Diversity and Aggressiveness of *Rhizoctonia solani* and *Rhizoctonia*-like Fungi on Vegetables in New York

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ABSTRACT

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Vegetable growers in New York, especially those growing table beets, have recently observed that the corn rotation is no longer effective in suppressing diseases caused by *Rhizoctonia solani* and *Rhizoctonia*-like fungi. To investigate this problem, 68 isolates of *Rhizoctonia solani* and *Rhizoctonia*-like fungi infecting vegetables in New York were isolated, characterized, and their pathogenicity on corn determined. Sequence analysis of the rDNA internal transcribed spacer region inferred 26 isolates to belong to *R. solani* anastomosis group (AG) 2-2 and 19 isolates to belong to AG 4. Remaining isolates belonged to AG 1, AG 2-1, AG 5, AG 11, *Ceratobasidium* AG (CAG) 2, CAG 6, and *Waitea circinata* var. *zeae*. This is a first report of AG 11 and *W. circinata* var. *zeae* recovered from naturally infected vegetables in New York. Pathogenicity tests on corn showed that the majority of isolates are pathogenic on corn, and isolates belonging to AG 2-2, AG 5, and AG 11 exhibited high aggressiveness. These results suggest that certain strains of *R. solani* and *Rhizoctonia*-like fungi infecting vegetables in New York have acquired the ability to infect corn. In addition, snap bean was inoculated with seven isolates exhibiting low to high aggressiveness on corn, and a correlation between aggressiveness on corn and snap bean was observed.

Rhizoctonia solani J.G. Kühn and *Rhizoctonia*-like fungi are geographically distributed worldwide and include some of the world's most devastating plant pathogens. Although different anastomosis groups (AGs) and species have different host ranges, most vascular plants are potential hosts of plant pathogenic *R. solani* or *Rhizoctonia*-like fungi (37,47). In New York, *R. solani* and *Rhizoctonia*-like fungi cause root and foliar diseases on various vegetables such as beans, table beets, carrots, and cabbage. Damage caused by these fungi in New York on vegetables has increased steadily during the past 10 years, and the sexual stage of *R. solani*, *Thanatephorus cucumeris*, was first reported in New York in 1990 on table beets (38). One factor contributing to the increased damage is thought to be the emergence of large numbers of aerially dispersed basidiospores (40). Another reason for the increased damage is thought to be changes in cultural practices, in which large tractors throw infested soil onto crowns of the plants as they cultivate fields for weed control (40).

Control of diseases caused by *R. solani* and *Rhizoctonia*-like fungi is difficult due to their soilborne nature, ability to persist as sclerotia, wide host range, and versatility (5,23,28,33,37). Today, many growers depend on fungicides to control these diseases, but fungicides are costly and must be applied at planting or early in the growing season to be effective (41). For vegetable growers, grain crop rotations are recommended and have been effective in suppressing diseases caused by *R. solani* and *Rhizoctonia*-like fungi (1,45). During the past few years, growers have reported that grain crop rotations have been ineffective. Increasing disease caused by these fungi and the limited control options available to growers warrant an in-depth investigation of this problem.

Among AGs of *R. solani*, those belonging to AG 1, AG 2, and AG 4 are documented to be pathogenic on corn (16,55,57,58). In particular, subgroups AG 1-IA and AG 2-2 have been identified to infect corn, and cultural type AG 2-IIIB has been reported to be the causal agent for root rot on corn in the United States (7,16,20,55). In China and Mississippi, AG 5 and AG 13, AGs generally not known to be pathogenic on corn, have been reported to infect corn as well (16,30,65,66). In Germany, a similar problem has been reported where high levels of infection on sugar beet were observed when it was in narrow rotation with corn (20). Their study

showed that isolates of AG 2-IIIB are capable of causing disease on sugar beet after surviving on corn (20).

Previous studies in this region showed that the dominant AGs present in western New York were AG 2-2 on table beets (88%) (39), AG 4 on snap beans (55%) (15), and AG 1 on cabbage (2). Other isolates found associated with table beets belonged to AG 5, AG 4, AG 2-1, and binucleate *Rhizoctonia* (= *Ceratobasidium* spp.) (39), whereas those from snap beans belonged to AG 1 and AG 2 (15). The presence of isolates belonging to AG 1, AG 2, and AG 4 suggests the possibility of corn-pathogenic isolates being present on other hosts. However, these studies were conducted more than 10 years ago, and a reassessment of the current population is necessary.

To investigate the ineffectiveness of the corn rotation in suppressing diseases caused by *R. solani* and *Rhizoctonia*-like fungi, the aim of this study was to characterize the *R. solani* and *Rhizoctonia*-like fungi infecting vegetables in New York and evaluate corn as a potential host. Currently, the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) is considered most appropriate in characterizing these fungi (18,54). DNA sequence data from the ITS region have been used to characterize unknown isolates of *R. solani* and *Rhizoctonia*-like fungi to AGs (27,29,32,46). Thus, this study was conducted to characterize *R. solani* and *Rhizoctonia*-like fungi that infect vegetables in New York using the ITS sequence and identify those strains that are pathogenic to corn.

