Infection of Sweetpotato by *Fusarium solani* and *Macrophomina phaseolina* Prior to Harvest

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Abstract

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The end rot disease complex, caused mainly by *Fusarium solani* and *Macrophomina phaseolina*, can be an important postharvest problem in sweetpotato. The disease develops a few weeks after storage roots are harvested and stored. Isolations attempted after harvest showed that the pathogens can be present inside the storage roots before symptoms appear. To determine how and when end rot pathogens enter sweetpotato storage roots, two greenhouse experiments were designed using tissue culture-derived plants free of *F. solani* and *M. phaseolina*. In one experiment, plants were grown in autoclaved soil, and 1 month after

transplanting, plants were inoculated at the soil line with either noninfested toothpicks or toothpicks infested with each fungus alone or combined. In the other experiment, plants were grown in noninfested soil or in soil infested with each fungus alone or combined. *F. solani* and *M. phaseolina* were isolated from roots, storage roots, and plant stems below the soil line, at the soil line, and 5 cm above the soil line in both experiments. This suggests these fungi are capable of invading sweetpotato plants and storage roots from infested soil, and can systemically colonize the plant from infected plant propagation material.

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is an important crop in the United States and internationally. Because of its high nutrient values, especially in vitamins and carbohydrates, it plays an important role in the human diet, principally in developing countries, where it is an essential source of food for humans and animals alike (34). Demand for sweetpotatoes has increased rapidly in recent years as people are becoming more health conscious and aware of the high nutrient value of this crop, and as availability of newer processed sweetpotato products such as fries, chips, and easy-to-prepare frozen products has increased.

Sweetpotato growers must now store sweetpotatoes for up to a year to be able to sufficiently supply and maintain their markets. Thus, losses that occur after harvest and after the full costs of production have been incurred are especially costly. Rotting sweetpotatoes may also produce the phytoalexins ipomeamarone and ipomeanols that have been implicated in deaths of cattle that were fed rotting sweetpotatoes culled from storage (7,34). Among the important postharvest disease complexes in sweetpotato are end rots, which are characterized by a visible dry decay at either or both ends of the storage roots. On some farms, these diseases have increased significantly in the last few years, causing significant loss during storage. The etiology of this disease complex is not fully understood, which makes it difficult to integrate methods of management and disease control.

End rots of sweetpotato storage roots are caused by *F. solani* (6), *M. phaseolina* (30), *Lasiodiplodia theobromae* (9), and/or *Diaporthe batatis* (14). Often, one fungus predominates; however, in recent isolations we detected *F. solani* and *M. phaseolina* simultaneously infecting the same lesions (W. L. da Silva, *personal communication*). *F. solani* and *M. phaseolina* were also isolated from healthy storage roots that did not show any end rot symptoms (11). These findings

indicated that these fungi are present inside of symptomless sweetpotato storage roots, suggesting that they are probably fungal endophytes in these roots, and become pathogens when conditions are favorable, as has been observed in other hosts (33).

Fusarium spp. and *M. phaseolina* can survive in crop residues and in the soil, from one season to another (7). In commercial U.S. sweetpotato production, storage roots are planted in beds to sprout, and then the sprouts are used as propagative material to establish field plantings. From transplanting, sweetpotato plants take around 4 months to produce storage roots ready for harvesting. Hence, during their entire life cycle sweetpotato plants are kept in contact with soil, creating different opportunities for end rot causal agents in the soil to penetrate the plant.

Most postharvest pathogens have been thought to infect storage roots through wounds, such as those created by the harvesting process (7,19,24). Although curing has long been established as an efficient practice to heal such wounds and thus reduce water loss and infections by several pathogens during storage (2,7), some growers have not invested in facilities to provide humidification and heating necessary to properly cure sweetpotatoes. Additional management strategies may be necessary to deal with infections by pathogens such as *F. solani* and *M. phaseolina* that enter storage roots before harvest. Understanding how and when those fungi invade sweetpotato storage roots are crucial steps to developing an effective end rot disease management program. This study was carried out to investigate how and when sweetpotato storage roots are invaded by the fungi *F. solani* and *M. phaseolina*.

Materials and Methods

Inoculum. Isolate CK-7 of *M. phaseolina* was isolated from an Evangeline sweetpotato storage root with tip rot at the Burden Center in Baton Rouge, LA (30.409 N, 91.113 W) in 2011. Isolate M-10 of *Fusarium solani* was isolated from a sweetpotato with Fusarium root rot in North Carolina and provided by J. W. Moyer (Department of Plant Pathology, North Carolina State University, Raleigh). Isolates were grown on potato dextrose agar (PDA), and the hyphal tip technique was performed to eliminate any possible contaminants. M-10 has been used for Fusarium root rot resistance screening in new sweetpotato lines and is preserved on silica gel crystals at -20°C. CK-7 was maintained on PDA.

