Identification, Isolation Frequency, and Pathogenicity of Anastomosis Groups of Binucleate *Rhizoctonia* spp. from Strawberry Roots

S. B. Martin

Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, 123 Huntington Street, P.O. Box 1106, New Haven 06504.

I thank Hilary Hudak and Mary Inman for excellent technical assistance, C. C. Green for assistance with the statistical analyses, and Terry Jones, Randy Vasauskas, Roger Pell, and Ralph Wetherall, Jr., for access to their farms. Also, the kindness of Dr. A. Ogoshi, Hokkaido University, in providing anastomosis group tester isolates is appreciated.

Accepted for publication 17 June 1987.

ABSTRACT

Martin S. B. 1988. Identification, isolation frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. Phytopathology 78:379-384.

Rhizoctonia spp. were isolated from roots of strawberries (*Fragaria* × *ananassa*) in Connecticut during the spring of 1985 and 1986 and the fall of 1986. *R. solani* (AG-5) was isolated infrequently (2.7% in 1985; 3.1% in 1986) in comparison with binucleate *Rhizoctonia* spp. Three anastomosis groups (AG) were identified among the binucleate *Rhizoctonia* spp.: AG A, AG G, and AG I. Isolates grouped in AG G were obtained more frequently than those in AG A or AG I in spring samples (42.9 vs. 26.1 and 15.4% in 1985, and 56.1 vs. 14.0 and 26.8% in 1986). There was a higher percentage of recovery of AG I compared with AG G or AG A in samples collected in fall 1986 (72.7 vs. 14.7 and 7.9%, respectively). Frequencies of isolation of isolates in particular anastomosis groups differed among farms and among fields within farms. Isolates obtained from different roots of the same plant usually belonged to the same anastomosis group. Isolates obtained from each anastomosis group were pathogenic, but at 15 C, those belonging to AG I induced significantly greater disease severity on strawberry than AG A or AG G isolates.

Additional key words: black root rot, *Ceratobasidium* sp., soilborne pathogens.

The black root rot complex of cultivated strawberries (*Fragaria* × *ananassa* Duchnesne) has been studied extensively (2,4–6,10–13,18–24). Root symptoms include the deterioration of the root cortex of perennial roots and loss of feeder rootlets. Many microorganisms, including fungi and nematodes (7), have been associated with diseased strawberry roots and have proven to be pathogens. Differentiation of these diverse pathogens and definitive association of specific pathogens or pathogen complexes with specific disease situations in the field have been difficult because of similar symptomatology of resultant diseases and continuous association of some of these diverse pathogens with strawberry roots.

Rhizoctonia spp. comprise a group of fungi that have been associated commonly with roots of strawberries and have been implicated as containing significant pathogens (5,6,10–12, 18–20,22–24). Most of the literature before 1960 mentioned *R. solani* Kühn as the species associated with diseased strawberry roots (4,10,11,24). In 1963, a new species, *R. fragariae* Husain and McKeen, was described as a pathogen of strawberries and was implicated in strawberry degeneration in Ontario (5,6). In 1967, Parmeter et al (17) reported that some fungi with *Rhizoctonia* anamorphic states resembled *R. solani*, but possessed predominantly binucleate hyphal cells. The teleomorphic states of certain of these binucleate *Rhizoctonia*-like fungi were identified as species of *Ceratobasidium* (17). However, *R. solani* has a *Thanatephorus* teleomorphic state (1). Parmeter et al (17) found isolates of *R. fragariae* from strawberry in California to belong in the group with binucleate hyphal cells.

Fungi that have been identified as binucleate *Rhizoctonia* spp. have been grouped by anastomosis (3,14,15) similar to *R. solani* (1). Burpee et al (3) identified seven anastomosis groups ("CAG" groups) among binucleate *Rhizoctonia* and placed one isolate of *R. fragariae* in CAG 2. Ogoshi et al (15) separated the binucleate *Rhizoctonia* spp. into at least 15 anastomosis groups ("AG" groups), and reported that isolates identified as *R. fragariae* belonged to one of three goups: AG A, AG G, or AG I (15). Ogoshi's results were based on isolates obtained from Japan, whereas Burpee's results were obtained with isolates from the United States.

