Rhizoctonia Decline: Studies on Hypovirulence and Potential Use in Biological Control

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ABSTRACT

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Virulence of a severely diseased isolate, 189a, of *Rhizoctonia solani* and its healthy counterpart, isolate 189HT5, was examined. In petri-plate tests using cabbage seedlings 189a killed only 12% of all seedlings whereas 189HT5 killed 100% of the seedlings. Coinoculation of plates with 189a and 189HT5 resulted in the killing of 38% of the seedlings. Pathogenicity trials in which soil was infested with *R. solani* showed 189a to be nonpathogenic to sugar beet seedlings; in contrast, 189HT5 caused 23% pre-emergence damping-off and 79% postemergence damping-off. Plants grown in soil infested with 189a and 189HT5 showed no significant amount of damping-off, which indicated complete biological control of the healthy, highly virulent 189HT5. The addition of viable, 189a mycelium to the seed furrow of soil previously infested with 189HT5, as blended or air-dried mycelium, resulted in a fivefold reduction in postemergence damping-off. However, introducing cultures of 189a into soil previously infested with 189HT5 as a broadcast treatment did not reduce damping-off. The survival of 189a and 189HT5 was tested under sterile and nonsterile soil conditions; 189a survived less than 1 mo, whereas 189HT5 survived 2 yr.

Additional key words: *Thanatephorus cucumeris*, survival, competitive saprophytic ability.

Rhizoctonia decline (2) is a degenerative disease of *Rhizoctonia solani* Kuehn [=*Thanatephorus cucumeris* (Frank) Donk]. In addition to the morphological and physiological changes exhibited by affected isolates, we found in preliminary studies that the level of virulence was markedly reduced (1). The use of such hypovirulent isolates of plant-pathogenic fungi in disease control is attractive, but few examples have been reported. Grente (3) described hypovirulent strains of *Endothia parasitica* and suggested that their natural spread was the primary reason that chestnut blight is no longer an important disease in some areas of Europe. More recently, Van Alfen et al. (7) showed hypovirulence to be due to a cytoplasmic factor, and that hypovirulent strains could be used in the control of chestnut blight in the United States.

In this report we present evidence that supports the hypothesis that a diseased, hypovirulent isolate of *R. solani* may be used in the control of plant disease caused by its healthy, highly virulent counterpart.

MATERIALS AND METHODS

Isolates.—All experiments involved isolate 189 (ATCC 13248), anastomosis group 1, of *R. solani* obtained from J. R. Parmeter, Jr. (6). Severely diseased cultures of 189 are designated 189a and healthy cultures obtained by hyphal-tip isolations are designated 189HT5 (2).

Media.—All cultures were maintained on potato-dextrose agar (PDA) at 24-28 C. Other media used in these studies include: acidified-PDA (PDA adjusted to pH 4.2-4.5 with 25% lactic acid), Weinhold's Medium A (8), Blue Ribbon malt extract broth (BRMEB) (2), PDA supplemented with 5% (w/v) wheat bran (PDA + bran), and water agar.

Seed.—Virulence of *R. solani* was tested using two hosts: cabbage (*Brassica oleracea* L. var. *capitata* 'Golden Acre') and sugar beet (*Beta vulgaris* L. 'USH9'). All seed prior to use were surface-disinfested by being immersed in a 1% solution of sodium hypochlorite for 10 min, rinsed with distilled water, and then air-dried. Prior to use cabbage seed were placed on moist filter paper for 24 hr to permit incipient germination.

Soils and soil tubes.—Two steam-pasteurized soils were used in pathogenicity studies: Yolo fine sandy loam and UC mix (type C, with fertilizer regime I, replacing calcium carbonate with an equal amount of gypsum) (4). Soil tubes were prepared using air-dried Yolo fine sandy loam supplemented with 5% (w/w) wheat bran. Approximately 10 g of this soil-bran mixture and 2 ml of distilled water were added to each test tube. The tubes were autoclaved at 121 C for 45 min on 2 successive days.

Petri-plate tests.—Isolates 189a and 189HT5 were grown on Weinhold's Medium A for 5 days at 25 C. Water-agar plates were inoculated with mycelial plugs (4 mm in diameter) of either isolate. Coinoculated plates were prepared by placing a mycelial plug of 189a on the water-agar surface and a plug of 189HT5 atop this plug.