Toothpick inoculation. In order to investigate if the pathogens, *F. solani* and *M. phaseolina*, move from infected mother plants to

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storage roots during growth and development of the plant, a greenhouse experiment was designed using vines from tissue culture plants (screened for the absence of *M. phaseolina* and *F. solani*). Individual Beauregard vine cuttings were transplanted in 15-cm-diameter clay pots filled with autoclaved soil mix (1 part river silt, 1 part sand, 1 part Jiffy Mix Plus [Jiffy Products of America Inc.], and Osmocote 14-14-14 [Scotts-Sierra Horticultural Products Company]) at 3.5 g/pot with 10 replicate plants per treatment. One month after transplanting, vines were mock inoculated with a sterile toothpick or inoculated with the specific fungus at the soil line, by a modification of the method of Pratt et al. (27). Tips (1.0 cm long) of 100 wooden toothpicks were autoclaved for 20 min in 250 ml of distilled water. They were then blotted and re-autoclaved in additional water to remove preservatives. The toothpick tips were autoclaved for a third time in 250 ml of 20% V8 juice. Finally, they were cooled in sterile petri dishes, and 30 toothpick tips were transferred individually to colonies of each fungus growing on PDA in petri dishes or to petri dishes with sterile PDA. After incubation for 7 days at 28°C, toothpick tips were removed, inserted into slits in vines of sweetpotato plants created at the soil line by using a flamed scalpel, and the inoculation site was wrapped with a piece of Parafilm to avoid desiccation of the wounded region. There were four treatments; plants mock inoculated with toothpick tips incubated on sterile PDA as a control (Control), plants inoculated with toothpick tips infested with *M. phaseolina* (Mp), plants inoculated with toothpick tips infested with *F. solani* (Fso), and plants inoculated with two toothpick tips, one infested with *F. solani* and another with *M. phaseolina* (Fso+Mp).

After 120 days, storage roots were harvested and washed with tap water to remove excess soil. Pencil roots (storage roots that uniformly enlarge to a diameter of about 1 cm and then cease enlarging), stems, and one storage root of each plant were collected for isolations as described below. The remaining storage roots were kept in paper bags, stored at 15°C, and analyzed after 3 months for end rot symptom development. Percentage of rotting storage roots infected with each of the pathogens was also determined. This experiment was performed in the summer (from May to September) of 2011 and repeated in the summer of 2012.

Soil infestation. This assay was performed to determine if the pathogens, *F. solani* and *M. phaseolina*, move from infested soil into the growing plant and/or storage roots. Vines from tissue culture (screened for the absence of *M. phaseolina* and *F. solani*) were planted in infested and noninfested soil. The soil was infested by a modified procedure (21) in which inoculum was produced in three metal pans (7 × 15 × 21 cm) lined with a double layer of aluminum foil. To each pan was added 150 g of cornmeal and 300 ml of warm 1% PDA. The pans were allowed to stand for 10 to 15 min, and 75 g of sand was thoroughly mixed with the cornmeal. Then, the pans were covered with two layers of aluminum foil and autoclaved for 1.5 h at 1.27 kg/cm² (18 psi) and 121°C. After cooling, the pans were inoculated with the agar from 1-week-old fungal PDA cultures, one pan with *M. phaseolina* and one with *F. solani*, and one pan was amended with sterile PDA. Agar from three petri dish cultures of each fungus was mixed in the sand-cornmeal of the corresponding pan with a sterile knife, and 50 ml of sterile distilled water was added. The pans were covered with aluminum foil and incubated for 4 weeks at 28°C. After the incubation period, the fungal inocula were spread on a tabletop for 2 days to air-dry. Dried inocula were stored individually in plastic bags at 4°C until needed. Cornmeal-sand inoculum of each fungus was mixed with sterile soil in a 15-cm-diameter clay pot to a concentration of approximately 10³ CFU/g (determined by serial dilution) of dry soil, and approximately 10 g of noninoculated cornmeal-sand was mixed in each control treatment pot. There were four treatments: pots with cornmeal-sand soil infested with *M. phaseolina* (Mp), pots with cornmeal-sand soil infested with *F. solani* (Fso), pots with cornmeal-sand soil infested with both fungi (Fso+Mp), and pots with noninfested cornmeal-sand soil (Control). One vine cutting of sweetpotato cultivar Beauregard was transplanted into each pot.

Sweetpotato plants and/or storage roots were sampled four times during the first year of this experiment (2011), at three developmental stages proposed by Villordon et al. (31) (SR1 = adventitious root initiation, SR2 = storage root initiation, and SR3 = storage root enlargement), and at harvest. Since transplants are wounded during cutting, the first sampling was done at SR1, 5 to 10 days after transplanting (DAT) to determine if the pathogens are able to penetrate the plant before an extensive root system develops. The plants were sampled above and below the soil line, and 5-cm-long segments were used for isolations as described below. The second isolations were performed at 15 to 20 DAT, at SR2, when cracks formed on the adventitious roots by the emergence of lateral roots might provide a natural entrance for the fungi. In addition to the isolations done as in the SR1, isolations were also attempted from the adventitious roots. The third sampling was attempted on plants at SR3, at 30 to 35 DAT, to investigate whether the fungi enter storage roots through lateral adventitious roots. The same locations were sampled as for the SR2 with the addition of one storage root per plant. The fourth sampling was attempted at harvest, and isolations were done as for the third sampling. In the second year (2012), samples were only collected at 1 week after transplanting and at harvesting. The stems, pencil roots, and storage roots were sampled as described below. In total, there were 10 plants sampled per treatment in each sampling time, and the remaining storage roots from the four samplings were kept in paper bags, stored at 15°C, and analyzed after 3 months for end rot symptom development. Percentage of rotting storage roots infected with each of the pathogens was determined from those remaining roots kept in storage.

