Mycelial Compatibility and Pathogenic Diversity Among *Sclerotium rolfsii* Isolates in the Southern United States

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Abstract

Xie, C., Huang, C.-H., and Vallad, G. E. 2014. Mycelial compatibility and pathogenic diversity among *Sclerotium rolfsii* isolates in the southern United States. *Plant Dis.* 98:1685-1694.

Sclerotium rolfsii is a soilborne fungus that causes southern blight on a wide range of plants in tropical and subtropical regions of the world. Eighty-four isolates collected from Florida, Georgia, Louisiana, South Carolina, Texas, and Virginia were paired and assigned to 23 mycelial compatibility groups (MCGs), of which 11 MCGs consisted of a single isolate. Isolates within an MCG typically originated from different hosts and different geographical areas, with the exception of MCG 11. In all, 13 of the 15 isolates in MCG 11 originated from peanut in Georgia and Florida, while the other 2 isolates originated from potato in Virginia and from the ornamental *Barlaeria cristata* in Florida. Significant differences in the size and number of sclerotia produced in vitro existed between isolates from peanut and other hosts. Nineteen isolates representative of the most common MCGs (1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

13, 14, 15, 16, 17, and 18) were tested for pathogenicity on tomato, pepper, and peanut. All isolates were pathogenic on all hosts but virulence differed significantly among isolates. Isolates collected from peanut were the most virulent on all three hosts compared with isolates collected from tomato and pepper. 'Georgia Green' peanut was more susceptible to peanut isolates from Georgia than to the other tested isolates. Of the two tomato entries, the commercial tomato 'Tygress' was less susceptible than the previously reported resistant breeding line 5635M to many of the *S. rolfsii* isolates tested, with the exception of the peanut isolates collected from Georgia. These initial findings suggest that considerable variation exists among *S. rolfsii* isolates throughout the southern United States, with some indications of specialization for the isolates collected from peanut.

Sclerotium rolfsii Sacc. (teleomorph *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.) was first described in Florida in 1892 by Rolfs on tomato (43). Based on host range, *S. rolfsii* is also one of the most destructive fungal pathogens, able to cause disease on 500 plant species across 100 plant families, including several crops of economic importance in Florida such as tomato, potato, pepper, cantaloupe, celery, carrot, cabbage, bean, eggplant, and peanut (3,16,31,38). Depending on the plant, disease caused by *S. rolfsii* is commonly referred to as southern blight, Sclerotium root rot, or southern stem rot (3). The disease has long been a major problem in the production of row crops such as peanut in the southern United States. However, the disease has become more problematic in vegetable production coinciding with the adoption of alternative fumigants to methyl bromide and the adoption of organic and other low-input production strategies (29,42,44,49). Losses attributed to this disease can be profound. For example, yield losses in peanut production to *S. rolfsii* typically do not exceed 25% and are around 7 to 10% annually on average but losses may reach to 80% in serious disease years in the southern United States (20). Annual economic loss of peanut associated with *S. rolfsii* alone was estimated at \$36.8 million in Georgia from 1988 through 1994 (20).

To effectively manage southern blight in infested areas, knowledge of the pathogen diversity would provide valuable information on population structure, dispersal, and distribution. Mycelial

compatibility refers to the ability of two strains of the same fungal species to fuse together and form a single colony, whereas incompatible strains cannot. Mycelial compatibility has been widely used to study potential genetic diversity within field populations of various fungi and is considered to be one of several events associated with vegetative incompatibility (21,27). Mycelial compatibility group (MCG) assignment is a popular method to describe *S. rolfsii* diversity, which is based on the hyphal interaction between different isolates. Isolates from the same MCG are assumed to have similar genetic background relative to isolates from different groups (9,24,27,38).

Mycelial compatibility has been used to assess genetic diversity among *S. rolfsii* populations in various global collections (1,12,15,19,23,25,32,34,39,40,46). Results from these previous studies show that populations of *S. rolfsii* in different geographic areas may have different levels of genetic variability. Recently, Remesal et al. (41) demonstrated that the combined sequence variation within *EF1a* and *RPB2* corresponded with the 12 MCGs previously assigned to 238 single-sclerotial isolates of *S. rolfsii* (40), similar to prior molecular studies (23,32,34) that supported the clonal nature of *S. rolfsii* isolates within MCGs. Knowledge of genetic diversity among *S. rolfsii* isolates from outbreaks occurring on important economic crops such as peanut and vegetables such as tomato in Florida and other southern states is essential for resistance breeding efforts, and important to determine whether any level of host specialization exists among isolates from different areas and hosts for considering potential crop rotation strategies. To date, no studies have assessed *S. rolfsii* diversity within the southern United States.

The objectives of this research were to (i) characterize *S. rolfsii* isolates collected from the southern United States for differences in cultural morphology and mycelial compatibility; (ii) test *S. rolfsii* isolates for differences in virulence and host adaptation against tomato, pepper, and peanut; and (iii) test the specificity of a previously reported resistant tomato line against diverse isolates of *S. rolfsii*. A specific emphasis was placed on the isolates from peanut and vegetable production areas to identify any possible relationship

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Accepted for publication 30 May 2014.

between their MCGs and host of origin, geographical origin, morphological features, and pathogenicity toward peanut, tomato, and pepper.

Materials and Methods

Fungal materials. Eighty-four isolates of *S. rolfssii* from Florida, Georgia, Louisiana, South Carolina, Texas, and Virginia were isolated from infected tissues of several hosts, including cantaloupe, tomato, eggplant, peanut, pepper, snap bean, sweet potato, watermelon, and several ornamental species. (Table 1). All isolates were purified from initial cultures by picking single sclerotia to increase

the isolate. All isolates were stored as dry sclerotia at room temperature. Individual colonies for subsequent experiments were initiated from single sclerotia that were disinfested with 75% ethanol prior to placing on the center of potato dextrose agar (PDA) plates. Cultures were then maintained at room temperature.