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Noninoculated plates served as controls. All water-agar plates were incubated for 2 days at 25 C. Ten cabbage seeds per plate were placed on the agar surface at the edge of the advancing colony. The plates were incubated at room temperature (23-27 C) under fluorescent lighting at 643-753 lx for 7 days and virulence was determined based on the number of seedlings killed, lesioned, or healthy.

Pathogenicity trials in soil.—UC mix in metal flats was infested with either 189a, 189HT5, or both; inoculum was prepared from cultures grown on plates of PDA + bran. These cultures were started, in the case of 189HT5, from a single mycelial plug, and for the slow-growing 189a, from homogenized mycelium spread over the agar surface. All plates were incubated 1-2 wk at 27 C. The cultures then were diced with a razor blade and one diced culture was added to each flat of UC mix. In flats simultaneously infested with 189a and 189HT5, one diced culture of each was added. All flats were placed in the greenhouse and the mix in each flat was mixed and watered on alternate days. Flats not infested, but otherwise treated exactly like the infested ones, served as controls. After 2 wk, sugar beet seeds were planted 2.5 cm deep at 50 seed per row, two rows per flat. Flats were maintained in the greenhouse at 23-30 C with a 12-hr photoperiod; when needed fluorescent light was used to extend daylength. Pathogenicity trials were conducted over a 2-yr period. Each trial consisted of four or five flats infested with either 189a, 189HT5, or 189a plus 189HT5 with an equal number of noninfested control flats. Virulence was based on the incidence of pre- and postemergence damping-off 21 days after planting. Data were analyzed according to Duncan's multiple range test, $P = 0.01$.

Biological control of Rhizoctonia solani.—The effect of diseased 189a on reducing the incidence of damping-off of sugar beets caused by healthy 189HT5 was tested by adding mycelium of 189a either as a broadcast treatment or seed-furrow application, to UC mix previously infested with 189HT5. For broadcast treatments, a 7-day-old, diced culture of 189a grown on PDA + bran was added to each flat 2 wk prior to planting. For seed furrow applications, flats were planted, but prior to covering the seed 189a was added either as a blended culture or as air-dried mycelial pieces (1-2 mm^2). Either one 7-day-old culture (grown on PDA + bran and blended in 50 ml water) or 1 g of air-dried mycelium (grown on BRMEB) of 189a was added per seed furrow. Both the blended and air-dried mycelium were viable, producing typical 189a cultures when plated on PDA.

Controls consisted of soil infested with (i) 189HT5 alone, (ii) 189HT5 plus autoclaved-blended or autoclaved-dried mycelium of 189a, and (iii) noninfested soil. All treatments and controls were replicated four times and virulence was based on the incidence of pre- and postemergence damping-off. Data were analyzed by Duncan's multiple range test, $P = 0.01$.

Survival of isolate 189 in soil.—Soil tubes were inoculated with either 189a or 189HT5 and stored at 23-27 C. Viability was determined by sprinkling soil samples from each tube onto PDA followed by incubation at 27 C for 14 days. In another test, two flats each of pasteurized UC mix and Yolo fine sandy loam were infested with 189a, 189HT5, or both, as previously described. The flats were kept on an open greenhouse bench and the soil was mixed once a week and watered as needed. Recovery of *R. solani* from this soil was done with killed sugar beet seed according to the isolation technique described by Papavizas et al. (5); samples were taken once a month from each flat.

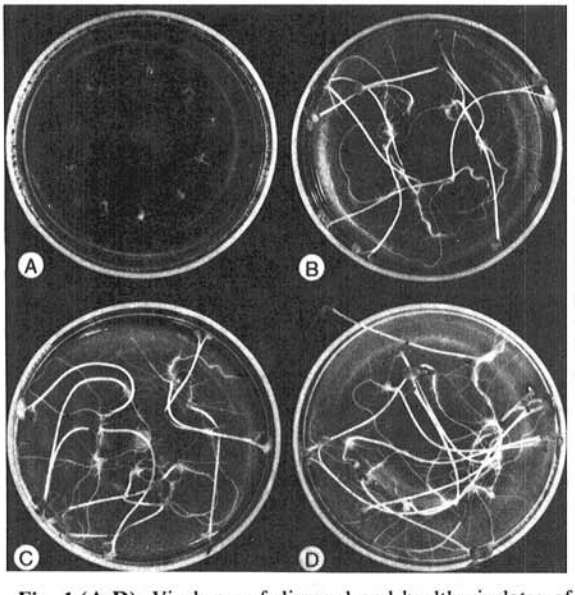

Fig. 1-(A-D). Virulence of diseased and healthy isolates of *Rhizoctonia solani* on cabbage. Seedlings in petri plates were inoculated with **A)** healthy isolate 189HT5, **B)** diseased isolate 189a, and **C)** isolate 189a plus isolate 189HT5. **D)** is non-inoculated. Each plate contains 10 cabbage seed.

