Biological Control of Rhizoctonia Root Rot of Snap Bean with Binucleate *Rhizoctonia*-like Fungi

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ABSTRACT

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Eleven isolates of binucleate *Rhizoctonia*-like fungi (BNR) and one isolate each of *R. zeae*, *Trichoderma hamatum*, and *T. harzianum* were studied as potential biocontrol agents of root rot of snap bean caused by *R. solani* in the greenhouse and field. Isolates of BNR reduced ($P = 0.05$) disease incidence and disease severity in four greenhouse experiments. Four selected isolates of BNR and the two isolates of *Trichoderma* species were then screened in soils naturally infested with *R. solani*. Four field experiments were conducted at two locations. Selected isolates of BNR significantly ($P = 0.05$) protected bean seedlings from Rhizoctonia root rot in one or more experiments. One BNR isolate (BN-160) significantly ($P = 0.05$) protected snap beans from Rhizoctonia root rot in all field experiments. Isolate TC-1 of *T. harzianum* protected bean seedlings in only one field experiment. Results indicate that isolates of BNR show potential as biocontrol agents of Rhizoctonia root rot of snap bean.

Several biological control systems for Rhizoctonia root rot of beans (*Phaseolus vulgaris* L.) caused by *Rhizoctonia solani* Kühn have been reported, but none has been effective under field conditions (5–9). Recently, however, Burpee and Goulty (2) demonstrated that when avirulent binucleate *Rhizoctonia*-like fungi (BNR) were used as biocontrol agents, the incidence of brown patch disease of creeping bentgrass caused by *R. solani* was reduced. Because BNR are frequently present in the rhizospheres of many plants (1,3,11–13), a study was undertaken to test the biocontrol potential of BNR against Rhizoctonia root rot of snap bean. Preliminary results of this work have been reported (4).

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MATERIALS AND METHODS

Test fungi consisted of 11 isolates of BNR, one of *R. zeae* Voorhees, one of *Trichoderma hamatum* (Bon.) Bainier, and one of *T. harzianum* Rifai (Table 1). A highly virulent isolate of *R. solani* (AC-1), assigned to anastomosis group 4 (AG-4), was used as the pathogen in greenhouse experiments. All fungi were grown on potato-dextrose agar (PDA) at 30 C for 3 days, and mycelial plugs were stored in sterile distilled water at room temperature.

Inoculum was produced by growing the test fungi on PDA as described, then transferring mycelial plugs to 250 g of moist sterile oat kernels (3:2, dry oats/distilled water, w/v) contained in 1-L Erlenmeyer flasks. Oat cultures were then incubated at 30 C for 10 days. Subsequently, colonized oat kernels were air-dried on a laboratory bench, and if not used immediately, they were stored in plastic bags at 5 C.

Several methods for screening biocontrol agents were tested in the greenhouse. Finally, the following method was adopted. Five kilograms of a pasteurized mixture of sandy loam soil/peat/washed sand (2:1:1, v/v/v) amended with 1.5 g of dried oat kernels colonized by the test fungus or uncolonized were dispensed into a greenhouse flat (35 × 25 × 7 cm). Seventy-seven seeds of snap bean cultivar Topcrop were planted 2.5 cm apart in each flat. Seeded flats were then infested with the pathogen by burying 0.15 g of *R. solani*-colonized

oat kernels 2 cm deep in the center. Subsequently, flats were placed on greenhouse benches, watered uniformly, and maintained at 30 ± 2 C under natural light.

Each flat represented a separate treatment, and treatments were arranged in a randomized complete block design with five replicates. Controls consisted of unamended soil mixture or soil mixture amended with uncolonized oat kernels.

Preemergence damping-off was recorded at seedling emergence, and disease severity was determined 2 wk after seeding by a subjective scale of 0–5, where 0 = no lesions on hypocotyl, 1 = lesions ≤2.5 mm long, 2 = lesions 2.5–5.0 mm long, 3 = lesions ≥5.0 mm long, 4 = lesions girdling plant and wilting visible on leaves, and 5 = seedlings damped-off or dead. Disease severity was determined in each flat by dividing the sum of individual scores by the number of seedlings observed. Disease incidence was estimated as the proportion of diseased seedlings per flat.

All greenhouse experiments were repeated once and analyzed statistically using analysis of variance. Multiple comparisons of means were performed using Fisher's least significant difference (FLSD) (10).

