HYPOVIRULENT *RHIZOCTONIA* SPP. ISOLATES FROM NEW ZEALAND SOILS PROTECT RADISH SEEDLINGS AGAINST DAMPING-OFF CAUSED BY *R. SOLANI*

B. SNEH[1], E. YAMOAH[2] and A. STEWART[2]

[1]*Department of Plant Sciences and Institute for Nature Conservation Research, The George S. Wise Faculty of Life Sciences, Tel-Aviv University Ramat Aviv, Tel-Aviv, Israel 69978*
[2]*National Centre for Advanced Bio-Protection Technologies, P.O. Box 84, Lincoln University*

Corresponding author: yamoahe@lincoln.ac.nz

ABSTRACT

Of the 206 *Rhizoctonia* spp. isolates obtained from 135 soil samples collected from different fields in the North and South Islands of New Zealand, 55% were pathogenic on radish (*Raphanus sativus* cv. Rex) seedlings. Only 27% of the isolates that were strongly pathogenic on radish were also pathogenic on ryegrass (*Lolium perenne*). While 13 of the 92 hypovirulent isolates provided >50% protection to radish seedlings against damping-off caused by *Rhizoctonia solani* in a screening experiment, only three provided >50% protection in the final, more detailed experiment. The best protective isolates, R85-10 and R30-8, consistently protected approximately 70% of radish seedlings in at least two separate experiments. There was no correlation between the growth rates of the hypovirulent *Rhizoctonia* spp. isolates and their percentage protection of radish seedlings against damping-off.
Keywords: *Rhizoctonia*, hypovirulent, damping-off, radish, biocontrol.

INTRODUCTION

Isolates of *Rhizoctonia* spp. differ in their ability to incite disease symptoms, or degree of virulence to their respective hosts. *Rhizoctonia* spp. isolates may be categorised according to their virulence over a continuum ranging from strongly virulent to avirulent isolates. Low virulent to avirulent isolates are considered hypovirulent. At some point along this continuum, hypovirulence ends and virulence begins (Van Alfen 1982). However, care must be taken when determining hypovirulence since low symptom severity can also be an indication of a plant induced resistance reaction, rather than actual disease.

Except for their inability to cause appreciable disease symptoms, hypovirulent isolates of the same plant pathogenic species are usually similar to virulent isolates in their ability and competence to colonise and occupy the same ecological niches on plant surfaces. Hence, some have the potential to compete successfully against their respective pathogens on host infection sites (Sneh 1990) or for nutrients, such as carbon and iron (Lemanceau & Alabouvette 1993). They may also protect the colonised plants against diseases caused by virulent isolates of the same species via other mechanisms, such as induction of plant resistance (Sneh & Ichielevich-Auster 1998), transmission of dsRNA mycoviruses (Van Alfen 1982) and mutating genes of pathogens to render them non-pathogenic epiphytic mutualists (Freeman & Rodriguez 1993).

Since 1984, there have been an increasing number of reports on the potential of non-pathogenic binucleate *Rhizoctonia* isolates of several different anastomosis groups (AGs) to protect seedlings of a wide variety of hosts against infection by virulent *Rhizoctonia* spp., belonging to different AGs, from Israel (Ichielevich-Auster et al. 1985b), USA

(Sneh & Ichielevich-Auster 1998), Canada (Burpee & Goulty 1984), Japan (Vilajuan-Abgona et al. 1996) and Australia (Harris et al. 1993a; Sneh 1998). Certain isolates also protected against *Pythium ultimum* var. *sporangiiferum* (Harris et al. 1993a), *P. aphanidermatum* and *Pseudomonas syringae* pv. *lachrymans* (Sneh & Ichielevich-Auster 1998). A few of these isolates also increased plant growth, which was expressed in increased yields (Sneh et al. 1986; Harris et al. 1993a) and also increased drought tolerance (Sneh & Ichielevich-Auster 1998).

Some effective non-pathogenic *Rhizoctonia* isolates grow more slowly than virulent ones (Ichielevich-Auster et al. 1985a). This may be a disadvantage to their use as biocontrol agents. To overcome this, non-pathogenic *Rhizoctonia* isolates should be applied in colonised cereal grain, close to the sown seeds. This rich food base increases their inoculum potential and provides them with a significant competitive advantage over the naturally existing virulent *Rhizoctonia* spp. propagules, which are frequently associated with relatively exhausted plant debris in soil (Sneh et al. 1986; Harris et al. 1993a). Thus, non-pathogenic *Rhizoctonia* hyphae can grow out vigorously to become the pioneer coloniser of the germinating seeds, roots and hypocotyls and form dense mycelial mats earlier, and at a higher inoculum potential than the virulent ones. The earlier the colonisation with the non-pathogenic isolates, the better the protection obtained (Harris et al. 1993b).

