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Biocontrol of *Rhizoctonia* damping-off of cucumber by non-pathogenic binucleate *Rhizoctonia*

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Abstract

Three isolates of binucleate *Rhizoctonia* (BNR) were tested for biological control of damping-off of cucumber seedlings caused by *Rhizoctonia solani* AG 2–2 and AG 4. BNR isolates L2 (AG Ba) and W1 and W7 (AG A) provided protection of 58 to 71% against virulent isolate C4 of AG 4 and 64 to 75% protection against virulent isolate RH 65 of AG 2–2. Varying protection was provided to the seedlings by the BNR isolates against the virulent *R. solani* from the two AGs depending on their combination. The BNR isolates did not vary in providing protection to the seedling when tested against virulent C4 when both isolates were inoculated using three different methods, *viz.* in water agar, combination of water agar and soil and using soil alone. Protection of 58 to 71% was provided by the isolates when inoculation was done on the hypocotyl using water agar, 62.8 to 75% using the combination of water agar and soil, and 75 to 85% when inoculation of both isolates was done in soil. Pre-incubation of BNR W7 or delayed inoculation of C4 (from 0.5 day to longer duration) using the different methods provided an increased protection to the seedlings to give complete inhibition of damping-off disease. Simultaneous inoculation of both BNR W7 and C4 using the three methods failed to provide protection to the seedlings. Among the BNR isolates, BNR W7 showed plant growth promotion in terms of significant increase in plant height ($P = 0.01$) and fresh weight ($P = 0.05$).

Introduction

Damping-off of seedlings caused by *Rhizoctonia solani* is responsible for considerable yield losses in a variety of crop plants. Effective and economical methods of control of this disease depends on the use of broad spectrum fungicides, but such measures establish imbalance in the microbial community which render it unfavorable for the activity of beneficial organisms [Lifshitz *et al.*, 1985]. Researchers have proposed the use of antagonists [Elad *et al.*, 1981] and non-pathogenic or hypovirulent strains of *Rhizoctonia* spp. [Ichielevich-Auster *et al.*, 1985; Harris *et al.*, 1993, 1994] for the control of seedling damping-off. Studies [Ichielevich-Auster *et al.*, 1985, Sneh *et al.*, 1986] conducted on the suppression of damping-off caused in cotton, radish and wheat seedlings by virulent isolates of *R. solani* and *R. zeae*, reported the efficacy of non-pathogenic *R. solani* (AG 4) in providing protection to the seedlings by 76–94%. Another related study [Harris *et al.*, 1994] was conducted in which bedding plants (*Capsicum*, *Celosia* and *Viola*) were protected from damping-off caused by *R. solani* AG 4 and AG 8 by binucleate *Rhizoctonia*. The studies showed the potential of the isolates as effective biocontrol agents and as good growth promoters.

The efficacy of binucleate *Rhizoctonia* to control other diseases caused by *R. solani* was also reported on creeping bentgrass [Burpee and Goulty, 1984], bean [Cardoso and Echandi, 1987a; 1987b], potato [Escande and Echandi, 1991] and sugarbeet [Herr, 1988]. A field experinment using binucleate *Rhizoctonia* to protect cucumber from damping-off was also conducted, but effective protection was attained when the isolate used as biocontrol agent was combined with fungicide metalaxyl [Cubeta and Echandi, 1991]. The

studies included different methods in cross-protection to control *R. solani* using binucleate *Rhizoctonia* in greenhouse and field experiments. The importance of this cross-protection phenomenon has been emphasized in relation to host-parasite interaction as well as microbial community [Lifshitz *et al.*, 1985]. Using greenhouse and field experiments, several workers have examined the practical use of cross-protection in controlling the above-mentioned diseases including seedling damping-off.

In this study, isolates of binucleate *Rhizoctonia* obtained from soil [Villajuan-Abgona *et al.*, 1993] previously planted to devil's tongue (BNR L2), grassland (BNR W1) and tomato (BNR W7) in Japan were tested for their efficacy to control seedling damping-off of cucumber caused by *R. solani* AG 4 through cross-protection tests. Protection provided by the isolates in experiments done under laboratory condition was compared with a greenhouse experiment to fully understand the nature of protection involved. A cucumber bioassay for testing suppression of seedling damping-off caused by virulent *R. solani* (AG 4) by hypovirulent isolates of binucleate *Rhizoctonia* was evaluated.