Isolation procedures. Isolations were attempted from different areas of the sweetpotato vines including the pencil roots, storage roots, the portion of the vine located below the soil line, and the portion of the vine from the soil line up to 5 cm above the soil line. All plant materials for isolations were surface-disinfested in 1% sodium hypochlorite for 10 min and placed on two different media: modified Peptone PCNB Agar (PPA) medium selective for *Fusarium* species (25,29) and RB selective medium for *M. phaseolina* (10). The pencil roots from each plant were chopped into small pieces and placed on each of the selective media. One storage root was selected from each plant, split in half longitudinally, and transverse strips were taken from both ends (distal and proximal) and from the center of the storage root and placed on each of the selective media. Segments from the vine of each plant were split in half, and one half was placed on each selective medium with the cut surface contacting the medium. After growing on the specific selective medium, fungal mycelia were transferred to PDA, hyphal tips were transferred to acquire single isolates, and the isolates were kept on PDA for further identification.

Isolate identification. The standard isolates, CK-7 and M-10, and one representative isolate of each group of other fusaria found in this study were identified by morphological criteria and confirmed by DNA sequence comparison. *Fusarium* spp. isolated from the experimental samples were identified based on morphological characters on PDA and on carnation leaf agar (CLA) (18,25). The identification was confirmed by comparing partial translation elongation factor 1- α (TEF 1- α) sequences acquired from one representative isolate of each group (19 isolates) with the FUSARIUM-ID database (13). The set of primers ef1 (5'-ATG GGT AAG GA(A/G) GAC AAG AC-3') and ef2 (5'-GGA (G/A)GT ACC AGT (G/C)AT CAT GTT-3') (26) were used for the partial gene amplification and sequencing. The partial TEF 1- α sequence of isolate M-10, used in the inoculations, was deposited in the GenBank database (accession number JX945169).

Isolates of *M. phaseolina* were identified by morphological characters on PDA (3) and confirmed by comparing the partial internal transcribed spacer region (ITS) sequences with the GenBank database using BLASTn at the National Center for Biotechnology Information website (<http://ncbi.nlm.nih.gov/blast>). The amplification and sequencing of the partial ITS region were performed using the primer set ITS1-(5'TCC GTA GGT GAA CCT

TGC GG 3') and ITS4-(5'TCC TCC GCT TAT TGA TAT GC 3') (32). The partial ITS sequences of the isolate CK-7, used in the inoculations, was deposited in the GenBank database (accession number JX945170).

DNA extraction, amplification, and sequencing. In preparation for DNA extraction, isolates were grown first on PDA plates for 7 days at 28°C in the dark. Approximately 100 mg of fresh mycelia was scraped from plates, placed in a 1.5-ml microfuge tube, macerated thoroughly with a micro-pestle, and allowed to stand for 6 to 10 min. DNA extraction and purification was performed using the GenElute Plant Genomic DNA Miniprep Kit following the manufacturer's protocol (Sigma-Aldrich Co., St. Louis, MO). Extracted DNA was quantified using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE), and samples in the range 1.8-2.0 ratio of absorbance at 260 and 280 nm were used as templates for polymerase chain reactions (PCR).

PCR reactions were done in a reaction mixture containing 5 µl of 5× thermophilic DNA buffer (Promega, Madison, WI), 0.5 µl of 10 mM deoxyribonucleoside triphosphates (dNTPs), 0.25 µl of *Taq* polymerase (0.5 U/µl), 3.33 µl of 25 mM MgCl₂, 1.25 µl of 10 mM of each primer, 1.0 µl of the 100 ng/µl of DNA template, and 12.42 µl of sterile distilled water. The PCR program included an initial denaturation period of 95°C for 2 min, 35 cycles of 95°C for 45 s, 53°C for 1 min, 72°C for 30 s, and a final extension period of 72°C for 5 min.

Amplicons were purified with QIAGEN's QIAquick PCR Purification Kit following the manufacturer's protocol (Qiagen Inc., Valencia, CA) and sequenced by the commercial contractor (Eton Bioscience Inc., Durham, NC). All sequences acquired were edited using BioEdit v7.1.11 software, annotated using CLC Sequence Viewer software (2013 CLC bio), and deposited in the GenBank database.

Pathogenicity test. All fungal isolates obtained from parts of the plants and storage roots were tested for pathogenicity in slices of sweetpotato storage roots. Whole asymptomatic sweetpotato

storage roots were washed with tap water, peeled, and surface disinfested with 1% NaOCl for 10 min. Slices 1 cm thick were aseptically cut from those roots and individually placed on top of moistened autoclaved filter paper that was placed individually in sterile glass petri plates. A 0.5-cm-diameter agar plug from a 24-h-old fungal colony growing on PDA was placed on the center of each sweetpotato slice, incubated for 2 weeks in the dark at 28°C, and evaluated for necrosis development.

Statistical analyses. All statistical analyses were performed using PROC GLIMMIX for generalized linear mixed models in SAS (version 9.3; Copyright 2002-2010 by SAS Institute Inc., Cary, NC). The data were arranged in binary distribution. Pairwise treatment comparisons were conducted on the independent variables using LS-Means comparison, and adjustments were performed using Tukey's honestly significant difference. All the LS-Means were transformed to percentages for presentation in the figures and tables.

In both experiments, data for each experimental unit consisted of binary values from each isolation site. Data were analyzed as a complete randomized design, with treatments consisting of toothpick inoculation type or soil infestation type. The independent variables were treatment, isolation sites, and time for the infested soil experiment, and isolation sites for the toothpick inoculation experiment. The response variables were the fungi isolated: *F. solani*, *M. phaseolina*, and others (other *Fusarium* species).

To evaluate the incidence of storage roots with end rot infected by each fungus, each storage root was used as an experimental unit and the presence or absence of each fungus was determined in a binary distribution. The independent variables were the treatments, and response variables were the fungi isolated from rotting storage roots (*F. solani*, *M. phaseolina*, and other fungi).

