Suppression of Damping-Off Caused by *Rhizoctonia* Species by a Nonpathogenic Isolate of *R. solani*

Meira Ichielevich-Auster, Baruch Sneh, Yigal Koltin, and Isaac Barash

ABSTRACT

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Among 107 isolates of *Rhizoctonia* spp. obtained from soil samples in Israel, 32 isolates were nonpathogenic to eleven hosts. The pathogenic and nonpathogenic isolates represented anastomosis groups (AG) 1, 2, 3, 4, 5, and 6 of *R. solani*, two groups of *R. zeae*, and three groups of binucleate *Rhizoctonia* spp. (AG-A, AG-F, and AG-K). A nonpathogenic isolate of *R. solani* (AG-4) suppressed damping-off caused in cotton, radish, and wheat seedlings by virulent isolates of *R. solani* and *R. zeae* by 76–94%.

The phenotypic diversity of isolates of *Rhizoctonia* spp. in soil is well documented (14,27). The characteristic features of *R. solani* Kühn [*Thanatephorus cucumeris* (Frank) Donk] include multinucleate hyphal cells and anastomosis groups (AG), seven of which have been reported (21–23,25–27). *R. zeae* Voorhees also contains multinucleate hyphae but differs from *R. solani* in sclerotial shape and color (24,31). The binucleate *Rhizoctonia* spp. contain representatives of *Ceratobasidium* spp. and have been reported under various species such as *R. fragariae* Husein and McKeen, *R. candida* Yamamoto, *R. oryza-sativa* Sadawa and *Corticium gramineum* Matsuura (26). Isolates that have been observed not to anastomose with any members of the AGs in *R. solani* or binucleate *Rhizoctonia* spp. have also been reported (3,26). The significance of AGs as independent genetic units is supported by studies on the divergence of DNA base composition (21).

The genus *Rhizoctonia* has a wide host range and isolates may vary in their virulence on different hosts (27). Kuninaga et al (23) detected a high incidence of isolates of *R. solani* (AG-6) in noncultivated soils in Japan. Isolates of this group were only marginally virulent, whereas isolates of AG-1, -2, and -5 exhibited a range from weakly to highly virulent. AG-3 isolates were reported to infect potatoes primarily (12), and AG-4 was common in the field and was highly virulent on a variety of hosts (27).

Reports by Castanho et al (9–11) suggested that biological control of *Rhizoctonia* spp. may be achieved through cytoplasmic transmission of a virulence-suppressive factor similar to the factors demonstrated for *Endothia parasitica* (Murr.) Anderson (1,20). However, the potential use of native nonpathogenic strains of *R. solani* for biological control has not been widely investigated. Recently, Burpee and Goulty (6) reported suppression of brown patch disease of creeping bentgrass by a nonpathogenic binucleate *Rhizoctonia* sp.

The objectives of the present work were to investigate the prevalence of nonpathogenic strains of *Rhizoctonia* in soil samples obtained from different locations in Israel, and to evaluate their potential use for biological control.

MATERIALS AND METHODS

Isolates and cultures. Strains of *R. solani*, *R. zeae*, and binucleate *Rhizoctonia* spp. were isolated from soil samples collected from 26 locations in Israel, according to the procedure described by Boosalis and Scharen (4). Cultures were maintained and stored at room temperature on potato-dextrose agar (PDA) and in dry soil cultures containing 4% (w/w) wheat bran (8). The cultures were characterized as *R. solani* by observing typical morphological features microscopically and determining the multinucleate nature by hematoxylin staining (27). Isolates with two nuclei per cell were characterized as binucleate *Rhizoctonia* spp. Isolates of *R. zeae* were identified according to the shape and color of the microsclerotia on PDA (24,31).

Anastomosis grouping. Anastomosis was tested by pairing isolates with representative testers of AGs 1 to 7 and AG-BI that were received from A. Ogoshi (Japan) and E. E. Butler (USA). The tests were performed on 2% tap water agar in 9-cm-diameter plates (28). Each pair of isolates was tested in a separate plate. Mycelial transfers from the margins of actively expanding young cultures on PDA were plated 2–4 cm apart in each plate. The cultures were incubated at room temperature until the advancing hyphae made contact and slightly overlapped. A 2- to 3-cm portion of the overlapped area was removed, mounted on a microscope slide, stained with 0.01% cotton blue and scanned for hyphal fusion. Cell-wall and cytoplasmic fusion were confirmed at ×500, as described by Parmeter et al (28).