Materials and methods

Isolates used

Strains of *Rhizoctonia* spp. were previously isolated from 48 soil samples collected from 13 locations in Gifu Prefecture, Japan using three different methods described in the previous study [Villajuan-Abgona *et al.*, 1993]. The isolates were tested for pathogenicity and three binucleate *Rhizoctonia* (BNR) (BNR W1 and W7 belonging to AG A and BNR L2 belonging to AG BA) were identified as hypovirulent to several crops (Chinese cabbage, carrot, lettuce, tomato, and onion) and non-pathogenic to cucumber and five of its varieties [Villajuan-Abgona *et al.*, 1993]. These three non-pathogenic BNR were tested in this study as biocontrol agents against damping-off disease on cucumber seedling, caused by virulent *R. solani*. *Rhizoctonia solani* AG 2–2 (RH65, GU-1) and AG 4 (C4, C9) isolated from soil, which were highly pathogenic to radish and cucumber and caused severe damping-off, were used as challenge isolates in one of the experiments, while *R. solani* C4 (AG 4) was used in experiments dealing with the different methods of inoculating the biocontrol agent and the pathogen. Each isolate was cultured on potato-dextrose agar (PDA) (Difco Laboratories, Detroit, Mich.) for 3 days at 25 °C in the dark. For experiments requiring delivery to soil, five to seven 5 mm mycelial disk of the isolates cut from the edges of three-day old cultures were added to 100 g moist autoclaved barley grain (1:1, dry barley grain/distilled water, w/v) contained in a 500 ml Erlenmeyer flask. The cultures were kept in darkness at 25 °C for 10 days and shaken regularly to aid even colonization. The infested barley grains were air-dried for 1 week and stored at 4 °C until needed.

Methods of inoculating biological control agent and the pathogen

Water agar method

Cucumber seeds were surface-sterilized in 70% Ethyl alcohol for 1 min followed by 2% sodium hypochlorite with 3 drops of Tween 20 (Polyoxyethylene sorbitan monolaureate) for 30 min. Then, rinsed three times with sterile distilled water (SDW) and pre-germinated between 2 layers of No. 1 filter papers (90 mm). Five seedlings were transferred to 2% water agar (WA) and allowed to grow for 2 days in a growth chamber (16 h photoperiod with a daytime illuminance of approx. $250/\mu E\ m^{-2} sec^{-1}$ and 75% RH) at 25 °C. A mycelial disk (3 mm) of the non-pathogenic BNR, taken from the advancing margin of a three-day old culture, was inoculated on the basal hypocotyl of the seedling and again incubated in the same growth chamber for 2 days. After this time, a 3 mm-mycelial disk of the virulent *R. solani*, grown in PDA the same way as the BNR, was inoculated on the same hypocotyl just beside the inoculated BNR. The treatments were done in three replicates, and the non-treated seedlings, seedlings inoculated with BNR alone and seedlings inoculated with virulent strain alone served as controls. Treated and control seedlings were kept in the same growth chamber. Disease severity was determined two weeks after inoculation of the virulent *R. solani* by a subjective scale of 0 = healthy, no lesions on the hypocotyl; 1 = one or two lesions <0.25 mm long; 2 = lesions <0.5 mm covering $<10\%$ of the hypocotyl; 3 = lesions >1.0 mm long and covering 10% to 100% of the hypocotyl and 4 = seedling is dead. Based on the disease severity rating, percent protection was estimated to measure the degree of protection provided by the biocontrol agent against the pathogen by the formula $[(A\text{-}B)/A] \times 100$ in which A represents the disease severity exhibited on the hypocotyl due to inoculation

of virulent *R. solani* alone while B is the disease severity exhibited on the hypocotyl due to co-inoculation of BNR and the virulent *R. solani*. The calculated values were analyzed statistically and means were compared among treatments.

Water agar/soil method

The biocontrol effectiveness of the BNR was further determined by slowly shifting the procedure from laboratory to the greenhouse by combining the water agar and soil method. The procedure for the water agar method was followed until the inoculation of BNR. After two days of incubation, water agar was carefully removed except those attached to the root portion of the seedlings and then, the seedling was carefully transplanted to 100 g of partially-sterilized soil (steam-air treated at 60 °C for 30 min) infested with 2% (w/w) ground barley grain completely colonized with virulent *R. solani*. The treated and control plants in three replicates, were kept in the same growth chamber and disease severity was similarly assessed. An additional experiment was done in which the incubation period of the BNR was varied from 0 day to 4 days prior to inoculation of virulent *R. solani*. Plant height and root length were also measured to determine the effect of BNR on seedling growth.