Results

Toothpick inoculation. Both *F. solani* and *M. phaseolina* were recovered from inoculated but not from noninoculated plants. Neither fungus caused discoloration or rotting symptoms in inoculated

Table 1. Percentage of fungi isolated from Beauregard sweetpotato plants 90 days after inoculation by inserting toothpicks infested with *Fusarium solani*, *Macrophomina phaseolina*, or both

Treatment ^y	Fungi isolated ^x					
	2011			2012		
	<i>F. solani</i>	<i>M. phaseolina</i>	Others	<i>F. solani</i>	<i>M. phaseolina</i>	Others
Control	0 b ^z	0 b	28 b	0 b	0 b	30 b
Fso	96 a	0 b	20 b	86 a	0 b	0 c
Mp	0 b	28 a	64 a	0 b	52 a	60 a
Fso+Mp	98 a	16 a	20 b	74 a	36 a	2 c
<i>P</i> > <i>F</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^x Others = other *Fusarium* species.

^y Mock-inoculated plants (Control), plants inoculated with *F. solani* (Fso), plants inoculated with *M. phaseolina* (Mp), and plants inoculated with *F. solani* and *M. phaseolina* (Fso+Mp).

^z Conservative Tukey-Kramer Grouping for Treatment Least Squares Means (Alpha = 0.05). Percentages with the same letter within the same column are not significantly different.

Table 2. Number of isolations of other *Fusarium* species recovered from different isolation sites^x in Beauregard sweetpotato plants 90 days after inoculation by inserting either infested toothpicks with *Fusarium solani*, *Macrophomina phaseolina*, or both; or 120 days after being planted in soil infested with those fungi alone or combined

<i>Fusarium</i> species	Toothpick inoculation experiment ^y					Soil infestation experiment ^z				
	Top	Soil line	Bottom	Roots	Storage roots	Top	Soil line	Bottom	Roots	Storage roots
<i>F. commune</i>	0	0	0	0	0	3	2	3	0	0
<i>F. concentricum</i>	0	0	0	0	0	0	0	0	0	1
<i>F. lateritium</i>	0	1	1	1	0	0	0	0	0	0
<i>F. oxysporum</i>	2	3	2	2	9	5	7	9	12	1
<i>F. pallidoroseum</i>	8	10	8	13	21	6	6	5	8	0
<i>F. proliferatum</i>	0	0	0	0	0	18	21	22	16	13
<i>F. solani</i>	5	7	5	11	13	21	18	24	20	12

^x Top (stem 5 cm above soil line), soil line (stem at soil line), bottom (stem 5 cm below soil line), roots (pencil roots), and storage roots.

^y Number of isolates derived from 40 attempted isolations per isolation site.

^z Number of isolates derived from 240 isolations attempted per isolation site, except for storage roots, for which 120 isolations were attempted.

plants. The percentage of recovery of each fungus did not differ among treatments where they were inoculated alone or where both fungi were inoculated together (Fso+Mp). However, *F. solani* was recovered over 70% of the time, while the recovery of *M. phaseolina* was below 53% in both years (Table 1). Interestingly, other *Fusarium* species (Others) were found in all treatments (Table 2), except in plants inoculated with *F. solani* alone in 2012 (Table 1). Also, the incidence of the other *Fusarium* species was lower in plants inoculated with *F. solani*, either alone or with *M. phaseolina*, and higher in plants inoculated with *M. phaseolina* alone (Table 1). These species were identified using morphological and sequence data, and the sequences obtained were edited, annotated, and deposited in the NCBI database (Table 3). The most frequently isolated were *F. proliferatum*, *F. pallidoroseum*, and *F. oxysporum*.

F. solani was recovered from all isolation sites tested in both years, and the rate of recovery did not differ among the isolation sites within a year (Table 4). On the other hand, *M. phaseolina* was recovered mainly from the stem near the soil line in both years. With the exception of the bottom region in 2012, *M. phaseolina* was not recovered or recovered at a low rate in the isolation sites above and below the soil line (Table 4). Other *Fusarium* species were isolated from all isolation sites in both years, and they were found in a higher percentage in the storage roots in 2011 than in 2012 (Table 4).

Over 30% of storage roots developed end rot symptoms after 3 months in storage from all treatments in both years. However, more rotting storage roots were observed in 2011 than in 2012 in

all treatments, and there were no statistical differences among treatments within year (Fig. 1). Neither *F. solani* nor *M. phaseolina* were isolated from rotting roots from the control treatment (Table 5). *F. solani* was isolated more often from rotting storage roots in the Fso treatment than in the Fso+Mp treatment in 2011; however, this difference was not statistically significant. In 2012, *F. solani* was isolated from 20% of the rotting roots in both Fso and Fso+Mp treatments. However, this percentage was not statistically different from the control and Mp treatments where *F. solani* was not isolated (Table 5). *M. phaseolina* was isolated from rotting storage roots from Mp and Fso+Mp treatments in both years, but the incidence was low and did not differ significantly from the failure to isolate *M. phaseolina* from the control or Fso treatments (Table 5). Furthermore, *F. pallidoroseum*, *F. concentricum*, *F. proliferatum*, and *Aspergillus* spp. (Other fungi) were frequently isolated from rotting storage roots from all treatments in both years (Table 5). However, these fungi failed to cause any significant necrosis or decay in sweetpotato storage root slice pathogenicity tests (data not shown).

