Differentiation of Populations of AG-2-2 of *Rhizoctonia solani* by Analysis of Cellular Fatty Acids

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ABSTRACT

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A population of *Rhizoctonia solani* representing AG-2-2 IIIB (mat rush type) and AG-2-2 populations isolated from corn, sugar beet, and turfgrass were analyzed for cellular fatty acid composition. The AG-2-2 IIIB population and populations of isolates from turfgrass and from sugar beet (AG-2-2 IV) were differentiated by cluster and principal component analyses of the percent composition of the fatty acids identified. A dendrogram of the four populations shows that AG-2-2 IIIB and the population from corn were closely related, and the population from turf was distinct from AG-2-2 IIIB and AG-2-2 IV.

Additional keywords: brown patch, *Juncus effusus*, St. Augustine grass.

Rhizoctonia solani Kühn (teleomorph = *Thanatephorus cucumeris* (A.B. Frank) Donk) is a cosmopolitan plant pathogen. It is a complex species varying in morphology, pathogenicity, and physiology among isolates (1). Attempts to divide *R. solani* into logical groupings fall into at least two categories: 1) hyphal anastomosis (21–23,27) and differences in pathogenicity, and 2) the physical appearance of cultures (13,21,23,30,35).

Hyphal anastomosis in *R. solani* was first described in 1921 by Matsumoto (19). Currently, 10 anastomosis groups (AG) are recognized (4,23,24), and one group (AG-2) is further divided into subgroups based on anastomosis frequency (21) and thiamine requirement (25,30). *R. solani* isolates characterized as AG-2 type 1 (AG-2-1) generally are pathogens of cruciferous species and are reported to be thiamine prototrophic (25,30). *R. solani* isolates characterized as AG-2 type 2 (AG-2-2) anastomose infrequently with isolates characterized as AG-2-1, and tested AG-2-2 isolates are auxotrophic for thiamine (25).

Ogoshi (23) recently proposed subdivision of AG-2-2 into two intraspecific groups (IIIB and IV) based on pathogenic specialization as reported by Watanabe and Matsuda (35). In Japan, *R. solani* AG-2-2 IIIB isolates cause sheath blight of cultivated mat rush (*Juncus effusus* L. var. *decipiens*) and false sheath blight of rice (*Oryza sativa* L.). AG-2-2 IV also occurs in Japan, where it causes crown and root rot of sugar beet (*Beta vulgaris* L.).

In the United States, *R. solani* isolates characterized as AG-2-2 cause several important diseases, including crown and root rot of sugar beet (36), crown and brace root rot of corn (*Zea mays* L.) (33), brown patch of St. Augustine grass (*Stenotaphrum secundatum* (Walter) Kuntze) (3,9,10), and root rot of soybean (*Glycine max* (L.) Merr.) (17).

The intraspecific groups (ISGs) within AG-2-2 (IIIB and IV) cannot be differentiated by anastomosis (21), thiamine requirement (25), DNA base composition (14), or ribosomal DNA restriction fragment length polymorphisms (34) but can be differentiated by DNA base sequence homology (15) and zymogram analysis (20). Based on isozyme analysis, a relationship between Ogoshi's AG-2-2 IV and *R. solani* isolates associated with crown and root rot of sugar beets in the United States was proposed (16).

The objective of this study was to compare the fatty acid composition of populations of AG-2-2 isolates representing AG-2-2 IIIB and populations of isolates recovered from diseased corn, sugar beet, and turfgrass. Fatty acid analysis has successfully differentiated closely related groups of bacterial plant pathogens (5–7), but it has not been used to differentiate closely related groups of fungi. Cultural appearance and thiamine requirements of isolates within these populations were also examined. Preliminary results have been published (11).