Pathogenicity tests. Pathogenic plants were evaluated as hosts for *Rhizoctonia* spp.: radish (*Raphanus sativus* L.), tomato (*Lycopersicon esculentum* Mill.), onion (*Allium cepa* L.), carrot (*Daucus carota* L. var. *sativa* DC.), lettuce (*Lactuca sativa* L.), cucumber (*Cucumis sativum* L.), cantaloupe (*Cucumis melo* L.), wheat (*Triticum aestivum* L.), and barley (*Hordeum sativum* L.). Isolates were grown on yeast extract (1%)-peptone (0.5%)-dextrose (0.5%) agar (1.7%) (YEDA), for 2 days at 26 ± 2 C. Agar disks (4-mm diameter) from the margin of the culture were placed in the center of 2% tap water agar plates and incubated for 2 days at 26 ± 2 C, and five seeds were then placed around the edge of the colony. All seeds were surface-disinfected in 1% sodium hypochlorite for 10 min, rinsed with sterile distilled water and aseptically blotted. The plates were incubated at 26 ± 2 C under fluorescent lighting with a photoperiod of 12 hr for 7–10 days before pathogenicity was recorded. Disease severity was rated on a scale of 0–5, based on the relative size of a necrotic area on the hypocotyl as follows: 0 = no disease, 1 = 1–10%, 2 = 11–30%, 3 = 31–50%, 4 = 51–80%,

and 5 = the entire hypocotyl infected. The average rating (disease severity index) was determined from tests with 45 seedlings per isolate. Isolates that produced a disease severity index between 0 and 1 were considered as essentially nonpathogenic. The pathogenicity test for cotton (*Gossypium hirsutum* L. 'SJ-2'), and bean (*Phaseolus vulgaris* L. Brittle Wax) were carried out as follows: seeds were germinated on moist filter paper for 3 days at 25 C. The seedlings were washed, surface disinfested in 1% NaClO solution for 1 min, rinsed with sterile distilled water, and placed on sterile moist filter paper in 9-cm-diameter plates. Hypocotyls were inoculated with 4-mm-diameter mycelial plugs taken from 2-day-old cultures. Disease severity was evaluated after 48 hr of incubation at 26 ± 2 C under continuous light. All the isolates were initially tested for pathogenicity on six different host plants, including radish, tomato, carrot, cotton, bean, and onion. To further characterize the virulence of the isolates, 43 of them, including all the nonpathogenic ones, were tested on five additional hosts: lettuce, cucumber, cantaloupe, wheat, and barley.

Isolate 521 (AG-4 - *R. solani*), which was nonpathogenic to all the hosts, was also tested in the field for 75 days on radish, cotton, carrot, lettuce, and potato. Soil temperatures were 26–32 C by day and 20–25 C at night. Inoculum was prepared on autoclaved presoaked wheat grain. Fifty seeds were placed per meter furrow at sowing time. Plants remained symptomless throughout the experiment.

Evaluation of disease suppression induced by nonpathogenic strains. Cultures of virulent or nonpathogenic strains of *Rhizoctonia* spp. were grown on medium A (29) in Roux bottles for 7 days at 27 C. The mycelial mats were rinsed with water, blotted dry, and weighed. This material was then blended for 15 sec with 200 ml of sterile water. The suspension made from one bottle (containing 4 g of mycelium) was thoroughly mixed with 4 kg of soil mix (raw sandy loam, vermiculite, and peat at 1:2:2, v/v) and placed in pots (15-cm diameter × 10 cm high). Uninfested soil mix was used as a control. Pots were incubated in a growth chamber at 26 ± 2 C with a 12-hr photoperiod at 5,200 lux. After 5 days of incubation, either cotton (10 seeds per pot), radish (10 seeds per pot), or wheat (45 seeds per pot) were sown. Three days after the seeds were sown in soil infested with the nonpathogenic strain, a mycelial suspension of a virulent strain (as a challenge pathogen) was either evenly spread on the soil, or mixed with a soil mix (1 g mycelium per kilogram of soil), to which the 3-day-old seedlings were transferred (five pots per treatment). All experiments were repeated at least three times. Damping-off incidence was recorded during 14 days.