This study was undertaken to isolate *Rhizoctonia* spp. from soil samples collected throughout New Zealand, screen them for pathogenicity on radish and evaluate the hypovirulent isolates for their ability to protect radish seedlings against damping-off caused by virulent *R. solani*.

MATERIALS AND METHODS

Isolation of non-pathogenic *Rhizoctonia* spp.

Soil samples were collected from 135 sites of the North and South Islands of New Zealand. *Rhizoctonia* spp. were isolated from the soil samples using the plant debris particles method (Boosalis & Scharen 1959). Soil samples (100 g) were suspended in 2 litres of tap water in an Erlenmeyer flask by thoroughly shaking the suspension and letting the soil particles settle down for several minutes. The plant debris particles floating on the surface were collected on a 60 mm mesh screen, washed with tap water and blotted dry on paper towel. Single particles of plant debris were plated on Tap Water Agar (TWA) plates containing 250 μg/ml chloramphenicol (six or more particles/plate). The plates were incubated at 25°C overnight. The hyphae growing from the particles were examined under a stereo microscope at low magnification for typical hyphal growth of *Rhizoctonia*. The typical growth of the hyphae includes branching near the distal septum of the cells, constriction of hyphae and formation of septa in a short distance from the origin of the hyphal branching (Sneh et al. 1991). *Rhizoctonia* spp. do not produce asexual spores. Hyphal tips were transferred to Potato Dextrose Agar (PDA) (Difco) plates containing 250 μg/ml chloramphenicol. The plates were incubated at 25°C for 2-3 days and pure cultures of *Rhizoctonia* were transferred to PDA plates.

Inoculum preparation and storage of *Rhizoctonia* spp.

Wheat grains (200 ml) were placed in a 1 litre Erlenmeyer flask with 600 ml tap water containing 250 μg/ml chloramphenicol. The water was heated to boiling and the flask was removed from the heater and left to settle for 10 min. The grains were then washed three times with tap water and the excess water was carefully strained to prevent the grains from sticking together in a clump during autoclaving. The grains were autoclaved for 1 h on each of 2 consecutive days. To replace water evaporated during the process, sterile water was added, mixed and strained prior to the second autoclaving. The sterile grains were aseptically placed in Petri dishes and inoculated with mycelial discs taken from the margins of *Rhizoctonia* cultures growing on PDA plates. The grains were incubated for 7-10 days at 25°C. During this period, the grains were shaken daily to prevent them from sticking together due to hyphal growth. The colonised grains were aseptically dried in a laminar flow hood for about 48 h and stored in cryovials at -20°C.

Pathogenicity testing

Colonised grains or agar disks (7-10 mm) taken from the margins of 3 day-old cultures growing on PDA were transferred to the centre of TWA plates and incubated for 3 days. Six pre-germinated seeds of radish (*Raphanus sativus* cv. Rex) and ryegrass (*Lolium perenne*) were placed on the margins of the *Rhizoctonia* colonies in separate plates. The pathogenicity of the isolates was evaluated after a further 6 and 14 days for radish and perennial ryegrass, respectively at 25°C. Disease severity was assessed visually and scored using a disease severity index (DSI) ranging from 0-5, where 0-1=<1 mm lesion; 2= 1-3 mm; 3=3-5 mm; 4=5-7 mm; 5=>7 mm or dead plant. Isolates causing no symptoms or very mild symptoms (0-0.3 DSI) were considered avirulent; isolates causing mild symptoms (0.4-1.9 DSI) were considered low virulent; isolates causing moderate symptoms (2-2.9 DSI) were considered moderately virulent; isolates causing severe symptoms (3-3.9 DSI) were considered virulent and isolates causing very severe symptoms (4-5 DSI) were considered strongly virulent. Avirulent and low virulent isolates were considered hypovirulent. The hypovirulent isolates were grown on TWA for 3-6 days and the radial growth was measured daily between day 2 and day 6. The growth rate was expressed in mm/day.

Testing of hypovirulent *Rhizoctonia* spp.

Protection experiments were set up to identify hypovirulent *Rhizoctonia* spp. capable of protecting radish seedlings against virulent *R. solani* (R73-13b). Pots (0.8 litre) filled with soil mix were watered with 4 mg/ml Ridomil (80 g/kg metalaxyl CIBA-GEIGY Ltd, Basle) suspension (to inhibit *Pythium*). One grain colonised with a hypovirulent *Rhizoctonia* isolate was placed in each of eight planting holes per pot. One 2-day-old radish seedling was then planted in each hole. The seedlings were watered regularly with distilled water and incubated in a growth room at 25°C and 660 μmols/m^2/s light at 16 h photoperiod. Two days after planting, the seedlings were challenged with the pathogen. Four grains colonised with the virulent *R. solani* were added to each pot (except for the non-treated control) at an equal distance (2 cm) from each seedling. The number of healthy plants was recorded 14 days later and the percentage protection was calculated using the relationship:

Protection (%) = [(C-B)/(A-B)] x 100

where: A=symptomless plants (i.e. plants with no visible lesion) in the untreated control, B=symptomless plants challenged with the pathogen and C=symptomless plants in soil infested with the hypovirulent isolate and challenged with the pathogen.