The role of *Rhizoctonia* spp. as root pathogens of strawberries has not been determined adequately under growing practices used in the Northeast United States, where plantings may be kept in production for as long as 6 yr. Recent discoveries have indicated the potential importance of distinguishing binucleate *Rhizoctonia* spp. from *R. solani* (3,14,15) and the usefulness of the anastomosis group concept for grouping isolates within *R. solani* (1) and binucleate *Rhizoctonia* spp. (3,14,15). It has become apparent that particular binucleate *Rhizoctonia* spp. may induce distinct diseases compared with those induced by *R. solani* on certain hosts. Furthermore, isolates within anastomosis groups of *R. solani* (1) and binucleate *Rhizoctonia* spp. (3,14,15) frequently exhibit varying degrees of host specialization. Determination of the predominant pathogenic *Rhizoctonia* spp. associated with particular plants is important for development of resistance in cultivars and in other forms of disease control. Therefore, the objectives of this study were to identify the *Rhizoctonia* spp. and anastomosis groups of isolates within species associated with strawberry roots; to determine the relative abundance of particular species and anastomosis groups of isolates within species; and to determine the pathogenicity and relative virulence of isolates among and between anastomosis groups. A preliminary report has been published (9).

MATERIALS AND METHODS

Isolate collection. Isolates of *Rhizoctonia* were obtained from roots of strawberries grown in commercial pick-your-own operations in Connecticut in the spring of 1985 and 1986 and the fall of 1986. In May 1985, strawberry roots were collected from five farms. Strawberry cultivars grown on these farms included Honeoye, Raritan, Redchief, Jewel, Allstar, Kent, and Canoga. On Farm I, located in southern Connecticut, seven fields were sampled, with plantings ranging in age from 1 to 6 yr. Five fields were sampled on Farm II, located in western Connecticut, with

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plantings ranging in age from 1 to 5 yr. On Farm III, located in central Connecticut, three 4- and 5-yr-old fields were sampled. Isolates were also obtained from two farms in northern Connecticut. One field on each of these farms was sampled. In May 1986, only Farms I, II, and III were sampled. Additional samples were collected from Farms I and III in November and December of 1986 to determine if there was a shift in frequencies of isolation of particular anastomosis groups under the cooler conditions. In this collection, five fields were sampled from Farm I, with five plants collected per field. Three fields were sampled from Farm III, with five plants collected per field.

A hierarchical sampling scheme was used. Five perennial roots per plant were assayed and isolation of one *Rhizoctonia* sp. isolate per root was attempted. As many as five isolates were collected from each of four plants within each field on the three farms. Isolates were labelled according to the plant number (1–4), field number, and farm (I, II, or III) from which they were obtained. Isolations were made by washing the soil from the roots using tap water. Individual perennial roots were selected and washed in sterile distilled water (three changes, approximately 1 min each), root segments (approximately 1 cm long) were blotted on sterile paper towels and placed on 2% water agar or acidified water agar in petri plates. After 2 to 3 days, hyphae resembling *Rhizoctonia* were transferred to plates containing Difco potato-dextrose agar (PDA) or PDA containing 20 μg/ml of rifampin and 100 μg/ml of streptomycin sulfate. Isolates were stored at 20–25 C on PDA slants in the laboratory.

Data from both the spring and fall samples were analyzed by analysis of variance as a completely hierarchical design to test for farm, field within farm, and plant within field differences for frequency of occurrence of each anastomosis group. Also, counts of isolates within anastomosis groups were compared using a chi-square test to determine if frequencies of occurrence of anastomosis group classes were equal within fields and across farms.

Isolate identification. Isolates were identified as *Rhizoctonia* by the typical hyphal branching and dolipore septa. Numbers of nuclei within hyphal cells of each *Rhizoctonia* spp. isolate were determined by using the fluorescent stain diamidino-2-phenylindole (DAPI) (8). Each isolate was cultured in petri plates containing approximately a 1-mm-thick layer of PDA for 3–5 days. Disks of mycelium and agar from the margins of cultures were taken, fixed in 3.5% formaldehyde, rinsed in sterile distilled water, stained in 1% DAPI for 4 min, and destained in sterile distilled water for 3 min. Hyphae were viewed with a Zeiss Universal microscope with UV epiillumination. The system used a Zeiss filter set No. 02, containing exciter filter G 365, dichromatic beam splitter FT 395, and barrier filter LP 420; a Neofluar 63× oil immersion objective was used. Nuclei in all isolates tested were observed clearly with this method. Isolates were identified as *R. solani* if hyphal cells were multinucleate and if the cultures eventually formed a brown coloration. Isolates were identified as binucleate *Rhizoctonia* spp. if they possessed the typical hyphal branching pattern, dolipore septa, and possessed predominantly binucleate hyphal cells, regardless of culture color.