MATERIALS AND METHODS

Isolate collection. One hundred and fifteen isolates of *R. solani* and *Rhizoctonia*-like fungi were recovered from symptomatic vegetable tissues throughout New York State. Host plants included pea, snap bean, dry bean, cabbage, carrot, and table beet. The fungi were isolated by placing small pieces of infected tissue on acidified water agar medium (pH 3.5). Prior to placement on media, infected tissue pieces were soaked in 10% bleach for 40 s to eliminate superficial contaminants, and the lesion margins were cut off using a sterile scalpel. Each isolate was established by making a hyphal tip transfer from the margin of a colony exhibiting typical colony

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Table 1. *Rhizoctonia solani* and *Rhizoctonia*-like fungi of New York characterized in this study

Isolate	Host plant	Location ^a	Date of collection
R1	Snap bean	Ontario	Fall 2005
R2	Snap bean	Ontario	Fall 2005
R3	Carrot	Yates	June 2006
R4	Cabbage	Seneca	June 2006
R5	Pea	Ontario	June 2006
R6	Pea	Ontario	June 2006
R7	Pea	Genesee	June 2006
R8	Table beet	Genesee	August 2004
R9	Carrot	Unknown (CNY)	June 2004
R10	Snap bean	Livingston	June 2006
R11	Snap bean	Livingston	June 2006
R12	Snap bean	Genesee	June 2006
R13 ^b	Snap bean	Genesee	June 2006
R14 ^b	Snap bean	Genesee	June 2006
R15	Carrot	Orleans	June 2006
R16	Carrot	Orleans	June 2006
R17	Carrot	Orleans	June 2006
R18	Carrot	Orleans	June 2006
R20	Snap bean	Orleans	June 2006
R21	Snap bean	Orleans	June 2006
R22	Snap bean	Orleans	June 2006
R25	Snap bean	Orleans	June 2006
R27	Table beet	Livingston	July 2006
R29	Table beet	Livingston	July 2006
R31	Table beet	Livingston	July 2006
R32	Table beet	Livingston	July 2006
R33	Table beet	Livingston	July 2006
R35	Dry bean	Livingston	July 2006
R36	Dry bean	Livingston	July 2006
R37	Dry bean	Livingston	July 2006
R39	Table beet	Livingston	July 2006
R41	Table beet	Livingston	July 2006
R43	Snap bean	Chemung	July 2006
R47	Table beet	Genesee	July 2006
R55	Snap bean	Genesee	July 2006
R57	Snap bean	Genesee	July 2006
R59	Snap bean	Ontario	July 2006
R60	Snap bean	Ontario	July 2006
R62	Table beet	Ontario	July 2006
R64	Snap bean	Genesee	July 2006
R65	Snap bean	Genesee	July 2006
R66 ^c	Table beet	Genesee	July 2006
R68 ^c	Table beet	Genesee	July 2006
R70 ^c	Table beet	Genesee	July 2006
R75	Snap bean	Ontario	July 2006
R77	Snap bean	Ontario	July 2006
R81	Table beet	Livingston	August 2006
R82	Table beet	Livingston	August 2006
R83	Table beet	Livingston	August 2006
R84	Table beet	Genesee	August 2006
R85	Table beet	Genesee	August 2006
R88	Table beet	Genesee	August 2006
R89	Table beet	Genesee	August 2006
R90	Table beet	Genesee	August 2006
R92	Snap bean	Genesee	August 2006
R93	Snap bean	Genesee	August 2006
R94	Snap bean	Genesee	August 2006
R100	Snap bean	Genesee	August 2006
R101	Snap bean	Genesee	August 2006
R104	Cabbage	Ontario	August 2006
R105	Snap bean	Ontario	July 2006
R106	Carrot	Orleans	August 2006
R107	Cabbage	Orleans	August 2006
R108	Cabbage	Orleans	August 2006
R110	Table beet	Genesee	August 2006
R112	Table beet	Genesee	August 2006
R113	Table beet	Genesee	August 2006
R115	Carrot	Yates	September 2006

^a Refers to the New York county where the vegetable field was located (CNY = Central New York).

^b Isolate was recovered from a field planted to table beet in 2005.

^c Isolate was recovered from a field planted to corn in 2005.

morphology and hyphal branching patterns of *Rhizoctonia* under a dissecting microscope. Isolates were stored in vials on potato dextrose agar (PDA) covered with mineral oil at 4°C. Sixty-eight isolates from different fields were chosen to represent *R. solani* and *Rhizoctonia*-like fungi causing disease on vegetables in New York for molecular characterization and pathogenicity tests (Table 1).

Molecular characterization. Individual isolates were grown on PDA at 27°C, and DNA was extracted using the Ultraclean Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA). The rDNA ITS region consisting of ITS1, 5.8S, and ITS2 was amplified using the polymerase chain reaction (PCR) with primers ITS1 and ITS4 for 22 isolates and ITS4 and ITS5 for 46 isolates that did not sequence well with ITS1 and ITS4 (Table 2) (69). Reactions for PCR amplifications were performed in a 50- μ l mixture containing 50 to 100 ng of template DNA, 0.2 μ M of each primer, 0.2 mM of each of the four dNTPs, 1.5 units of *Taq* DNA Polymerase (New England BioLabs, Inc., Ipswich, MA), and 1 $\times$ ThermoPol Buffer containing 10 mM KCl, 20 mM Tris-HCl, and 2 mM MgSO₄ (New England BioLabs). Cycle parameters were an initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. A 4- μ l aliquot of each PCR product was run electrophoretically on a 1% agarose gel to confirm amplification. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). The ITS region was sequenced at the Life Sciences Core Laboratories Center of Cornell University using Big Dye Terminator chemistry and the AmpliTaq-FS DNA Polymerase on the Automated 3730 DNA Analyzer (Table 2) (Applied Biosystems, Foster City, CA).

Sequencing results of many isolates exhibited overlapping peaks in the fluorescent peak trace chromatograms of DNA sequence data. For such isolates, PCR products were cloned using the TOPO TA Cloning Kit for Sequencing with One Shot TOP10 Chemically Competent *E. coli* (Invitrogen, Carlsbad, CA). For each isolate, one plasmid with a confirmed insertion was purified using the Wizard *Plus* Minipreps DNA Purification System (Promega, Madison, WI), and sequenced as described above. The clones were sequenced in both directions using the ITS4 and ITS5 primers (Table 2) (69).

