Identity and Pathogenicity of Two *Marasmius* Species from the Sterile White Basidiomycete Complex

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ABSTRACT

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Three isolates with white mycelium, rhizomorphic strands, clamp connections, and dolipore septa belonging to the plant-pathogenic group known as the sterile white basidiomycetes (SWB) were isolated from bermudagrass (*Cynodon dactylon*) and cultured in flasks on living sweet corn (*Zea mays*) and snap bean (*Phaseolus vulgaris*) plants grown in vitro. Sporophores of *Marasmius graminum* and *M. rotula* were formed in flasks containing the isolate designated as SWB 1. Reisolations from the pilei of both *Marasmius* spp. were identical to the original SWB 1, which indicates that either this culture was contaminated with both species or the isolate has two teleomorphic states. Pathogenicity tests with the three original isolates and reisolations from the two sporophores were conducted with sweet corn, snap bean, pearl millet (*Pennisetum glaucum*), pigeon pea (*Cajanus cajan*), sorghum (*Sorghum vulgare*), and rye (*Secale cereale*). The five isolates were slightly virulent to all six crops in the greenhouse, but results from the field were more ambiguous and showed fewer significant differences compared with the control. SWB fungi were reisolated from all six crops in the greenhouse and field experiments.

A sterile white basidiomycete (SWB) infecting agricultural crops has been reported by several researchers (1–4,9, 10). The SWB isolates resemble *Sclerotium rolfsii* Sacc. in culture but do not form sclerotia. Although distinctive cultural characters are observed in some SWB isolates, these fungi have not been identified because basidiocarps have not been observed.

Fungi in the SWB complex are pathogenic on many crop species (1). Susceptible grasses include sweet corn (*Zea mays* L.), sorghum (*Sorghum vulgare* Pers.), and rye (*Secale cereale* L.). Sumner et al (11) found lesions caused by a SWB on hypocotyls of lima bean (*Phaseolus lunatus* L.) and snap bean (*P. vulgaris* L.). Kaiser et al (4) observed that pigeon pea (*Cajanus cajan* (L.) Huth) was susceptible to a SWB. Pathogenicity tests indicated that an isolate from bermudagrass (*Cynodon dactylon* (L.) Pers.) did not differ from an SWB isolated from corn (13).

The identity of the SWB remained uncertain. This study was conducted to induce sporulation of SWB isolates to determine their identity and to compare the pathogenicity and host range of the original and derived isolates in greenhouse and field studies.

MATERIALS AND METHODS

Isolates tested and cultural methods. Three isolates of the SWB were used: 1) 44-S-1, from the bermudagrass forage cultivar Tifton 44 near Cordele, Georgia, in June 1988; 2) T419, from the bermudagrass turf cultivar Tifton 419 at the Sunset Country Club in Moultrie, Georgia, in May 1988; and 3) SWB 1, from Tifton 419 at Bull Creek Sod Farms near Moultrie in July 1989. Two additional isolates, Mush 1 and Mush 2, reisolated from sporophores formed in flasks containing the SWB 1 culture, were included in the pathogenicity tests. All isolates were cultured on malt extract agar (MEA) (12 g of malt extract, 20 g of agar, and 1,000 ml of demineralized water).

For in vitro inoculations, 2-L Erlenmeyer flasks containing three peat moss pellets and 150 ml of MEA were autoclaved for 35 min and allowed to cool. Seed of snap bean (cv. Blue Lake) and sweet corn (cv. Snowbelle) were surface-disinfected in 0.525% aqueous sodium hypochlorite solution for 2 min. Peat pellets in each of six flasks were planted with either corn or snap bean, and two flasks containing each crop were inoculated with mycelial plugs from one of the three original isolates.

All sporophores growing on the plants were collected, and reisolations were made onto MEA. Isolates that formed sporophores were identified by standard mycological procedures (5). Reisolations from sporophores were obtained by plating excised portions of the pileus on MEA. Mycelium growing from the tissue was subcultured and grown at 25 C for 5 days. Cultural and hyphal characteristics of new isolates were compared with the original to confirm similarity.