Binucleate isolates were separated into anastomosis groups by pairing them with known tester isolates of binucleate *Rhizoctonia* spp. Anastomosis group tester isolates and their origins have been reported (14). Isolates from strawberry and tester isolates from Japan were cultured separately on PDA for 2 days. Disks of mycelium and agar (2 mm diameter) were cut from the margins of these actively growing cultures. A mycelial disk of an unknown isolate and an appropriate tester strain were placed with the mycelium side down, approximately 3-cm apart, on an agar-coated slide. Slides were placed on sterile bent glass rods in plastic petri plates containing 5 ml of sterile distilled water for humidification. Plates were enclosed in plastic bags and incubated at 20–25 C until hyphae overlapped 3–5 mm (about 3 days). Hyphae were observed microscopically (100–430×) to determine if anastomosis had occurred. Anastomosis was considered positive if hyphae of the two isolates fused without discernible cell death or if fusion occurred with apparent cell death resulting in imperfect anastomosis (1). Simple contact of hyphae was scored negatively. Isolates that were not assigned to an anastomosis group initially were retested. Isolates that were multinucleate and corresponded to the characteristics of *R. solani* were similarly tested for anastomosis with tester isolates of *R. solani* in AG 1, AG 2-1, AG 2-2, AG 3, AG 4, and AG 5 from the author's collection.

Pathogenicity. Dormant strawberry plants, cultivar Honeoye, were heat treated in water at 54 C for 4 min (18) to eliminate contaminating *Rhizoctonia* spp. from the roots and crowns. These plants were grown in the greenhouse in an artificial peat-perlite mix that had been autoclaved previously for 1 hr. Runners from these plants were rooted into artificial mix contained in 10-cm-diameter pots. The pots with the runners were supported 25 cm above the greenhouse bench to prevent contamination. Runners rooted readily in this mix and plants that developed were used for pathogenicity tests 4–8 wk after rooting. Roots from runner plants were indexed for *Rhizoctonia* contamination by plating approximately 10 feeder roots from each plant on 2% water agar.

Four experiments were conducted to estimate the pathogenicity of binucleate *Rhizoctonia* spp. Experiments 1 and 2 used 10 randomly selected isolates chosen from a group of isolates collected in May 1985. The anastomosis groups of these isolates were undetermined before inoculation, but afterwards were determined to consist of binucleate isolates with five in AG A and five in AG I. These isolates were cultured on sterile, moist, tall fescue seed (*Festuca arundinacea* Schreb.). Ten grams of seed was added to each 125-ml Erlenmeyer flask, moistened with 15 ml of distilled water, and sterilized by autoclaving for 1 hr on 2 consecutive days. Two disks of agar (1 cm diameter) with actively growing mycelium of each isolate were added to the fescue seed medium and incubated in the lab for 2 wk. Field soil, previously fumigated with methyl bromide, was infested with colonized fescue seed at the rate of 1 g of infested seed per 4 kg of soil. A single runner plant was washed free of artificial mix and transplanted into the infested field soil. A control treatment consisted of plants transplanted into uninfested soil. There were five replicate pots per isolate arranged in a randomized complete block design in the greenhouse (25–>30 C) for experiment 1. The same isolates were tested in a Percival Model PT-80 growth chamber set at 25 C with a 12-hr day/night cycle for experiment 2. Plants were fertilized once after 1 wk with 200 ml per pot of a soluble 20-20-20 (N, P, and K) fertilizer with approximately 3 g of fertilizer per liter of water. After a 4-wk incubation, the soil was washed gently from the roots. The percentage of root rot was estimated visually by comparison with root systems of uninoculated plants having white to tan roots and no lesions. The roots and tops were also weighed.