MCGs. For grouping isolates based on mycelial compatibility, fresh mycelial discs (5 mm in diameter) were cut from the edge of an active colony (4 to 5 days old), transferred to new 100-by-15-mm petri dishes containing 25 ml of PDA, and incubated at room temperature (24 to 28°C). Three isolates per plate were spaced 2 to 2.5 cm apart and visually examined after 5 to 8 days for the pres-

Table 1. Characteristics of sclerotia and mycelial compatibility group (MCG) designation for *Sclerotium rolfssii* isolates sorted by geographic origin and host of origin^a

Origin, host, isolate ^b	Original name	Sclerotia color ^c	Year isolated	MCG number	Sclerotia diameter (mm) ^d	Number of sclerotia ^e
Florida						
Tomato						
SR 1	...	Tan	2009	1	0.56 ± 0.03	1,273 ± 146
SR 2	...	Brown	2009	1	0.73 ± 0.02	648 ± 128
SR 3	...	Tan	2009	2	0.93 ± 0.09	341 ± 105
SR 4	...	Tan	2009	2	0.96 ± 0.05	219 ± 60
SR 5	...	Tan	2009	1	0.72 ± 0.03	839 ± 73
SR 6	...	Tan	2009	3	0.82 ± 0.08	576 ± 192
SR 7	...	Brown	2009	1	0.94 ± 0.13	434 ± 104
SR 8	...	Brown	2009	4	0.88 ± 0.08	492 ± 57
SR 9	...	Brown	2009	5	0.89 ± 0.08	500 ± 134
SR 10	...	Tan	2009	6	1.02 ± 0.16	526 ± 316
SR 11	...	Brown	2009	6	0.94 ± 0.17	577 ± 309
SR 12	...	Brown	2009	7	1.02 ± 0.06	367 ± 17
SR 13	...	Dark brown	2009	6	1.17 ± 0.05	163 ± 24
SR 14	...	Tan	2009	6	0.85 ± 0.05	432 ± 57
SR 15	...	Tan	2009	8	1.04 ± 0.04	357 ± 84
SR 16	...	Tan	2009	6	0.90 ± 0.09	380 ± 27
SR 17	...	Tan	2009	8	0.99 ± 0.15	311 ± 68
SR 18	...	Brown	2009	3	0.96 ± 0.12	461 ± 107
SR 19	...	Brown	2009	9	0.82 ± 0.12	731 ± 42
SR 20	...	Tan	2009	8	0.82 ± 0.20	424 ± 65
SR 22	...	Tan	2010	10	0.86 ± 0.16	466 ± 50
SR 47	...	Tan	2010	6	0.99 ± 0.10	1,201 ± 154
SR 48	...	Tan	2010	8	0.96 ± 0.06	752 ± 77
SR 49	...	Tan	2010	6	1.02 ± 0.06	935 ± 104
SR 50	...	Tan	2010	1	0.95 ± 0.03	605 ± 85
SR 51	...	Tan	2010	20	0.85 ± 0.04	802 ± 51
SR 52	...	Tan	2010	10	0.96 ± 0.04	598 ± 21
SR 69	...	Tan	2010	22	0.86 ± 0.03	1,258 ± 33
SR 72	...	Tan	2010	8	0.90 ± 0.13	869 ± 63
SR 49	...	Tan	2010	6	1.02 ± 0.06	935 ± 104
Pepper						
SR 21	...	Tan	2009	2	1.02 ± 0.09	528 ± 46
SR 73	...	Tan	2010	21	0.93 ± 0.08	291 ± 25
SR 105	...	Tan	—	8	0.60 ± 0.03	1,009 ± 129
SR 920	...	Tan	—	14	0.64 ± 0.01	914 ± 34
Peanut						
SR 754	...	Dark brown	1998	11	1.27 ± 0.09	91 ± 6
SR 876	...	Tan	—	12	—	—
SR 877	...	Tan	2003	12	1.45 ± 0.15	87 ± 4
SR 948	...	Tan	2004	11	0.93 ± 0.04	228 ± 18
SR 68	4835	Brown	2010	11	1.46 ± 0.24	379 ± 48
Watermelon						
SR 978	...	Light brown	1995	2	0.57 ± 0.08	797 ± 27
SR 70	...	Tan	2010	8	0.91 ± 0.05	1,427 ± 128
Cantaloupe						
SR 71	...	Tan	2010	8	0.97 ± 0.13	1,159 ± 45
Pumpkin						
SR 23	...	Brown	1998	5	0.90 ± 0.04	516 ± 233
Ruellia simplex						
SR 24	...	Tan	2003	5	0.94 ± 0.03	502 ± 249
Iris sp., pod						
SR 979	...	Tan	2008	12	1.60 ± 0.25	77 ± 16

(continued on next page)

^a The value — is used when the pertinent information is unknown.

^b Geographic origin, host of origin, and isolate name.

^c Based on growth of isolates on standard 100-by-15-mm petri-dishes of potato dextrose agar.

^d Mean sclerotia diameter ± standard deviation ($n = 15$).

^e Mean sclerotia produced from a single colony on a petri-dish ± standard deviation ($n = 3$).

ence of an aversion or a barrage zone (Fig. 1A and B). Pairings were marked as incompatible when an antagonistic barrage zone was observed between two paired isolates and assigned to different groups. Pairings were considered compatible when mycelia from two isolates intermingled without a barrage zone between them, in which case the isolates were placed into the same group. Eventually, a representative tester isolate was designated for each MCG to streamline testing of later isolates. Each test was conducted twice.

Morphological characterization. A single sclerotium was placed in the center of a petri dish containing PDA and incubated at room temperature. During incubation, the colony was characterized as either raised or flat, and sclerotia characteristics such as the

size, color, and the number of sclerotia produced was recorded. After 4 weeks, 15 mature sclerotia were randomly picked from each petri dish and their diameter was measured using the right-angle method (36) with an optical microscope at $\times 40$ magnification. Each isolate had three replicate petri dishes.