Pathogenicity tests. *Greenhouse.* Isolates T419, 44-S-1, SWB 1, Mush 1, and Mush 2 were grown in 3% cornmeal-sand (CMS) mixture (100 g of sand, 3 g of cornmeal, and 15 ml of distilled water) for 2 wk in flasks on a laboratory bench. Six agricultural crops with known susceptibility to SWBs were tested: sweet corn (cv. Snowbelle), snap bean (cv. Blue Lake), pearl millet (*Pennisetum glaucum* (L.) R.Br. cv. Tifleaf 2), pigeon pea (cv. Indian ICPL 85010), sorghum (cv. Tift MR 88), and rye (cv. Wrens Abruzzi). Even though it was the source of isolates T419, 44-S-1, and SWB 1, bermudagrass was not included as a test crop; stolons are difficult to surface-sterilize, and root and plant damage derived from stolons is difficult to compare with that derived from seed.

Pots (20 × 100 cm) containing Dothan loamy sand (Thermic Arenic Paleaquult) were treated with aerated steam (65 C) for 25 min. For each of the five isolates and the control, 55 L of soil was mixed with 110 ml of CMS inoculum and 2 L of soil was added to each of 24 pots. Seeds of the six crops were planted in each of four pots and covered with an additional 250 ml of soil. For the control pots, no cornmeal-sand inoculum was added and only 2.25 L of sterile soil and seed were used. The experimental design was a randomized complete block with four replicates per treatment.

Soils were watered daily to maintain soil moisture above 50% of the available moisture capacity. The first stand count was made immediately after plant emergence, followed by weekly counts over a 21-day period. During each stand count, dead plants were removed and necrotic tissue was placed in 70% (v/v) aqueous ethyl alcohol for 3 min, then on 2% water agar. Actively growing cultures were transferred to MEA plates and compared with the original isolates. Plants were removed on the 21st day after emergence and rated for hypocotyl and root disease severity on a scale of 1–5, where 1 = <2%, 2 = 2–10%, 3 = 11–50%, and 4 = >50% discoloration and necrosis on roots and hypocotyls and 5 = dead plant. Emergence and postemergence damping-off were analyzed by least-squares analysis of variance. Sums of squares were partitioned into effects due to replication, crop, fungus, and crop × fungus interactions. Final root and

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hypocotyl damage ratings, including ratings for dead plants removed for fungal reisolations, were analyzed. Sums of squares were partitioned into effects due to replication, crop, fungus, crop × fungus interactions, and plant within crop × fungus combinations. Means were compared with Fisher's LSD.

Field studies. The five isolates were compared in the field for pathogenicity to the same sweet corn, snap bean, pearl millet, pigeon pea, sorghum, and rye cultivars used in the greenhouse experiment. Field plots were disk-harrowed 10–15 cm deep, then moldboard-plowed 25–30 cm deep. Then, 448.4 kg/ha of a preplant fertilizer (Super Rainbow 5-10-15 plus micronutrients) and 224.2 kg/ha of $K_2SO_4 \cdot MgSO_4$ (22% K_2O, 22% S, 11% Mg) were applied and rototilled 15 cm into the soil, and the beds were prepared for planting.

Two tests were established in Dothan loamy sand as randomized complete blocks with four replications. Each plot was a single bed with one row (3 m × 15 cm), irrigated as needed by a solid-set overhead sprinkler. In the first test, sweet corn, snap bean, pearl millet, and pigeon pea were planted by hand in the plots on 2 May 1990. Corn was planted 5–8 cm deep in furrows at a rate of 20 seed per plot with 15-cm spacing. The other three crops were planted at the same depth but at a rate of 40 seed per plot with 7.5-cm spacing. Each SWB isolate was grown on CMS in sterile flasks as in the greenhouse studies. Approximately 40 ml of the inoculum was distributed evenly over the furrow plots. Immediately after inoculum was added, soil was raked over the furrow to cover the seed to a depth of 8 cm. In the second test, the same five isolates were used with sorghum and rye, which were planted on 6 June 1990 in a field adjacent to the first test. Similar cultural methods were used for both fields. Each plot was side-dressed with an equivalent of 45 kg/ha of ammonium nitrate 4 wk after planting.