Phylogenetic analysis. To infer species, AGs, and subgroups; reference sequences and the outgroup sequence (*Athelia rolfsii*) from the study conducted by Sharon et al. (54) were obtained from GenBank (Table 3). Additional sequences for AG 13, AG BI, and *Ceratobasidium* AGs, CAG 1 through CAG 7, were added (Table 3)

(9,18). A reference sequence for CAG 2 was not available; therefore a sequence from AG A (18) was used to represent CAG 2, because the two groups are known to be equivalent between the Japanese and American AGs (Table 3) (16). Sequences of collected isolates and reference sequences were aligned using Clustal X (1.81) (62) and its default parameters. Sequence alignment was adjusted manually by visual examination using MacClade 4.08 (31).

The sequence data set was analyzed using two analyses, Bayesian inference and neighbor-joining. For Bayesian inference, the Hasegawa-Kishino-Yano plus Gamma (HKY+G) model (19) was chosen as the appropriate evolutionary model by Modeltest 3.7 (44) using the Akaike Information Criterion (3). The output parameters (number of substitution types = 2, among-site rate variation = gamma, transition/transversion rate ratio = 1.27, state frequencies [A, C, G, T] = 0.27, 0.21, 0.16, 0.36, proportion of invariable sites = 0, gamma shape = 0.35) were entered into MrBayes 3.2.1 (48). Two million generations were run and trees were sampled every 100 generations. The first 2,000 trees were discarded as the burn-in, and a strict consensus tree was generated. Both neighbor-joining and Bayesian inference analyses included 833 characters and were unrooted. *A. rolfsii* was positioned as the outgroup after constructing the trees. For neighbor-joining analysis, the appropriate distance model was chosen according to the guidelines described in the *MEGA* version 4 manual (61) based on calculating pair-wise distances (*d*) using the Jukes-Cantor distance model (22) and the transition/transversion ratio (*R*). Since $d < 0.3$ ($0.00 < d < 0.272$) and *R* was low ($R = 1.164$), the Jukes-Cantor distance model (22) was selected. A neighbor-joining tree (49) was generated using PAUP* ver. 4.0b10 (60) with bootstrap values based on 1,000 replicates.

Pathogenicity evaluation on corn in the greenhouse. To determine whether the representative isolates from New York were capable of causing disease on corn, a series of greenhouse bioassays were performed. Untreated seeds of sweet white corn cultivar Silver Princess were planted in 3.8×21 cm cone-tubes (Stuewe & Sons, Inc., Tangent, OR). Approximately 200 cm³ of pasteurized soil (60°C for 30 min) was placed in each tube and planted with two seeds of corn. Inoculum of the selected isolates was prepared by growing them on PDA for 5 to 7 days. Seven or 8 days after planting, the soil adjacent to the stem of corn seedlings was removed and a colonized PDA disk (8 mm diameter) was placed in the pocket against the lower stem. Noncolonized PDA disks were placed next to the stems of the negative controls. After inoculation, the disks were covered with soil to prevent drying. An

Table 2. Polymerase chain reaction (PCR) primer combinations, cloning status (Y = cloned, N = not cloned), sequencing primer(s), and GenBank accession numbers for individual isolates used in the molecular characterization

Isolate	PCR primers	Cloned	Sequencing primer ^a	GenBank accession no.
R1	ITS4 - ITS5	Y	ITS4, ITS5	EU591747
R2	ITS1 - ITS4	N	ITS1	NA
R3	ITS1 - ITS4	N	ITS1	EU591748
R4	ITS1 - ITS4	N	ITS4	EU591749
R5	ITS4 - ITS5	Y	ITS4, ITS5	EU591750
R6	ITS4 - ITS5	Y	ITS4, ITS5	EU591751
R7	ITS1 - ITS4	N	ITS1	EU591752
R8	ITS4 - ITS5	Y	ITS4, ITS5	EU591753
R9	ITS1 - ITS4	N	ITS1	EU591754
R10	ITS1 - ITS4	N	ITS1	EU591755
R11	ITS1 - ITS4	N	ITS1	EU591756
R12	ITS1 - ITS4	N	ITS1	EU591757
R13	ITS4 - ITS5	Y	ITS4, ITS5	EU591758
R14	ITS4 - ITS5	N	ITS4	EU591759
R15	ITS4 - ITS5	Y	ITS4, ITS5	EU591760
R16	ITS4 - ITS5	Y	ITS4, ITS5	EU591761
R17	ITS4 - ITS5	Y	ITS4, ITS5	EU591762
R18	ITS4 - ITS5	Y	ITS4, ITS5	EU591763
R20	ITS4 - ITS5	Y	ITS4, ITS5	EU591764
R21	ITS1 - ITS4	N	ITS1	EU591765
R22	ITS1 - ITS4	N	ITS1	EU591766
R25	ITS4 - ITS5	Y	ITS4, ITS5	EU591767
R27	ITS4 - ITS5	Y	ITS4, ITS5	EU591768
R29	ITS4 - ITS5	Y	ITS4, ITS5	EU591769
R31	ITS1 - ITS4	N	ITS1	EU591770
R32	ITS1 - ITS4	N	ITS4	EU591771
R33	ITS1 - ITS4	N	ITS1	EU591772
R35	ITS1 - ITS4	N	ITS1	EU591773
R36	ITS4 - ITS5	Y	ITS4, ITS5	EU591774
R37	ITS4 - ITS5	N	ITS4	EU591775
R39	ITS4 - ITS5	Y	ITS4, ITS5	EU591776
R41	ITS4 - ITS5	Y	ITS4, ITS5	EU591777
R43	ITS4 - ITS5	Y	ITS4, ITS5	EU591778
R47	ITS4 - ITS5	Y	ITS4, ITS5	EU591779
R55	ITS1 - ITS4	N	ITS4	EU591780
R57	ITS4 - ITS5	Y	ITS4, ITS5	EU591781
R59	ITS1 - ITS4	N	ITS4	EU591782
R60	ITS4 - ITS5	N	ITS4	NA
R62	ITS4 - ITS5	N	ITS4	EU591783
R64	ITS4 - ITS5	Y	ITS4, ITS5	EU591784
R65	ITS4 - ITS5	Y	ITS4, ITS5	EU591785
R66	ITS4 - ITS5	Y	ITS4, ITS5	EU591786
R68	ITS4 - ITS5	Y	ITS4, ITS5	EU591787
R70	ITS4 - ITS5	Y	ITS4, ITS5	EU591788
R75	ITS4 - ITS5	Y	ITS4, ITS5	EU591789
R77	ITS1 - ITS4	N	ITS4	EU591790
R81	ITS4 - ITS5	Y	ITS4, ITS5	EU591791
R82	ITS1 - ITS4	N	ITS4	EU591792
R83	ITS4 - ITS5	Y	ITS4, ITS5	EU591793
R84	ITS4 - ITS5	Y	ITS4, ITS5	EU591794
R85	ITS4 - ITS5	Y	ITS4, ITS5	EU591795
R88	ITS4 - ITS5	Y	ITS4, ITS5	EU591796
R89	ITS4 - ITS5	Y	ITS4, ITS5	EU591797
R90	ITS4 - ITS5	Y	ITS4, ITS5	EU591798
R92	ITS1 - ITS4	N	ITS4	EU591799
R93	ITS4 - ITS5	N	ITS4, ITS5	EU591800
R94	ITS4 - ITS5	Y	ITS4, ITS5	EU591801
R100	ITS4 - ITS5	Y	ITS4, ITS5	EU591802
R101	ITS4 - ITS5	Y	ITS4, ITS5	EU591803
R104	ITS4 - ITS5	Y	ITS4, ITS5	EU591804
R105	ITS4 - ITS5	Y	ITS4, ITS5	EU591805
R106	ITS1 - ITS4	N	ITS4	EU591806
R107	ITS4 - ITS5	Y	ITS4, ITS5	EU591807
R108	ITS1 - ITS4	N	ITS4	EU591808
R110	ITS1 - ITS4	N	ITS4	EU591809
R112	ITS4 - ITS5	Y	ITS4, ITS5	EU591810
R113	ITS4 - ITS5	N	ITS4, ITS5	EU591811
R115	ITS4 - ITS5	Y	ITS4, ITS5	EU591812