Plant materials. Plant materials included ‘Tygress’ (Seminis Vegetable Seeds, Inc., St. Louis), a commercial determinate fresh-market round tomato produced in Florida; the advanced breeding line 5635M, previously characterized as resistant to *S. rolfii* (26) (obtained from Dr. J. Scott, University of Florida); ‘TomCat’ (Siegers Seed Co., Holland, MI), a commercial sweet bell pepper produced in Florida; and ‘Georgia Green’ (7), a commercial peanut

Table 1. (continued from preceding page)

Origin, host, isolate ^b	Original name	Sclerotia color ^c	Year isolated	MCG number	Sclerotia diameter (mm) ^d	Number of sclerotia ^e
<i>Barlaeria cristata</i>						
SR 980	...	Tan	2008	12	1.49 $\pm$ 0.15	49 $\pm$ 11
SR 113	...	Dark brown	—	11	1.21 $\pm$ 0.13	151 $\pm$ 28
SR 74	...	Brown	—	17	1.06 $\pm$ 0.04	777 $\pm$ 137
<i>Pothos</i>						
SR 63	102709-762	Light brown	2010	21	1.19 $\pm$ 0.03	241 $\pm$ 13
<i>Vanda</i>						
SR 64	113009-789	Light brown	2010	21	1.24 $\pm$ 0.06	153 $\pm$ 12
SR 65	113009-793	Brown	2010	21	1.26 $\pm$ 0.13	133 $\pm$ 22
Georgia						
Peanut						
SR 27	WM109	Brown	2009	11	1.28 $\pm$ 0.04	226 $\pm$ 94
SR 28	WM209	Tan	2009	11	1.28 $\pm$ 0.24	239 $\pm$ 32
SR 29	WM309	Tan	2009	11	1.45 $\pm$ 0.05	146 $\pm$ 15
SR 30	WM409	Tan	2009	13	1.41 $\pm$ 0.04	142 $\pm$ 6
SR 31	WM509	Tan	2009	11	1.31 $\pm$ 0.03	152 $\pm$ 19
SR 32	WM609	Tan	2009	11	1.22 $\pm$ 0.02	294 $\pm$ 16
SR 33	WM709	Tan	2009	11	1.44 $\pm$ 0.05	170 $\pm$ 31
SR 34	WM809	Tan	2009	11	1.58 $\pm$ 0.06	97 $\pm$ 17
SR 35	WM909	Brown	2009	8	0.75 $\pm$ 0.04	1,130 $\pm$ 90
SR 36	WM1009	Brown	2009	13	1.34 $\pm$ 0.05	170 $\pm$ 12
SR 37	WM042	Tan	2004	11	1.45 $\pm$ 0.20	228 $\pm$ 28
SR 38	WM91-8	Tan	1991	16	0.99 $\pm$ 0.04	759 $\pm$ 25
SR 39	WM00-10	Dark brown	2000	11	1.20 $\pm$ 0.02	267 $\pm$ 74
SR 40	SR18	Tan	1993	17	1.80 $\pm$ 0.05	66 $\pm$ 16
SR 66	...	Brown	2010	11	1.62 $\pm$ 0.13	509 $\pm$ 70
Tomato						
SR 58	...	Tan	2010	10	0.90 $\pm$ 0.11	1,152 $\pm$ 222
Snap bean						
SR 67	...	Tan	2010	17	1.15 $\pm$ 0.24	427 $\pm$ 115
Louisiana						
Sweet potato						
SR 25	LA-SR1	Tan	2009	15	1.00 $\pm$ 0.06	529 $\pm$ 242
SR 26	LA-SR2	Tan	2009	5	0.92 $\pm$ 0.09	531 $\pm$ 45
South Carolina						
Pepper						
SR 41	SR20	Tan	2010	18	0.76 $\pm$ 0.20	794 $\pm$ 78
SR 42	SR21	Tan	2010	1	0.85 $\pm$ 0.05	381 $\pm$ 61
Eggplant						
SR 43	SR22	Brown	2010	1	0.82 $\pm$ 0.08	463 $\pm$ 40
Snap bean						
SR 44	SR23	Brown	2010	10	1.00 $\pm$ 0.02	348 $\pm$ 279
SR 45	SR24	Tan	2010	10	1.01 $\pm$ 0.09	371 $\pm$ 42
Virginia						
Snap bean						
SR 59	...	Tan	2009	17	1.59 $\pm$ 0.13	313 $\pm$ 59
Pepper						
SR 61	...	Tan	2010	13	1.07 $\pm$ 0.05	966 $\pm$ 44
SR 60	...	Tan	2009	17	1.49 $\pm$ 0.11	370 $\pm$ 21
Potato						
SR 62	...	Tan	2010	11	1.44 $\pm$ 0.08	313 $\pm$ 51
Texas						
Peanut						
SR 53	...	Tan	2010	21	1.45 $\pm$ 0.15	—
SR 54	...	Tan	2010	23	1.11 $\pm$ 0.02	949 $\pm$ 73
SR 55	...	Tan	2010	21	1.91 $\pm$ 0.12	168 $\pm$ 2
Tomato						
SR 56	...	Tan	2010	22	1.06 $\pm$ 0.07	649 $\pm$ 210
Unknown						
SR 46	...	Tan	2010	19	0.95 $\pm$ 0.12	638 $\pm$ 62

with moderate resistance to *S. rolfii* and commonly produced in Florida and Georgia. Plants were grown in a sterilized peat potting mixture (Speedling Inc., Sun City, FL), fertilized as needed with Miracle-Gro water-soluble plant food (The Scotts Company LLC, Marysville, OH) every 7 days, and maintained in a temperature-controlled greenhouse at $30 \pm 5^\circ\text{C}$ and natural daylight. Tomato and pepper seed were germinated in Speedling trays. Four weeks after sowing, seedlings were transplanted into 10-cm-diameter plastic pots, one plant per a pot. Peanut seed were germinated directly in 15-cm-diameter plastic pots, two seedlings in each pot.