Soil infestation. *F. solani* was frequently recovered from plants in the Fso and Fso+Mp treatments in both years (Table 6). *F. solani* recovery was significantly greater from the Fso than from the Fso+Mp treatment in 2011, but there was no difference between the treatments in 2012 (Table 6). Percent recovery of *M. phaseolina* was not significantly different between the treatments Mp and Fso+Mp in 2011, whereas in 2012 its recovery was greater from the plants inoculated with *M. phaseolina* alone than from the combined inoculation with *F. solani* (Table 6). Additionally, *M. phaseolina* was found in about 10% of the plants from the Fso

Table 3. *Fusarium* isolates from this study, for which the TEF 1-alpha gene was partially sequenced and deposited in the NCBI Database

Isolate	Species	Isolation site	Isolation source	GenBank accession number
F1	<i>F. pallidoroseum</i> ^y	Plant stem	Exp_Infested Soil/Treat_Mp	KC820972
F7	<i>F. pallidoroseum</i> ^y	Plant stem	Exp_Infested Soil/Treat_Mp	KC820973
F9	<i>F. pallidoroseum</i> ^y	Plant stem	Exp_Infested Soil/Treat_Control	KC820974
G2	<i>F. oxysporum</i>	Storage root	Asymptomatic Storage Root	KC820980
G3	<i>F. oxysporum</i>	Storage root	Asymptomatic Storage Root	KC820981
G5	<i>F. oxysporum</i>	Plant stem	Exp_Infested Soil/Treat_Control	KC820979
G6	<i>F. nygamai</i>	Plant stem	Exp_Toothpick/Treat_Mp	KC820978
G7	<i>F. proliferatum</i>	Plant stem	Exp_Toothpick/Treat_Mp	KC820975
G9	<i>F. commune</i>	Storage root	Surface Rot	KC820971
F-89.021 ^z	<i>F. denticulatum</i>	Leaf tissue	Chlorotic Leaf Distortion	KC820969
C	<i>F. proliferatum</i>	Plant stem	Exp_Infested Soil/Treat_Fso+Mp	KC820976
D	<i>F. concentricum</i>	Storage root	Exp_Toothpick/Treat_Control	KC820970
E	<i>F. proliferatum</i>	Storage root	Surface Rot	KC820977
F2	<i>F. solani</i>	Plant stem	Exp_Infested Soil/Treat_Mp	KC820963
F3	<i>F. solani</i>	Plant stem	Exp_Infested Soil/Treat_Mp	KC820964
F4	<i>F. solani</i>	Plant stem	Exp_Infested Soil/Treat_Mp	KC820965
F5	<i>F. solani</i>	Plant stem	Exp_Infested Soil/Treat_Mp	KC820966
F6	<i>F. solani</i>	Plant stem	Exp_Infested Soil/Treat_Mp	KC820967
F8	<i>F. solani</i>	Plant stem	Exp_Infested Soil/Treat_Mp	KC825359
B	<i>F. solani</i>	Rotting storage root	Exp_Infested Soil/Treat_Fso	KC820968

^y Synonyms *F. incarnatum* and *F. semitectum*.

^z Not from this study.

Table 4. Percent isolation of fungi from different isolation sites in Beauregard sweetpotato plants 90 days after inoculation by inserting infested toothpicks with *Fusarium solani*, *Macrophomina phaseolina*, or both

Isolation site ^x	Fungi isolated					
	2011			2012		
	<i>F. solani</i>	<i>M. phaseolina</i>	Others ^y	<i>F. solani</i>	<i>M. phaseolina</i>	Others ^y
Top	50 a ^z	10 b	15 b	33 a	13 bc	23 a
Soil line	50 a	38 a	25 b	38 a	45 a	28 a
Bottom	50 a	8 b	13 b	45 a	30 ab	28 a
Roots	48 a	0 b	38 b	45 a	18 bc	30 a
Storage roots	45 a	0 b	75 a	40 a	5 c	8 a
<i>P</i> > <i>F</i>	0.9889	<0.0001	<0.0001	0.7640	<0.0001	0.1142

^x Top (stem 5 cm above soil line), soil line (stem at soil line = inoculation site), bottom (stem 5 cm below soil line), roots (pencil roots), and storage roots.

^y Others (other *Fusarium* species).

^z Conservative Tukey-Kramer Grouping for Isolation Site Least Squares Means (Alpha = 0.05). Percentages with the same letter within the same column are not significantly different.

treatment, which was not significantly different from the failure to isolate it from the control plants (Table 6). Other *Fusarium* species were isolated from all treatments in both years and, except for the Fso+Mp treatment in 2012, were isolated more frequently from treatments that did not include Fso (Table 6).

Except for a lower frequency of isolation from storage roots in 2011, the rate of *F. solani* recovery was not statistically different among isolation sites (Table 7). In contrast, *M. phaseolina* was recovered at higher frequency at isolation sites located in the in-

fested soil in both years (Table 7). In aggregate, other *Fusarium* species were isolated from all parts of the plants, and their isolation rate was not significantly different among isolation sites (Table 7). Although recovery rates of *F. solani*, *M. phaseolina*, and others differed slightly among sampling times in 2011, they did not significantly increase from week 1 to harvest (Table 8). In 2012, *F. solani* was recovered at the same rate in week 1 and at harvest, while there was a small but significant increase in recovery of *M. phaseolina* and others from week 1 to harvest (Table 8).

Fig. 1. Total percentage of sweetpotato storage roots in **A**, 2011, and **B**, 2012, that developed end rot symptoms after 90 days of storage following toothpick inoculation with *Fusarium solani* (Fso), *Macrophomina phaseolina* (Mp), *F. solani* and *M. phaseolina* (Fso+Mp), or mock-inoculation with sterile toothpicks (Control). Error bars represent Conservative Tukey-Kramer Grouping for Treatments Least Squares Means (Alpha = 0.05). Percentages within year were not significantly different.