^a Primers were from White et al. (69).

individual tube represents a replication, and each test consisted of four replications per isolate. Pathogenicity tests were conducted at four time-points, and negative controls were included each time. A subset of isolates was tested each time, and each isolate was tested twice. Thus, a total of 16 observations for each isolate and 32 observations for the negative control were made. After 2 weeks of incubation, plants were carefully removed from the soil and the roots were washed. Disease severity was assessed at the lower stem using a rating scale from 0 to 5, where 0 = no symptoms observed (healthy), 1 = lesions smaller than 2 mm, 2 = lesions larger than 2 mm, 3 = girdling lesion on crown tissue, 4 = rotted mesocotyl causing wire-stem symptoms, and 5 = dead seedling. After harvest, pieces of symptomatic corn tissue from several treatments were placed on acidified PDA to ascertain the presence and recovery of the same *R. solani* or *Rhizoctonia*-like fungi used in the inoculation.

Pathogenicity evaluation on snap beans in the greenhouse. This experiment was conducted to compare aggressiveness of *R. solani* and *Rhizoctonia*-like isolates on corn and on a vegetable, snap bean cultivar Hystyle. Seven isolates (R18, R20, R25, R31, R39, R47, and R62) that showed variable aggressiveness on corn were chosen to inoculate snap bean plants. Snap bean seeds treated with Captan, Maxim, and Thiram were planted in 10-cm-diameter clay pots (4 seeds per pot) filled with pasteurized soil. Inoculum of the collected isolates was prepared by growing the isolates on PDA for 10 days. Eleven days after planting, snap bean seedlings were inoculated as described above for corn seedlings. An individual pot represented a replication, and there were four replications per isolate. The test was conducted only once. After 2 weeks of incubation, plants were carefully removed from the soil and the roots and stems were washed. Disease severity was assessed at the lower stem using a rating scale of 0 to 3, where 0 = no symptoms (healthy), 1 = superficial lesions, 2 = sunken distinct lesions, and 3 = rotted lower hypocotyl tissues showing initial symptoms of wire-stem.

Statistical analysis. Data from both corn and snap bean experiments were analyzed using SAS software Version 9 (SAS Institute Inc., Cary, NC). The ordinal data did not have a normal distribution; therefore they were analyzed using the non-parametric methodology of Brunner et al. (6) as described by Shah and Madden (52). For analyses on corn, data were pooled from the two tests to obtain overall results. One-way analyses were performed to separately assess disease severity as influenced by individual isolates for the first analysis and by AGs or subgroups for the second analysis. PROC RANK was used to obtain midranks followed by PROC MIXED to

calculate test statistics and significance levels. Estimated relative treatment effects were calculated using $p_{\text{hat}}_i = 1/N\{R_{\text{bar}}_i - 1/2\}$ where i is the treatment, N is the number of observations, and R is the mean rank. Confidence intervals were calculated using LD_CI macro (52). The analysis on snap bean was done similarly; however, because the incidence of infection on snap bean was relatively low, only infected plants were included in the analysis.

RESULTS

Phylogenetic analysis. In the Bayesian inference tree, collected isolates were inferred to belong to an AG, subgroup, or species if they formed a cluster including a reference sequence supported by a posterior probability value (PP) of 90% or higher and were considered closely related to a group if supported by a PP lower than 90%. The analysis inferred the identification of 61 isolates: 26 isolates (38%) were AG 2-2, 19 isolates (28%) were AG 4, 6 isolates (9%) were AG 1, 3 isolates (4%) were AG 2-1, 2 isolates (3%) were AG 5, 2

isolates were *Waitea circinata* var. *zeae*, 1 isolate (1%) was AG 11, 1 isolate (1%) was CAG 2, and 1 isolate (1%) was CAG 6 (Fig. 1). For the remaining 7 isolates, 5 isolates were closely related to CAG 2 (PP 38%) and the identity of 2 isolates (R20 and R59) could not be determined (Fig. 1). As for identifying isolates to further subgroup of cultural type within AG 2-2: 5 isolates belonged to AG 2-2IIIB, 1 isolate was closely related to AG 2-2IIIB (PP 86%), 16 isolates were closely related to AG 2-2IV (PP 80% and PP 64%), and 4 isolates were closely related to AG 2-2LP (PP 84%). Within AG 4: 1 isolate belonged to AG 4-HGI, 1 isolate was closely related to AG 4-HGI (PP 77%), 15 isolates belonged to AG 4-HGII, and 1 isolate was closely related to AG 4-HGII (PP 58%). Within AG 1: 2 isolates belonged to AG 1-IB and 4 isolates belonged to AG 1-IC (Fig. 1).