Pathogenicity tests. A single isolate representative of each MCG group was used for pathogenicity tests. Isolates were initially cultured on PDA at room temperature for 3 to 4 weeks. Mature sclerotia were then collected and air dried for at least 7 days before beginning tests. Plants were inoculated at 8 weeks after germination by wrapping 10 sclerotia around the stem of each plant just above the soil line with tape (Fig. 2A). The experiment was arranged as a completely randomized design with 16 replicate plants per isolate, including a set of noninoculated plants ($n = 16$) as a control. Plants were maintained under greenhouse conditions for 4 weeks. Disease symptoms were recorded every 7 days after inoculation using a visual scale of 0 to 6 for the severity of wilting, where 0 = no symptoms, 1 = less than 12.5% wilted foliage, 2 = 12.5 to 25% wilted foliage, 3 = 25 to 50% wilted foliage, 4 = 50 to 75% wilted foliage, 5 = 75 to 100% wilted foliage, and 6 = dead plant. Pathogenicity tests were conducted twice.

After the first series of experiments was completed, the response of tomato plants to the various isolates could not be ascertained, because all plants quickly succumbed to all *S. rolfii* isolates when 10 sclerotia per a plant were used (*data not shown*). To better ascertain the effect of inoculum load on disease severity, a separate series of tomato pathogenicity tests was conducted. Different inoculation levels of 2, 4, 6, or 8 sclerotia per tomato plant were tested on both Tygress and 5635M using isolates SR 1, 29, and 41 from tomato, pepper, and peanut, respectively, that varied in viru-

lence from previous studies. Each treatment had 10 individual plants and the experiment was conducted twice. Based on the results of these trials, the tomato pathogenicity trials were repeated using 4 sclerotia per plant with 15 plants per isolate; this experiment was also conducted twice.

Data analysis. Treatments were arranged as a completely randomized design for statistical analyses. Because results from repeated experiments were consistent and no significant interactions between experiments and other fixed effects were detected in the initial analyses, data were pooled and experiments and replication within experiments were included as random effects in the final statistical analysis. Disease severity data from all experiments were analyzed using a nonparametric procedure for the analysis of ordinal data in factorial experiments (10,47). The overall effect of *S. rolfii* isolate on the severity of disease on either pepper, peanut, or tomato was analyzed by the analysis of variance type statistic of ranked data using the PROC MIXED procedure in SAS (version 9.3; SAS Institute, Cary, NC) to generate relative marginal effects (RMEs) and the LD_CI macro to generate 95% confidence intervals (10,47). The RME values were calculated from the equation $\text{RME} = (R - 0.5)/N$, where N is the total number of experimental units in the analysis and R is the mean treatment ranking. Differences among *S. rolfii* isolates in the size and number of sclerotia produced in vitro were also analyzed using the PROC MIXED procedure in SAS (version 9.3; SAS Institute). Count data from the number of sclerotia produced in vitro were log transformed prior to the analysis. For all data, experiments and replications within experiments were treated as random effects within analyses. Linear contrasts were performed to test specific differences between groups of isolates within models.

Results

MCGs. An aversion zone was observed between incompatible isolates after 7 to 10 days of growth. As initial contact between isolates occurred, hyphae lysed and died, leaving a zone of clearing (Fig. 1A). Colony boundaries of compatible isolates could not be easily discerned over the same period of time (Fig. 1B). In total,

Fig. 1. Paired isolates of *Sclerotium rolfii* exhibiting **A**, an incompatible mycelial reaction and **B**, a compatible mycelial reaction; and overhead and side profiles, respectively, of *S. rolfii* **C** and **E**, isolate SR 1 from tomato and **D** and **F**, isolate SR 754 from peanut showing differences in mycelium growth observed on potato dextrose agar plates.

Fig. 2. Symptoms of southern blight caused by *Sclerotium rolfii* using **A**, the stem inoculation method with foliar wilt and blight symptoms observed on **B**, pepper; **C**, tomato; and **D**, peanut.

the 84 isolates of *S. rolfii* were assigned into 23 MCGs based on pairing isolates with testers. Eleven MCGs (4, 7, 9, 14, 15, 16, 18, 19, 20, 22, and 23) consisted of a single isolate, whereas MCG 11 was the largest group, consisting of 15 isolates. Of the 15 peanut isolates from Georgia, 10 belonged to MCG 11; in comparison, the other isolates from vegetable and ornamental plants were distributed into 13 different MCGs (Table 2).

Morphological characterization. The isolates of *S. rolfii* varied in colony morphology, the average diameter of sclerotia, and the average number of sclerotia produced in vitro. Most isolates produced sclerotia that were tan in color but a few isolates formed light-brown, brown, or dark-brown sclerotia (Table 1). All strains consistently formed sclerotia, with the exception of SR 876, which sometimes produced no sclerotia or as few as 10 sclerotia per petri dish. Average sclerotia size was 0.56 to 1.91 mm in diameter, with the smallest sclerotia produced by tomato isolate SR 1 and the largest produced by peanut isolate SR 55. Significant differences existed among isolates in the average sclerotia size and average number of sclerotia produced in vitro ($P < 0.0001$ and 0.0001 , respectively). In general, isolates originating from peanut produced larger sclerotia in vitro than those originating from other hosts. Of the 22 peanut isolates, 19 were in the top one-third of all isolates based on sclerotia diameter. As a group, sclerotia produced by peanut isolates were significantly larger than those produced by isolates from tomato ($P < 0.0001$) and pepper ($P = 0.0282$) (Table 3), while the difference in sclerotia size between tomato and pepper isolates was not significant ($P = 0.1193$). Sclerotia size was different among MCGs ($P < 0.0001$), mostly corresponding to host

of origin. The largest group, MCG 11, consisted of 15 isolates, 13 from peanut and 2 from potato and the ornamental *Barlaeria cristata*, and produced significantly larger sclerotia compared with MCGs 1, 8, and 21, with an average diameter of 1.35 mm. Isolate SR 38 in MCG 16 that was collected from a peanut leaf was incompatible with other peanut isolates. Sclerotia of SR 38 were distinctly smaller than other peanut isolates but similar in size to other non-peanut isolates.