MATERIALS AND METHODS

Source of isolates. Forty *R. solani* isolates, representing AG-2-2 IIIB and AG-2-2 IV, as well as isolates from diseased corn, sugar beet, and turf identified as AG-2-2, were analyzed (Table 1). Two Japanese isolates, from sugar beet (2-24-040) and sugar beet tare soil (2-24-042), characterized as AG-2-2 IV (21), were included for comparison. Two isolates from diseased soybean in Illinois, 2-2C-009 and 2-2C-010, were included for comparison to the population of isolates from corn. Isolate Rhs 36 (2-2C-001) was originally isolated from peanut. Isolate Rhs 36B was a reisolation of Rhs 36 obtained from corn roots by Sumner. Two sources of isolate Rhs 36B (2-2C-002 and 2-2C-003) were included for comparison.

Cultural appearance. Characterization of cultural appearance 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 (28), zonation, pigmentation of agar, and sclerotial formation at 4 and 14 days.

Thiamine requirement. The thiamine requirement of the 40 isolates was determined using previously described methods (25). Two isolates of AG-2-1, Anderson 48 (8) and Butler 456 (2), were included as controls.

The basal medium (glucose asparagine medium [GA]) consisted of 10 g of D-glucose, 2 g of L-asparagine, 0.5 g of $MgSO_4 \times 7H_2O$, 1 g of KH_2PO_4, 0.2 mg of $Fe(NO_3)_3 \times 9H_2O$, 0.2 mg of $ZnSO_4 \times 7H_2O$, and 0.1 mg of $MnSO_4 \times 7H_2O$ in 1 L of distilled deionized water. Thiamine hydrochloride (10^{-5} M, 3.4 mg/L) was added after autoclaving. Inoculum for the GA cultures was grown on GA (without thiamine) plus 20 g of agar (GAA). Inoculated flasks were incubated in the dark at 28 C for 14 days, after which mycelial mats were removed, dried at 100 C for 24 h,

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and weighed. The experiment was repeated twice. Analysis of variance, using the Statistical Analysis System (SAS Institute, Cary, NC), was performed on the dry weights.

Cellular fatty acid analyses. As previously described (12), 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 repeated twice with three replications per run. Analysis of variance, using SAS, was performed on percent FAME compositions and on mycelial fresh weights. Mean separation was accomplished by the Waller-Duncan *K*-ratio *t* test ($K = 100$, $P = 0.05$). 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.

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 percent of total samples that contained the 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 peaks represented in very low concentrations or peaks detected only once or twice from being used to create a library entry.

A fatty acid library entry was generated for each population and was used to compare the populations. Each population library entry was the mean fatty acid composition from 60 samples (10 isolates with six replicate samples per isolate). Fatty acid compositions of the samples used to create each population library entry were compared to each of the population library entries. The two closest matches, based on similarity indices, were given. The similarity index (a value between 0.0 and 1.0) indicated how closely the fatty acid composition of an individual sample matched the mean fatty acid composition of a library entry (29). This index reflected the sample's distance in n-dimensional space (Gaussian distance) from the mean fatty acid composition of the closest library entry.

Dendrograms of the population library entries and the isolate library entries were constructed using cluster analysis to produce unweighted pair matchings based on fatty acid composition. A dendrogram was used to depict relatedness of pairs of entries. The Euclidian distance was the distance in two-dimensional space between populations when their fatty acid compositions were compared.

Challenge isolates. Each population library entry was challenged with an additional 10 isolates (two replicate samples per isolate) from their respective populations to determine if "un-

