Characterization of Populations of *Rhizoctonia solani* AG-3 from Potato and Tobacco

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ABSTRACT

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Historically, *Rhizoctonia solani* anastomosis group 3 (AG-3) was regarded as host specific, composed of isolates causing black scurf of potato. Recently, isolates of *R. solani* AG-3 were reported causing target spot of tobacco in South Africa. In the United States, isolates causing target spot of tobacco were identified as AG-2-2. Our anastomosis tests indicated that isolates of *R. solani* from diseased tobacco in North Carolina belong to AG-3. Furthermore, the tobacco isolates, like AG-3 from potato, were prototrophic for thiamine, whereas isolates of AG-2-2 were auxotrophic for thiamine. Cluster and principal component analyses based on cellular fatty acids indicated that AG-3 isolates from tobacco were distinct from AG-3 isolates from potato. Isolates from tobacco had uniform cultural appearance, whereas isolates from potato showed cultural variation. Isolates of *R. solani* from potato were not pathogenic on tobacco plants, and isolates from tobacco were not pathogenic on potato plants.

Historically, isolates of *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (A.B. Frank) Donk) characterized as anastomosis group 3 (AG-3) were associated with black scurf disease of potato (2,4,6). More recently, other isolates of *R. solani* AG-3 have been found causing leaf blight of tomato (7), brown spot of eggplant (11), and Rhizoctonia leaf spot of tobacco (13).

In the United States, target spot of tobacco (*Nicotiana tabacum* L.) caused by *R. solani* was first observed in North Carolina in 1984 (18) and was later reported in Kentucky, South Carolina, Tennessee, and Virginia (19). Shew and Main (18,19) described the isolates of *R. solani* from target spot lesions on tobacco as belonging to AG-2-2. Isolates from tobacco leaves are very uniform in cultural morphology (17). Other *R. solani* isolates with diverse morphology have been associated with damping-off, root rot, and sore shin (stem rot) of tobacco. These isolates, associated with nonfoliar symptoms of tobacco, were characterized as AG-1, AG-2-2, and AG-4 (but not AG-3) (17).

Although isolates of *R. solani* AG-2-2 generally are considered to cause root rots (2,14) and are known to cause crown and root rot of sugar beet (22) and crown and brace root rot of corn (21), they also have been associated with foliar diseases, such as brown patch of St. Augustine grass (10) and sheath blight of mat rush (14).

The objective of the current study was to reassess the identity of isolates of *R. solani* collected from target spot lesions on tobacco leaves from North Carolina. We used anastomosis-group affinity, cultural appearance, thiamine requirement, whole-cell fatty acid analysis, and pathogenicity to further characterize these isolates.

MATERIALS AND METHODS

Source of isolates. We analyzed 20 isolates of *R. solani* from potato and 20 isolates from tobacco (Table 1). Isolates from potato included 12 from sclerotia on potato tubers grown in the Red River Valley of Minnesota and North Dakota, seven from diseased potato stems and tubers in Alaska, and one from Japan. Tobacco isolates were collected by Shew and Main (18,19) from target spot lesions on plants growing in North Carolina.

Anastomosis. Ten isolates of *R. solani* from target spot lesions on tobacco leaves were tested for their anastomosis-group affinity with two sets of testers: a U.S. set of AG-1 through -5 and AG-8 and -9, and a Japanese set of AG-1 through -7. Two isolates of AG-2-1, Anderson 48 (9) and Butler 458 (1), were included in the anastomosis tests. Also included were 12 previously described (20) isolates of AG-2-2: 2-2B-002, 2-2B-003, and 2-2B-

010 (AG-2-2 IIIB), 2-24-004, 2-24-020, and 2-24-042 (AG-2-2 IV), 2-2C-001, 2-2C-009, and 2-2C-010 (AG-2-2 Corn), and 2-23-002, 2-23-012, and 2-23-013 (AG-2-2 Turf). Representative isolates designated as testers for the potato population (AG-3) and for the tobacco population are noted in Table 1.

Cultural appearance. Characterization of cultural appearance of isolates was carried out on potato-dextrose agar (Difco Laboratories, Detroit, MI) amended with 50 mg/L each of streptomycin sulfate and penicillin G (PDA+). Cultures were incubated at 28 C in the dark and were evaluated for color (16), zonation, and formation of sclerotia at 4 and 14 days.