Competition for nutrients on the host between the nonpathogenic and the virulent strains. Cotton seedlings were grown for 3 days in a soil mix infested with the nonpathogenic isolate 521 and transferred to soil infested with virulent isolate 82. Nutrients in the form of medium A (34) or an extract of cotton hypocotyls (20 g of three-day-old cotton hypocotyls blended for 1 min in 50 ml of H_2O and passed through four layers of cheesecloth) were added directly to hypocotyls in soil (0.5 ml per seedling) one, two, or three times per day for 3 days following transfer (30 plants per pot, three pots per treatment). Damping-off incidence was recorded during the following 14 days.

Possible suppression of diseases caused by other pathogens. *Pythium aphanidermatum* (Edson) Fitzp. on cucumber, *Macrophomina phasolina* (Tassi) Goid. on melon, *Sclerotium rolfsii* Sacc. on bean, *Fusiarum oxysporum* (Schlecht.) f. sp.

TABLE 1. Protection by nonpathogenic isolate 521 (AG-4) of *Rhizoctonia solani* of cotton and radish seedlings from damping-off caused by virulent isolates of *Rhizoctonia* spp.

Virulent isolate[c]	AG[d]	Symptomless plants[a] (%)							
		Cotton[b]				Radish[b]			
		Isolate 521		Protection[e] (%)	LSD (P = 0.01)	Isolate 521		Protection[e] (%)	LSD (P = 0.01)
		Not present	Present			Not present	Present		
Uninfested	—	100	100	—	—	100	100	—	—
13	4	—	—	—	—	0.0	76.3	76.3	3.76
56	4	0.0	78.7	78.7	4.42	—	—	—	—
82	4	0.0	81.5	81.5	4.55	0.0	94.2	94.2	4.23
363	1	13.8	86.5	84.5	9.88	14.7	82.1	79.0	4.57

[a] Plants with no damping-off symptoms.
[b] Seeds were sown in soil infested with the nonpathogenic strain (isolate 521) (1 g/kg soil). Three days after sowing, a suspension of the virulent strain (1 g/kg soil) was evenly spread on the soil surface (25 seeds per pot, five pots per treatment). Damping-off incidence was recorded during 14 days after addition of the virulent strain.
[c] Isolates 13, 56, and 82 = *R. solani*, and isolate 363 = *R. zeae*.
[d] Anastomosis group.
[e] Percent protection = $[(C - B)/(A - B)] \times 100$ in which A = percentage of symptomless plants in the uninfested control; B = symptomless plants in soil infested with the virulent strain, and C = symptomless plants in soil infested with the avirulent strain and then challenged with the virulent isolate.

TABLE 2. Protection by nonpathogenic isolates of *Rhizoctonia solani* of wheat seedlings from damping-off caused by a virulent isolate of *R. solani*[w]

Virulent isolate 13	Symptomless plants[x] (%)					
	Nonpathogenic isolate 521			Pathogenic isolate 82		
	Not present	Present	Protection[y] (%)	Not present	Present	Protection[y] (%)
Not present	96.7 a[z]	97.5 a	—	96.7 a	81.7 b	—
Present	45.0 b	93.3 b	93.4	45.0 d	55.8 c	20.9

[w] Seeds were sown in noninfested soil, soil infested with nonpathogenic isolate 521, and soil infested with strain 82 (weakly virulent to wheat, but virulent to other crops) (1 g mycelium/kg soil). Seedlings were transferred 3 days after sowing (30 seedlings per pot, five pots per treatment), to noninfested soil or to soil infested with virulent isolate 13 (1 g mycelium per kilogram of soil). Damping-off was recorded during 21 days after transfer. When seeds were sown in soil infested with the virulent strain, only 12.5% of the plants remained free of damping-off.
[x] Plants with no damping-off symptoms.
[y] Percent protection = $[(C - B)/(A - B)] \times 100$ in which A = percentage of healthy plants in the noninfested control, B = percentage of symptomless plants in soil infested with the virulent strain, and C = percentage of symptomless plants in soil infested with the avirulent strain and then challenged with the virulent strain.
[z] Numbers for each avirulent isolate followed by the same letter are not significantly different (P = 0.01) by two-way ANOVA and MSR.