Another experiment (experiment 3) was conducted using three isolates each of AG A and AG I. Experimental conditions were the same as experiment 2, except that the inoculated plants were incubated at 15 C for 8 wk. Plants in experiment 3 were fertilized as outlined previously at weeks 2 and 5. Disease severity estimates were based on a visual rating of percent root rot and weights of roots and tops.

A fourth pathogenicity test used 24 additional isolates, including nine isolates of AG A, eight of AG G, and seven of AG I tested in the growth chamber at 15 C. The virulence of each isolate was further evaluated in this experiment by calculating the percent decrease in root volume. The root volume of each plant was determined at the time of planting into the infested soil and 4 wk later by measuring the displacement of water in a graduated cylinder. Percent root rot was estimated and root and top weights were measured. Selected portions of roots from each treatment were plated as before to compare reisolated *Rhizoctonia* cultural types with the initial isolate. Data were analyzed by analysis of variance as randomized complete block designs and all model effects were considered random. Residual plots were examined to determine if a transformation was necessary to meet the assumptions of analysis of variance; these examinations did not indicate a need for transformation of data before analysis of variance. Means were separated by single degree-of-freedom linear contrasts to test for mean differences in pathogenicity among anastomosis groups.

RESULTS

Isolation and identification. All isolates collected from root isolation with hyphal branching typical of *Rhizoctonia* spp. (16) possessed dolipore septa *Rhizoctonia* spp. were commonly associated with greater than 70% of both perennial and feeder roots of strawberries from fields that had been in strawberry cultivation at least 1 yr previous to sampling. A relatively low incidence of isolation (20%) of *Rhizoctonia* spp. was found from roots sampled in 1985 from a previously fallow field on Farm I. One hundred fifty isolates were obtained from five farms in 1985, but only 4/150 (2.7%) were identified as *R. solani*, and these were grouped in AG 5. All other isolates were identified as binucleate *Rhizoctonia* spp. Within the binucleate isolates, three anastomosis groups were detected: AG A, AG G, and AG I. Of these groups, AG G was isolated from 79/150 (42.9%) plants, AG A from 41/150 plants (26.1%), and AG I from 26/150 (15.4%) of the plants.

Isolates belonging to AG G were culturally distinct from isolates belonging to AG A or AG I. AG G isolates turned dark brown in PDA culture similar to isolates of *R. solani* and also formed aggregates of monilioid cells resembling sclerotia on the surface of the mycelium within 2 wk of seeding of the plates. Isolates of AG A or AG I were white to light tan in color and did not form sclerotia, although tufts of monilioid cells were present occasionally. Isolates of AG A and AG I were similar in appearance until the cultures were more than 4 wk old. After this time, AG I isolates retained a strong zonation and white color, whereas AG A cultures turned tan in color with less zonation.

Isolates were obtained similarly in spring 1986 but were grouped according to farms of origin, fields within farms, plants within fields, and isolates within plants. *R. solani* was again detected infrequently (5/164, or 3.1%) in comparison with binucleate *Rhizoctonia* spp. (Table 1). As in 1985, binucleate isolates were assigned to one of three anastomosis groups: AG A, AG G, or AG I. AG G isolates were detected most frequently (92/164 or 56.1%), followed by AG I (44/164 or 26.8%) and AG A (23/164 or 14%). When isolations were made from different roots of the same root system, all isolates from a particular plant belonged to a single AG group in 33 of 43 (76.7%) plants sampled. There was no case in which isolates of all three AG groups (AG A, AG G, and AG I) were detected on roots of the same plant (Table 1). Analysis of variance of counts indicated a significant difference ($P = 0.01$) in

TABLE 1. Counts of isolates belonging to anastomosis groups (AG)[a] of binucleate *Rhizoctonia* spp. from strawberry roots, spring 1986