In the neighbor-joining tree, collected isolates were inferred to belong to an AG, subgroup, or species if they formed a cluster including a reference sequence sup-

Table 3. Reference sequences used in this study to characterize *Rhizoctonia solani* and *Rhizoctonia*-like fungi isolated in New York

Species	AG/subgroup	GenBank accession no.	Origin	Reference
<i>Rhizoctonia solani</i>	AG 1-IA	AY270010	Soybean, Brazil	(12)
		AB122133	Unknown	(64)
	AG 1-IB	AB122139	Unknown	(64)
		AF308626	Bean, Dominican Republic	(17)
	AG 1-IC	AB122142	Unknown	(64)
		U19951	Unknown, France	(51)
	AG 1-ID	AB122128	Unknown	(64)
		AB122130	Unknown	(64)
	AG 2-1	U57729	<i>Pinus</i> , Canada	(51)
		AY154317	Unknown	(26)
	AG 2-1-2t	AB054850	Tulip, Netherlands	(10)
		AB054852	Tulip, Netherlands	(10)
	AG 2-2LP	AB054866	Zoysia grass, Japan	(10)
		AJ238163	Zoysia grass, Japan	(50)
	AG 2-2IIIB	AJ238160	Zoysia grass, Japan	(50)
		AF354116	Matrush, Japan	(18)
	AG 2-2IV	AJ238166	Maize, Japan	(50)
		AB000014	Sugar beet, Japan	(25)
	AG 2-3	AY270014 ^a	Unknown	(12)
		U57740	Soybean, Japan	(51)
	AG 2-4	AB054871	Soybean, Japan	(10)
		AB054878	Maize, USA	(10)
	AG 2-BI	AB054879	Carrot, USA	(10)
		AB054880	Carrot, USA	(10)
	AG 3TB	AB054873	Soil, Japan	(10)
		AB054875	Soil, Japan	(10)
	AG 3PT	AF153774	Tabacco, USA	(43)
		AB000004	Tabacco, USA	(25)
	AG 4-HGI	AB019023	Soil, Australia	(24)
		AB019017	Potato, USA	(24)
	AG 4-HGII	AB000007	Spinach, Japan	(25)
		AY152704	Tomato, Brazil	(26)
	AG 4-HGIII	AB000006	Sugar beet, Japan	(25)
		AY154308	Unknown	(26)
	AG 5	AY154659	Spinach, Brazil	(26)
		DQ102449	Soil, Israel	(53)
	AG 6-HGI	AF153778	Unknown	(43)
		AF354113	Sugar beet, Japan	(18)
		AB000019	Soil, Japan	(25)
		AF354102	Soil, Japan	(18)

(continued on next page)

^a The GenBank accession number is not listed in the publication but inferred to by GenBank.

ported by a bootstrap value (BS) of 95% or higher, and were considered closely related to a group if supported by a BS lower than 95%. Using this criterion, the neighbor-joining analysis supported the inferences suggested by the Bayesian inference analysis for 61 isolates. The remaining 7 isolates were closely related to CAG 2 supported by a BS 87% (Fig. 1). As for identifying isolates to further subgroup or cultural type, within AG 2-2: 10 isolates belonged to AG 2-2IV; within AG 4: 1 isolate belonged to AG 4-HGI and 15 isolates were closely related to AG 4-HGII (BS 81%); and within AG 1: 4 isolates belonged to AG 1-IC and 2 isolates belonged to AG 1-IB. In comparison to the Bayesian tree, the neighbor-joining tree was not as resolved and did not permit tentative inferences about subgroups and cultural types for the remaining isolates within AG 2-2 and AG 4.

Pathogenicity evaluation on corn in the greenhouse. Disease severity on corn caused by *R. solani* and *Rhizoctonia*-like fungi isolated in New York was evaluated using relative treatment effects and their 95% confidence intervals (CI) (Table 4).

Among the 32 observations of the negative control, 2 plants were minimally infected with a disease rating of 1. This was potentially due to contamination through splashing of soil during watering, and the 95% CI of the negative control was used to infer the chance of contamination. Sixty-one isolates were considered pathogenic to corn; whereas pathogenicity of the remaining 7 isolates (R20, R62, R60, R65, R18, R59, and R36) could not be confirmed as it was within the range of the chance of contamination. There were significant differences in disease severity on corn caused by individual isolates (Table 4). In addition, there were significant differences in disease severity on corn when the isolates were grouped according to inferred AG/subgroup/species of *R. solani* and *Rhizoctonia*-like fungi (Fig. 2). AG 2-2 isolates were the most aggressive, although not significantly different from AG 5 and AG 11, and CAG 2 isolates were the least aggressive on corn (Fig. 2).

Pathogenicity evaluation on snap bean. Disease severity on snap bean caused by the selected 7 isolates of *R. solani* and *Rhizoctonia*-like fungi collected

in New York was evaluated using relative treatment effects and their 95% CI (Table 4). All isolates tested were pathogenic to snap bean. Also, there were significant differences in disease severity caused by the selected isolates on snap bean. Isolate R18 was the least aggressive on snap beans, whereas isolate R39 was the most aggressive (Table 4).