Stand counts in both field studies were taken weekly for 3 wk. Dead or dying plants were collected and plated for fungal reisolations. Isolations were made as previously described. Isolates were compared with the original cultures for similarity in colony morphology. At 6 wk after planting, all plants were cut at the ground surface and fresh weights were obtained for both tests. The plant material was dried in forced air for 5 days and reweighed.

Data were transformed to make the means and variances independent and to achieve homogeneity of variances. Fresh and dry weight data were adjusted by $\log_{10}$ transformation, and arcsine transformation was used for stand counts (plants emerged per seed planted). All data were subjected to analysis of variance and mean separation determined by Fisher's LSD ($P = 0.05$). Treatment effects were compared on stand counts and fresh and dry weights of the six crops.

Table 1. Analysis of variance of effects of soil infested with sterile white basidiomycete isolates on emergence and postemergence damping-off of six crops

Source	df	Mean squares: Emergence	Mean squares: Postemergence damping-off
Replication	3	3.86**[z]	0.04
Crop	5	25.42**	10.89**
Fungus	5	1.16	0.88
Crop × fungus	25	1.41	0.85
Error	105	1.03	0.63

[z] Significant at $P = 0.01$.

Table 2. Analysis of variance of hypocotyl and root damage of treatments with soil infested with sterile white basidiomycete isolates to six crops in the greenhouse

Source	df	Mean squares
Replication	3	2.74
Crop	5	34.78**[z]
Fungus	5	8.58**
Crop × fungus	25	1.81
Plant (crop × fungus) (error a)	158	1.46**
Error b	439	0.45

[z] Significant at $P = 0.01$.

Table 3. Mean separations of hypocotyl and root discoloration of six crops caused by six fungi in greenhouse evaluations

Crop	Damage[y]	Fungus	Damage
Snap bean	4.05 a[z]	44-S-1	3.32 a
Pigeon pea	3.15 b	SWB 1	3.29 a
Corn	3.02 bc	Mush 1	3.23 ab
Sorghum	2.96 c	T419	3.10 bc
Rye	2.54 d	Mush 2	2.97 c
Pearl millet	2.41 d	Control	2.49 d
LSD (0.05)	0.19		0.18

[y] Rated on a scale of 1–5, where 1 = <2%, 2 = 2–10%, 3 = 11–50%, and 4 = >50% discoloration and necrosis and 5 = dead plant.
[z] Numbers followed by the same letter are not significantly different according to Fisher's LSD.

Table 4. Effects of sterile white basidiomycete isolates on stands of rye and sorghum (3 m × 15 cm plots) planted 2 May 1990

Isolate	Percentage of living plants per sown seed: 14 June	19 June	25 June
44-S-1	81.0 ab[z]	63.8 c	81.7 a
T419	82.8 ab	65.8 bc	80.6 a
Mush 1	79.7 b	72.6 ab	79.2 a
SWB 1	85.1 a	63.9 c	77.8 a
Mush 2	82.2 ab	73.4 a	75.2 a
Control	82.2 ab	75.4 a	74.1 a

[z] Analysis and means separation performed using arcsine transformation. Numbers followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

RESULTS AND DISCUSSION

The in vitro study to induce sporulation of three SWB isolates was successful with isolate SWB 1. From this culture, sporophores of both *Marasmius graminum* (Lib.) Berk. & Broome and *M. rotula* (Scop.:Fr.) Fr. were observed growing from the sweet corn root systems. Both sporophores, however, were not observed in the same flask. The teleomorphs formed after approximately 3 mo of in-

Table 5. Effects of sterile white basidiomycete isolates on fresh and dry weights of rye and sorghum[y] 6 wk after emergence in the field