On average, isolate SR 980 produced the fewest number of sclerotia at 49 sclerotia per petri dish, while isolate SR 70 produced the greatest number of sclerotia at 1,427 per petri dish. Of 84 isolates, 59 produced a large number of sclerotia, with an average of >300 sclerotia per petri dish; 7 isolates formed fewer than 100 sclerotia per petri dish, including isolates SR 34, 40, 754, 877, and 880 from peanut (Table 1). The number of sclerotia produced in vitro only differed significantly between MCGs 11 and 21 ($P < 0.0001$) but not between MCG 11 and MCG 1 or between MCG 11 and MCG 8 (Table 3).

Colonies from all isolates consisted of white mycelial growth. However, when compared with other isolates grown on PDA, the mycelia of peanut isolate colonies remained flat along the media surface, giving the colony a thin, compact morphology (Fig. 1D and F). In contrast, colonies of most other isolates produced abundant aerial hyphae, giving the colony a fluffy appearance (Fig. 1C and E).

Pathogenicity tests. Isolates of *S. rolfii* collected from tomato ($n = 7$), peanut ($n = 5$), pepper ($n = 4$), sweet potato ($n = 1$), pumpkin ($n = 1$), and snap bean ($n = 1$) were pathogenic on tomato

Table 2. *Sclerotium rolfii* isolates sorted by mycelial compatibility group (MCG)

MCG	N ^a	Isolates ^b
1	7	SR 1*, SR 2, SR 5, SR 7, SR 42, SR 43, SR 50
2	5	SR 3, SR 4, SR 18, SR 21*, SR 978
3	3	SR 6, SR 47, SR 49
4	1	SR 8*
5	4	SR 9, SR 23*, SR 24, SR 26
6	5	SR 10, SR 11, SR 13, SR 14*, SR 16
7	1	SR 12*
8	9	SR 15, SR 17, SR 20, SR 35, SR 48, SR 70, SR 71, SR 72, SR 105*
9	1	SR 19*
10	6	SR 22, SR 44*, SR 45, SR 52, SR 56, SR 58
11	15	SR 27, SR 28, SR 29*, SR 31, SR 32*, SR 33, SR 34, SR 37, SR 39, SR 62, SR 66, SR 68, SR 113, SR 754, SR 948
12	4	SR 876, SR 877, SR 979, SR 980
13	4	SR 30*, SR 36, SR 61, SR 883
14	1	SR 920*
15	1	SR 25
16	1	SR 38*
17	5	SR 40*, SR 59, SR 60, SR 67, SR 74
18	1	SR 41*
19	1	SR 46
20	1	SR 51
21	6	SR 53, SR 55, SR 63, SR 64, SR 65, SR 73
22	1	SR 69
23	1	SR 54

^a Number of isolates in each group.

^b Isolates followed by an asterisk were used in pathogenicity studies.

Table 3. Linear contrasts for the diameter of sclerotia and the number of sclerotia produced in vitro by *Sclerotium rolfii* isolates collected from tomato, pepper, and peanut

Contrasts ^a	Sclerotia diameter		Sclerotia number	
	F	P value	F	P value
Tomato isolates versus peanut isolates	56.02	<0.0001	66.16	<0.0001
Tomato isolates versus pepper isolates	2.45	0.1193	156.11	<0.0001
Pepper isolates versus peanut isolates	4.90	0.0282	50.26	<0.0001
MCG 11 versus MCG 1	29.59	<0.0001	0.00	0.9561
MCG 11 versus MCG 8	41.88	<0.0001	0.00	0.9486
MCG 11 versus MCG 21	37.02	<0.0001	75.00	<0.0001

^a Mycelial compatibility group (MCG) 11 contains 14 peanut isolates, 1 snap bean isolate, and 1 potato isolate; MCG 1 contains 5 tomato isolates, 1 pepper isolate, and 1 eggplant isolate; MCG 8 contains 5 tomato isolates, 1 pepper isolate, 1 peanut isolate, 1 watermelon isolate, and 1 cantaloupe isolate; and MCG 21 contains 3 peanut isolates, 1 pepper isolate, 1 *Pothos* isolate, and 1 *Vanda* isolate.

(Tygress and 5635M), pepper, and peanut (Fig. 2), causing stem lesions and some level of wilt. The time required for initial wilt symptoms to appear across hosts ranged from 4 to 10 days after inoculation, or 7 days on average. Peanut plants required the longest time (10 days after inoculation) for initial wilt symptoms to appear. On occasion, some pepper plants developed stem lesions from *S. rolfisii* but never exhibited any wilt. Overall, the temporal development of symptoms was very uniform across isolates on each host. No symptoms of wilt were observed on noninoculated plants.

Symptoms on tomato (Tygress and 5635M) were more severe than those exhibited on pepper or peanut. Although severity among isolates on tomato differed statistically in the first trial using 10 isolates per tomato plant, significant differences between Tygress and breeding line 5635M were not detected (*data not shown*). Additional experiments found that tomato cultivar ($P = 0.0010$) and varying inoculum load ($P < 0.0001$) from two, four, six, or eight sclerotia per tomato plant had a significant effect on wilt severity, with no significant interaction between isolate and inoculum load (Table 4). An inoculum load of six or eight sclerotia per plant produced higher disease compared with two and four sclerotia per plant (Table 5). Based on these findings, an inoculum load

of four sclerotia per plant was chosen for subsequent pathogenicity tests on tomato, which detected a significant cultivar–isolate interaction ($P = 0.0017$; Table 6). Although the median disease severity ratings across isolates was similar for both Tygress tomato and breeding line 5635M at 28 days following inoculation, mean disease severity ranks across isolates for 5635M were significantly higher ($P < 0.0001$) than on Tygress (Table 7). As a group, isolates collected from peanut were more virulent than those collected from either pepper or tomato when used to inoculate tomato plants based on contrasts of mean disease severity rankings (Table 8). Although differences in mean disease severity rankings between tomato and pepper isolates were also significant ($P = 0.0176$), the overall difference was minimal compared with the peanut isolates (Fig. 3C). The interaction between tomato cultivar and isolate on mean disease severity rankings was significant ($P = 0.0017$; Table 6), with 13 of 19 isolates producing significantly lower mean disease severity rankings on Tygress tomato compared with breeding line 5635M (Fig. 3C).