TABLE 1. Isolates of *Rhizoctonia solani* AG-2-2 analyzed in this study

Isolate	AG/ISG	Host/Origin	Isolate reference	Source of isolate[a]
2-2B-001	AG-2-2 IIIB	mat rush, Japan	Ogoshi B-60	2
2-2B-002	AG-2-2 IIIB	mat rush, Japan	Butler 456	1
2-2B-003	AG-2-2 IIIB	mat rush, Japan	Ogoshi C-330	5
2-2B-004	AG-2-2 IIIB	sugar beet, Japan	Ogoshi BV-43	5
2-2B-005	AG-2-2 IIIB	*Monochoria*, Japan	Ogoshi C-61	5
2-2B-006	AG-2-2 IIIB	*Robinia*, Japan	Ogoshi C-104	5
2-2B-007	AG-2-2 IIIB	soil, Japan	Ogoshi C-109	5
2-2B-008	AG-2-2 IIIB	rice, Japan	Ogoshi C-148	5
2-2B-009	AG-2-2 IIIB	rice, Japan	Ogoshi C-354	5
2-2B-010	AG-2-2 IIIB	rice, Japan	Ogoshi C-355	5
2-24-003	AG-2-2	sugar beet, ND	Windels 7-S-45	7
2-24-004	AG-2-2	sugar beet, MN	Windels 12-T-1	7
2-24-005	AG-2-2	sugar beet, MN	Windels 12-T-9	7
2-24-006	AG-2-2	sugar beet, MN	Windels 12-T-10	7
2-24-007	AG-2-2	sugar beet, MN	Windels 13-T-8	7
2-24-009	AG-2-2	sugar beet, MN	Windels 18-T-2	7
2-24-012	AG-2-2	sugar beet, MN	Windels 85-34-A3	7
2-24-020	AG-2-2	sugar beet, ND	Windels 88-32-4	7
2-24-040	AG-2-2 IV	sugar beet, Japan	Ogoshi BV-41	1
2-24-042	AG-2-2 IV	sugar beet, Japan	Ogoshi A-19	1
2-2T-002	AG-2-2	St. Augustine grass, TX	Jones TR-2	3
2-2T-003	AG-2-2	St. Augustine grass, TX	Stone 4	3
2-2T-005	AG-2-2	St. Augustine grass, TX	Milberger 5	3
2-2T-006	AG-2-2	St. Augustine grass, TX	Milberger 6	3
2-2T-007	AG-2-2	St. Augustine grass, TX	Mata 1	3
2-2T-010	AG-2-2	St. Augustine grass, TX	Milberger 1	3
2-2T-012	AG-2-2	St. Augustine grass, TX	Colbaugh 3	3
2-2T-013	AG-2-2	St. Augustine grass, TX	Stone 6	3
2-2T-014	AG-2-2	St. Augustine grass, TX	Mata 4	3
2-2T-018	AG-2-2	St. Augustine grass, TX	Stone 7	3
2-2C-001	AG-2-2	peanut, GA	Sumner Rhs 36	7
2-2C-002	AG-2-2	corn, GA	Sumner Rhs 36B	4
2-2C-003	AG-2-2	corn, GA	Sumner Rhs 36B	7
2-2C-004	AG-2-2	corn, GA	Sumner 672-IV All	6
2-2C-005	AG-2-2	corn, GA	Sumner 680-4I8	7
2-2C-006	AG-2-2	corn, GA	Sumner 707 III-8	7
2-2C-007	AG-2-2	corn, GA	Sumner 707 IV-13	7
2-2C-008	AG-2-2	corn, GA	Sumner 87-150-1728	6
2-2C-009	AG-2-2	soybean, IL	Liu 62-D	5
2-2C-010	AG-2-2	soybean, IL	Liu 65-L	5

[a] 1 = E. E. Butler, Univ. CA, Davis; 2 = D. E. Carling, Univ. AK, Palmer; 3 = P. F. Colbaugh, TX Agric. Exp. Stn., Dallas; 4 = R. K. Jones, Univ. MN, St. Paul; 5 = Z. Liu, Univ. IL, Urbana; 6 = D. R. Sumner, Univ. GA, Tifton; 7 = C. E. Windels, Univ. MN, Crookston.

known" isolates would be matched to the correct population library entry. The 10 isolates from sugar beet were from Minnesota and North Dakota. Six of the isolates from turf were from Texas, two were from Missouri, and two were from South Carolina. Because 10 additional isolates representing AG-2-2 IIIB were unavailable, the corn and soybean isolates were used to challenge the AG-2-2 IIIB library entry. Similarity indices were used to determine the relationship between "unknown" isolates and a library entry.