lycopersici Synder & Hansen, *F. oxysporum* f. sp. *radicis*, and *Verticillium dahliae* Kleb. on tomato, were tested against nonpathogenic isolate 521 for disease suppression, as outlined above. In addition, suspensions (10^5 spores per milliliter) of airborne pathogens, *Oidium* sp. on carrot and *Alternaria macrospora* Zimm. on cotton, were sprayed on 3-wk-old seedlings, grown in soil mix with the nonpathogenic *Rhizoctonia* (521). Plants were covered with plastic bags for 24 hr and leaf spots were observed during the following 3 wk.

Statistical analysis. One-way ANOVA and LSD for data in Table 1, two-way analysis and MSR for data in Table 2, and the nonparametric method of Kruskal-Wallis for data in Table 3 (30) were performed.

RESULTS

Anastomosis grouping and pathogenicity of isolates. Representatives of AGs-1, -2, -3, -4, -5, and -6 of *R. solani* were identified among 107 isolates. Strains of the multinucleate *R. zeae* and the binucleate *Rhizoctonia* sp. (AG-A, -F, and -K according to Ogoshi, 26), were also found. Thirty-two isolates of *Rhizoctonia* spp. were essentially nonpathogenic to all of the eleven hosts tested (19).

Attempts to transfer a hypovirulent factor from isolate 521 (AG-4) to the virulent isolate 82 by hyphal anastomosis, in a manner similar to that used for *Endothia parasitica* (1,32) or as suggested by Castanho et al (10), failed to yield a hypovirulent isolate 82. However, hyphal-tip isolations from the virulent strain 82 (200 tips) of *R. solani* (AG-4) occasionally (1%) yielded nonpathogenic cultures. Those cultures were weak and did not survive after six or seven additional transfers on PDA. Similar attempts to isolate virulent isolates by hyphal tips from the nonpathogenic isolate 521 (200 tips) failed to yield virulent cultures (15).

Radish, carrot, lettuce, cotton, and potato plants grown in soil in the field infested with the nonpathogenic isolate 521 remained symptomless throughout the growing period while the fungus was isolated routinely from hypocotyls and roots.

Disease suppression experiments. Preliminary experiments indicated that isolate 521 (AG-4) provided the most efficient protection from damping-off among the eight avirulent strains tested. Results summarized in Table 1 and Fig. 1 indicate that protection of cotton and radish seedlings, infested with the nonpathogenic isolate 521, against three virulent isolates of *R.*

solani was between 79 and 94%. Protection of 79–84% was also obtained when the seedlings were challenged by inoculation with a virulent isolate of *R. zeae*. Protection induced by isolate 521 was also achieved after the seedlings infected with the avirulent strain were transferred into pots containing soil infested with the challenge virulent strains (Tables 2 and 3). The nonpathogenic isolate was reisolated routinely on tap water agar from seedling hypocotyls. Isolate 82 of *R. solani*, which was virulent to carrot, radish, bean, lettuce, cucumber, onion, and cotton (20) but nonpathogenic to wheat, was compared with strain 521 for protecting wheat seedlings. Results summarized in Table 2 indicate that the nonpathogenic isolate 521 was a much more effective protector in wheat (93%) than was isolate 82 (21%).

The nonpathogenic isolate 521 failed to induce protection to the following hosts challenged by their respective pathogens: *Macrophomina phaseolina* on melon; *Pythium aphanidermatum* on cucumbers; *Sclerotium rolfsii* on beans; *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis* on tomato; *Oidium* sp. on carrot; and *Alternaria macrospora* on cotton leaves.

Competition for nutrients. The addition of nutrients did not reduce protection provided by the nonpathogenic isolate (Table 3).

DISCUSSION

These results indicate that the natural population of *Rhizoctonia* consists of virulent and avirulent strains and that avirulent strains are not rare. Most of the AGs contained nonpathogenic isolates.