Soil method

To test the performance of the biocontrol agents in soil, a greenhouse experiment was conducted. Paper pot set [manufactured by Nippon Beet Sugar Co. Ltd., Japan for growing sugar beet seedlings (*Beta vulgaris* L.)] which could be separated into individual pots (6.5 cm depth × 1.5 cm diameter/pot) were autoclaved and filled with approx. 16 g of BNR-amended soil (2% w/w, ground barley grain inoculum) and sown with two surface-sterilized cucumber seeds. The seedlings were allowed to grow until 7 days and then transferred, together with the BNR-amended soil, to test pots (6.5 cm depth × 5 cm diameter) containing 100 g *R. solani*-infested soil. The treatments and the control plants (4 replicates) were kept in the greenhouse for two weeks at 25 ± 2 °C. Disease severity and growth parameters were measured as described above. Another experiment was done with 0, 1, 2, 4 and 7 day-incubation of BNR prior to inoculation of virulent *R. solani*.

Data analysis

The experiments were carried out in a Randomized Complete Block Design (RCBD) and statistical analysis was done using Microstat Analysis Program Release 2.0 (Ecosoft, Inc., 1984). Analysis of variance (ANOVA) was performed for the assessed data on disease severity and calculated values of percent protection in all experiments to test for the significance of the treatments. Treatment means obtained for disease severity were compared using Fisher's Least Significant Difference (LSD) at $P = 0.05$ and $P = 0.01$ while comparison for the calculated values for percent protection was done using Duncan's Multiple Range Test (DMRT) at $P = 0.05$.

Results

Protection of cucumber seedlings by non-pathogenic BNR against Rhizoctonia *damping-off caused by* R. solani *AG 2–2 and* R. solani *AG 4*

Hypovirulent BNR isolates L2, W1 and W7 protected seedlings grown on water agar variably according to the combination of hypovirulent BNR and pathogenic *R. solani* isolates (Table 1, Fig. 1). Seedling protection against pathogenic isolate C4 (AG 4), ranged between 58 to 71% protection, while only 32 to 42% protection was provided against C9 (AG 4). Low protection (47.2 to 52%) was provided against isolate GU-1 (AG 2–2), while better protection of 68 to 75% was recorded against RH 65 (AG 2–2). The three BNR isolates did not differ in their ability to protect the seedlings from damping-off caused by *R. solani*. Since effective and stable protection was also provided by the combination of BNR W7 as the biocontrol agent and *R. solani* C4 of AG 4 as the challenge isolate from other experiments (data not shown), this combination was used in subsequent experiments.

Different methods of inoculating BNR isolates and the pathogen

Using the water agar method, 58 to 71% protection was obtained by inoculation of hypovirulent BNR isolates with agar disks directly on the hypocotyl of seedlings two days before challenge-inoculation with *R. solani*. *R. solani* inoculated on the same hypocotyl just beside the BNR isolates failed to incite severe disease on the BNR-inoculated seedlings as compared with the non-inoculated control. Slightly higher protection (62.8 to 75%) was provided by inoculation of the hypovirulent

Table 1. Protection of cucumber seedlings (cv. *Jibai*) grown in water agar[1], against damping-off caused by pathogenic *R. solani* by non-pathogenic isolates of binucleate *Rhizoctonia*

Virulent isolates	AG[2]	BNR L2				BNR W1				BNR W7			
		−[3]	+[3]	Prot.[4] (%)	LSD[5]	−[3]	+[3]	Prot.[4] (&)	LSD[5]	−[3]	+[3]	Prot.[4] (%)	LSD[5]
Uninfested		0.0	0.0			0.0	0.0			0.0	0.0		
C4	4	3.8	1.6**	58.0ab	1.15	3.6	1.1**	69.5a	2.49	3.8	1.1**	71.0a	1.24
C9	4	4.0	2.7*	32.0c	1.24	3.5	2.0*	42.0b	1.50	4.0	2.7*	32.5c	1.70
RH65	2-2	3.0	0.9**	68.0a	0.99	3.2	0.9**	71.8a	1.14	3.5	0.9**	75.0a	1.49
GU-1	2-2	3.5	1.8*	48.5b	0.74	3.6	1.7*	52.0b	0.89	3.6	1.9*	47.2b	1.08

[1] Two-day old cucumber seedlings were grown in 2% water agar, inoculated with 3 mm mycelial disks of non-pathogenic BNR and virulent *R. solani* isolates. BNR isolates were inoculated 2 days prior to inoculation of virulent *R. solani*.