Thiamine requirements. Thiamine requirements of 10 isolates from tobacco and three from potato were determined using previously described methods (20). Two isolates of AG-2-1 (Anderson 48 and Butler 458) and three isolates of AG-2-2 (2-2B-002, 2-24-004, and 2-2T-002) previously described as prototrophic and auxotrophic, respectively (20), were included as controls. The experiment was repeated twice. Analysis of variance (ANOVA) was performed on the dry mycelial weight with the ANOVA procedure found in the Statistical Analysis System (SAS Institute, Cary, NC).

Cellular fatty acid analyses. As previously described (20), cultures were grown on PDA+ at 28 C in the dark for 4 days. A 32-cm^2 mycelial mat was removed, weighed, and analyzed. Fatty acid methyl ester (FAME) compositions were determined for each isolate. Each extraction and analysis was performed twice with three replicates per run. ANOVA, using SAS, was performed on percent FAME compositions. Mean separation was accomplished by a Student's t test ($P = 0.05$). Principal component analysis, using SAS, was performed on percent FAME composition.

Library generation. Library Generation Software (Microbial ID, Inc., Newark, DE) was used to develop two fatty acid libraries: an isolate library and a population library. Each library entry was a set of data points representing the mean percent composition of the fatty acids found in the samples used to create that entry.

Isolate library entries were created and used to compare isolates within and between populations. A mean of the fatty acid composition from six separate extractions of harvested mycelia from each isolate was calculated. Fatty acids used to create a library entry were required to be reproducibly detectable in the isolates. For a fatty acid to be used in creating a library entry, the mean fatty acid percent composition multiplied by the percentage of total samples that contained that fatty acid was required to exceed 0.25. Fatty acids failing to reach this quality threshold were excluded from use in creating the library entry. This prevented fatty acids represented in very low concentrations or detected only once or twice from being used to create a library entry.

Potato and tobacco entries were generated in a population library for comparisons between the populations. Each entry in the population library represented the mean fatty acid composition from 120 samples (20 isolates with six replicate samples per isolate).

A dendrogram of the isolate library entries was constructed by first computing a resemblance matrix, using Euclidean distance coefficients, from the pairwise comparisons of each isolate with every other isolate based on qualitative and quantitative differences in fatty acid composition. Using cluster analysis, a dendrogram was constructed by the unweighted pair-group method with arithmetic averages. A dendrogram was used to depict relatedness of pairs of entries. The Euclidean distance was the distance in two-dimensional space between isolates when their fatty acid compositions were compared.

Pathogenicity. Three isolates each from potato and from target spot lesions from tobacco leaves were used in pathogenicity studies. Pathogenicity to potato was determined on potato sprouts (cultivar Green Mountain) by the methods of Carling and Leiner (5). Seed tuber pieces were placed on a 10-cm layer of soil near the bottom of 6- × 25-cm black plastic tubes. Inoculum, consisting of five agar disks 7 mm in diameter, was placed approximately 2 cm above the seed. Tubes were placed in a dark growth room at 12.8 C. Plants were harvested approximately 55 days after planting. Roots and shoots were washed free of soil, and disease assessments were determined separately on roots and shoots.

Sprout damage was rated on a 0–4 scale: 0 = no damage, no lesions; 1 = minor damage, one to several lesions less than 5 mm long; 2 = intermediate damage, lesions greater than 5 mm long and girdling of some sprouts; 3 = major damage, large lesions, girdling, and death of most sprouts; and 4 = all sprouts killed. Root damage was rated on a similar 0–4 scale: 0 = no damage, no lesions or rot; 1 = minor damage, one to several lesions less than 5 mm long; 2 = intermediate damage, lesions greater than 5 mm long, some roots girdled, and much dead tissue, 3 = major damage, lesions large, and most root tissue dead; and 4 = all roots rotted and dead or no roots present.

Four replicates of each treatment were placed in a randomized complete-block design. The experiment was performed twice. Data were analyzed by ANOVA, and means were separated with Duncan's multiple range test.