Isolates which protected >50% of the challenged seedlings were selected for more detailed experimentation. All experiments were conducted in a randomised complete block design with six replicates. Results were analysed by analysis of variance and mean separation was based on least significant difference (LSD) tests at the P<0.05 level.

RESULTS

Pathogenicity tests

A total of 206 *Rhizoctonia* spp. isolates were obtained from 135 soil samples. Ninety-two isolates were hypovirulent on radish seedlings, with 36 of these isolates being avirulent (representing 17% of the total number of *Rhizoctonia* spp. isolates) and 56 (28%) being low virulent. The remaining 114 isolates were pathogenic (i.e. 39 moderately virulent, 22 virulent and 53 strongly virulent). Thus, approximately 45% of the isolates obtained from soils in New Zealand were hypovirulent (DSI 0-1.9) while 55% were pathogenic (DSI 2-5) on radish.

Fifteen strongly virulent and 50 hypovirulent isolates were selected for further testing with perennial ryegrass. Seven days after the perennial ryegrass seedlings were placed on the TWA plates with the *Rhizoctonia* spp. isolates, only one isolate (R73-13b) had caused plant death. An additional three isolates, R10-3, R33-1 and R85-2, which were strongly virulent on radish, also killed the ryegrass seedlings after a further 7 days. No other isolates virulent on radish were virulent on ryegrass. Hence, only 27% of the 15 isolates that were strongly pathogenic on radish were also pathogenic on ryegrass. None of the 50 isolates, which were hypovirulent on radish, was pathogenic on the perennial ryegrass.

Screening of hypovirulent isolates

In the first experiment, 13 hypovirulent isolates provided >50% protection to the radish seedlings against the pathogen (summary data of only 10 isolates are presented in Table 1), while 100% of the plants in the non-inoculated control pots were healthy and 100% of the plants were killed in the pathogen control. The best isolate was R85-10, which provided 75% protection of radish seedlings against the pathogen. In the second experiment, the best isolates, R85-10, R30-8 and R41-3, protected 68, 67 and 51%, respectively, of the radish seedlings (Table 1). The percentage of healthy plants in the non-inoculated control was 98%, while that in the pathogen-inoculated control was only 6%. Further testing of isolate R85-10 showed 69% protection of radish seedlings against damping-off. Isolate R85-10 was obtained from a soil sample collected in the Waikato, North Island, whilst isolates R30-8 and R41-3 were from Lincoln and Waimate, respectively, in the South Island.

TABLE 1: Protection (%) of radish seedlings against damping-off caused by virulent *R. solani* (R73-13b) with hypovirulent *Rhizoctonia* spp. isolates.

Hypovirulent isolate	Exp. 1[1]	Exp. 2[1]
R85-10	75	68
R30-8	63	67
R41-3	50	51
XR135-3	50	42
R36-7	50	40
R36-11	50	40
R104-4	50	27
X72-5b	50	20
R98-1	50	19
R32-3	50	15
LSD ($P < 0.001$)		17.9
SE		6.3

[1]The non-inoculated controls had 100 and 98% healthy plants, while the pathogen controls had 0 and 6 % healthy plants in experiments 1 and 2, respectively.

Growth rate of the hypovirulent isolates

Half (51%) of the hypovirulent isolates had growth rates greater than 10.0 mm/day (data not presented). The highest growth rate was 13.3 mm/day, while the lowest growth rate was 5.0 mm/day. The best protective isolates R85-10, R30-8 and R41-3 had growth rates of 11.7, 7.3 and 10.0 mm/day, respectively compared to 13.3 mm/day for the virulent isolate, R73-13b. There was no correlation between the growth rates of the hypovirulent *Rhizoctonia* spp. isolates and their percentage protection of radish seedlings against damping-off ($r^2 = 0$).

DISCUSSION

In a previous study, a variety of crop plants were inoculated with a number of soil isolates of *Rhizoctonia* spp. Radish seedlings were by far the most susceptible plant species, being infected by 85% of the isolates (Ichielevich-Auster et al. 1985a). Therefore, radish seedlings were selected as the most appropriate indicator species for the identification of non-pathogenic *Rhizoctonia* spp. isolates.