On peanut, the four *S. rolfisii* isolates collected from peanut produced the highest mean disease severity rankings (Table 8; Fig. 3B) compared with those collected from tomato ($P < 0.0001$) or pepper ($P < 0.00001$); no significant difference was observed between tomato and pepper isolates ($P = 0.5412$). The same trend was observed on pepper plants, with the isolates collected from peanut causing higher mean disease severity rankings than those collected from tomato or pepper ($P = 0.0001$ and 0.0053 , respectively; Table 8; Fig. 3A).

Discussion

Previous studies of *S. rolfisii* found that MCG diversity can vary greatly within a specific host or within a limited geographical area (23,39,40,46). This study confirmed this conclusion. For example, seven isolates of *S. rolfisii* collected from a natural outbreak in a single tomato field at the Gulf Coast Research and Education Center, Wimauma, FL were differentiated into three MCGs. Isolates in MCG 5 originated from four different host plants. Several studies have demonstrated that isolates from the same MCG are genetically more similar than those from different MCGs (1,12,23,32,41). Therefore, the distribution of 31 tomato isolates from Florida grouped into 13 different MCGs suggests that a high level

Table 4. Test statistics based on the mean ranks of southern blight severity on ‘Tygress’ tomato and breeding line 5635M, 28 days after inoculating with *Sclerotium rolfisii* isolates SR 1, 29, and 41 at four different levels of inoculum per plant

Effect	Analysis of variance-type statistics ^a			
	df _{Num}	df _{Den}	F	P value
Isolate	1.98	387	151.71	<0.0001
Inoculum ^b	2.99	387	16.52	<0.0001
Cultivar	1.00	387	11.04	0.0010
Isolate × inoculum	5.80	∞	0.53	0.7861
Isolate × cultivar	1.98	∞	0.19	0.8254
Inoculum × cultivar	2.99	∞	0.84	0.4692
Isolate × inoculum × cultivar	5.80	∞	0.51	0.7966

^a Represents data from two separate experiments; df_{Num} = numerator degrees of freedom and df_{Den} = denominator degrees of freedom.

^b Individual plants were inoculated with either 2, 4, 6, or 8 sclerotia.

Table 5. Median, mean ranks (*R*), and relative marginal effects (RMEs) calculated for the severity of southern blight on ‘Tygress’ tomato and breeding line 5635M 28 days after being inoculated with 2, 4, 6, or 8 sclerotia of *Sclerotium rolfisii* isolates SR 1, 29, or 41^a

Isolate, inoculum ^b	Tygress			5635M		
	Median	<i>R</i> ^c	RME (95% CI)	Median	<i>R</i> ^c	RME (95% CI)
SR1						
8	1.0	195	0.42 (0.32–0.53)	1.0	255	0.55 (0.44–0.65)
6	1.0	205	0.44 (0.36–0.53)	1.0	229	0.49 (0.39–0.60)
4	1.0	167	0.36 (0.27–0.46)	1.0	180	0.39 (0.31–0.47)
2	0	108	0.23 (0.15–0.34)	1.0	138	0.30 (0.22–0.39)
SR29						
8	4.0	343	0.74 (0.65–0.81)	5.0	371	0.80 (0.74–0.85)
6	4.0	365	0.79 (0.72–0.83)	5.0	383	0.82 (0.76–0.87)
4	4.0	339	0.73 (0.66–0.79)	4.0	337	0.73 (0.60–0.82)
2	4.0	284	0.61 (0.50–0.71)	4.0	331	0.71 (0.59–0.81)
SR41						
8	1.0	199	0.43 (0.34–0.52)	1.0	204	0.44 (0.34–0.55)
6	1.0	180	0.39 (0.29–0.49)	1.0	211	0.45 (0.35–0.56)
4	0.5	142	0.31 (0.22–0.41)	1.0	170	0.37 (0.29–0.45)
2	0	84	0.18 (0.13–0.26)	1.0	164	0.35 (0.27–0.44)
All						
8	4.0	246	0.59 (0.53–0.66)	4.0	276	0.53 (0.47–0.59)
6	4.0	250	0.59 (0.52–0.65)	4.0	274	0.54 (0.47–0.60)
4	2.0	216	0.49 (0.43–0.56)	1.0	229	0.47 (0.40–0.53)
2	0	158	0.45 (0.39–0.52)	1.0	211	0.34 (0.28–0.41)

^a Results represent data from two independent experiments.

^b Isolate and inoculum level. ‘All’ represents data combined across all three isolates.