DISCUSSION

Molecular characterization of *R. solani* and *Rhizoctonia*-like fungi in New York showed the presence of a diverse population with isolates belonging to AG 1, AG 2-2, AG 4, AG 5, AG 11, CAG 2, CAG 6, *W. circinata* var. *zeae*, and several isolates closely related to CAG 2 (Fig. 1). The dominant groups were AG 2-2 (38%) and AG 4 (28%). As most isolates were collected from table beets and snap beans, this finding is consistent with results reported previously from New York (15,39). This is the first report of *W. circinata* var. *zeae* isolated from vegetable tissues, specifically from naturally infested carrot and snap bean plants in western New York. *W. circinata* is generally known to be pathogenic on grasses including corn (55); however, *W. circinata* var. *zeae* has been found to cause disease on onions and has been isolated from naturally infected soybean and bean in Turkey (13,14). This was also the first time that AG 11 (isolate R22) was isolated in this region. AG 11 has been reported to be pathogenic on wheat and lupine (11,16,59), but isolate R22 was collected from snap bean.

Isolates from AGs that are documented to be pathogenic on corn, AG 1, AG 2, and AG 4, were present. Within these AGs, we specifically sought the presence of subgroups/cultural types that are well-documented to be pathogenic on corn, AG 1-IA and AG 2-IIIB. No isolates of AG 1-IA were detected; however, 6 isolates of AG 2-IIIB were recovered in this investigation.

Among the different AGs, CAGs, and *Rhizoctonia*-like fungi that were examined, *R. solani* AG 2-2 isolates were most aggressive on corn, AG 4 isolates were moderately aggressive, and binucleate CAG 2 isolates were least aggressive (Fig. 2). These results are in agreement with those reported previously by Ithurrart et al. (20). The earliest observations of the ineffectiveness of corn rotation in reducing severity of *R. solani* and *Rhizoctonia*-like diseases on vegetables in New York were made by beet growers, and this study shows that most isolates recovered from table beets belong to AG 2-2. Additionally, AG 2-2 has been confirmed to produce the sexual state, *T. cucumeris*, on table beets in commercial fields in New York (39). This suggests the possibility that AG 2-2 isolates of *R. solani* are evolving pathogenicity traits to corn through sexual recombination. In combination with the isolation of

Table 3. (continued from preceding page)

Species	AG/subgroup	GenBank accession no.	Origin	Reference
<i>Waitea circinata</i>	AG 6-GV1	AF153780	Unknown, Japan	(43)
		AF153782	Soil, Japan	(43)
	AG 6-GV2	AY154304	Unknown	(26)
		AF354104	Soil, Japan	(18)
	AG 6-GV3	AF153788	Wheat, South Africa	(43)
		AF153790	Wheat, Tanzania	(43)
	AG 6-GV4	AF153785	Leaf litter, Australia	(43)
		AF153787	Leaf litter, Australia	(43)
	AG 7	AF354100	Soil, USA	(18)
		AB000003	Soil, Japan	(25)
	AG 8	AF354068	Barley, Australia	(18)
		AF153797	Soil, Australia	(43)
		AB000011	Wheat, Australia	(25)
	AG 9	AF354108	Potato, USA	(18)
		AF354065	Potato, USA	(18)
	AG 10	AF354071	Barley, Australia	(18)
		AF153800	Soil, Australia	(43)
	AG 11	AF153802	Lupine, Australia	(43)
		AY154313	Unknown	(26)
	AG 12	AF153804	<i>Pterostylis acuminata</i>	(43)
		AF153805	<i>Pterostylis acuminata</i>	(43)
	AG 13	AB275645	Cotton, USA	(9)
		AB275642	Cotton, USA	(9)
	AG BI	AF354110	Soil, Japan	(18)
		AB000044	Soil, Japan	(25)
	<i>W. circinata</i> var. <i>zeae</i>	AB213594	Soil, Japan	(63)
		AB213597	Unknown	(63)
	<i>W. circinata</i> var. <i>agrostis</i>	AB213575	Creeping bentgrass, Japan	(63)
		AB213577	Kentucky bluegrass, Japan	(63)
	<i>W. circinata</i> var. <i>circinata</i>	AB213582	Creeping bentgrass, Japan	(63)
		AB213581	Unknown	(63)
	<i>W. circinata</i> var. <i>oryzae</i>	AJ000195	Rice, Japan	(21)
		AB213589	Rice, Japan	(63)
<i>Ceratobasidium</i> spp.	CAG 1	AF354086	Turfgrass, USA	(18)
	AG A (CAG 2)	AF354092	Soil, Japan	(18)
	CAG 3	AF354080	Peanut, USA	(18)
	CAG 4	AF354081	Soybean, USA	(18)
	CAG 5	AF354082	Cucumber, USA	(18)
	CAG 6	AF354083	<i>Erigeron</i> , USA	(18)
	CAG 7	AF354084	<i>Pittosporum</i> , USA	(18)
<i>Athelia rolfsii</i>	<i>A. rolfsii</i>	AY684917	Unknown	Unpubl.

Fig. 1. Bayesian inference tree generated from the rDNA ITS1-5.8S-ITS2 region of the nuclear ribosomal DNA of reference sequences and collected isolates. Bayesian posterior probability values greater than 50% are indicated in *italics*. Reference sequences are indicated with GenBank accession numbers followed by the species, anastomosis group (AG), or subgroup designation. New York isolates are shaded in gray and indicated by the isolate number and its host of origin. Inferred identifications are indicated on the right. The part of the neighbor-joining tree that resulted in different inferences or resolution to the Bayesian inference tree is indicated in the boxes with bootstrap values.