Only 27% of the isolates that were pathogenic on radish were also pathogenic on perennial ryegrass, while all of the isolates that were non-pathogenic on radish were also non-pathogenic on ryegrass. Cereals, in particular, are infected by a relatively low percentage of *Rhizoctonia* spp. isolates (Ichielevich-Auster et al. 1985a). A high percentage of hypovirulent isolates (45%) were obtained from New Zealand soils, compared to ca 28% in previous overseas studies (Sneh 1998). However, in this study,

only a small percentage of those hypovirulent isolates were able to protect plants against *Rhizoctonia* diseases. Isolates R85-10 and R30-8 consistently protected ca 70% of the radish seedlings. These isolates have the potential for use as biocontrol agents against damping-off caused by *Rhizoctonia*. Hypovirulent *Rhizoctonia* spp. isolates from soils in Israel and the USA have provided higher protection percentages (75-95%) (Ichielevich-Auster et al. 1985b; Sneh & Ichielevich-Auster 1998). Few of these protective isolates were also capable of promoting plant growth and increasing crop yields in the field (Sneh et al. 1986; Harris et al. 1993b).

There was no correlation between *in vitro* growth rate of hypovirulent isolates and their disease protection ability. Among the protective hypovirulent isolates, a higher growth rate would be advantageous for faster and more extensive colonisation of the infection sites on the host surface before the approaching hyphal tips of the pathogen. The properties that provide the hypovirulent isolates with the ability to protect plants against the disease and the genes regulating these processes are still to be determined.

REFERENCES

Boosalis, M.G.; Scharen, A.L. 1959: Methods for microscopic detection of *Aphanomyces euteiches* and *Rhizoctonia solani* and for isolation of *Rhizoctonia solani* associated with plant debris. *Phytopath. 49*: 192-198.

Burpee, L.L.; Goulty, L.G. 1984: Suppression of brown patch disease of creeping bentgrass by isolates of non-pathogenic *Rhizoctonia* spp. *Phytopath. 74*: 692-694.

Freeman, S.; Rodriguez, R. 1993: Genetic conversion of fungal plant pathogen to non-pathogenic, epiphytic mutualist. *Science 260*: 75-78.

Harris, A.R.; Schisler, D.A.; Neate, S. 1993a: Culture of *Rhizoctonia solani* and binucleate *Rhizoctonia* spp. on organic substrates for inoculation of seedlings in containers. *Soil Biol. Biochem. 25*: 337-341.

Harris, A.R.; Schisler, D.A.; Neate, S.; Ryder, M.H. 1993b: Suppression of damping-off caused by *Rhizoctonia solani* and growth promotion in bedding plants by binucleate *Rhizoctonia* spp. *Soil Biol. Biochem. 25*: 263-268.

Ichielevich-Auster, M.; Sneh, B.; Koltin, Y.; Barash, I. 1985a: Pathogenicity, host specificity and anastomosis groups of *Rhizoctonia* spp. isolated from soils in Israel. *Phytoparasitica 13*: 103-112.

Ichielevich-Auster, M.; Sneh, B.; Koltin, Y.; Barash, I. 1985b: Suppression of damping-off caused by *Rhizoctonia* spp., by non-pathogenic *R. solani*. *Phytopath. 75*: 1080-1084.

Lemanceau, P.; Alabouvette, C. 1993: Suppression of *Fusarium* wilt by fluorescent *Pseudomonads*: Mechanisms and applications. *Biocontrol, Sci. Technol. 3*: 219-234.

Sneh, B. 1990: Mechanisms involved in protection of infection sites. *In*: Baker, R.; Dunn, P. *ed*. New Directions in Biological Control: Alternatives for suppressing agricultural pests and diseases. Alan Liss Inc., New York. Pp. 653-662.

Sneh, B. 1998: Use of non-pathogenic or hypovirulent fungal isolates to protect plants against closely related fungal pathogens. *Biotechnol. Adv. 16*: 1-32.

Sneh, B.; Burpee, L.L.; Ogoshi, A. 1991: Identification of *Rhizoctonia* species. APS Press, St. Paul, Minnesota. 133 p.

Sneh, B.; Ichielevich-Auster, M. 1998: Induced resistance of cucumber seedlings caused by some non-pathogenic *Rhizoctonia* (np-R) isolates. *Phytoparasitica 26*: 27-38.

Sneh, B.; Zeidan, M.; Ichielevich-Auster, M.; Barash, I.; Koltin, Y. 1986: Increased growth responses induced by a non-pathogenic *Rhizoctonia solani*. *Can. J. Bot. 64*: 2372-2378.

Van Alfen, N.K. 1982: Biology and potential for disease control with hypovirulence of *Endothia parasitica*. *Ann. Rev. Phytopath. 20*: 349-362.

Villajuan-Abgona, R.; Kageyama, K.; Hyakumachi, M. 1996: Biocontrol of damping-off of cucumber by non-pathogenic binucleate *Rhizoctonia*. *European J. Plant Path. 102*: 227-235.