Isolate	Fresh weight (g)	Dry weight (g)
44-S-1	488.13 (1.75) b[z]	85.00 (1.37) b
T419	435.63 (2.06) ab	84.50 (1.65) ab
Mush 1	578.25 (2.10) ab	116.13 (1.70) ab
SWB 1	396.50 (1.97) ab	95.13 (1.59) ab
Mush 2	541.88 (1.98) ab	107.63 (1.59) ab
Control	630.75 (2.23) a	129.75 (1.84) a

[y] Planted 6 June 1990 in 3 m × 15 cm plots.
[z] Means of untransformed data ($\log_{10}$ transformed means). Numbers followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

cubation. All corn plants were killed by the pathogens within 2 wk after inoculation. Tehon (12) and Young (14) reported that *M. inoderma* (Berk.) Singer was a pathogen of maize, and the researchers were able to get isolates to sporulate in vitro to confirm their identity. Pont (6) later identified *M. g. brevispora* Dennis and *M. sacchari* Wakk. var. *hawaiiensis* Cobb as pathogens causing root rot and stalk rot of maize.

Because the two *Marasmius* spp. growing from corn roots in our study came from isolate SWB 1, either the culture contained a mixture of species or the somatic hyphae that initiated the teleomorphic states were expressing themselves as two distinct species. Cultures of both species looked identical to the original isolate, having clamp connections, dolipore septa, white mycelium, and rhizomorphic strands. It would be useful to conduct studies with Mush 1 (*M. rotula*) and Mush 2 (*M. graminum*) using DNA procedures to determine if the original isolate SWB 1 can sporulate as two different taxa or if the original culture was contaminated. In addition, several hyphal-tip isolates could be examined for their ability to produce either teleomorph.

The sporophores identified as *M. rotula* had the following characteristics: pileus <1.0 cm, white to whitish, dark at disk, and pleated to furrowed or umbilicate; lamellae whitish, widely spaced, with collar nearest stipe; stipe up to 2.0 cm long, glabrous, dark brown, becoming black with age; spores 5.0 × 11.0 (12.0) μm. Two sporophores formed in vitro on dead sweet corn roots. The sporophores identified as *M. graminum* had the following characteristics: pileus up to 2.0 cm, rusty brown to brownish orange, pleated to furrowed or umbilicate, cuticle containing abundant brown cystidia; lamellae whitish and widely spaced; stipe up to 3.0 cm long, glabrous, brownish to black with age with brown more toward upper portion; spores 5.0 × 11.0 μm. Ten sporophores formed in vitro on dead sweet corn roots.

In the greenhouse experiment, replications and crops were significant sources of variation in emergence (Table 1). Crops were a significant source of variation in postemergence damping-off. The fungi did not differ greatly in effects on emergence or postemergence damping-off.

When considering the damage caused by the fungal isolates, both fungus and crop were significant sources of variation (Table 2). Across all crops, greater damage was caused by isolates 44-S-1, SWB 1, and Mush 1 (Table 3). The control showed significantly less hypocotyl and root discoloration than any of the fungal treatments. The *M. rotula* isolate (Mush 1) caused greater damage to the crops than did the *M. graminum* isolate (Mush 2).

Snap bean was most susceptible to damage, while pearl millet and rye were most resistant (Table 3). All isolates infected all six crops. Previous research (1-3,8,10,11) also has indicated that isolates of the SWB and other unidentified basidiomycetes can have a wide host range.

In the first field test, some differences in stand among treatments were seen during the first sampling date (Table 4), which was primarily an emergence count. After the last date, only Mush 2 reduced the stand significantly. During stand counts, dying plants were collected and all five SWBs were routinely isolated from gray to blackish gray plant tissue. Sumner et al (10) observed that the SWB in their study caused similarly colored lesions. In the control plots, *S. rolfsii* was routinely isolated from the plants, which probably contributed to reduced stand counts in all the plots and was a confounding variable. *S. rolfsii* and SWB fungi were isolated from dead and dying plants. There were no significant differences among treatments for fresh and dry plant weights (Table 5).