[2] Anastomosis grouping

[3] − = absence or uninoculation of BNR; + = presence or inoculation of BNR. Values in columns represent mean disease severity index; 0 = no lesion on the hypocotyl; 1 = one or two lesions <0.25 mm long; 2 = lesions <0.5 mm long covering $<10\%$ of the hypocotyl; 3 = lesions >1.0 mm long covering 10% to 100% of the hypocotyl; 4 = seedling is dead.

[4] Percent protection = $[(A-B)/A] \times 100$ in which A = disease severity exhibited on the seedling hypocotyl due to the presence of virulent *R. solani* alone; B = disease severity exhibited on the hypocotyl in the presence of both BNR and virulent isolates. Values followed by the same letter are not significantly different at $P = 0.05$ according to DMRT.

[5] LSD values differ in each combination. * and ** represent significant values at $P = 0.05$ and $P = 0.01$, respectively.

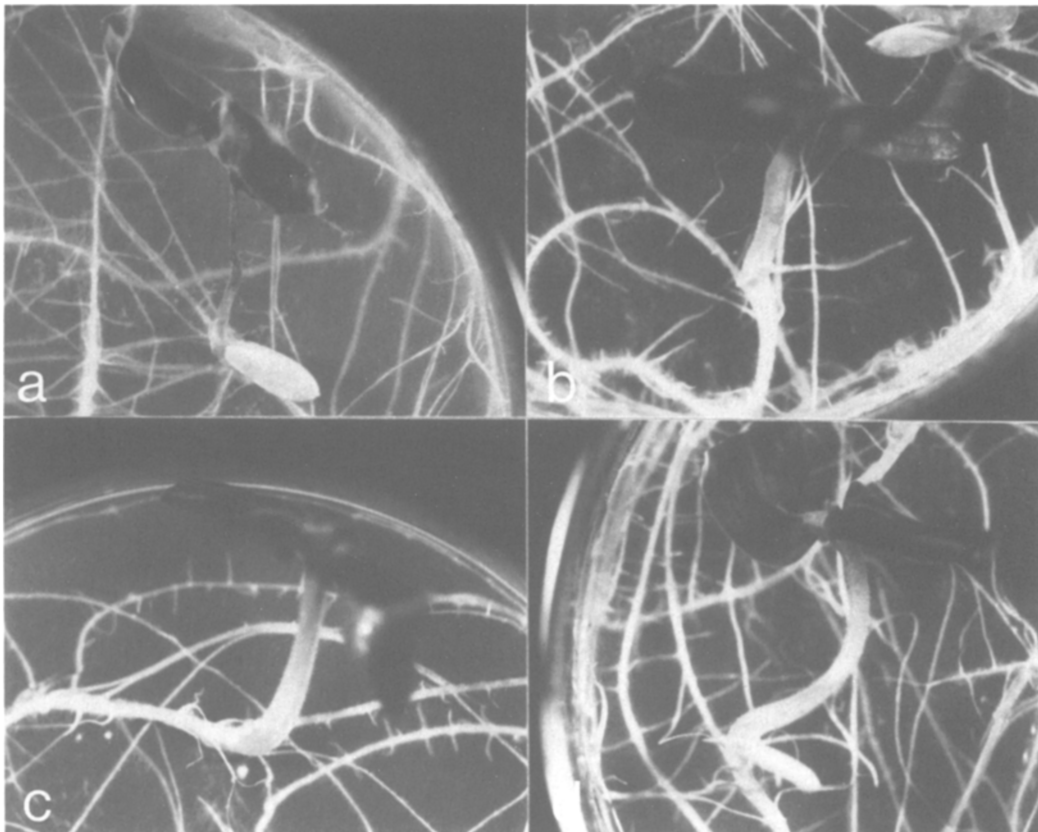

Fig. 1. Protection of cucumber seedlings from *Rhizoctonia* damping-off by non-pathogenic BNR isolates using the water agar method. The seedling hypocotyls a. inoculated with mycelial disk of *R. solani* alone, b. treated with mycelial disks of non-pathogenic BNR and challenge-inoculated after 2 days with mycelial disk of virulent *R. solani* (AG 2-2 or AG 4), c. not treated with any of the isolates (control), and d. treated with mycelial disk of the non-pathogenic BNR alone.