Table 5. Percent isolation of fungi from sweetpotato storage roots that were rotted after 3 months in storage at 15°C^x

Treatment ^y	Fungi isolated					
	2011			2012		
	<i>F. solani</i>	<i>M. phaseolina</i>	Other fungi	<i>F. solani</i>	<i>M. phaseolina</i>	Other fungi
Control	0 b ^z	0 a	73 a	0 a	0 a	35 a
Fso	70 a	0 a	20 b	20 a	0 a	35 a
Mp	0 b	8 a	39 ab	0 a	6 a	28 a
Fso+Mp	40 ab	20 a	40 ab	20 a	8 a	40 a
<i>P</i> > <i>F</i>	<0.0001	0.2610	0.0535	0.0349	0.3915	0.8822

^x Storage roots were derived from Beauregard sweetpotatoes that were inoculated by inserting infested toothpicks with *Fusarium solani*, *Macrophomina phaseolina*, or both.

^y Plants inoculated with *F. solani* (Fso), plants inoculated with *M. phaseolina* (Mp), plants inoculated with *F. solani* and *M. phaseolina* (Fso+Mp), and noninoculated plants (Control).

^z Conservative Tukey-Kramer Grouping for Isolated Fungi Least Squares Means (Alpha = 0.05). Percentages with the same letter within the same column are not significantly different.

Table 6. Percent isolation of fungi isolated from Beauregard sweetpotato plants 120 days after being planted in soil infested with *Fusarium solani*, *Macrophomina phaseolina* or both

Treatment ^x	Fungi isolated					
	2011			2012		
	<i>F. solani</i>	<i>M. phaseolina</i>	Others ^y	<i>F. solani</i>	<i>M. phaseolina</i>	Others ^y
Control	0 c ^z	0 b	26 b	0 b	0 c	53 a
Fso	92 a	0 b	1 c	94 a	2 c	3 b
Mp	0 c	58 a	47 a	0 b	76 a	10 b
Fso+Mp	79 b	55 a	4 c	96 a	53 b	51 a
<i>P</i> > <i>F</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^x Noninfested soil (Control), soil infested with *F. solani* (Fso), soil infested with *M. phaseolina* (Mp), and soil infested with *F. solani* and *M. phaseolina* (Fso+Mp).

^y Others = other *Fusarium* species.

^z Conservative Tukey-Kramer Grouping for Treatments Least Squares Means (Alpha = 0.05). Percentages with the same letter within the same column are not significantly different.

While *M. phaseolina* was recovered less frequently from the storage roots in 2011, the analysis of the interaction effects between treatments and isolation sites revealed a tendency of *M. phaseolina* to be isolated from parts of the plants that were in contact with the infested soil (bottom, roots, and storage roots). On the other hand, *F. solani* and others were distributed throughout the plant (data not shown).

End rot symptoms developed in stored storage roots from all treatments in 2011 and 2012 (Fig. 2). There was a higher incidence of end rots in storage roots from 2011 than from 2012 in all treatments, and end rot incidence was significantly different among

treatments in 2011, in which the Fso treatment had the highest incidence (Fig. 2).

End rots occurred in the control treatment each year, but neither *F. solani* nor *M. phaseolina* was isolated from any of these roots (Table 9). *F. solani* was isolated more often in both years from rotting storage roots from the Fso treatment than rotting storage roots from the Fso+Mp treatment. Furthermore, the overall isolation rate of *F. solani* was much higher in 2011 than in 2012 (Table 9). Although the recovery of *M. phaseolina* from rotting storage roots did not differ statistically among treatments in 2012, it was isolated slightly more often from the Mp than from the Fso+Mp

Table 7. Percent isolation of fungi from different isolation sites in Beauregard sweetpotato plants 120 days after being planted in soil infested with *Fusarium solani*, *Macrophomina phaseolina*, or both

Isolation site ^x	Fungi isolated					
	2011			2012		
	<i>F. solani</i>	<i>M. phaseolina</i>	Others ^y	<i>F. solani</i>	<i>M. phaseolina</i>	Others ^y
Top	43 a ^z	16 c	17 a	41 a	16 b	25 a
Soil line	43 a	27 bc	20 a	49 a	34 ab	25 a
Bottom	49 a	36 ab	23 a	50 a	41 a	33 a
Roots	50 a	42 a	20 a	49 a	41 a	30 a
Storage roots	19 b	11 c	18 a	50 a	30 ab	40 a
<i>P</i> > <i>F</i>	<0.0001	<0.0001	0.6893	0.8017	0.0038	0.4039

^x Top (stem 5 cm above the soil line), soil line (stem at the soil line), bottom (stem 5 cm below the soil line), roots (pencil roots), and storage roots.

^y Others = other *Fusarium* species.

^z Conservative Tukey-Kramer Grouping for Isolation Site Least Squares Means (Alpha = 0.05). Percentages with the same letter within the same column are not significantly different.

Table 8. Percent isolation of fungi from Beauregard sweetpotato plants at different times after being planted in soil infested with *Fusarium solani*, *Macrophomina phaseolina*, or both

Sampling time ^w	Fungi isolated					
	2011			2012		
	<i>F. solani</i>	<i>M. phaseolina</i>	Others ^x	<i>F. solani</i>	<i>M. phaseolina</i>	Others ^x
1 Week	47 ab ^y	28 ab	15 b	46 a	25 b	9 b
2 Weeks	50 a	33 ab	30 a	ND ^z	ND	ND
3 Weeks	34 b	21 b	9 b	ND	ND	ND
Harvest	44 ab	33 a	27 a	49 a	39 a	46 a
<i>P</i> > <i>F</i>	0.0127	0.0218	<0.0001	0.5253	0.0048	<0.0001

^w Week 1 (1 week after transplanting), week 2 (2 weeks after transplanting), week 3 (3 weeks after transplanting), and harvest (120 days after transplanting).