Tobacco, cultivar K-326, was seeded (approximately 200 seedlings per pot) in 20-cm-diameter pots containing a soil mix of sandy loam/builders sand/Metro-Mix 220 (W. R. Grace & Co., Construction Products Div., Cambridge, MA) (1:1:1). Ten days after seeding, soil was infested with *R. solani* by placing approximately 0.6 g of colonized rice 1-cm below the soil surface in the center of each pot. Sterile rice grains served as the control. Inoculum was prepared by inoculating twice-autoclaved rice grains (50 g of rice per 25 ml of water) with agar plugs containing

TABLE 1. Isolates of *Rhizoctonia solani* analyzed in this study

Isolate	AG	Host and origin	Isolate reference	Source of isolate[y]
3-P-001[z]	AG-3	Potato, Japan	Ogoshi ST-11-6	5
3-P-002[z]	AG-3	Potato, MN	Anderson P-42	5
3-P-003[z]	AG-3	Potato, MN	Johnk P1GF-A	4
3-P-005	AG-3	Potato, MN	Johnk P2GF-A	4
3-P-007	AG-3	Potato, MN	Johnk P3GF-A	4
3-P-008	AG-3	Potato, MN	Johnk P3GF-B	4
3-P-009	AG-3	Potato, ND	Gudmestad P310-88	2
3-P-010	AG-3	Potato, ND	Gudmestad P361-88	2
3-P-011	AG-3	Potato, ND	Gudmestad P114	2
3-P-014	AG-3	Potato, ND	Gudmestad P329-88	2
3-P-018	AG-3	Potato, ND	Gudmestad P53	2
3-P-019	AG-3	Potato, ND	Gudmestad P157-88	2
3-P-023	AG-3	Potato, ND	Gudmestad P302-88	2
3-P-024	AG-3	Potato, AK	Carling M16	1
3-P-025	AG-3	Potato, AK	Carling SCL 5	1
3-P-026	AG-3	Potato, AK	Carling M69	1
3-P-028	AG-3	Potato, AK	Carling BS69	1
3-P-029	AG-3	Potato, AK	Carling L38	1
3-P-032	AG-3	Potato, AK	Carling SCL 24	1
3-P-033	AG-3	Potato, AK	Ogoshi ST-11-6	1
3-T-001[z]		Tobacco, NC	Shew-Sampson	3
3-T-002[z]		Tobacco, NC	Shew-Craven	3
3-T-003[z]		Tobacco, NC	Shew-Columbus	3
3-T-004		Tobacco, NC	Shew-Wilson	3
3-T-005		Tobacco, NC	Shew-Edgecombe	3
3-T-007		Tobacco, NC	Shew-Guilford	3
3-T-008		Tobacco, NC	Shew-Granville	3
3-T-009		Tobacco, NC	Shew-Halifax	3
3-T-010		Tobacco, NC	Shew-Vance	3
3-T-011		Tobacco, NC	Shew-1602	3
3-T-012		Tobacco, NC	Shew-1606	3
3-T-013		Tobacco, NC	Shew-1609	3
3-T-014		Tobacco, NC	Shew-1636	3
3-T-015		Tobacco, NC	Shew-1600	3
3-T-016		Tobacco, NC	Shew-1607	3
3-T-017		Tobacco, NC	Shew-1610	3
3-T-019		Tobacco, NC	Shew-1643	3
3-T-021		Tobacco, NC	Shew-1667	3
3-T-023		Tobacco, NC	Shew-1670	3
3-T-024		Tobacco, NC	Shew-1671	3

[y] 1 = D. Carling, Univ. AK, Palmer; 2 = N. Gudmestad, ND State Univ., Fargo; 3 = H. D. Shew, NC State Univ., Raleigh; 4 = J. Stevens Johnk, Univ. MN, St. Paul; and 5 = C. E. Windels, Univ. MN, Crookston.

[z] Isolates chosen as anastomosis tester isolates.

a single isolate of *R. solani*. Inoculated rice was incubated at 22–26 C for 14 days prior to use. Tests were conducted over a 21-day period in a greenhouse maintained at 25–35 C.

Disease was rated as percent seedlings killed 10 and 14 days after infesting soil. At 21 days, 15 seedlings were removed from each pot and rated for the presence of root, stem, or leaf symptoms. Five of the 15 seedlings were washed to remove all soil, surface-sterilized in 0.5% NaOCl for 30 s, and placed on 2% water agar. The percentage of plants that yielded *R. solani* was recorded.

There were four replicate pots arranged in a randomized complete-block design for each of the six isolates. The experiment was performed twice. Data were analyzed by ANOVA (SAS), and differences among isolates were determined by Waller-Duncan *K*-ratio *t* test ($K = 100$, $P = 0.05$).

