a wide variety of plant species.

LITERATURE CITED

1. Anderson, N. A. 1977. Evaluation of the *Rhizoctonia* complex in relation to seedling blight of flax. Plant Dis. Rep. 61:140-142.
2. Beard, J. B. 1982. Turf Management for Golf Courses. Burgess Publishing Co., Minneapolis. 642 pp.
3. Blakeman, J. P. 1981. Microbial Ecology of the Phylloplane. Academic Press, London. 502 pp.
4. Brodie, I. D. S., and Blackman, J. P. 1976. Competition for exogenous substrates in vitro by leaf surface microorganisms and germination of conidia of *Botrytis cinerea*. Physiol. Plant Pathol. 9:227-239.
5. Burpee, L. L. 1980. *Rhizoctonia cerealis* causes yellow patch of turfgrasses. Plant Dis. 64:1114-1116.
6. Burpee, L. L., Sanders, P. L., Cole H., Jr., and Sherwood, R. T. 1980. Pathogenicity of *Ceratobasidium cornigerum* and related fungi representing five anastomosis groups. Phytopathology 70:843-846.
7. Butler, E. E. 1957. *Rhizoctonia solani* as a parasite of fungi. Mycologia 49:354-373.
8. Cole, H., Jr., Warren, C. G., and Sanders, P. L. 1978. Field procedures for evaluating fungicides for control of Pythium blight of turfgrasses. Pages 85–86 in: Methods for Evaluating Plant Fungicides, Nematicides, and Bactericides. E. I. Zehr, ed. American Phytopathological Society, St. Paul, MN. 141 pp.
9. Deacon, J. W. 1976. Biological control of the take-all fungus, *Gaeumannomyces graminis*, by *Phialophora radicicola* and similar fungi. Soil Biol. Biochem. 8:275-283.
10. Dodman, R. L. 1970. Factors affecting the penetration phase of infection by *Rhizoctonia solani*. Pages 116–121 in: Root Diseases and Soil-borne Pathogens. T. A. Toussoun, R. V. Bega, and P. E. Nelson, eds. University of California Press, Berkeley. 252 pp.
11. Fokkema, N. J. 1981. Fungal leaf saprophytes, beneficial or detrimental? Pages 433–454 in: Microbial Ecology of the Phylloplane. J. P. Blakeman, ed. Academic Press, London. 502 pp.
12. Horsfall, J. G., and Cowling, E. G. 1978. Phytopathometry: The measurement of plant disease. Pages 120-136 in: Plant Disease. An Advanced Treatise. Vol. 1. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York. 465 pp.
13. Hurd, B., and Grisham, M. P. 1983. Characterization and pathogenicity of *Rhizoctonia* spp. associated with brown patch of St. Augustinegrass. Phytopathology 73:1661-1665.
14. Martin, S. B., Campbell, C. L., and Lucas, L. T. 1983. Horizontal distribution and characterization of *Rhizoctonia* spp.in tall fescue turf. Phytopathology 73:1064-1068.
15. Murray, D. I. L., and Burpee, L. L. 1984. *Ceratobasidium cereale* sp. nov. the teleomorph of *Rhizoctonis cerealis*. Trans. Br. Mycol. Soc. 82:170-172.
16. Ploetz, R. C., and Mitchell, D. J. 1983. Rapid identification of isolates of *Rhizoctonia* spp. from a field multi-cropped with rye and soybean under reduced tillage. (Abstr.) Phytopathology 73:813.
17. Ploetz, R. C., and Mitchell, D. J. 1983. Pathogenicity to rye and soybean by *Rhizoctonia* spp. isolated from a field multi-cropped under reduced tillage. (Abstr.) Phytopathology 73:813-814.
18. Sanders, P. L., Burpee, L. L., and Cole, H., Jr. 1978. Preliminary studies on binucleate turfgrass pathogens that resemble *Rhizoctonia solani*. Phytopathology 68:145-148.
19. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw Hill, New York. 633 pp.
20. Sumner, D. R., and Bell, D. K. 1982. Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zeae*. Phytopathology 72:86-91.