Table 2. Effect of different inoculation methods on the protection of cucumber seedlings (cv. *Jibai*) against damping-off caused by virulent *R. solani* C4 (AG 4) by non-pathogenic binucleate *Rhizoctonia*

Methods	Seedling age (days)	BNR L2				BNR W1				BNR W7			
		−[1]	+[1]	Prot.[2] (%)	LSD[3]	−[1]	+[1]	Prot.[2] (%)	LSD[3]	−[1]	+[1]	Prot.[2] (%)	LSD[3]
Water agar[4]	21	3.8	1.6**	58.0b	1.15	3.6	1.1**	69.5b	2.49	3.8	1.1**	71.0b	1.24
Water agar/soil[5]	21	3.7	1.3*	64.8ab	1.98	3.5	1.3*	62.8b	1.74	4.0	1.0**	75.0ab	1.15
Soil[6]	21	4.0	1.0**	75.0a	1.83	4.0	0.6**	85.0a	1.68	4.0	0.6**	85.0a	1.68

[1] − = absence or unamended with BNR; + = presence or amended with BNR. Values in columns represent mean disease severity index; 0 = no lesion on the hypocotyl; 1 = one or two lesions <0.25 mm long; 2 = lesions <0.5 mm long covering <10% of the hypocotyl; 3 = lesions >1.0 mm long covering 10% to 100% of the hypocotyl; 4 = seedling is dead.

[2] Percent protection = [(A-B)/A] × 100 in which A = disease severity exhibited on the seedling hypocotyl due to the presence of virulent *R. solani* alone; B = disease severity exhibited on the hypocotyl in the presence of both BNR and virulent isolates. Values followed by the same letter are not significant at $P = 0.05$ according to DMRT.

[3] LSD values differ in each combination. * and ** represent significant values at $P = 0.05$ and $P = 0.01$, respectively.

[4] Two-day old cucumber seedlings were grown in 2% water agar, inoculated with 3 mm mycelial disks of non-pathogenic BNR and virulent *R. solani*. BNR isolates were inoculated 2 days prior to inoculation of virulent *R. solani*.

[5] Two-day old cucumber seedlings were grown in 2% water agar, inoculated with 3 mm mycelial disks of non-pathogenic BNR. After 2 days, the BNR-treated seedlings were transplanted to 100 g partially-sterilized soil amended with 2% ground barley grain colonized with virulent *R. solani*. Uninfested seedlings were transplanted to the same amount of unamended partially-sterilized soil.

[6] Cucumber seeds were sown in 16 g partially-sterilized soil in paper pot amended with 2% ground barley grain colonized with non-pathogenic BNR. The seedlings were transplanted to 100 g of partially-sterilized soil in plastic pots infested with the same amount of inoculum of virulent *R. solani* after 7 days.

Fig. 2. Cucumber seedlings protected from *Rhizoctonia* damping-off using the soil method. The BNR-treated seedlings were challenged after 7 days with the pathogen by transferring to soil infested with the virulent *R. solani* C4 (AG 4).

BNR isolates with agar disk on the hypocotyl and challenge-inoculated by transfer to soil infested with the pathogen (Table 2). The best protection (75 to 85%) ($P = 0.05$) was obtained with the soil method where cucumber seeds were sown in soil, seedlings were allowed to grow with the hypovirulent BNR isolates

Table 3. Protection of cucumber seedlings against post-emergence damping-off caused by virulent *R. solani* C4 (AG 4) by non-pathogenic binucleate *Rhizoctonia* using the water agar method[1]

Pre-incubation of BNR on hypocotyl[2] (days)	Seedling age (days)	Disease index[3]		Prot.[4] (%)
		Without BNR W7	with BNR W7	
0	19	4.0	4.0	0.0d
0.5	19.5	2.4	1.4*[5]	41.7c
1	20	2.4	1.2*	49.5bc
2	21	3.8	1.6**	58.0b
4	23	3.0	1.2**	59.0b
6	25	3.0	0.8**	73.4a