RESULTS

Cultural appearance. At 14 days, the isolates from turf looked most similar to each other and were visually distinct from AG-2-2 IIIB, corn, and sugar beet isolates. The cultural appearance of isolates from turf showed no zonation, were generally chestnut brown to dark brown, and caused a dark-brown discoloration of the media. Sclerotia were not observed after 4 days. At 14 days, sclerotia, when present, were lighter in color than mycelia, and some had droplets of liquid on their surface.

At 4 and 14 days, cultural appearance varied between and within the isolates representing the populations from AG-2-2 IIIB, corn, and sugar beet. Half of the isolates formed concentric zones; half did not. Growth varied from appressed to aerial. Sclerotia, when present, varied from forming crustlike zones to being scattered and embedded in the agar. Sclerotial color varied from buff to dark brown, with some sclerotia having droplets of liquid formed on their surface. The color of the cultures varied from buff to dark brown, and the media was not discolored in any of the cultures. The variability in cultural appearance of these populations made it difficult to assign isolates to their correct population on this basis alone.

TABLE 2. Mycelial dry weight (g) after 14 days of populations of *Rhizoctonia solani* AG-2 on glucose asparagine medium, with or without thiamine added

R. solani populations	GA (+)[w]	GA (−)[x]	*P*-value[y]
AG-2-2 IIIB (mat rush)	0.110[z]	0.050	<0.001
AG-2-2 IV (sugar beet)	0.150	0.020	<0.001
AG-2-2 (turfgrass)	0.070	0.020	<0.001
AG-2-2 (corn)	0.180	0.030	<0.001
AG-2-1	0.230	0.230	$=0.54$

[w] GA(+) = glucose asparagine medium plus 10^{-5} M thiamine hydrochloride.
[x] GA(−) = glucose asparagine medium.
[y] *P*-value ($\alpha = 0.05$) of *t* test between GAA plus thiamine and GAA.
[z] Mean of 10 isolates tested in three replications.

Thiamine requirements. Isolates representing AG-2-2 IIIB, corn, sugar beet, and turf populations were thiamine auxotrophic. Mycelial dry weight differed significantly ($P < 0.001$) between each of the four populations grown for 14 days on GAA supplemented with and on GAA not supplemented with thiamine (Table 2). Both AG-2-1 isolates attained similar growth on GAA with and without thiamine added.

Fatty acid analyses. Mean fresh weights of mycelia harvested from 4-day-old cultures, grown on PDA+, of each population differed significantly ($P = 0.05$) and averaged plus or minus standard: 0.35 g ±0.17 (AG-2-2 IIIB), 0.55 g ±0.14 (AG-2-2 sugar beet), 0.85 g ±0.27 (AG-2-2 corn/soybean), and 0.47 g ±0.14 (AG-2-2 turf) (data not shown). Significant differences ($P = 0.05$) between isolates within a population also were observed.

Ten fatty acids were present in all four populations of *R. solani* AG-2-2 isolates (Table 3). Three of these fatty acids (linoleic-

TABLE 3. Percent composition of cellular fatty acids identified in populations of *Rhizoctonia solani* AG-2-2 grown on potato-dextrose agar for 4 days at 28 C in the dark