Location[b] and field	Plant number 1 AG A	1 AG G	1 AG I	2 AG A	2 AG G	2 AG I	3 AG A	3 AG G	3 AG I	4 AG A	4 AG G	4 AG I
Farm I												
Field 1	0	1	3	0	4	0	0	4	1	...	...	...
Field 2	0	5	0	0	0	4	0	3	2	0	2	1
Field 3	0	5	0	1	1	0	0	5	0	0	5	0
Field 4	0	3	0	0	0	5	0	0	4	0	2	0
Farm II												
Field 1	1	0	2	0	0	4	0	1	0	0	5	0
Field 2	1	0	2	4	0	0		(4/4 *R.s.*[c])		0	3	0
Field 3	0	3	0	0	4	0	0	1	2	...	...	...
Field 4	0	0	5	4	0	0	5	0	0		(1/1 *R.s.*)	
Field 5	0	1	2	0	0	2	1	0	1	0	0	4
Farm III												
Field 1	0	5	0	0	5	0	4	0	0	...	...	...
Field 2	0	4	0	0	4	0	0	5	0	bn[d]	bn	bn
Field 3	1	0	0	1	4	0	0	4	0	0	3	0

[a] Anastomosis group designations are those of Ogoshi (15).
[b] See text for location of individual farms.
[c] *R.s.* = *Rhizotonia solani*. These were identified as AG 5.
[d] Three binucleate *Rhizoctonia* spp. isolates were not assigned to an AG group.

TABLE 2. Counts of isolates belonging to anastomosis groups (AG)[a] of binucleate *Rhizoctonia* spp. from strawberry roots, fall 1986

Location[b] and field	Plant number 1 AG A	1 AG G[a]	1 AG I	2 AG A	2 AG G	2 AG I	3 AG A	3 AG G	3 AG I	4 AG A	4 AG G	4 AG I	5 AG A	5 AG G	5 AG I
Farm I															
Field 1	0	0	2	0	0	1	0	0	2	0	0	3	0	*[c]	2
Field 2	0	1	1	3	0	0	0	1	2	1	0	2	0	1	2
Field 3	0	0	3	*	*	*[c]	0	0	2	0	0	3	0	0	4
Field 4	2	0	0	0	0	2	0	0	4	0	0	3	0	0	2
Field 5	0	0	2	0	0	3	0	0	3	0	1	2	0	0	2
Farm III															
Field 1	0	2	0		(1/1	*R.s.*)[d]	0	0	3	1	0	3	0	2	0
Field 2	0	2	0	1	0	3	bn	bn	bn[e]	...	...	...	0	1	3
Field 3	...	...	...	0	2	0	...	...	...	...	...	...	...	...	...

[a] Anastomosis group designations are those of Ogoshi (14).
[b] See text for location of individual farms.
[c] These isolates were identified as *R. zeae*.
[d] *R.s.* = *R. solani* AG 5.
[e] These binucleate *Rhizoctonia* spp. were not assigned to an AG group.

frequency of occurrence of particular AG groups among plants.

Frequencies of detection of isolates belonging to AG A, AG G, and AG I were significantly different among farms and within farms (chi-square test, 4 df; chi-square = 42.75). For instance, only a single isolate of AG A was detected on Farm I, whereas 16/59 isolates from Farm II were assigned to AG A (Table 1). There were no AG I isolates recovered from Farm III in spring samples.

Isolates obtained from the fall-collected samples were predominantly AG I (Table 2). When data from both farms were pooled, the frequencies of isolation were as follows: AG I—72.7%; AG G—14.8%; AG A—7.9%; and *R. solani* (AG 5)—1.1%. *R. zeae* Voorhees was isolated from roots of one plant from Farm III, and an unidentified binucleate *Rhizoctonia* was also detected in the spring and fall of 1986 from roots of one plant (per sample) from Farm III. These unidentified *Rhizoctonia* isolates could not be assigned to a particular anastomosis group.

Pathogenicity. Isolates of binucleate *Rhizoctonia* spp. were pathogenic to roots of the strawberry cultivar Honeoye, rotting feeder roots off at the point of attachment to perennial roots, and causing sunken, sharply defined lesions on the primary perennial roots. *Rhizoctonia* isolates that were culturally identical to the original isolate were isolated from infected roots. There was a wide range in disease severity induced by the isolates tested in the pathogenicity experiments. Overall, disease severity under greenhouse temperatures (25–>30 C) (experiment 1) was relatively low, ranging from 0.6 to 10% root rot severity (Table 3) as compared with the disease severity induced by the same isolates when tested in the growth chamber at 15 C (experiment 2), when root rot ranged from 1.8 to 60% severity (Table 4). When disease severity was analyzed according to AG at 15 C, the AG I isolates induced significantly more disease than the AG A isolates (Table 4, experiment 1). Root rot induced by AG A isolates at 15 C ranged from 1.8 to 20% and by AG I isolates from 13.6 to 60%. Significant differences in root rot severity were reflected in root weight and total weight data. Top weight was not reduced after the 4-wk incubation period, and these data are not presented. The differences noted in experiment 1 were seemingly due to an increase in disease severity of plants inoculated with AG I isolates. Experiment 2, in which plants were incubated 8 wk instead of 4 wk, indicated wider differences in disease severity associated with isolates of AG A or AG I. In this experiment, the average disease severity induced by three AG A isolates was 26.6 compared with 82.3% for three AG I isolates. These differences in root rot severity were reflected in weight data.