RESULTS

Petri-plate tests.—Healthy 189HT5 was highly virulent to cabbage seedlings whereas 189a was weakly virulent (Fig. 1, Table 1). Isolate 189a also caused a great reduction in the virulence of 189HT5 in plates coinoculated with the two isolates (Fig. 1, Table 1).

Pathogenicity trials in soil.—The incidence of damping-off of sugar beet seedlings was extensive in soil infested with 189HT5, whereas little or no damping-off

TABLE 1. Effect of Rhizoctonia decline on the virulence of *Rhizoctonia solani* on cabbage in petri-plate tests[a]

Inoculum	No. of seed plated (total)	Cabbage seedling condition		
		Killed (%)	Lesioned (%)	Healthy (%)
189HT5 (healthy)[b]	650	100	0	0
189a (diseased)[b]	620	12	23	65
189a + 189HT5[c]	390	38	40	22
None	400	0	0	100

[a]Data from eight separate tests.

[b]Isolate 189a is a severely diseased isolate; 189HT5 is a healthy isolate derived from 189a and hyphal-tipped five successive times.

[c]Test plates coinoculated with 189a and 189HT5.

occurred in soil infested with 189a alone, 189a plus 189HT5, or noninfested soil (Table 2). Thus, isolate 189a was essentially nonpathogenic and the highly virulent 189HT5 was controlled by coinfestation with 189a.

Biological control of R. solani.—The addition of viable 189a to the seed furrow of soil containing 189HT5 as either blended or air-dried mycelium resulted in considerably less seedling damping-off. The incidence of preemergence damping-off in soil infested with 189HT5 alone and soil containing 189HT5 plus 189a was not significantly different ($P = 0.01$) (Table 3); however, the presence of 189a resulted in significantly less postemergence damping-off. The addition of autoclaved mycelium of 189a resulted in significantly more pre- and postemergence damping-off than in soil containing only 189HT5 ($P = 0.01$). This probably was due to the addition of autoclaved mycelium of 189a which served as a food base for 189HT5. The addition of 189a as a broadcast treatment did not significantly reduce disease caused by 189HT5.

Survival of isolate 189 in soil.—Isolate 189a was not recovered from greenhouse soils or soil tubes 1 or 2 mo, respectively, after their addition to soil. In contrast, 189HT5 survived 2 yr in soil tubes and was readily isolated from greenhouse soils after 6 mo, at which time the experiment was terminated. From soil coinfested with 189a and 189HT5, we were unable to recover *R. solani* after 1 mo; thus the results were similar to those for 189a. This inability to isolate *R. solani* from soil containing a mixture of 189a and 189HT5, indicates that 189HT5 was probably converted to the 189a-type isolate.

DISCUSSION

Biological control of *R. solani* could probably not be attained by attempting to establish a diseased isolate like 189a in a field soil. Whereas 189a survived on agar media, it failed to survive in sterile or pasteurized soil for more than 1 mo. The ephemeral nature of 189a in soil probably is related to its slow growth, failure to produce sclerotia, and its low competititve saprophytic ability.

Thus, it is not surprising that 189a was nonpathogenic in pathogenicity trials using soil, even though tests in petri plates indicated that it was at least weakly virulent. This situation is probably because mycelium of 189a died in the soil prior to planting or the level of viable mycelium was so low and sparsely distributed that it caused no damping-off and could not be recovered.

How then does 189a cause reductions in seedling disease? According to our results biological control of healthy 189HT5 by 189a occurs only when both are actively growing. Presumably this allows anastomosis to

TABLE 2. Virulence of diseased and healthy isolates of *Rhizoctonia solani* on sugar beets[a]

Isolate	Sugar beet seedlings[d] Emerged (no.)	Killed (no.)	Healthy (no.)	Postemergence damping-off (%)
None	1,866 y	1 y	1,865 y	0 y
189a (diseased)[b]	1,850 y	2 y	1,848 y	0 y
189a + 189HT5[c]	1,872 y	52 y	1,820 y	3 y
189HT5 (healthy)[b]	1,374 z	1,085 z	289 z	79 z

[a]A UC soil mix infested with one culture of each isolate and planted with 50 seed/row, two rows/flat; results from five separate trials.