^x Others = other *Fusarium* species.

^y Conservative Tukey-Kramer Grouping for each Sampling Time Least Squares Means (Alpha = 0.05). Percentages with the same letter within the same column are not significantly different.

^z Not determined, plants were not sampled at these times.

Fig. 2. Soil infestation experiment, **A**, 2011, and **B**, 2012. Total percentage of sweetpotato storage roots with end rot symptoms in soil infested with *Fusarium solani* (Fso), soil infested with *Macrophomina phaseolina* (Mp), soil infested with *F. solani* and *M. phaseolina* (Fso+Mp), or non-soil-infested (Control). Error bars represent Conservative Tukey-Kramer Grouping for Treatments Least Squares Means (Alpha = 0.05). Percentages with the same letter within year are not significantly different.

treatment in 2011 (Table 9). Additionally, *F. pallidroseum*, *F. concentricum*, and *Aspergillus* spp. were frequently isolated from rotting storage roots from all treatments in both years (Table 9), but they did not cause necrosis or decay in sweetpotato storage root slice pathogenicity tests (data not shown).

Discussion

Both *F. solani* and *M. phaseolina* can colonize sweetpotato plants without causing symptoms and are capable of moving from the vines of the plant to storage roots. Storage roots infected by *F. solani*, used as 'seed', were also found to produce infected sprouts (22). Since propagation material, sweetpotato vine cuttings and/or 'seed' roots, can be a substantial source of initial inoculum, future research should consider finding means to eliminate this inoculum.

The finding that both fungi can infect sweetpotato plants and storage roots from infested soil indicates that controlling the population of these fungi in the soil would also be an essential approach to reducing initial inoculum. In fact, in a separate experiment, we discovered that soil fumigated with 1% chloropicrin has potential to reduce the incidence of *M. phaseolina* in symptomless sweetpotato storage roots (W. L. da Silva and C. A. Clark, unpublished). Soil fumigation should be more extensively investigated as a tactic to diminish the potential of infested soil serving as a primary source of inoculum.

Given the extensive opportunities for infection to occur despite efforts to produce pathogen-free propagating material and reduce populations in soil, preventing infection now seems a more daunting task than previously recognized (1,5). Thus, management of sweetpotato end rots should also involve management of production and storage practices to avoid conditions that favor disease development. End rots appear on sweetpotato storage roots more frequently after the roots have been stored for a period of 3 months or longer. The storage roots often begin to shrivel at the ends at this time, possibly due to loss of moisture. Curing, which promotes wound healing on storage roots, was thought to reduce postharvest disease by promoting wound periderm and preventing invasion of roots by wound pathogens (2). Despite the fact that pathogenic fungi such as *F. solani* and *M. phaseolina* may already be inside of storage roots at the time of harvest, curing has been shown to substantially reduce the incidence of end rots. Curing can have varied and complex effects on sweetpotato storage roots, but it is well known to reduce water loss from the roots during storage (7). Future research should investigate whether the extent of water loss from storage roots during storage affects susceptibility to end rots.

Although it is now clear that *F. solani* and *M. phaseolina* have alternative avenues of ingress into sweetpotatoes in addition to invasion through wounds that occur during harvest, such harvest-related wounds may still provide a major site of entrance for these pathogens. Thus, curing can still reduce the incidence of infection that occurs at harvest in addition to other benefits. Furthermore, the presence of pathogens inside of symptomless storage roots does

not necessarily mean that end rot disease will develop, as pathogenic isolates of fungi were isolated from symptomless stored roots, and in resistance screening, roots from inoculated susceptible plants often fail to develop the disease (C. A. Clark, unpublished).

Recently, Arancibia et al. (1) suggested that in Mississippi the incidence of tip rot, a restricted type of end rot, is linked with ethylene-induced stress, and it is increased by preharvest foliar applications of ethephon, which is a defoliant used in potato (*Solanum tuberosum*). It has also been observed that storage roots grown in greenhouse conditions, as well as those grown in flooded soils, are more likely to develop end rot diseases (W. L. da Silva and C. A. Clark, unpublished). Likewise, pathogen genotypes, plant cultivars, plant nutrition, storage root desiccation, and storage conditions may all play a significant role in the development of end rot diseases in sweetpotato storage roots. In our experiments, *F. solani* and *M. phaseolina* were isolated from most of the rotting roots derived from treatments where these fungi were inoculated either alone or together; no known pathogens were isolated from the end rots in the control treatments. In fact, in many isolation attempts, nothing grew from the rotting tissue on PDA after being incubated for over 2 weeks. These observations suggest that either some organisms were present but failed to grow on PDA or that some rotting symptoms in sweetpotato storage roots may result from physiological breakdown, in addition to that caused by recognized fungal pathogens.

F. solani and *M. phaseolina* were commonly isolated simultaneously from inside sweetpotato plants, and they did not seem to influence the spread of each other in plants. This helps to clarify why both pathogens have frequently been isolated at the same time from sweetpotato storage roots with end rots. Sweetpotatoes produce several compounds in response to fungal infection, and these have occasionally been associated with poisoning of cattle fed rotting cull sweetpotatoes (8). Some studies suggest that the most acutely toxic compounds which induce lung edema, the ipomeanols, are specifically formed by enzymatic modifications carried out by *F. solani* (4). The fact that *F. solani* may be present in multiple infections with other pathogens such as *M. phaseolina* may explain why the ipomeanols were found in lesions from many different pathogens (5).