[1] Two-day old cucumber seedlings were grown in 2% water agar, inoculated with 3 mm mycelial disk of non-pathogenic BNR W7 and virulent *R. solani*. BNR W7 was inoculated 2 days prior to inoculation of virulent *R. solani*.
[2] Non-pathogenic BNR W7 remained on the seedling hypocotyl, kept in growth chamber (16 h photoperiod with $250/\mu E\ m^{-2}s^{-1}$) at 25 °C.
[3] Values in columns represent mean disease severity index; 0 = no lesion on the hypocotyl; 1 = one or two lesions <0.25 mm long; 2 = lesions <0.5 mm long covering <10% of the hypocotyl; 3 = lesions >1.0 mm long covering 10% to 100% of the hypocotyl; 4 = seedling is dead.
[4] Percent protection = [(A-B)/A] × 100 in which A = disease severity exhibited on the seedling hypocotyl in the presence of virulent *R. solani* alone; B = disease severity exhibited on the hypocotyl in the presence of both BNR and virulent *R. solani*. Values followed by the same letter are not significantly different at $P = 0.05$ according to DMRT.
[5] * and ** represent significant values at $P = 0.05$ and $P = 0.01$ respectively, based on ANOVA.

Table 4. Protection of cucumber seedlings against post-emergence damping-off caused by virulent *R. solani* C4 (AG 4) by non-pathogenic binucleate *Rhizoctonia* using the water agar/soil method[1]

Pre-incubation of BNR on hypocotyl[2] (days)	Seedling age (days)	Disease index[3]		Prot.[4] (%)
		Without BNR W7	With BNR W7	
0	19	4.0	4.0	0.0d
0.5	19.5	2.2	0.6*[5]	72.7c
1	20	2.2	0.3**	86.3b
2	21	2.3	0.3**	86.9b
4	23	3.0	0.0**	100.0a

[1] Two-day old cucumber seedlings were grown in 2% water agar, inoculated with 3 mm mycelial disk of BNR W7. After incubation, the BNR-treated seedlings were transplanted to 100 g partially-sterilized soil amended with 2% (w/w) ground barley grain colonized with virulent *R. solani*. Uninfested seedlings were transplanted to the same amount of unamended sterile soil.
[2] Non-pathogenic BNR W7 remained on the seedling hypocotyl, kept in growth chamber (16 h photoperiod with $250/\mu E\ m^{-2}s^{-1}$) at 25 °C.
[3] Values in columns represent mean disease severity index; 0 = no lesion on the hypocotyl; 1 = one or two lesions <0.25 mm long; 2 = lesions <0.5 mm long covering <10% of the hypocotyl; 3 = lesions >1.0 mm long covering 10% to 100% of the hypocotyl; 4 = seedling is dead.
[4] Percent protection = [(A-B)/A] × 100 in which A = disease severity exhibited on the seedling hypocotyl in the presence of virulent *R. solani* alone; B = disease severity exhibited on the hypocotyl in the presence of both BNR and virulent *R. solani*. Values followed by the same letter are not significantly different at $P = 0.05$ according to DMRT.
[5] * and ** represent significant values at $P = 0.05$ and $P = 0.01$ respectively, based on ANOVA.

and then challenged after 7 days by transferring to soil infested with the pathogen (Fig. 2). Control seedlings not inoculated with the hypovirulent BNR were highly susceptible to damping-off caused by *R. solani* (AG 4).

Table 5. Protection of cucumber seedlings against post-emergence damping-off caused by virulent *R. solani* C4 (AG 4) by non-pathogenic binucleate *Rhizoctonia* using the soil method[1]

Pre-incubation of BNR on hypocotyl[2] (days)	Seedling age (days)	Disease index[3]		Prot.[4] (%)
		Without BNR W7	With BNR W7	
0	14	4.0	3.8	5.0e
1	15	3.0	2.5*[5]	16.7d
2	16	3.4	2.2*	35.3c
4	18	4.0	1.8**	55.0b
7	21	4.0	0.5**	87.5a