TABLE 3. Pathogenicity[a] of isolates belonging to two anastomosis groups of binucleate *Rhizoctonia* spp. to strawberry in the greenhouse, experiment 1

Isolate	AG[b]	Root wt (g)	Total wt (g)	Root rot (%)[c]
S6	A	4.1	11.9	0.6
R23	A	3.3	8.8	7.6
S7	A	4.0	9.7	4.6
S24	A	4.0	10.3	3.0
S2	A	3.5	9.2	2.2
R105	I	3.2	9.6	10.6
R113	I	4.9	12.7	2.8
R153	I	4.6	12.4	2.2
R92	I	4.3	10.9	1.8
R109	I	4.2	11.0	4.8
Control	···	4.4	11.1	1.8
LSD ($P = 0.05$)		3.3	2.0	5.4
Overall means[d]				
AG A (5 isolates)		3.8	10.0	3.6
AG I (5 isolates)		4.2	11.3	4.4
Control		4.4	11.1	1.8
Contrasts[e]				
Within AG A		ns	ns	ns
Within AG I		ns	ns	*
AG A vs. AG I		ns	*	ns

[a] Plants of the cultivar Honeoye were grown in 10-cm-diameter pots containing fumigated field soil infested with a single isolate. Values are means of five replications (pots) arranged in a randomized complete block design.
[b] Anastomosis group designations are those of Ogoshi (14).
[c] Percent root rot estimated visually.
[d] Overall means are means combined for all isolates within an anastomosis group.
[e] Mean separation using single degree-of-freedom linear contrasts; ns = not significant, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.

TABLE 4. Pathogenicity[a] of isolates belonging to two anastomosis groups of binucleate *Rhizoctonia* spp. to strawberry roots in the growth chamber (15 C)

		Root wt (g)		Total wt (g)		Root rot (%)[c]	
Isolates	AG[b]	Experiment 2	Experiment 3	Experiment 2	Experiment 3	Experiment 2	Experiment 3
S6	A	4.8	6.4	11.9	10.1	1.8	4.0
R23	A	4.9	5.7	12.6	9.3	4.0	15.3
S2	A	4.4	5.7	11.0	8.7	7.2	5.8
S7	A	4.8	···	11.9	···	16.2	···
S24	A	4.8	···	11.8	···	20.0	···
R105	I	3.1	2.4	9.6	5.7	39.0	81.3
R92	I	1.5	1.6	7.5	4.4	60.0	93.3
R109	I	4.1	2.0	11.2	5.3	15.4	71.3
R113	I	4.0	···	11.6	···	13.6	···
R153	I	4.0	···	10.7	···	22.0	···
Control	···	4.1	6.8	11.4	10.3	9.4	2.5
LSD ($P = 0.05$)		1.2	2.6	2.9	4.3	16.8	20.8
Overall means[d]							
AG A		4.7	5.1	11.8	8.5	9.8	26.6
AG I		3.3	1.8	10.1	4.9	30.0	82.3
Control		4.1	6.8	11.4	10.3	9.4	2.5
Contrasts[e]							
Within AG A		ns	**	ns	ns	ns	*
Within AG I		**	ns	*	ns	**	*
AG A vs. AG I		**	**	**	**	**	**

[a] Plants of the cultivar Honeoye were grown in 10-cm-diameter pots containing fumigated field soil infested with a single isolate. Values are means of five replications (pots) arranged in a randomized complete block design in a growth chamber.
[b] Anastomosis group designations are those of Ogoshi (14).
[c] Percent root rot estimated visually.
[d] Overall means are means combined for all isolates within an anastomosis group.
[e] Mean separation using single degree-of-freedom linear contrasts; ns = not significant, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.