The biocontrol effectiveness of selected isolates, BN-105, BN-160, CB-3, CB-4, and TC-1, was tested in the field in 1984 and 1985 at Clayton and Hendersonville, NC. In 1984, two experiments initiated on 15 May and 15 June were conducted in adjacent fields (81% sand, 15% silt, 4% clay, <1% organic matter, and pH 5.9) at Clayton, NC. These fields were heavily infested with *R. solani* (mainly AG-1 and AG-4) and had been cropped previously with snap beans for three growing seasons. In 1985, one experiment was conducted at Clayton in a field with the same soil characteristics as the ones described before and in another at Hendersonville (69% sand, 27% silt, 4% clay, 1% organic matter, and pH 6.5). This last field also was heavily infested with *R. solani* and was previously cropped with snap beans and cabbage.

Treatments were arranged in a randomized complete block design with five replicates. Each plot consisted of four 3-m rows spaced 1 m apart with 0.6-m alleys between plots. Biocontrol agents were applied in-furrow before planting as colonized oat kernels at the rate of 15 g/m of row, and snap bean

Table 1. Fungal isolates evaluated for control of Rhizoctonia root rot of snap bean

Fungus	Isolate	Source	Origin	Received from[a]
Trichoderma hamatum	T95	Soil	South America	4
T. harzianum	TC-1	Soil	North Carolina	1
Rhizoctonia zeae	ARKI	Soil	Arkansas	3
BNR[b]	CB-3	Unknown	Unknown	3
BNR	CB-4	Unknown	Unknown	3
BNR	CB-39	Unknown	Unknown	3
BNR	BN-67	*Juniperus* sp.	North Carolina	2
BNR	BN-80	*Poa* sp.	New York	2
BNR	BN-89	Fescue	North Carolina	2
BNR	BN-105	Fescue	North Carolina	2
BNR	BN-157	Fescue	North Carolina	2
BNR	BN-160	Fescue	North Carolina	2
BNR	BN-164	Fescue	North Carolina	2
BNR	BN-8401	Soil	North Carolina	1

[a] 1 = Authors, local isolation; 2 = S. B. Martin and L. T. Lucas, North Carolina; 3 = A. S. Windham and L. T. Lucas, North Carolina; and 4 = R. Baker, Colorado.
[b] Binucleate *Rhizoctonia*-like fungi.

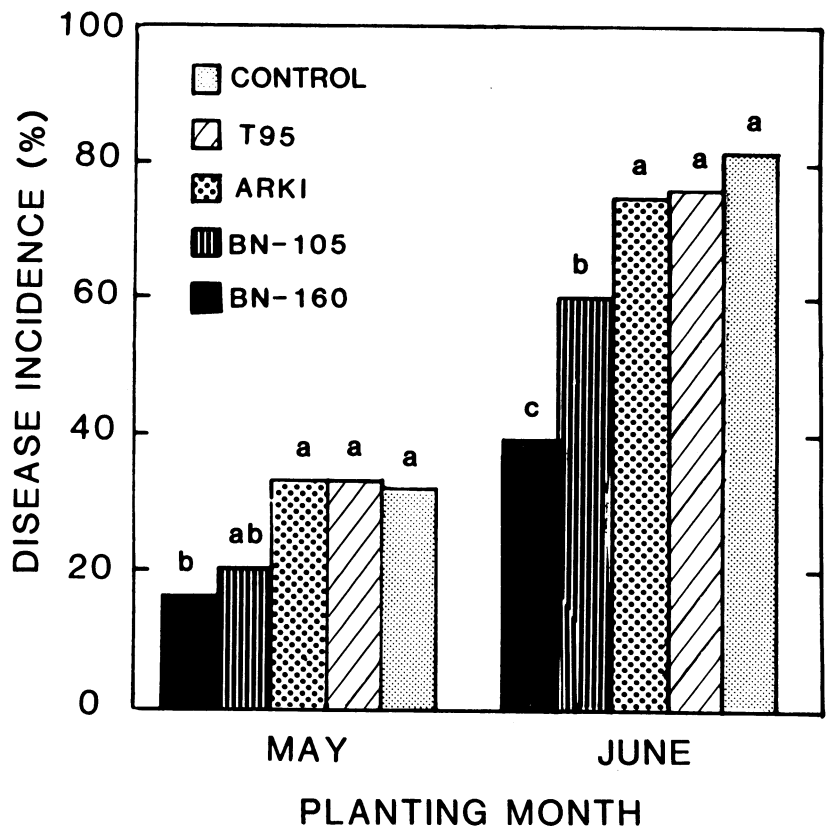

Fig. 1. Disease incidence in snap bean seedlings (cultivar Topcrop) planted in May or June of 1984 in a field naturally infested with *Rhizoctonia solani* and treated with isolates of binucleate *Rhizoctonia*-like fungi (BN-160 and BN-105), *R. zeae* (ARKI), and *Trichoderma hamatum* (T95). Diseased plants were counted 3 wk after planting. Data are means of five replicates. Bars with the same letter represent statistically ($P = 0.05$) similar results according to Fisher's least significant difference test.