RESULTS

Anastomosis affinity. Ten isolates from target spot lesions on tobacco were paired with two isolates each of testers AG-1 through AG-9 (from the United States and Japan). No anastomosis was seen in pairings of tobacco isolates with AG-1, AG-2-1, AG-2-2, AG-4, AG-5, AG-6, AG-7, AG-8, and AG-9. Because of the suspected relationship to AG-2-2, 10 isolates from tobacco were paired with additional isolates from populations belonging to AG-2-2. Pairings of tobacco isolates with isolates representing populations of AG-2-2 IIIB, IV, Corn, and Turf all showed no anastomosis. Only pairings of isolates from target spot lesions on tobacco with AG-3 isolates from potato anastomosed. A category 2 (3) or "k" type (8) of anastomosis reaction occurred when isolates from the target spot lesions were paired with the isolates from potato.

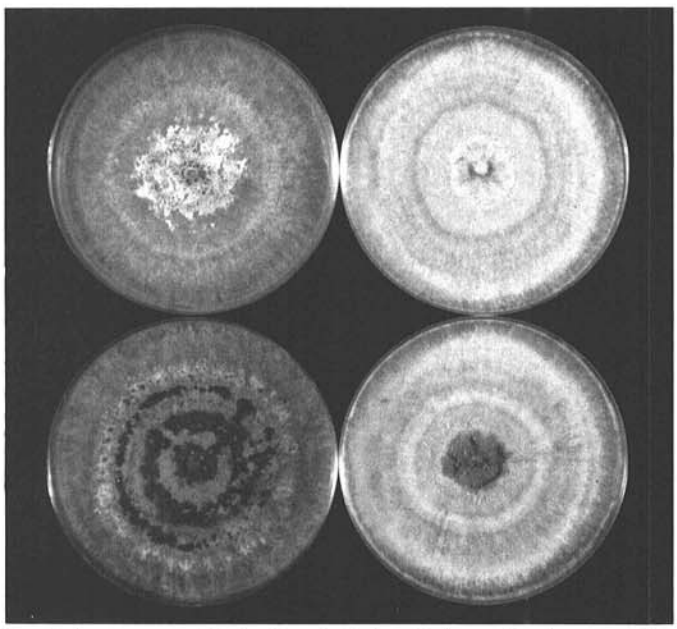

Fig. 1. Appearance of 4- (top) and 14-day-old (bottom) cultures of *Rhizoctonia solani* AG-3 isolated from potato (left) and tobacco (right). Cultures were grown on potato-dextrose agar in the dark at 28 C.

Cultural appearance. At both 4 and 14 days after inoculation on PDA+, there were distinct differences in appearance between cultures of isolates from potato and tobacco (Fig. 1). At 4 days, cultures isolated from potato generally were buff in color with no zonation and no pigmentation of the agar. Mycelial growth at both 4 and 14 days was appressed with tan to brown sclerotia formed on the agar surface or, with some isolates, embedded in the agar.

At 4 days, cultures isolated from tobacco generally were olive buff in color, with light zonation and no pigmentation of the agar. All but two of the isolates produced a few clay-colored sclerotia concentrated near the center of the culture. At both 4 and 14 days, growth was appressed, and some of the isolates showed a depression of the agar where sclerotia were present. At 14 days, all isolates from tobacco had a similar light brown, zonate appearance.

Thiamine requirements. Growth on GA (glucose asparagine medium) with or without thiamine did not differ significantly for the three AG-3 isolates from potato ($P = 0.49$), the 10 isolates from tobacco ($P = 0.89$), or the two isolates of AG-2-1 ($P = 0.54$). Mean mycelial dry weights were 0.15 ± 0.04 and 0.12 ± 0.07 g ($n = 3$) for the potato isolates and 0.15 ± 0.03 and 0.15 ± 0.02 g ($n = 10$) for the tobacco isolates on GA with thiamine and GA alone.

Fatty acid analyses. Twelve fatty acids were consistently present in the populations of isolates of *R. solani* from potato and tobacco (Table 2). Three of these fatty acids (linoleic-18:2 cis 9, 12; oleic-18:1 cis 9; and palmitic-16:0) comprised 93.6–95.2% of the cellular fatty acids identified. Fatty acids present in minor amounts in these isolates included: myristic (14:0), pentadecanoic (15:0), palmitoleic (16:1 cis 9), 9-heptadecanoic (17:1 cis 9), heptadecanoic (17:0), 2-hydroxypalmitic (16:0 20H), stearic (18:0), the dimethyl acetyl derivative of 11-octadecanoic acid (18:1 cis 11 dma), and an unknown fatty acid with an equivalent chain length (ECL) of 18.201 (20). The ECL was derived from the retention times of straight chain saturated fatty acids (20). ANOVA showed no effect of experimental runs ($P = 0.93$) on fatty acid composition. Therefore, data were combined for further statistical analyses.