Expression of virulence by any pathogen population is generally a continuum between two extremes; high virulence and avirulence. At some point along the continuum, however, hypovirulence ends and virulence begins (32). Low virulent to avirulent isolates are considered hypovirulent. Hypovirulence may be nontransmissible or transmissible, as in the case of *Endothia parasitica* (32). However, the term "hypovirulence" is sometimes limited to the the transmissible hypovirulence (13). The avirulent isolate in the present study induced no disease symptoms, even in extreme conditions. It may, therefore, be considered to be a nontransmissible hypovirulent isolate.

The results of tests conducted to verify whether nonpathogenic isolates can provide protection against virulent isolates of *Rhizoctonia* spp. have shown that strong protection was induced by the avirulent isolate 521 of *R. solani* on such diverse plant species as cotton, radish, and wheat (Tables 1 and 3). These results suggest its potential use for biological control. The protection phenomenon was demonstrated in plants grown in soil infested by the nonpathogenic isolate and then transferred into soil infested with the virulent isolate. This suggests that the avirulent isolate was acting in or on the plant. Moreover, protection could be obtained

TABLE 3. Protection by the nonpathogenic isolate 521 of *Rhizoctonia solani* of cotton seedlings from damping-off caused by virulent isolate 82 of *R. solani* as affected by supplemental nutrients[w]

Planted in soil with 521	Transferred to soil with 82	Protection (%) and nutrient supplement[x]		
		None	Medium A	Hypocotyl extract
None	None	—[y]	—	—
None	Nonpathogenic	—	—	—
None	Virulent	0 a[z]	0 a	0 a
Nonpathogenic	Nonpathogenic	—	—	—
Nonpathogenic	Virulent	93 b	90 b	93 b

[w] Surface-disinfested cotton seeds were planted in either untreated soil or soil mixed with 1 g of mycelium of the nonpathogenic isolate per kilogram of soil. Three days later, all seedlings were transferred to soils treated as indicated in the second column. Disease incidence was recorded during 14 days after transfer. Numbers represent percent protection, calculated as explained in Tables 1 and 2. The experiment was repeated twice with similar results (30 plants per pot, three pots per treatment).

[x] Aliquots of 0.5 ml of either medium A or an extract of 3-day-old cotton seedlings added to hypocotyls of each plant (one, two, or three times per day) during each of the 3 days following seedling transfer.

[y] All seedlings in the uninoculated soil, or inoculated with only the nonpathogenic isolate, remained symptomless throughout the experimental period.

[z] Numbers followed by the same letter are not significantly different ($P = 0.001$) by the Kruskal-Wallis test (30).
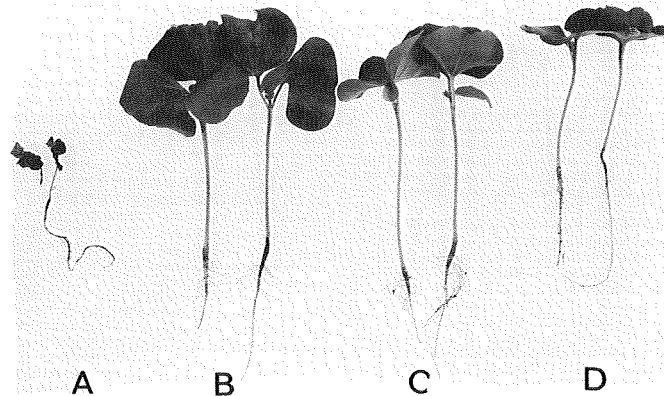

Fig. 1. Protection of cotton seedlings induced by nonpathogenic isolate 521 of *Rhizoctonia solani* against damping-off caused by virulent strain 56 of *R. solani*. Left to right: **A**, inoculated with the virulent isolate; **B**, inoculated with the nonpathogenic isolate and 3 days later challenged with the virulent isolate; **C**, inoculated with the nonpathogenic isolate; and **D**, uninoculated control.

between the strains of *R. solani* and *R. zeae* (Table 1), which suggests that cytoplasmic compatibility is not required for protection. These results emphasize the lack of specificity among *Rhizoctonia* spp. in protection against damping-off. Similarly, protection against Fusarium wilt in tomatoes has been induced by a different forma specialis of *F. oxysporum* (35). However, resistance to *Fusarium* spp. was also induced by several root or foliar pathogens (17). Our results indicated that *Rhizoctonia* could not provide protection against other pathogens.