Table 4. Median and relative treatment effects (p) with 95% confidence intervals (CI) of disease severity ratings obtained for individual isolates from the corn and snap bean pathogenicity tests^a

Isolate	AG	Host plant	Corn			Snap bean		
			Median	p ^b	95% CI for p ^b	Median	p ^b	95% CI for p ^b
Neg. control	NA	NA	0	0.151	(0.131, 0.17)	0	0.269	(0.228, 0.31)
R20	CAG 2	Snap bean	0	0.166	(0.111, 0.221)	1	0.516	(0.401, 0.631)
R62	CAG 2	Table beet	0	0.191	(0.087, 0.295)	1	0.487	(0.376, 0.599)
R60	CAG 2	Snap bean	0	0.207	(0.131, 0.283)			
R65	AG 4	Snap bean	0	0.207	(0.131, 0.283)			
R18	<i>W. circinata</i> var. <i>zeae</i>	Carrot	0	0.22	(0.142, 0.297)	1	0.419	(0.313, 0.525)
R59	CAG 2	Snap bean	0	0.22	(0.142, 0.297)			
R36	CAG 2	Dry bean	0	0.26	(0.163, 0.357)			
R37	CAG 2	Dry bean	0	0.26	(0.173, 0.348)			
R15	AG 2-1	Carrot	0	0.273	(0.187, 0.36)			
R16	AG 2-1	Carrot	0	0.291	(0.188, 0.393)			
R9	AG 4	Carrot	0.5	0.301	(0.209, 0.394)			
R77	CAG 6	Snap bean	0.5	0.301	(0.209, 0.394)			
R113	CAG 2	Table beet	0.5	0.308	(0.197, 0.418)			
R14	AG 4	Snap bean	1	0.312	(0.231, 0.393)			
R104	AG 2-1	Cabbage	0.5	0.316	(0.218, 0.415)			
R12	AG 4	Snap bean	1	0.329	(0.233, 0.425)			
R112	AG 4	Table beet	0.5	0.332	(0.228, 0.435)			
R94	AG 4	Snap bean	0	0.344	(0.213, 0.474)			
R21	CAG 2	Snap bean	1	0.372	(0.271, 0.473)			
R110	AG 4	Table beet	1	0.39	(0.257, 0.523)			
R5	AG 4	Pea	1	0.393	(0.282, 0.503)			
R55	AG 4	Snap bean	1.5	0.413	(0.319, 0.507)			
R92	AG 4	Snap bean	1	0.424	(0.345, 0.503)			
R107	AG 1	Cabbage	2	0.452	(0.334, 0.571)			
R108	AG 1	Cabbage	2	0.452	(0.334, 0.571)			
R105	AG 2-2	Snap bean	2	0.462	(0.328, 0.597)			
R115	AG 2-2	Carrot	1	0.468	(0.33, 0.605)			
R6	AG 4	Pea	1.5	0.477	(0.381, 0.573)			
R17	AG 1	Carrot	2	0.478	(0.372, 0.584)			
R106	AG 4	Carrot	2	0.478	(0.372, 0.584)			
R43	AG 4	Snap bean	2	0.484	(0.403, 0.565)			
R83	AG 2-2	Table beet	1.5	0.491	(0.33, 0.651)			
R4	AG 1	Cabbage	2	0.525	(0.471, 0.579)			
R13	<i>W. circinata</i> var. <i>zeae</i>	Snap bean	2	0.54	(0.443, 0.636)			
R101	AG 4	Snap bean	2	0.552	(0.467, 0.638)			
R35	AG 5	Dry bean	2	0.553	(0.44, 0.667)			
R68	AG 2-2	Table beet	2	0.555	(0.415, 0.695)			
R3	AG 1	Carrot	2	0.558	(0.487, 0.628)			
R82	AG 4	Table beet	2	0.564	(0.487, 0.642)			
R90	AG 2-2	Table beet	2	0.564	(0.43, 0.697)			
R31	AG 2-2	Table beet	3	0.572	(0.412, 0.732)	1	0.518	(0.389, 0.647)
R22	AG 11	Snap bean	2	0.574	(0.435, 0.713)			
R70	AG 2-2	Table beet	2	0.574	(0.438, 0.711)			
R10	AG 4	Snap bean	2	0.59	(0.501, 0.679)			
R32	AG 2-2	Table beet	2.5	0.593	(0.453, 0.734)			
R93	AG 1	Snap bean	2	0.599	(0.49, 0.708)			
R57	AG 4	Snap bean	2	0.606	(0.522, 0.689)			
R33	AG 2-2	Table beet	2	0.617	(0.516, 0.718)			
R66	AG 2-2	Table beet	2	0.631	(0.496, 0.765)			
R88	AG 2-2	Table beet	2	0.633	(0.51, 0.755)			
R11	AG 4	Snap bean	2	0.64	(0.536, 0.744)			
R64	AG 4	Snap bean	2	0.646	(0.558, 0.733)			
R81	AG 2-2	Table beet	2	0.646	(0.543, 0.748)			
R89	AG 2-2	Table beet	2	0.654	(0.553, 0.755)			
R84	AG 2-2	Table beet	3	0.68	(0.551, 0.809)			
R1	AG 2-2	Snap bean	2.5	0.697	(0.597, 0.798)			
R7	AG 5	Pea	2	0.697	(0.612, 0.783)			
R29	AG 2-2	Table beet	2.5	0.717	(0.608, 0.826)			
R41	AG 2-2	Table beet	3	0.727	(0.61, 0.844)			
R27	AG 2-2	Table beet	2.5	0.732	(0.656, 0.808)			
R85	AG 2-2	Table beet	3	0.733	(0.653, 0.814)			
R100	AG 2-2	Snap bean	3	0.775	(0.699, 0.851)			
R47	AG 2-2	Table beet	3	0.777	(0.674, 0.88)	1	0.505	(0.379, 0.632)
R2	AG 2-2	Snap bean	3	0.785	(0.694, 0.876)			
R8	AG 2-2	Table beet	3.5	0.792	(0.699, 0.884)			
R25	AG 2-2	Snap bean	3	0.813	(0.739, 0.887)	1	0.518	(0.389, 0.647)
R75	AG 2-2	Snap bean	3.5	0.818	(0.735, 0.901)			
R39	AG 2-2	Table beet	4	0.898	(0.872, 0.924)	2	0.795	(0.703, 0.886)

^a Isolates are ranked in ascending order of relative treatment effects from the corn pathogenicity test. Each isolate is accompanied by the inferred identification of anastomosis group (AG), subgroup, or species and the host of isolation. Disease severity ratings on corn were determined on a scale of 0 to 5, and those on snap bean were determined on a scale of 0 to 3.