Although the fatty acid compositions of the populations were qualitatively similar, quantitative differences were observed. ANOVA indicated that there were significant differences ($P = 0.001$) between isolates within a population. ANOVA followed by mean separation, using a Student's *t* test, indicated that there were significant differences ($P = 0.05$) between the populations from potato and tobacco in all 12 detected fatty acids (Table 2).

Cluster analysis by unweighted pairwise matching resulted in the dendrogram shown in Figure 2. Potato isolates clustered together, with a Euclidean distance of 2.1, and tobacco isolates clustered together, with a Euclidean distance of 3.8. These two populations clustered together, with a Euclidean distance of 6.6.

Principal component analysis, using all 12 detected fatty acids, confirmed the distinct separation between isolates representing AG-3 potato and tobacco (Fig. 3). The first three of the 12 principal components of this analysis accounted for 85.2% of the variation in the data. Principal component 1 accounted for 52.6% of the variability; principal component 2 accounted for 20.7% of the variability.

Library generation and validation. A fatty acid library entry was created for each population. Seven of 12 fatty acids were reproducibly detected and met the threshold criteria (0.25). These

TABLE 2. Percent composition of cellular fatty acids identified in populations of *Rhizoctonia solani* from potato and tobacco

	Fatty Acid (%)											
Host	14:0	15:0	16:1 cis 9	16:0	17:1 cis 9	17:0	16:0 20H	18:2 cis 9,12	18:1 cis 9	18:0	ECL 18.201	18:1 cis 11 dma
Potato	0.31[z]	1.30	0.85	6.48	0.85	0.19	0.07	78.35	10.41	0.45	0.48	0.27
Tobacco	0.26	1.42	1.10	8.37	1.55	0.48	0.25	72.83	12.44	0.91	0.26	0.11

[z] Mean of 20 isolates, with six replicate extractions per isolate, grown on potato-dextrose agar for 4 days at 28 C in the dark. There were significant differences ($P = 0.0001$) between the two populations in each of the 12 fatty acids detected.

seven fatty acids were used in the creation of both population library entries. They included 15:0; 16:1 cis 9; 16:0; 17:1 cis 9; 18:2 cis 9, 12; 18:1 cis 9; and 18:0. Qualitative differences in the presence of specific fatty acids also were used in generating library entries. These included 14:0 (used in potato), 17:0 (used in tobacco), 18:1 cis 11 dma (used in potato), and ECL 18.201 (used in potato).

Pathogenicity. ANOVA showed no effect of experimental runs in either the potato test ($P > 0.05$) or the tobacco test ($P > 0.05$); therefore, data were pooled in each test for subsequent analyses.

Isolates of *R. solani* from diseased potatoes induced disease in potato shoots and roots (Table 3) but caused no damage to tobacco plants (Table 4). *R. solani* was isolated from tobacco plants inoculated with two of the potato isolates (Table 4), but no symptoms were present. The percentage of plants infected was much higher among the tobacco isolates, with 40–75% of plants infected.

Isolates of *R. solani* from target spot lesions of tobacco induced disease in tobacco (Table 4) but caused little damage to potato sprouts and no damage to potato roots (Table 3). Few tobacco plants had symptoms after 7 days (data not shown), but abundant mycelium was visible in the developing canopy of the seedlings. Disease began as leaf lesions on many plants and developed rapidly between days 7 and 14, with many plants completely destroyed by day 10. No hymenia were observed in any of the pots.

DISCUSSION

Historically, AG-3 has been considered a homogeneous group causing black scurf of potato and has been cited as the example of host specificity within *R. solani*. Only in the past few years have isolates of *R. solani* characterized as AG-3 been found on other solanaceous crops, including tomato, eggplant, and tobacco (7,11,13).

Isolates from diseased tobacco in North Carolina had been characterized as AG-2-2 by Shew and Main (18). They reported infrequent anastomosis of tobacco isolates with the AG-2-2 tester, Rhs 36B from corn (20). Meyer et al (13) reported that isolates of *R. solani* from tobacco leaf spots in South Africa were AG-3. Our anastomosis tests indicated that isolates of *R. solani* from target spot lesions on tobacco leaves in the United States also were AG-3.