^c Mean ranks of southern blight severity were determined for each cultivar–isolate–inoculum level interaction and used to calculate RMEs based on the formula $RME = (R - 0.5)/N$, where N = total experimental units in the analysis ($N = 464$). Confidence intervals (CIs) for RMEs were calculated with the LD_CI macro (10). Southern blight severity was rated using an ordinal scale of 0 to 5, in which 0 = no symptoms, 1 = less than 12.5% wilted foliage, 2 = 12.5 to 25% wilted foliage, 3 = 25 to 50% wilted foliage, 4 = 50 to 75% wilted foliage, 5 = 75 to 100% wilted foliage, and 6 = dead plant.

of genetic diversity exists among *S. rolfii* isolates in Florida. In contrast, 10 of 15 peanut stem isolates collected from Georgia belonged to the same MCG, although they were collected from different peanut fields in Georgia over a 10-year period. This result suggests that the level of diversity of *S. rolfii* among Georgia peanut fields is relatively low. This difference in population structure between tomato and peanut isolates may be the result of differences in cropping history or even due to differences in the diversity of the crop. In Georgia, Georgia Green peanut was the main commercial standard, accounting for approximately 85% of the peanut acreage in Georgia (50). However in Florida, tomato production utilizes a diverse array of tomato types and varieties with varying levels of genetic diversity, which may hinder any selective pressure on the *S. rolfii* population. However, it is also possible that some of these differences may represent regional differences due to cropping practices, the introduction of novel isolates, or the result of

sampling bias. Molecular markers are a useful tool for directly evaluating genetic diversity and phylogenetic relationships within and between species (23,25,28,32,34,41). Further studies using molecular markers are required to improve our understanding of population structure and dispersal of *S. rolfii* isolates throughout the southern United States.

In this study, peanut isolates collected in Georgia produced larger sclerotia on PDA than those collected from other hosts, similar to results reported for peanut isolates in Vietnam (25). Le et al. (25) reported that three peanut isolates in one MCG produced sclerotia approximately two times larger than sclerotia from two other groups that included isolates from both peanut isolates and tomato or other isolates. Okabe et al. (34) also described a group of *S. rolfii* isolates that produced larger, irregularly shaped sclerotia with stipes compared with other groups of isolates, and Punja and Sun (39) suggested that these isolates be reclassified as *S. delphinii* instead of *S. rolfii*. Punja and Damiani (36) demonstrated that *S. delphinii* could be distinguished from *S. rolfii* based on larger sclerotia size (0.75 to 1.80 mm), reddish-brown sclerotia color, and generally lower levels of oxalic acid and pectinase enzymes than *S. rolfii*. Although the peanut isolates in this study did produce larger sclerotia than the other isolates, they were not irregularly shaped and were not reddish-brown in color as previously reported (36) but, rather, were spherical in shape and tan to dark brown in color.

Earlier works demonstrated that differences in virulence exist among isolates of *S. rolfii* (13,40,44,48), similar to the findings of this study. Georgia peanut isolates always exhibited the highest level of disease severity compared with the other isolates, regardless of the host plant. In contrast, tomato and pepper isolates dif-

Table 6. Test statistics based on the mean ranks of southern blight severity caused by *Sclerotium rolfii* isolates on ‘Tygress’ tomato and breeding line 5635M using 4 sclerotia per plant

Effect	Analysis of variance-type statistics ^a			
	df _{Num}	df _{Den}	F	P value
Cultivar	18	1,102	42.87	<0.0001
Isolate	1	1,102	63.42	<0.0001
Cultivar × isolate	∞	1,102	2.48	0.0017

^a Represents data from two separate experiments; df_{Num} = numerator degrees of freedom and df_{Den} = denominator degrees of freedom.

Table 7. Median and mean ranks (*R*) calculated for the severity of southern blight on ‘TomCat’ pepper, ‘Georgia Green’ peanut, ‘Tygress’ tomato, and tomato breeding line 5635M plants 28 days after being inoculated^a

Isolate ^b	Pepper		Peanut		Tygress		5635M	
	Median	<i>R</i> ^c	Median	<i>R</i> ^c	Median	<i>R</i> ^c	Median	<i>R</i> ^c
SR 1	1.0	192	1.0	257	1.0	412	1.0	569
SR 5	1.0	214	1.0	205	1.0	427	1.0	494
SR 8	1.0	299	1.0	296	1.0	442	1.0	677
SR 12	4.5	320	1.0	195	1.0	412	1.0	449
SR 14	2.0	290	1.0	284	1.0	412	1.0	585
SR 19	1.0	296	1.0	234	1.0	412	1.0	465
SR 47	1.0	265	2.0	340	1.0	412	1.0	505
SR 21	4.0	316	1.0	275	1.0	412	1.0	533
SR 41	1.0	297	1.0	217	1.0	412	1.0	501
SR 105	1.0	229	1.0	195	1.0	472	1.0	601
SR 920	4.0	324	1.0	283	1.0	545	1.0	635
SR 29	5.0	331	3.0	418	5.0	826	5.0	756
SR 30	5.0	466	5.0	515	5.0	937	5.0	987
SR 32	5.0	359	4.0	517	5.0	819	5.0	902
SR 40	5.0	443	5.0	511	5.0	906	5.0	827
SR 23	1.0	256	1.0	219	1.0	427	1.0	477
SR 25	1.0	274	1.0	216	1.0	412	1.0	581
SR 38	1.0	258	1.0	212	1.0	427	1.0	620
SR 44	4.0	355	2.0	396	1.0	449	1.0	574

^a Results represent data from 2 independent experiments.

^b Mean ranks of southern blight severity was determined for each isolate or each cultivar-isolate level interaction and used to calculate relative marginal effects (RMEs) based on the formula $RME = (R - 0.5)/N$, where N = total experimental units in the analysis (N = 608 for pepper and peanut and N = 1,140 for tomato). Confidence intervals for RMEs were calculated with the LD_CI macro (10). Southern blight severity was rated for using an ordinal scale of 0 to 5, in which 0 = no symptoms, 1 = less than 12.5% wilted foliage, 2 = 12.5 to 25% wilted foliage, 3 = 25 to 50% wilted foliage, 4 = 50 to 75% wilted foliage, 5 = 75 to 100% wilted foliage, and 6 = dead plant.

^c Isolates of *S. rolfii* included SR1, 5, 8, 12, 14, 19, and SR 47 originating from tomato; SR 21, 41, 105, and 920 from pepper; SR 29, 30, 32, and SR 40 from peanut; SR 23 from pumpkin; SR 25 from sweet potato, SR 38 from peanut leaf; and SR 44 from snap bean.