Table 2. Effects of soil treatments with different fungi on incidence and severity of Rhizoctonia root rot of snap bean in the greenhouse in 1984

Fungus isolate[w]	Disease incidence (%)	Disease severity[x]
Control	85.7 a[y]	3.83 a
BN-80[z]	78.0 ab	3.47 ab
T95	71.0 ab	3.45 ab
BN-67	70.3 b	3.26 ab
BN-164	66.5 bc	2.40 abc
ARKI	56.0 bc	2.13 abc
BN-105	55.0 c	1.62 bc
BN-157	54.5 c	2.32 abc
BN-89	53.5 c	1.96 abc
BN-160	33.2 d	0.70 c

[w] Inoculum of biocontrol candidate was added at 0.3 g of colonized oat kernels per kilogram of soil mixture. Uninfested control soil mixture received 0.3 g of sterile oat kernels per kilogram of soil mixture.
[x] Disease severity scale ranged from 0 = no disease to 5 = damped-off or dead seedlings.
[y] Means of two experiments with five replicates each. Values followed by the same letter do not differ significantly from each other ($P = 0.05$) according to Fisher's least significant difference test.
[z] BN, T, and ARKI represent isolates of binucleate *Rhizoctonia*-like fungi, *Trichoderma*, and *R. zeae*, respectively.

cultivar Topcrop was planted at the rate of 25 seeds per meter of row. Control treatments consisted of untreated rows or rows that received uncolonized sterile oats. In Hendersonville, the field was broadcast-treated with 2.3 L a.i./ha of metalaxyl before planting to reduce incidence of Pythium root rot. Disease incidence and severity were estimated in each plot (as previously described) 3 wk after planting. Data were analyzed as described for the greenhouse experiments.

RESULTS

In the greenhouse, Rhizoctonia root rot was evident at seedling emergence (about 5 days after planting) and increased progressively in a radial fashion, reaching the border of the flat within 10–15 days. Preemergence damping-off was limited to a few seedlings around the site of initial infestation with the pathogen.

In 1984, isolates BN-67, BN-89, BN-105, BN-157, BN-160, BN-164, and ARKI significantly ($P = 0.05$) reduced disease incidence; isolates BN-105 and BN-160 also reduced disease severity compared with the control (Table 2). Disease incidence and severity were correlated ($P = 0.05$) in these experiments ($r = 0.96$).

In 1985, isolates BN-160, CB-3, and CB-4 significantly reduced disease incidence and disease severity, whereas BN-89, BN-105, BN-157, *T. hamatum* (T95), and *T. harzianum* (TC-1) did not protect the seedlings. Isolates BN-8401 and CB-39 increased disease incidence and disease severity (Table 3).

In 1984, two experiments were conducted at Clayton, NC, one in May and the other in June. In May, isolate BN-160 reduced ($P = 0.05$) disease incidence in spite of the cool weather, which was not conducive for disease development. In June, isolates BN-105 and BN-160 reduced ($P = 0.05$) disease incidence (Fig. 1).

In 1985, two experiments were conducted, one at Clayton and the other at Hendersonville. At Clayton, isolates BN-160 and CB-3 reduced ($P = 0.05$) disease incidence (Fig. 2). At Hendersonville, isolates BN-105, BN-160, CB-4, CB-3, and TC-1 also reduced ($P = 0.05$) disease incidence (Fig. 2).

Diseased plants collected at Clayton and Hendersonville yielded mostly isolates of *R. solani* belonging to AG-4.

DISCUSSION

The greenhouse screening method described in this paper was useful in identifying BNR isolates with potential for biocontrol of Rhizoctonia root rot of snap bean. Isolates BN-105, BN-160, CB-3, and CB-4, selected for field testing on the basis of their high protective ability in the greenhouse, reduced ($P = 0.05$) Rhizoctonia root rot of snap bean in one or more field experiments.

Selected isolates varied in their ability to protect snap beans from root rot. Isolates BN-160 protected bean plants in all field experiments, whereas isolates BN-105, CB-3, and CB-4 protected plants in two, two, and one experiment, respectively.

Treating the soil with metalaxyl before planting reduced the incidence of Pythium root rot and apparently increased the effectiveness of the biocontrol agents. In Hendersonville, where the soil was treated with metalaxyl before planting, all five biocontrol agents significantly ($P = 0.05$) reduced Rhizoctonia root rot, and the level of protection was higher than in any other field experiment.