Differences in disease severity among anastomosis groups were confirmed in experiment 4, in which plants were inoculated with 24 additional arbitrarily selected isolates (nine AG A, eight AG G, and seven AG I) and incubated for 4 wk at 15 C. Isolates within AG I induced significantly greater disease severity than isolates within AG A or AG G at 15 C (Table 5). The average percent root rot induced by AG A isolates was 11.9%, AG G—10.3, and AG I—33.9%. There was no significant difference in disease severity between the mean of AG A isolates versus the mean of AG G isolates. Inoculation with AG I isolates resulted in significantly less root growth than AG A or AG G isolates, reflected in root volume measurements.

DISCUSSION

Binucleate *Rhizoctonia* spp. comprised greater than 90% of the *Rhizoctonia* spp. from a combined sample size of nearly 400 isolates examined in this study. *R. solani*, previously reported as a cause of black root rot (4,10,11,24), was isolated infrequently in comparison with binucleate *Rhizoctonia* spp. Isolates of *R. solani* from strawberry in the present study were all found to belong to AG 5. A similar result was obtained by Molot et al (12) who recently reported a low frequency of isolation of *R. solani* (8%) compared with *R. fragariae* (92%) from strawberry roots in France but did not report the anastomosis groups of either *R. solani* or *R. fragariae* from their study.

Among the binucleate *Rhizoctonia* spp., at least three distinct anastomosis groups were identified: AG A, AG G, and AG I. Although other researchers have referred to "clonal types" of *Rhizoctonia* from strawberries, isolates generally have been referred to only as *R. fragariae* (5,6,18,19) or *Ceratobasidium* sp. (21,22) without reference to anastomosis groups.

There were several interesting results associated with sampling from different farms and fields within farms at different times of the year. First, when several isolates were collected from different roots of the same plant, they were predominantly of the same anastomosis group. Secondly, there was an apparent shift in the frequency of detection of particular anastomosis groups isolated at different times of the year since AG I isolates were isolated more frequently from fall-collected than from spring-collected roots, when AG G isolates predominated. This phenomenon was observed from two farms; however, on Farm III, AG I was not detected in spring-collected samples but was predominant in fall-collected samples. Exactly when and why this apparent shift in isolation frequency of AG I occurred was not determined in this study. The apparent replacement of AG G and/or AG A by AG I

TABLE 5. Pathogenicity[a] of isolates belonging to three anastomosis groups of binucleate *Rhizoctonia* spp. to strawberry roots in the growth chamber (15 C), experiment 4

Isolates	AG[b]	Root vol[c] increase (%)	Root wt (g)	Total wt (g)	Root[c] rot (%)
R268	A	68.0	11.1	21.3	7.5
S61	A	63.2	12.2	22.7	6.3
R5	A	47.6	12.9	25.1	10.0
R9	A	55.2	14.3	26.2	11.3
R71	A	62.1	11.2	22.5	7.0
R67	A	63.0	13.5	23.9	11.3
S10	A	60.2	13.4	24.5	6.3
R204	A	53.6	8.8	18.7	25.2
R48	A	60.6	12.2	22.1	22.5
R107	G	51.2	9.7	20.2	20.0
R95	G	50.2	8.5	19.8	20.0
R151	G	68.5	11.3	22.3	7.5
R223	G	62.4	10.9	21.8	5.3
R123	G	64.5	12.9	23.3	7.5
R33	G	62.7	12.9	23.5	6.5
R85	G	68.6	11.0	21.5	7.8
R24	G	61.1	12.7	25.5	7.5
R64	I	44.6	10.3	21.6	45.0
R62	I	47.8	9.3	20.5	37.5
R57	I	50.4	9.8	21.3	35.0
R130	I	58.0	10.3	22.1	18.8
R52	I	42.3	9.8	18.7	46.3
R113	I	53.3	12.6	25.5	26.3
R118	I	64.4	12.5	24.2	28.8
Control	...	60.1	11.2	22.9	1.8
LSD ($P = 0.05$)		13.3	3.3	ns	14.7
Overall means[d]					
AG A (9 isolates)		59.3	12.2	23.0	11.9
AG G (8 isolates)		61.2	11.2	22.2	10.3
AG I (7 isolates)		51.5	10.6	21.9	33.9
Control		60.1	11.2	22.9	1.8
Contrasts[e]					
Within AG A		ns	ns	ns	ns
Within AG G		ns	ns	ns	ns
Within AG I		ns	ns	ns	*
AG A vs. AG G		ns	ns	ns	ns
AG A vs. AG I		**	*	ns	**
AG G vs. AG I		**	ns	ns	**