R. solani population	Fatty Acid (%) 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 cisll dma
AG-2-2 IIIB (mat rush)	0.3 a[z]	0.6 b	1.1 a	8.0 c	0.5 c	0.1 a	0.23 a	77.5 c	10.0 b	0.9 b	0.5 b	0.4 a
AG-2-2 IV (sugar beet)	0.2 b	0.7 a	1.0 a	7.1 d	0.7 a	0.0 b	0.03 c	81.8 a	7.5 d	0.4 c	0.3 c	0.2 c
AG-2-2 (turfgrass)	0.1 c	0.7 a	0.7 c	9.4 a	0.3 d	0.0 b	0.00 d	72.6 d	14.1 a	1.0 a	0.6 a	0.3 b
AG-2-2 (corn)	0.3 a	0.6 b	0.9 b	8.4 b	0.6 b	0.1 a	0.12 b	77.8 b	9.3 c	1.0 a	0.3 c	0.2 c

[z] Mean of 10 isolates with six replicate extractions per isolate. Values in each column followed by different letters are significantly different, Waller-Duncan *K*-ratio *t* test, $P = 0.05$.

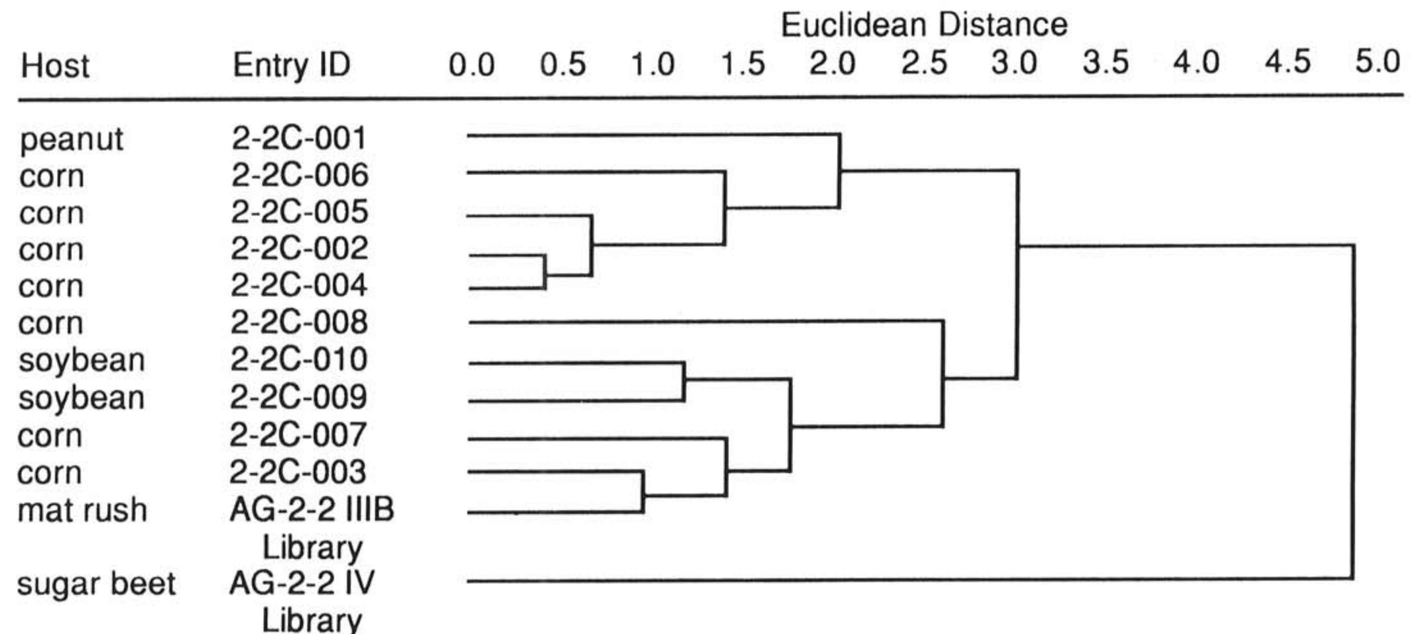

Fig. 1. Dendrogram of isolate library entries of *Rhizoctonia solani* AG-2-2 Corn populations, based on percent fatty acid composition. Population library entries for AG-2-2 IIIB and AG-2-2 IV were included for comparison.