The mechanism by which BNR isolates reduced root rot was not identified. Nevertheless we observed, as did Burpee and Goulty (2), that the BNR used in this study were not hyperparasitic and did not inhibit *R. solani* through antibiosis. We also observed that BN-160, the most consistent and stable protector, was easily reisolated from uninfected, unsterilized bean roots and stems, but it could not be recovered from the same material after treatment with 0.5% NaOCl for 30 sec. This observation suggests that although the fungus

Table 3. Effects of soil treatments with different fungi on incidence and severity of Rhizoctonia root rot of snap bean in the greenhouse in 1985

Fungus isolate[w]	Disease incidence (%)	Disease severity[x]
CB-39[y]	99.5 a[z]	3.32 a
BN-8401	88.0 ab	4.04 a
BN-157	80.7 bc	3.66 a
BN-89	78.7 bcd	3.61 a
ARKI	78.5 bcd	3.59 a
BN-105	75.5 cd	3.44 a
Control	70.5 cd	3.12 a
TC-1	67.5 d	3.36 a
CB-3	36.4 e	1.51 b
CB-4	32.2 e	1.41 b
BN-160	28.7 e	1.09 b

[w] Inoculum was added at the rate of 0.3 g of colonized oat kernels per kilogram of soil mixture. Uninfested control soil mixture received 0.3 g of sterile oat kernels per kilogram of soil mixture.

[x] Disease severity scale ranged from 0 = no disease to 5 = damped-off or dead seedlings.

[y] BN and CB, TC-1, and ARKI represent isolates of *Rhizoctonia*-like fungi, *Trichoderma*, and *R. zeae*, respectively.

[z] Means of two experiments with five replicates each. Values followed by the same letter do not differ significantly from each other ($P = 0.05$) according to Fisher's least significant difference test.

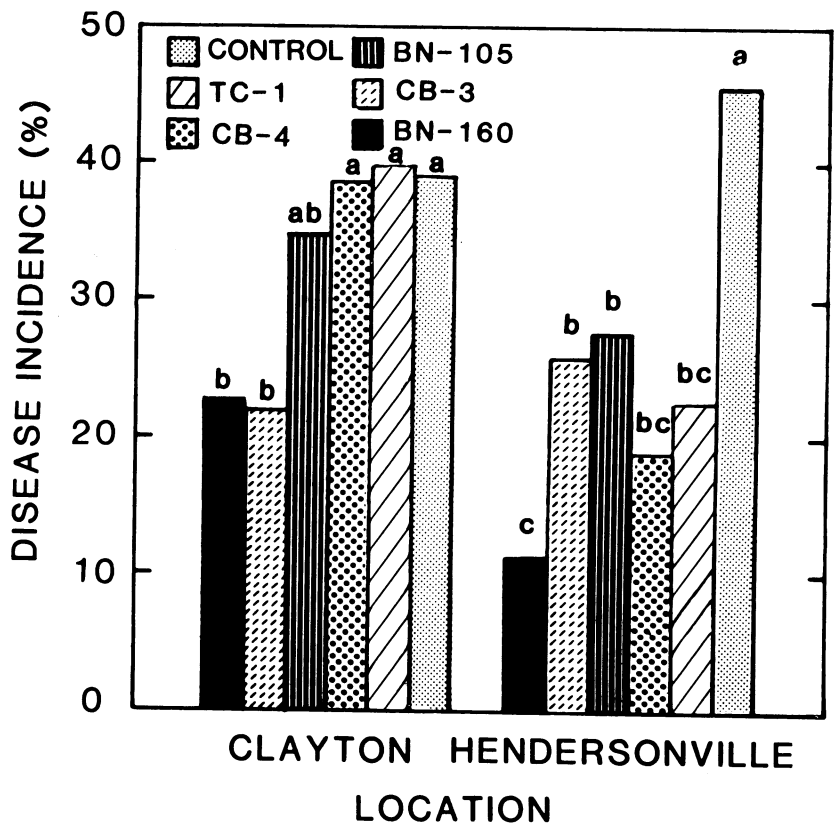

Fig. 2. Disease incidence in snap bean seedlings (cultivar Topcrop) planted in two different locations in June of 1985 in fields naturally infested with *Rhizoctonia solani* and treated with isolates of binucleate *Rhizoctonia*-like fungi (BN-160, BN-105, CB-3, and CB-4) and *Trichoderma harzianum* (TC-1). Disease was estimated 3 wk after planting. Data are means of five replications. Bars with the same letter represent statistically ($P = 0.05$) similar results according to Fisher's least significant difference test.

colonizes the surfaces of the roots and stems, it may not have to penetrate the plant to protect it. Therefore, nutrient competition and/or host-induced resistance may play important roles as mechanisms of action in this system. The hypothesis of host-induced resistance is most attractive, because some BNR isolates are indeed pathogenic in some host plants (1,3,12). As mentioned before, some BNR isolates colonize the surfaces of the bean seedlings; therefore, their close association with the host may induce some undetectable physiological process that activates a defensive response by the plant that could render it more resistant to the pathogen.

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