[1] Cucumber seeds were sown in 16 g partially-sterilized soil in paper pot amended with 2% (w/w) ground barley grain colonized with BNR W7, transplanted to 100 g of partially-sterilized soil in plastic pots infested with the same amount of inoculum of virulent *R. solani*. Non-treated seedlings were transplanted to the same amount of uninfested partially-sterilized soil.
[2] Non-pathogenic BNR W7 remained on the seedling hypocotyl, kept in growth chamber (16 h photoperiod with $250/\mu E\,m^{-2}s^{-1}$) at 25 °C.
[3] Values in columns represent mean disease severity index; 0 = no lesion on the hypocotyl; 1 = one or two lesions <0.25 mm long; 2 = lesions <0.5 mm long covering <10% of the hypocotyl; 3 = lesions >1.0 mm long covering 10% to 100% of the hypocotyl; 4 = seedling is dead.
[4] Percent protection = $[(A-B)/A] \times 100$ in which A = disease severity exhibited on the seedling hypocotyl in the presence of virulent *R. solani* alone; B = disease severity exhibited on the hypocotyl in the presence of both BNR and virulent *R. solani*. Values followed by the same letter are not significantly different at $P = 0.05$ according to DMRT.
[5] * and ** represent significant values at $P = 0.05$ and $P = 0.01$ respectively, based on ANOVA.

Protection of cucumber seedlings against damping-off caused by R. solani *C4 (AG 4) as affected by pre-incubation time with hypovirulent BNR W7*

Pre-incubation periods of ≥ 0.5 days using BNR W7 mycelium inoculated on the hypocotyl of seedlings grown in water agar and similarly challenge – inoculated with *R. solani*, resulted in a significant reduction in disease severity among the BNR-amended seedlings as compared with the control (Table 3) ($P = 0.05$). At 0.5 day (12 h) pre-incubation of BNR, 41.7% protection was provided. Protection gradually increased with longer pre-incubation periods. The same trend was observed when seedling hypocotyls were pre-inoculated with mycelium and challenge – inoculated with pathogenic *R. solani* in soil (Table 4). Pre-incubation with the mycelium of BNR on seedling hypocotyl at 0.5 day (12 h) with pathogenic *R. solani* in soil resulted in 72.7% protection. When the pathogenic *R. solani* was applied after 1 and 2 days incubation of BNR W7, 86% protection was obtained and prolonging its incubation period to 4 days resulted to complete protection (100%). However, BNR W7 failed to provide protection to the seedlings when both isolates were applied simultaneously. Increasing pre-incubation time of the seedlings in BNR W7-infested soil (Table 5) from 0 to 7 days resulted in increase in protection from 5 to 87.5%.

Effect of non-pathogenic BNR isolates on growth of cucumber seedlings

Among the BNR isolates, only BNR W7 induced a highly significant plant growth promotion. It was expressed in increase in plant height (20.8%), fresh weight (24%) and dry weight (45.9%) as compared to control plants. Root length was not affected by inoculation of BNR W7.

Discussion

BNR isolates L2, W1 and W7 were previously selected [Villajuan-Abgona *et al.*, 1993] for further studies due to their high ability to protect cucumber seedlings effectively against damping-off caused by pathogenic *R. solani* AG 2-2 and AG 4. Protection efficiency of the same non-pathogenic isolates varied against different isolates of pathogenic *Rhizoctonia* (AG 2-2 and AG 4). These results support the findings reported by others [Burpee and Goulty, 1984; Cardoso and Echandi, 1987a; Herr, 1988]. Protection efficiency induced by the BNR may vary due to some uncontrollable factors [Burpee and Goulty, 1984; Cardoso and Echandi, 1987a]. The present study has indicated that non-pathogenic BNR isolates protect seedlings against pathogenic strains of *R. solani* belonging to

Table 6. Effect of non-pathogenic BNR isolates on growth of cucumber seedlings[1]

Isolates	Plant height[2] (cm)	Root length[3] (cm)	Fresh weight[4] (g)	Dry weight[4] (g)
BNR L2	5.17	14.97	1.06	0.12
BNR W1	5.44	14.08	1.23	0.14
BNR W7	5.97**[5]	22.34	1.65*	0.20**
Control (no NBR)	4.94	21.84	1.33	0.13
LSD				
($P = 0.01$)	0.58	5.22	0.30	0.05
($P = 0.05$)	0.81	9.61	0.41	0.07

[1] Data were taken on 21 day-old seedlings grown in growth chamber (16 h photoperiod with daytime illuminance of approx. $250/\mu E\ m^{-2}s^{-1}$) at 25 °C. Cucumber seeds were sown in 16 g partially-sterilized soil in paper pot amended with 2% (w/w) ground barley grain colonized with BNR W7, transplanted to 100 g partially-sterilized soil in plastic pots infested with the same amount of inoculum of virulent *R. solani*. Non-treated seedlings were transplanted to the same amount of uninfested partially-sterilized soil.
[2] Measurement was taken from the basal portion to the growing point.
[3] Measurement includes lateral roots and taproot attached to the basal stem.
[4] Measurement includes the whole plant (leaves, stem and roots).
[5] * and ** represent significant values at $P = 0.05$ and $P = 0.01$, respectively.

different AGs. These results support those obtained by Ichielevich-Auster *et al.* [1985] in which 76 to 94% protection was provided to cotton, radish and wheat by avirulent isolate 521 against *R. solani* and *R. zeae*, suggesting the irrelevance of cytoplasmic compatibility and lack of specificity in protection against different AGs among *Rhizoctonia* spp.