18:2 cis 9, 12; oleic-18:1 cis 9; and palmitic-16:0) comprised 95.5–96.4% of the cellular fatty acids identified. Other fatty acids present in these isolates included: myristic (14:0), pentadecanoic (15.0), palmitoleic (16:1 cis 9), 9-heptadecenoic (17:1 cis 9), stearic (18:0), the dimethyl acetyl derivative of 11-octadecenoic acid (18:1 cis 11 dma), and an unknown fatty acid with an equivalent chain length (ECL) of 18.201 (12). The ECL was derived from the retention times of straight chain saturated fatty acids (12). Analysis of variance showed no effect of experimental runs ($P = 0.93$) on fatty acid composition. Therefore, data were combined for further statistical analyses. Analysis of variance showed no effect of replicate plates ($P = 0.98$) on fatty acid composition for any of the 12 detected fatty acids. Therefore, data were combined for further statistical analyses.

Although the fatty acid compositions of the populations were qualitatively similar, quantitative differences were observed. Analysis of variance indicated that there were significant differences ($P = 0.001$) between isolates. Analysis of variance followed by mean separation, using the Waller-Duncan t test, indicated that there were significant differences ($K = 100$, $P = 0.05$) between certain populations in all 12 detected fatty acids (Table 3).

Dendrogram analysis clustered isolate 2-2C-001 (from peanut) with certain AG-2-2 isolates from corn, with a Euclidean distance of 2.03 (Fig. 1). The two Rhs 36B isolates (2-2C-002 and 2-2C-003) from different sources were clustered, with a Euclidean distance of 3.04 (Fig. 1).

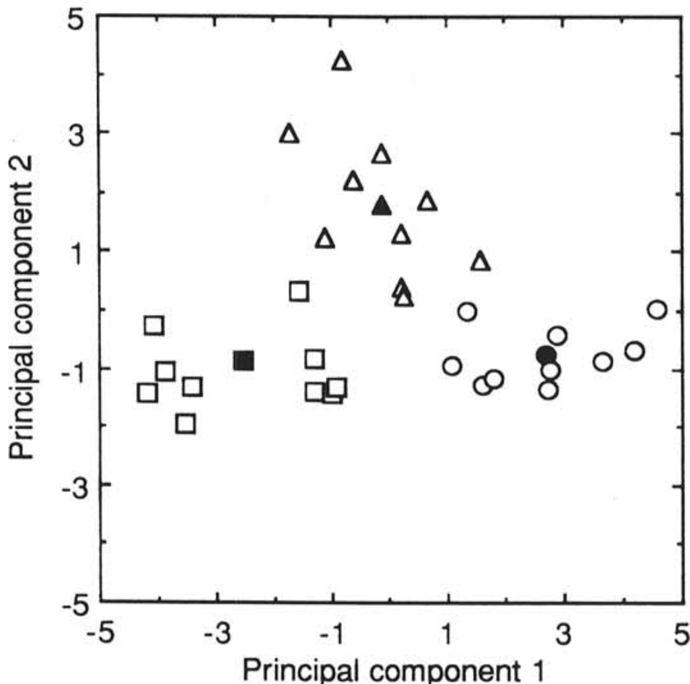

Fig. 2. Plot of the first two principal components derived from the fatty acid percent compositions (of all detected fatty acids) of *Rhizoctonia solani* AG-2-2 isolates, representing AG-2-2 IV (□), AG-2-2 IIIB (△), and AG-2-2 Turf (○). The solid symbols represent the library mean for each population.

Dendrogram analysis clustered the two AG-2-2 isolates from soybean (2-2C-009 and 2-2C-010) with certain AG-2-2 isolates from corn, with a Euclidean distance of 1.66 (Fig. 1). Therefore, for the remainder of the analyses, the AG-2-2 isolates from corn and soybean were combined and referred to as the AG-2-2 Corn population. This entire population also clustered closely with the AG-2-2 IIIB library entry, with a Euclidean distance of approximately 3.06 (Fig. 1).