We found that isolates from both potato and target spot lesions on tobacco leaves were prototrophic for thiamine, a characteristic of isolates belonging to AG-3 (15). This provided additional evidence that the isolates from target spot lesions belong to AG-3. Previously, we showed that isolates of AG-2-2 were auxotrophic for thiamine (20).

Although data on anastomosis-group affinity and thiamine requirement supported the grouping of isolates from target spot lesions into AG-3, fatty acid analysis indicated that isolates from tobacco were distinct from isolates from potato. Principal component analysis and cluster analysis of 20 isolates from potato

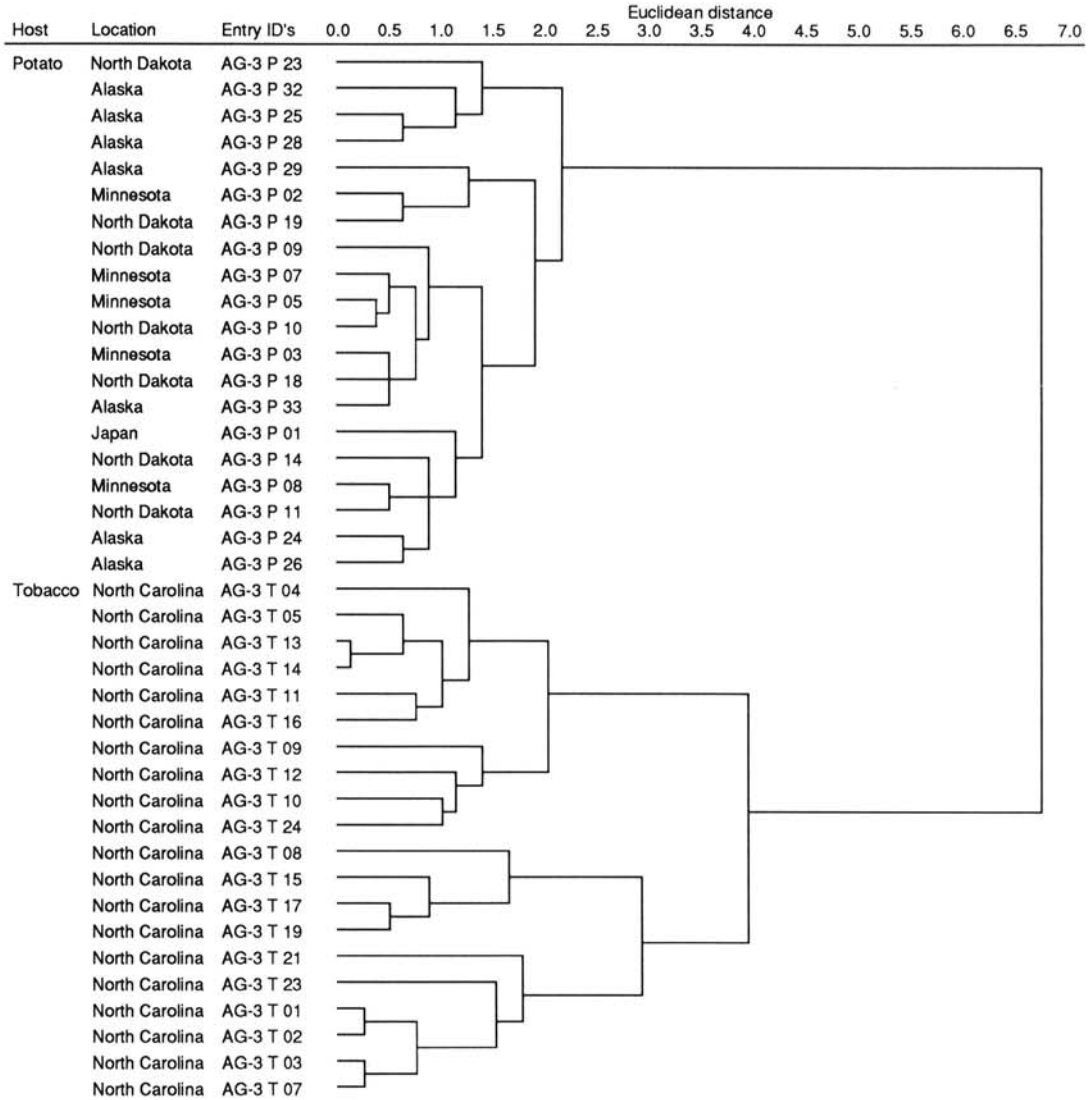

Fig. 2. Dendrogram of isolate library entries of *Rhizoctonia solani* AG-3 from potato and tobacco, based on percent fatty acid composition.

and 20 isolates from tobacco showed that all isolates from the same host were more closely related to each other than to any isolates from the other host.