^b Relative treatment effects were calculated by performing one-way analyses using the nonparametric method for ordinal data described by Shah and Madden (52).

W. circinata var. *zeae* from symptomatic vegetables, these findings suggest the ineffectiveness of the corn rotation may be due to an expansion in host range. Isolates of AG 2-2 may have acquired the ability to infect corn, while isolates of *W. circinata* var. *zeae* may have gained the ability to infect vegetables. Further investigation of the host range of these isolates is necessary to confirm these hypotheses. AGs or species previously considered to be nonpathogenic on corn were not recovered in this investigation.

Our results also revealed differences in AGs recovered from different hosts. Most isolates recovered from table beets belonged to AG 2-2, and most isolates recovered from cabbage belonged to AG 1. However, isolates recovered from carrot and snap bean belonged to several AGs. This suggests that certain hosts are susceptible to specific AGs, whereas other hosts are susceptible to a wide array of AGs (Fig. 1, Table 4).

The pathogenicity test on snap beans suggested a potential correlation between aggressiveness of the collected isolates on corn and snap bean. Isolates R18, R20, and R62 exhibited relatively low aggressiveness on corn; whereas isolates R25, R31, R39, and R47 were higher in aggressiveness. Similarly, isolates R18 and R62 were low in aggressiveness on snap bean; whereas isolates R25, R31, and R39 exhibited higher aggressiveness (Table 4). Due to the small number of isolates that were tested for aggressiveness on snap bean, the correlation between the aggressiveness of the isolates on corn and snap bean should be further tested before definitive conclusions are made. If such correlation between aggressiveness on corn and snap bean or other vegetables can be proven with a larger number of isolates and under

field conditions, then it can be recommended to avoid corn as a rotation crop for snap bean or other vegetables where *R. solani* and *Rhizoctonia*-like fungi are prevalent.

R. solani and *Rhizoctonia*-like fungi traditionally have been grouped in the form genus *Rhizoctonia*, a taxon that includes a heterogeneous mix of polyphyletic fungi grouped by general vegetative features (16,56). Teleomorphs, hyphal anastomosis reactions, and nuclear conditions have been studied to better understand *Rhizoctonia* systematics (4,34,35,42,67,68), but the segregation of these fungi is still not well-defined and their taxonomic ambiguity was reflected in our phylogenetic analysis. In both the Bayesian and neighbor-joining trees, reference sequences of AG 2 and CAGs did not group with other reference sequences representing the same group. As for AG 2 subgroups that did not cluster together, Kuninaga et al. (25) reported similar results and suggested that subgroups within AG 2 may be phylogenetically distant. There are different opinions about whether AG 2 subgroups should be considered as distinct AGs or not (8). Unlike other AG subgroups that are determined by pathogenicity, morphology, and biochemical properties, AG 2 subgroups are determined by hyphal fusion frequency (8,36,51), a criterion similar to that used in characterizing AGs. Taking this into consideration, AG 2 subgroups may be more representative of distinct AGs than subgroups, and our analyses support this hypothesis. As for the CAG reference sequences that did not group together, Gonzalez et al. (18) reported similar results and the taxonomy of *Ceratobasidium* and *Thanatephorus* is still under debate. A more rigorous, multilocus approach is necessary to clarify the phylogeny of these

two genera. Our analyses suggest the use of the rDNA ITS sequence in either Bayesian inference or neighbor-joining to be robust enough to identify isolates to species and AG. However, in order to classify isolates to subgroup or AG 2-2 cultural type, Bayesian inference appears to be more predictive than neighbor-joining (Fig. 1).

Our results suggest that *R. solani* and *Rhizoctonia*-like fungi that infect vegetables in western New York, especially AG 2-2 isolates, are capable of infecting corn as well. Visual observations of symptoms caused by these isolates and their reisolation from inoculated seedlings clearly indicate that corn serves as a host plant. The results from our experiments suggest that corn should be avoided as a rotation crop especially when AG 2-2 is present in the field. In fact, disease severity caused by isolates R66, R68, and R70 (all characterized as AG 2-2) that were collected from a field that was in corn rotation during 2005 were as high as or higher than that caused by isolates R13 and R14 (characterized as *W. circinata* var. *zeae* and AG 4, respectively) that were collected from a field that was in table beet during 2005 (Table 4). Following up this study with larger sample sizes from each AG may provide a better understanding of when corn rotations should be avoided due to the presence of certain genotypes of *R. solani* and *Rhizoctonia*-like fungi in the field. In this study we looked at one sweet corn cultivar, but it would be interesting to see if certain corn cultivars are resistant to infection by *R. solani* and *Rhizoctonia*-like fungi. Additionally, in contrast to the controlled environment of a greenhouse, pathogenicity studies should be conducted in the field to simulate natural conditions. Future investigations to evaluate the effectiveness of other cereals as rotation crops against *R. solani* and *Rhizoctonia*-like fungi are also needed.

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Fig. 2. Disease severity on corn caused by *Rhizoctonia solani* and *Rhizoctonia*-like isolates collected in New York, when grouped by inferred anastomosis group (AG), subgroup, or species. Disease severity ratings were determined on a scale of 0 to 5. Relative treatment effects were calculated using the nonparametric method for ordinal data described by Shah and Madden (52). Bars indicate 95% CI of relative treatment effects. Different letters indicate significant differences between groups using the 95% CI. Group 0 indicates the negative control and group W.c.v.z. indicates *Waitea circinata* var. *zeae*.

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