The AG-2-2 IV isolates from Japan (2-24-040 and 2-24-042) and the AG-2-2 isolates from sugar beets in the United States were very closely related, with a Euclidean distance of 1.0 (data not shown). Therefore, for the rest of the analyses, the sugar beet isolates from Japan and those from the United States were combined and referred to as the AG-2-2 IV population.

Principal component analysis using all 12 detected fatty acids showed a close but distinct relationship between isolates representing AG-2-2 IIIB, AG-2-2 IV, and AG-2-2 Turf (Fig. 2). The first two of the 12 principal components of this analysis accounted for 68.2% of the variation in the data. Principal component 1 accounted for 52.3% of the variability; principal component 2 accounted for 15.9% of the variability.

Library generation and validation. A fatty acid library entry was created for each population. Six of 12 fatty acids were reproducibly detected and met the threshold criteria (0.25). These six fatty acids were used in the creation of all four population library entries. They include 15:0; 16:1 cis 9; 16:0; 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 AG-2-2 IIIB and AG-2-2 Corn), 17:1 cis 9 (used in AG-2-2 IIIB, AG-2-2 IV, and AG-2-2 Corn), 18:1 cis 11 dma (used in AG-2-2 IIIB), and ECL 18.201 (used in AG-2-2 IIIB and AG-2-2 Turf).

A dendrogram of the population library entries, generated from reproducibly detectable fatty acids, showed that AG-2-2 IIIB and AG-2-2 Corn are very closely related, with a Euclidian distance of 0.78 (Fig. 3). The AG-2-2 IV library entry, with a Euclidian distance of 4.88, is less closely related to the AG-2-2 IIIB or AG-2-2 Corn library entries. The AG-2-2 Turf library entry, with a Euclidian distance of 8.92, is distinct from the other library entries (Fig. 3).

Challenge isolates. After each population library entry was created, an additional 10 isolates from each population were used to challenge the library entries. Although data are not shown, of 10 additional sugar beet isolates, all were correctly matched to the AG-2-2 IV library entry, with similarity indices of 0.803–0.970. Of 10 additional turf isolates, all were matched to the AG-2-2 Turf library entry, with similarity indices of 0.567–0.872. Because 10 additional isolates representing AG-2-2 IIIB were unavailable, the corn and soybean isolates were used to challenge the AG-2-2 IIIB library entry. These 10 isolates were matched to the AG-2-2 IIIB library entry, with similarity indices of 0.696–0.973.

DISCUSSION

Our results suggest that analysis of the cellular fatty acids from *R. solani* populations representing AG-2-2 IIIB and AG-2-2 IV can be used to differentiate pathological types within an anas-

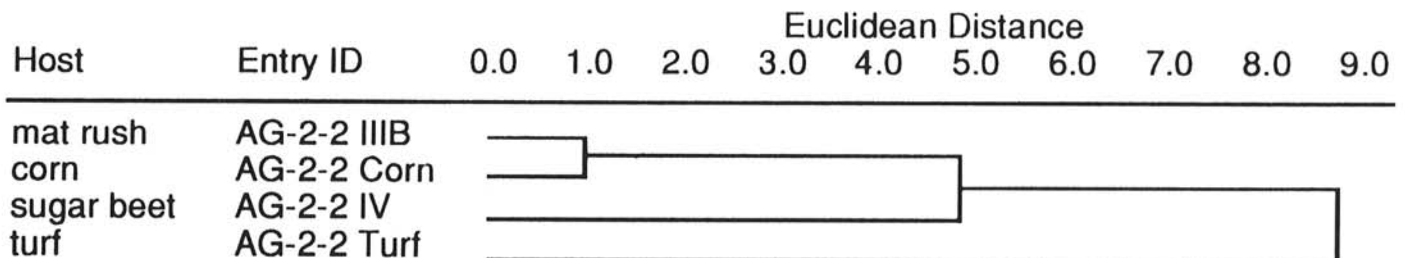

Fig. 3. Dendrogram of library entries of *Rhizoctonia solani* AG-2-2 populations representing AG-2-2 IIIB, AG-2-2 IV, AG-2-2 Corn, and AG-2-2 Turf, based on percent fatty acid composition.