Table 8. Linear contrasts of *Sclerotium rolfii* isolates collected from tomato, pepper, and peanut based on mean ranks of disease severity on ‘Tygress’ tomato and breeding line 5635M using 4 sclerotia per plant, and of ‘Georgia Green’ peanut and ‘TomCat’ pepper using 10 sclerotia per plant

Effect	Tomato		Peanut		Pepper	
	F	P value	F	P value	F	P value
Tomato isolates versus pepper isolates	5.63	0.0176	0.39	0.5412	1.21	0.2845
Peanut isolates versus tomato isolates	432.22	<0.0001	61.60	<0.0001	22.86	0.0001
Peanut isolates versus pepper isolates	228.11	<0.0001	55.25	<0.0001	9.88	0.0053

ferred in virulence among the four different host plants. Georgia Green peanut was considered resistant to stem rot caused by *S. rolfsii* in 1999 (7,8), and was used for approximately 85% of the peanut acreage in Georgia in 2007 (50). It is quite possible that new, more virulent isolates of *S. rolfsii* emerged that were better adapted to Georgia Green and other resistant peanut varieties. Ansari and Agnihotri (2) reported a positive correlation between oxalic acid production and the virulence of *S. rolfsii* isolates. The larger sclerotia size of Georgia peanut isolates may be another result of this process that enhanced the virulence by secreting a

larger volume of oxalic acid and other cell-wall-degrading enzymes (5,6,11,30,37,45). It is also possible that a new, more virulent isolate was introduced in this area and became predominant but additional genetic studies are needed to elucidate this further.

Host resistance to *S. rolfsii* has been reported in peanut, cowpea, pepper, alfalfa, and tomato (4,8,14,17,22,26,35); however, the actual mechanisms conferring resistance among these hosts is unclear. Documented sources of resistance to southern blight in tomato are limited. To date, wild Peruvian accessions PI126932 and PI126432 of *S. pimpinellifolium* are the only source. Six breeding

Fig. 3. Estimated relative effects (REs) and relative marginal effects (RMEs) based on the analysis of mean ranks for the severity of wilt caused by 19 isolates of *Sclerotium rolfsii* 28 days after inoculating **A**, 'Tom Cat' pepper; **B**, 'Georgia Green' peanut; and **C**, tomato breeding line 5635M and 'Tygress' tomato plants. Isolates of *S. rolfsii* included SR1, 5, 8, 12, 14, 19, and SR 47 originating from tomato; SR 21, 41, 105, and 920 from pepper; SR 29, 30, 32, and SR 40 from peanut; SR 23 from pumpkin; SR 25 from sweet potato; SR 38 from peanut leaf; and SR 44 from snap bean. Vertical bars on each column represent the 95% confidence intervals. An asterisk above bars in C denotes a significant difference in wilt severity ranks between the tomato cultivar and breeding line ($\alpha = 0.05$).

lines (5635M, 5707M, 5719M, 5737M, 5876M, and 5913M) were released from Texas A&M University, each one containing *S. pimpinellifolium* in their genetic background (26). Incorporating resistance against *S. rolsfii* into cultivated tomato cultivars would provide a cost-effective and environmentally safe method for managing this disease. Bhakta (4) found that one of the six Texas breeding lines, 5913M, was resistant to peanut isolate WM 609 (corresponding to SR 32 in this study) of *S. rolsfii* from Georgia but susceptible to Florida tomato isolate GCT-1 (corresponding to SR 1 in this study), whereas breeding line 5635M was resistant to both. In contrast, in this study, 5635M was susceptible to peanut isolates, including WM 609 used in Bhakta's study. The peanut isolates from Georgia caused higher levels of disease severity than the other isolates on Tygress and 5635M, including tomato isolates from Florida. Interestingly, Tygress was more resistant toward the non-peanut isolates than 5635M, suggesting that resistance in tomato may be isolate specific. However, additional studies will need to be conducted. Because tomato isolates collected in Florida were less virulent on Tygress, a commercial cultivar grown in Florida, future studies should assess the susceptibility of other commercial tomato cultivars so that growers could potentially choose more tolerant cultivars for production in problematic areas.

Some pepper plants with severe stem lesions did not develop any symptoms of wilt. Similar observations were made for the southern-blight-resistant pepper 'Golden California Wonder', which was reported to contain resistance conferred by a single recessive gene (18). Interestingly, the response of TomCat pepper varied across isolates from highly resistant (SR 1, 5, and 105) to highly susceptible (SR 30 and 40). In highly resistant interactions with SR 1, 5, and 105, some pepper plants did not exhibit any symptoms of wilt 4 weeks after inoculation, even though they developed severe stem lesions after inoculation. Subsequent studies should investigate these potential differences across a larger group of pepper cultivars and breeding lines.

The phase-out of methyl bromide has brought more attention to diseases caused by soilborne pathogens such as southern blight from growers and researchers alike (29,33,42,44). Although southern blight has traditionally been considered a minor disease of tomato production in Florida, it now has the potential to become more problematic. This study evaluated population structure of the pathogenic fungus *S. rolsfii* and observed that high levels of genetic diversity existed among tomato isolates in Florida based on MCG, with evidence of either host or geographic specialization among peanut isolates in Georgia. Tomato pathogenicity tests detected evidence of resistance but suggest that it may be specific to certain groups of isolates, which could make breeding efforts more challenging. However, some commercial tomato and pepper cultivars, such as Tygress and TomCat, respectively, may already have some level of resistance to isolates common to Florida and should be investigated further.

Acknowledgments

We thank T. Brenneman for providing isolates SR 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, and 40; A. Gevens for providing SR 978; M. Elliott for providing SR 979 and SR 980; D. Ferrin for providing SR 25 and SR 26; A. Palmateer for providing SR 63, 64, and 65; T. Kucharek for providing SR 876, 877, 920, and 948; A. P. Keinath for providing SR 41, 42, 43, 44, and 45; J. Woodward for providing SR 53, 54, 55, and 56; and M. Paret for providing SR 71, 72, 73, and 74.

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