Isolates from potato and tobacco also are distinctly different in pathogenicity. Isolates from potato were nonpathogenic on tobacco, and those from tobacco were nonpathogenic on potato in our tests. This is the first report of complete host specificity between populations within any of the proposed ISGs (intraspecific groups) of *R. solani* (14).

Liu et al (12) reported that the isolates from tobacco were more closely related to AG-2-1 than to AG-2-2. They analyzed isozyme patterns of 10 of the tobacco isolates included in this study. However, Liu et al (12) did not include any documented AG-3 isolates in their isozyme analysis. Our anastomosis tests clearly place isolates of *R. solani* from target spot lesions on tobacco in the United States into AG-3. However, cultural appearance, fatty acid composition, and pathogenicity tests indicate unique differences between the tobacco and potato populations.

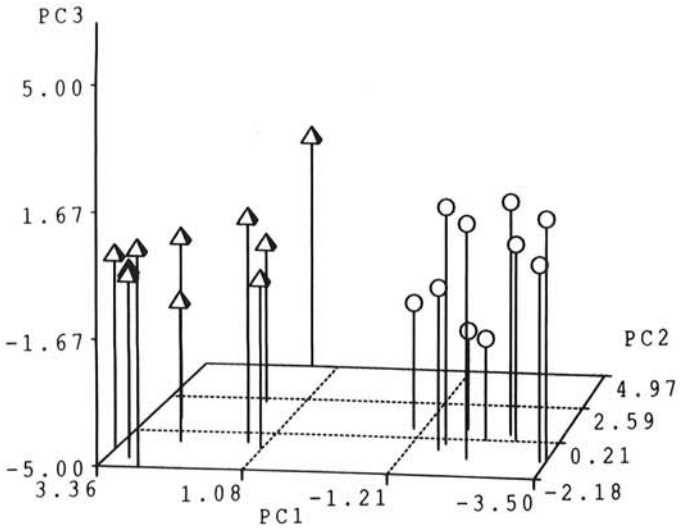

Fig. 3. Three-dimensional plot of the first three principal components derived from the fatty acid percent compositions (of all detected fatty acids) of isolates of *Rhizoctonia solani* AG-3 representing tobacco (△) and potato (○). X-axis is principal component 1 (PC1), y-axis is principal component 2 (PC2), and z-axis is principal component 3 (PC3).

TABLE 3. Damage to sprouts and roots of inoculated potato plants by isolates of *Rhizoctonia solani* AG-3 collected from tobacco or potato

	Damage assessment[y]	
Isolate	Sprout	Root
Tobacco		
3-T-001	0.25 a[z]	0.00 a
3-T-002	0.13 a	0.00 a
3-T-003	0.00 a	0.00 a
Potato		
3-P-024	3.25 b	3.13 b
3-P-029	3.25 b	3.00 b
3-P-033	3.38 b	2.88 b
Uninoculated Control	0.00 a	0.00 a

[y] Damage assessment for sprouts and roots made on a scale of 0–4, in which 0 = no damage, no lesions and 4 = all sprouts (roots) dead.
[z] Values followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

TABLE 4. Development of disease on tobacco seedlings inoculated with potato or tobacco isolates of *Rhizoctonia solani* AG-3

	Percentage of infected plants killed[y]		
Isolate	Day 10	Day 14	Percent infected
Potato			
3-P-001	0 a	0 a	0 a[z]
3-P-002	0 a	1 a	20 b
3-P-003	0 a	1 a	20 b
Tobacco			
3-T-001	34 b	60 b	45 c
3-T-002	23 b	35 b	65 c
3-T-003	46 b	58 b	70 c

[y] Values in each column followed by different letters are significantly different, Waller-Duncan K-ratio t test, $P = 0.05$.
[z] Percentage of plants that yielded *R. solani* on water agar on day 21. Values followed by different letters are significantly different, Waller-Duncan K-ratio t test, $P = 0.05$.

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