The three inoculation methods (water agar, soil, and a combination of the two) were useful for evaluating the potential of the non-pathogenic BNR as biocontrol agents. The methods established the potential of the three binucleate *Rhizoctonia* isolates to suppress damping-off on cucumber caused by *R. solani*. Using the water agar method, aseptic inoculation of the mycelium of BNR and the pathogen at the same site on the hypocotyl showed the efficacy of the BNR isolates in suppression of the disease incited by the pathogen (Table 2). Although the BNR-treated plants were under high disease pressure in the different assay systems, protection was still provided by the BNR isolates to prevent the pathogen to cause the disease. In the water agar method, although the pathogen was directly applied to the hypocotyl surface, 50% protection was still provided by the BNR. Increased protection upon addition of soil suggested that, the soil might have reduced the disease pressure incited by the pathogen. The increased protection provided by pre-incubation of BNR isolates 2 days before challenge-inoculation of the pathogen suggests a role for prior colonization of the hypocotyl surface and hyphal lysis of *R. solani* in disease suppression. This was confirmed by the microscopic observation done on the hypocotyl surface which showed physiological changes on the hyphae of both isolates (data not shown). However, when pairing was done among BNR isolates and *R. solani* in culture in the absence of the host, hyphal interaction was not observed, and these findings support earlier reported observations [Burpee and Goulty, 1984; Cardoso and Echandi, 1987b; Ichielevich-Auster *et al.*, 1985].

Pre-incubation of the non-pathogenic BNR or delayed application of the pathogenic *Rhizoctonia* was tested due to the failure of the BNR to protect the seedlings when both were applied simultaneously. In previous studies, *Rhizoctonia* root rot of snap bean was significantly reduced after simultaneous inoculation of *R. solani* AG 4 [Cardoso and Echandi, 1987b]. Similar results were obtained by Harris *et al.* [1994] when bedding plants were protected against *Rhizoctonia* damping-off when binucleate *Rhizoctonia* was applied together with *R. solani* AG 4 and AG 8. In *Persea* species, lesion development was significantly reduced at a site of non-pathogen inoculation if stems were challenge-inoculated after 48 h with *P. cinnamomi* and *P. citricola* [Dolan *et al.*, 1986]. Several reasons may account for the differences in achieving protection from the biocontrol agent obtained by different researchers and the reason might include the differences in the mechanism of disease suppression involved in the varying system. Herr

[1988] showed disease control in 8 days post-planting application of a binucleate *Rhizoctonia* to sugarbeet, indicating more than simple persistence of the biological control agent in soil until *R. solani* infestation, was likely to be involved. The author surmised that this enhanced biocontrol effect may have been a consequence of improved establishment of the biocontrol agent on the plant surface, specifically on potential infection courts. A similar result was obtained in a study conducted by Cardoso and Echandi [1987b] in which treatment of bean seedlings with BNR, 24 h before inoculation with *R. solani* inhibited formation of infection cushions by *R. solani* and this suggested a BNR-induced metabolic response by the seedling which suppresses *R. solani* at the infection site. This might also be a possible explanation for the gradual increase in the protection provided by BNR W7 to the seedlings using the three methods, and final inhibition of the disease after thorough colonization of the host surface.

Results of the present study support previous reports where a non-pathogenic *R. solani* (No. 521, AG 4) improved plant growth in a variety of crops [Sneh *et al.*, 1986], and two non-pathogenic binucleate *Rhizoctonia* isolates increased dry weights of *Capsicum* shoots [Harris *et al.*, 1993].

The mechanisms involved in the disease suppression in the present study have not yet been studied. Histological study on the extent of host tissue penetration by the non-pathogenic BNR and subsequent interactions with the challenge pathogen are needed to fully assess the mechanism of protection.

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