[a] Plants of the cultivar Honeoye were grown in 10-cm-diameter pots containing fumigated field soil infested with a single isolate. Values are means of five replicates (pots) arranged in a randomized complete block design in a growth chamber.
[b] Anastomosis group designations are those of Ogoshi (14).
[c] Percent root rot was estimated visually.
[d] Overall means are means combined for all isolates within an AG.
[e] Mean separation using single degree-of-freedom linear contrasts; ns = not significant, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.

could be in direct response to lower temperatures, or there could be a differential response to exudates from the strawberry roots in response to lower temperatures and shorter days as the plants approach dormancy. In this regard, Husain and McKeen (6) provided some evidence of stimulation of *R. fragariae* (AG A) by amino acids that were exuded from strawberry roots in greater quantities at 5 and 10 C than at 20 or 30 C.

Pathogenicity tests indicated that binucleate *Rhizoctonia* spp. from each anastomosis group are pathogens of strawberries. However, this is the first evidence, to my knowledge, of differential pathogenicity among the anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry. These results should be considered preliminary, as tests were conducted on a single cultivar of strawberry. Although disease severity induced by isolates among all anastomosis groups generally was greater at cooler temperatures, AG I isolates, collectively, were found to induce greater disease severity than the collective isolates of AG A or AG G at 15 C. Although the AG I population mean for disease severity was significantly greater than that for the AG A population, there were a few AG A isolates that induced disease severity as great as isolates in the AG I population. This suggests genetic variability for pathogenicity within populations of anastomosis groups.

Husain and McKeen (5) demonstrated an increase in disease severity at 5 and 10 C compared with 20 and 30 C. However, they apparently tested only a single isolate of *R. fragariae* (5). The isolate ATCC 14691, deposited by Husain and McKeen (5), was later assigned to CAG 2 by Burpee (3). Ogoshi still later equated CAG 2 with AG A in his system (14). Therefore, Husain and McKeen apparently worked with a single, virulent isolate of AG A and did not report variation in culture morphology among isolates of *R. fragariae*, probably indicating that AG I was not included in their studies. Ribeiro (18) tested additional isolates of *R. fragariae* later and noted differences in disease severity associated with particular isolates, but did not correlate these differences with cultural types that might correspond with the AG identified in this study.

An intriguing aspect of previous work (12,18,19) regards the reported mycorrhizal relationship of *R. fragariae* with strawberry roots (18,19). Difficulties were encountered in freeing plants of *Rhizoctonia* infestation for experimental purposes (control group) as reported by Ribeiro (18), indicating the close association of these fungi with strawberry roots. Ribeiro (18) reported similarities of strawberry root infections by *R. fragariae* with *Rhizoctonia*-orchid mycorrhizal associations, including an increase in top growth of plants inoculated with some isolates. My experiments did not show increased top growth in response to infection by a wide range of isolates of three AG, but my experiments were conducted at 15 C, whereas Ribeiro's work was conducted at higher temperatures in the greenhouse. Likewise, Molot et al (12) also recently reported increased top growth in plants of the cultivar Sequoia when inoculated with a particular isolate of *R. fragariae*, but this same isolate reduced top growth of plants representing five other cultivars; incubation temperatures were not reported.

The interactions of *Rhizoctonia* spp. with strawberries are apparently complex, but the confirmation of different AG among the binucleate *Rhizoctonia* spp. provides a logical framework for further studies. Although the isolates identified were collected from Connecticut fields, these same anastomosis groups have been found in Japan (14). Researchers in other localities should attempt to identify the anastomosis groups of isolates of binucleate *Rhizoctonia* spp. from strawberry. These data are necessary to further evaluate the role of these fungi as pathogens or as growth-promoting endophytes of strawberries alone and in combination with other stresses, both biotic (e.g., nematodes and other fungi) and abiotic (e.g., winter injury).

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