Extensive infection by both *F. solani* and *M. phaseolina* occurred during the first week after transplanting, probably through wounds on the cut transplants. There was little additional infection at later dates that coincided with the developmental stages proposed by Villordon et al. (31), but this may in part be due to the fact that such a high proportion of plants was already infected. The timing and location of infections could be further investigated by using strains of the pathogen transformed either with genes for green fluorescent protein or red fluorescent protein. Hyphae of the pathogen could be located within the tissue by fluorescence microscopy, and this could also be used to differentiate pathogen growth arising from different times of infection.

Table 9. Percent isolation of fungi from sweetpotato storage roots that were rotted after 3 months in storage at 15°C^x

Treatment ^y	Fungi isolated					
	2011			2012		
	<i>F. solani</i>	<i>M. phaseolina</i>	Other fungi	<i>F. solani</i>	<i>M. phaseolina</i>	Other fungi
Control	0 c ^z	0 b	31 a	0 b	0 a	21 a
Fso	89 a	0 b	0 a	25 a	0 a	10 a
Mp	0 c	42 a	15 a	0 b	10 a	18 a
Fso+Mp	39 b	31 ab	0 a	13 ab	0 a	6 a
P > F	<0.0001	0.0007	0.0247	0.0056	0.0306	0.2489

^x Storage roots were derived from Beauregard sweetpotatoes plants that were planted in soil infested with *Fusarium solani*, *Macrophomina phaseolina*, or both.

^y Noninfested soil (Control), soil infested with *F. solani* (Fso), soil infested with *M. phaseolina* (Mp), and soil infested with *F. solani* and *M. phaseolina* (Fso+Mp).

^z Conservative Tukey-Kramer Grouping for Isolated Fungi Least Squares Means (Alpha = 0.05). Percentages with the same letter within the same column are not significantly different.

The greater recovery of *F. solani*, especially at sites distant to the site of inoculation, compared to *M. phaseolina* suggests that *F. solani* is more aggressive in colonizing Beauregard sweetpotato plants than *M. phaseolina*. *F. solani* was commonly recovered from all parts of the plants in both experiments, while *M. phaseolina* was mostly recovered from the inoculation sites and from plant parts exposed to infested soil. Furthermore, the fact that *F. solani* is more aggressive at colonizing sweetpotato plants suggests that it might be more likely than *M. phaseolina* to induce end rots when environmental conditions favor their development.

The dramatically reduced frequency of isolation of other *Fusarium* species from treatments in which *F. solani* was present suggests that *F. solani* has some means of competitively colonizing the plant and reducing colonization by the other fusaria. In contrast, *M. phaseolina* seems to have a positive biological interaction with other *Fusarium* species, as these species were isolated more often in some treatments where *M. phaseolina* was inoculated. Further inoculation studies in which the other *Fusarium* species are inoculated alone or simultaneously with *F. solani* or *M. phaseolina* should be conducted to test these interactions.

Isolates of *F. solani* recovered from noninoculated plants did not cause significant rot in inoculated storage root slices. These *F. solani* isolates differed significantly from the *Fusarium* root and stem canker isolate (M-10) used in this research. While M-10 colonies produced a blue to blue-green pigment, sparse aerial mycelia, abundant micro- and macroconidia in PDA, and aerial microconidia from long monophialides, matching the morphological characteristics described by Clark (6), the isolates found in our studies had a cream color on the upper surface of PDA and also produce microconidia on mono- and polyphialides (data not shown). The identification of these isolates was confirmed to be *F. solani* by comparing their TEF gene sequences with the FUSARIUM-ID database (13). These isolates should be further characterized, as many isolates from the *F. solani* species complex (FSSC) have been reassigned to new *Fusarium* species (23).

Even though *F. oxysporum* and *F. pallidroseum* (synonyms *F. incarnatum* and *F. semitectum*) have been found to cause surface rot in sweetpotato storage roots (15,28), isolates of these species collected in this study did not cause significant symptoms on inoculated sweetpotato slices. Furthermore, in end rot surveys, different species of *Fusarium* were isolated from rotting tissues, but did not reproduce the disease following inoculations (W. L. da Silva and C. A. Clark, unpublished). The role, if any, of these endophytic fusaria in end rot diseases of sweetpotatoes is thus unclear. It is possible that they become pathogenic to plants under certain stresses, which has been observed in other plant species (12,20). A more in-depth study should be conducted to investigate what these fusaria species are doing in the plant. Possibly, some of them may have some means of competition with fungal species that cause end rot symptoms in storage roots. If so, this behavior could be explored to promote resistance in sweetpotato plants against infection of those pathogens, such as was done in other pathosystems (16,17).

In this research, we provide evidence that infection of sweetpotato storage roots by the pathogens *F. solani* and *M. phaseolina* can occur prior to harvest and several months before manifestation of end rots in storage. However, sweetpotato farming is already a very expensive and labor-intensive activity, and adding more processes or costs to try and reduce infection may be prohibitive. As part of a separate study (1,5), it has been shown that curing reduces end rot incidence and that preharvest variables such as exposure to ethephon or flooding can increase end rot incidence. It appears that understanding and managing the preharvest and postharvest environments may have the greatest potential for minimizing the impact of end rots.

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