Recent studies suggest that induced immunity to plant disease by cross-protection may involve elicitation of phytoalexins, lignification or suberization (17,18,29,35). Such responses of the plant may also interfere with the pectolytic enzyme activity which plays an essential role in the pathogenicity of *Rhizoctonia* spp. (33). The stability of avirulence in certain strains of *Rhizoctonia* spp., as well as the high efficiency of isolate 521 in inducing protection as compared to other isolates, could involve, among other mechanisms, an elicitation of natural plant-resistance responses.

A major question is whether hypovirulence in *Rhizoctonia* spp. depends on host-pathogen interactions or can be preserved by the fungal strain regardless of the host plant. The fact that many of the virulent strains were also hypovirulent to some host plants suggests that hypovirulence may depend on host-pathogen interactions. However, existence of strains of *Rhizoctonia* in which hypovirulence is governed merely by properties of the fungal strain itself cannot be excluded in view of the saprophytic nature and genetic diversity of *Rhizoctonia* spp. (27). The stability of some nonpathogenic strains is indicated by the fact that no virulent strains were recovered from hyphal tips of the nonpathogenic isolate 521, whereas nonpathogenic isolates were recovered from hyphal tips of the virulent strain 82. Those nonpathogenic cultures were weak and did not survive after six to seven transfers. The hypovirulence could not be transmitted from strain 521 to strain 82. The proof for stability of nonpathogenic strains is crucial for cases in which such isolates will be considered for use in biological control.

A degenerative disease was detected in some isolates of *R. solani* by Castanho and Butler (9). This disease was transmitted cytoplasmically and reduced the severity of the disease in greenhouse tests (10). The degenerative effect was suggested to be related to the multiple forms of dsRNA which were present in the diseased hypovirulent isolates, but absent in the virulent isolates (11). In contrast to the senescent hypovirulent isolates of *R. solani* described by Castanho et al (9–11), the native nonpathogenic isolates described in the present study exhibit a normal growth rate that does not differ significantly from virulent isolates. Therefore, they might be fit to compete with virulent isolates under natural conditions. In addition, dsRNA and virus particles were detected in the virulent strain 82, and neither dsRNA nor virus particles were detected in the nonpathogenic isolate 521 (15). These characteristics indicate that the nature of avirulence as well as the suppression of virulence in the two reported cases are distinct.

Recently, Burpee and Goulty (6) reported that a nonpathogenic binucleate *Rhizoctonia* suppressed brown patch disease caused on leaves of creeping bentgrass by a pathogenic binucleate *Rhizoctonia* sp. They indicated that in the absence of hyperparasitic (as was shown for *Rhizoctonia* spp. in certain cases [6,7]) and antibiotic effects, competition for nutrients or host-induced resistance would appear to be plausible mechanisms of suppression. Although no experimental attempts were made to indicate positively which of the mechanisms play the role in disease suppression, they suggested that it is likely to be competition for nutrients provided by the leaf exudates. This was supported by citing previous publications that indicate the role of host exudates on infection cushion formation by *Rhizoctonia* sp. (14), and on antagonism to *Botrytis cinerea* on leaf surfaces by bacteria and yeasts (5,16).

In the present work, no hyperparasitic or antibiotic effects were observed. On the contrary, isolates 521 and 82 were cytoplasmically compatible and formed via anastomosis a new strain in which two different characteristics from the two parental hyphae were combined (15). The ability of the limiting factor of an added candidate to nullify or ameliorate inhibition or suppression, provides the evidence for the operation of the factor in competition (2). Repeated addition of external nutrients to the hosts' hypocotyls did not reduce the protective effect (Table 3). This indicates that the protection probably was not induced by competition for nutrients. Therefore, the plausible mechanism is more likely to be induced resistance or cross-protection. Further work is needed to determine positively the nature of disease suppression induced by the nonpathogenic isolate of *R. solani*.

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