In the second field test with rye and sorghum, reported previously to be susceptible to the SWBs (10), there were significant differences among treatments during the first two stand counts but no differences for the last (Table 6). Total counts during the last sampling indicated late germination of the seed as of 25 June. As in the first test, SWBs and *S. rolfsii* were reisolated from the plant tissue, and only *S. rolfsii* and *Fusarium* spp. were isolated from control plants. There were differences in fresh and dry weights among treatments (Table 7) for rye and sorghum, but the control differed significantly only from isolate 44-S-1.

The results indicate that the *Marasmius* isolates were similar to the SWBs, causing moderate to severe infection under controlled conditions in the greenhouse, where other organisms (e.g., *S. rolfsii*) were not present to compete for substrate. Bell and Sumner (1) observed similar results with greenhouse vs. field tests, but their isolates were not available for this research. In our greenhouse study, SWBs were more damaging to roots and hypocotyls after emergence than at emergence. Under actual field conditions, the SWBs, including Mush 1 and Mush 2, were pathogenic but were not more effective in killing plants than other naturally occurring organisms such as *S. rolfsii*. The stand counts in the control plots were often the same as in the treated plots because of pathogens occurring naturally in the field soil. Sumner et al (10) reported that an SWB isolate was more virulent than *S. rolfsii* at lower temperatures on rye, sorghum, and snap bean. The field studies reported here were conducted during very hot weather, which potentially limited infection.

Our identification of sporophores of *Marasmius* spp. from a pathogenic fungus is consistent with reports of the pathogenic potential of this genus. Basidiomycete species within the mushroom-forming genus *Marasmius* have been reported to be pathogenic on numerous hosts. For example, Young (14) observed that a *Marasmius* species was parasitic on small grains in Illinois. Sabet et al (7) isolated a basidiomycete that attacked maize and was shown by in vitro studies to be a *Marasmius* species. Later, two species of *Marasmius* were shown to cause root and stalk rots of maize (6).

In conclusion, sporophores of *M. rotula* and *M. graminum* were formed from isolate SWB 1. The SWB fungi may apparently consist of a complex of basidiomycetes with similar cultural characters. These basidiomycetes may all belong only to the genus *Marasmius* or may consist of several as of yet unre-

Table 6. Effects of sterile white basidiomycete isolates on stands of sweet corn, pearl millet, pigeon pea, and snap bean (3 m × 15 cm plots) planted 2 May 1990

Isolate	Percentage of living plants per sown seed		
	12 May	19 May	26 May
44-S-1	86.0 a[z]	83.3 a	82.8 a
T419	85.2 ab	81.2 a	82.0 a
Mush 1	85.3 ab	83.0 a	81.5 a
SWB 1	84.3 b	82.2 a	83.8 a
Mush 2	85.0 ab	81.4 a	77.8 b
Control	85.0 ab	83.8 a	82.3 a

[z] Arcsine transformation used for analysis. Numbers followed by the same letter are not significantly different according to Student's *t* test ($P = 0.05$).

Table 7. Effects of sterile white basidiomycete isolates on fresh and dry weights of sweet corn, pearl millet, pigeon pea, and snap bean[y] 6 wk after emergence in the field

Isolate	Fresh weight (g)	Dry weight (g)
44-S-1	1,844.07 (2.99) a[z]	277.13 (2.29) a
T419	1,623.00 (2.98) a	257.13 (2.27) a
Mush 1	1,751.13 (3.00) a	297.56 (2.29) a
SWB 1	1,727.69 (2.98) a	284.63 (2.29) a
Mush 2	1,674.21 (2.96) a	261.75 (2.23) a
Control	1,790.63 (2.97) a	288.88 (2.26) a

[y] Planted 2 May 1990 in 3 m × 15 cm plots.
[z] Means of untransformed data ($\log_{10}$ transformed means). Numbers followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

ported genera. The identification of the two species suggests that the SWB 1 isolate was a mixed culture. This is the first report linking the SWB with specific teleomorphs.

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