[b]Isolate 189a is a severely diseased isolate; 189HT5 is a healthy isolate derived from 189a and hyphal-tipped five successive times.

[c]Soil was coinfested with isolates 189a and 189HT5.

[d]Values followed by different letters in the same column are significantly different, according to Duncan's multiple range test, $P = 0.01$.

TABLE 3. Treatment of seed furrows with different types of hypovirulent 189a mycelium to control seedling disease in soil infested with the virulent isolate 189HT5 of *Rhizoctonia solani*

Treatment[a]	Sugar beet seedlings[b] Emerged (no.)	Killed (no.)	Healthy (no.)	Postemergence damping-off (%)
Control (noninfested)	368 x	1 w	367 w	0 w
189HT5 + air-dried 189a	261 y	12 w	250 x	5 wx
189HT5 + blended 189a	272 y	41 wx	231 x	15 x
189HT5 alone	254 y	193 y	61 y	80 y
189HT5 + autoclaved, blended 189a	69 z	59 xy	10 z	86 yz
189HT5 + autoclaved, air-dried 189a	99 z	94 z	5 z	95 z

[a]Soil previously infested with healthy isolate 189HT5; diseased isolate 189a added to the seed furrow as either a blended culture or air-dried mycelial pieces (1-2 mm^2). One culture blended in 50 ml of water or 1 g of dried mycelium was added to each seed furrow. Sugar beet seed planted 50 seed/furrow, two furrows/flat. There were four flats/treatment.

[b]Values followed by different letters in the same column are significantly different, $P = 0.01$, according to Duncan's multiple range test.

occur between the two, resulting in the rapid conversion of the 189HT5 into the disease-type (2). In so doing, the virulence of the 189HT5 is reduced, since seedling disease is less. Total biological control resulted when 189a and 189HT5 were added to soil together. In this case both isolates were actively growing. Similarly, the addition of viable 189a to the seed furrow in soil previously infested with 189HT5 resulted in significant reductions in disease. The failure to control disease caused by 189HT5 with broadcast treatments of 189a was probably due to the fact that although isolate 189a was viable, isolate 189HT5 was not actively growing in the soil; thus, contact and conversion of the 189HT5 did not occur.

We have been unable to transmit the disease agent from 189a to other field isolates of *R. solani* (2) and thus all of our information on pathogenicity is based on one isolate. Nevertheless, our results support the principle that hypovirulent strains of plant pathogens can be exploited in disease control. Isolate 189a is only one of several diseased isolates of *R. solani* that we have encountered during the course of our work. Conceivably, there are more versatile diseased isolates than 189a which could be used to control disease caused by the predominant strains of *R. solani* in a given crop area.

LITERATURE CITED

1. CASTANHO, B., and E. E. BUTLER. 1975. A transmissible and infectious disorder of Rhizoctonia solani. Proc. Am. Phytopathol. Soc. 2:112. (Abstr.).
2. CASTANHO, B., and E. E. BUTLER. 1978. Rhizoctonia decline: a degenerative disease of Rhizoctonia solani. Phytopathology 68:1505-1510.
3. GRENTE, M. J. 1965. Les formes hypovirulentes d' Endothia parasitica et les espoirs de lutte contre le chancre du chataignier. C. R. Seances Acad. Agric. Fr. 51:1033-1037.
4. MATKIN, O. A., P. A. CHANDLER, and K. F. BAKER. 1957. Components and development of mixes. Pages 86-107 *in* K. F. Baker, ed. The U. C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23, 332 p.
5. PAPAVIZAS, G. C., P. B. ADAMS, R. D. LUMSDEN, J. A. LEWIS, R. L. DOW, W. A. AYERS, and J. G. KANTZES. 1975. Ecology and epidemiology of Rhizoctonia solani in field soil. Phytopathology 65:871-877.
6. PARMETER, J. R., JR., R. T. SHERWOOD, and W. D. PLATT. 1969. Anastomosis grouping among isolates of Thanatephorus cucumeris. Phytopathology 59:1270-1278.
7. VAN ALFEN, N. K., R. A. JAYNES, S. L. ANAGNOSTAKIS, and P. R. DAY. 1975. Chestnut blight: biological control by transmissible hypovirulence in Endothia parasitica. Science 189:890-891.
8. WEINHOLD, A. R., T. BOWMAN, and R. L. DODMAN. 1969. Virulence of Rhizoctonia solani as affected by nutrition of the pathogen. Phytopathology 59:1601-1605.