tomosis group. Isolates from different hosts, classified as AG-2-2 IIIB (21), had similar fatty acid profiles (similarity indices of 0.858–0.995) but were distinct from sugar beet isolates (AG-2-2 IV) collected from widely separated geographic areas (Japan and the United States).

The two populations isolated from diseased corn and from warm-season turf have not been placed yet in a specific ISG. We have chosen to designate these populations AG-2-2 Corn and AG-2-2 Turf because these populations represent pathological groupings, not taxonomic entities. Fatty acid analysis of the corn and soybean isolates showed a close relationship between the two. In addition, the corn and soybean isolates were closely related to the AG-2-2 IIIB population of isolates. Our data support the findings of Liu et al (16), who placed isolates from corn and soybean in ISG AG-2-2 IIIB based on isozyme analysis. However, pathogenicity data of corn isolates on rice and mat rush are needed to further support the grouping of corn isolates into the ISG AG-2-2 IIIB.

In Japan, Oniki et al (26) reported that brown patch disease of turf is caused by AG-2-2 IIIB. In the United States, brown patch of St. Augustine grass has been associated with an unspecified AG-2-2 (3,9,10). Fatty acid profiles of *R. solani* AG-2-2 isolates from diseased turf were distinct from the fatty acid profiles of ISGs AG-2-2 IIIB and AG-2-2 IV isolates. Differences in fatty acid composition and cultural appearance indicate that the turf population should not be assigned to either of the currently recognized AG-2-2 ISGs.

The AG-2-2 isolates from turf had a distinct cultural appearance that was useful but not reliable in assigning isolates to this population. The cultural appearance of isolates representing AG-2-2 IIIB and AG-2-2 IV were more variable, and this characteristic was unreliable in assigning isolates to the proper ISG.

AG-2-2 isolates previously tested for thiamine requirement were auxotrophic (25). In our study, isolates of AG-2-2 IIIB, AG-2-2 IV, AG-2-2 Corn, and AG-2-2 Turf populations exhibited 3.5–7.5 times more growth in the presence of supplemental thiamine. This evidence confirms previous findings (17,25) that *R. solani* AG-2-2 isolates are auxotrophic for thiamine. We believe thiamine response may be more useful than anastomosis frequency in distinguishing AG-2-1 from populations of AG-2-2. However, AG-9 isolates respond differentially to thiamine without exhibiting differences in anastomosis frequency (4).

Two additional *R. solani* AG-2-2 populations have been reported in the United States but were not examined in this study. They include isolates causing cavity spot of carrot (8) and leaf spot of tobacco (31). To date, we have been unable to obtain an adequate population of isolates from carrots. Analysis of isolates from tobacco is currently underway.

The relationships between AG-2-2 populations observed in this study were consistent with the relationships observed by Liu and Sinclair (18). Liu and Sinclair studied the relationship between these populations using isozyme polymorphism and DNA restriction analyses on many of the same isolates we used in our study. In their analyses, they reported three distinct groups within AG-2-2: AG-2-2 IIIB, AG-2-2 IV, and a newly defined group including the same *R. solani* isolates from diseased turf used in this study (18). Liu and Sinclair (18) reported that *R. solani* isolates causing leaf spot of tobacco are related to AG-2-1. Preliminary results in our laboratory indicate that the isolates from leaf spot lesions on tobacco belong to AG-3 (32).

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