Morphological and Pathogenic Characterization of *Sclerotium rolfsii*, the Causal Agent of Southern Blight Disease on Common Bean in Uganda

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Abstract

Over the last 5 years, Southern blight caused by *Sclerotium rolfsii* Sacc. has superseded root rots caused by pathogens such as *Fusarium* and *Pythium* spp. as a major constraint of common bean (*Phaseolus vulgaris* L.) production in Uganda. Although *S. rolfsii* is prevalent in all bean-growing regions of Uganda, there is a lack of information about its diversity and pathogenicity to guide the development of effective management strategies. In total, 348 *S. rolfsii* isolates were obtained from bean fields in seven agroecological zones of Uganda, with the following objectives: to characterize their morphology, based on mycelial growth rate, mycelium texture, and number of sclerotia; and to determine the pathogenicity of 75 selected isolates on five common bean varieties in artificially inoculated soils in a screenhouse. We found that mycelial growth rate and the

number of sclerotia produced on artificial media varied among agroecological zones but not within a zone. The five bean varieties tested were found to be susceptible to *S. rolfsii*, including varieties MLB49-89A and RWR719 that are resistant to *Fusarium* and *Pythium* root rots, respectively. Preemergence damping-off ranged between 0 and 100%, and disease severity index ranged between 4.4 and 100%. The widespread and high levels of *S. rolfsii* virulence on varieties of common bean indicate that management intervention is urgently required to help reduce losses incurred by Ugandan smallholder farmers.

Keywords: dry bean, *Phaseolus vulgaris*, preemergence damping-off, *Sclerotium* root rot, Southern stem rot

The pathogen *Sclerotium rolfsii* Sacc. (teleomorph *Arthelia rolfsii* (Curzi) C. C. Tu & Kimbr.) has a wide host range comprising more than 500 plant species and causes Southern blight disease, which results in significant crop yield losses worldwide. For example, it caused 80% yield losses in peanut (*Arachis hypogaea* L.) between 1988 and 1994 in the United States that led to economic losses of \$36.8 million (Franke et al. 1998). Southern blight, which is also termed sclerotium root rot or southern stem rot, was first identified on tomato (*Solanum lycopersicum* L.) in 1892 (Rolfs 1892) and is now known to occur across most crop types, particularly in warm regions (Aycok 1966; Domsch 1980; Farr et al. 1989). For example, the disease has been reported on common bean (*Phaseolus vulgaris*) in Uganda (Paparu et al. 2018), India (Mahadevakumar et al. 2015), and Italy (Garibaldi et al. 2013); maize (*Zea mays*) in Pakistan (Yasmin et al. 1984); soy bean (*Glycine max*) in Nigeria (Akem and Dashiell 1991); potato (*S. tuberosum*) in Italy (Garibaldi et al. 2006); okra (*Abelmoschus esculentus*) in Côte d'Ivoire (Koné et al. 2010); and sesame (*Sesamum indicum* L.) in Mexico (Hernández-Morales et al. 2018).

Key management strategies for Southern blight disease typically include the removal and destruction of infected plants which serve as sources of inoculum, the treatment of plants and seed with fungicides (e.g., Metalaxyl), and inclusion of nonhost plants in crop rotations. Other options include soil solarization (Chellemi 2002; Flores-Moctezuma et al. 2006), use of inorganic and organic soil fertility amendments (Bonanomi et al. 2007; Bulluck and Ristiano 2002; Flores-Moctezuma et al. 2006), and cultivation of resistant host plants (Woodward et al. 2008).

Symptoms of Southern blight include gray and water-soaked lesions on the stem above the soil line; plant surface mycelia and sclerotia; seed rot or preemergence damping-off; seedling root rot; and stem, fruit, tuber, and pod rots (Mullen 2001). *Sclerotium rolfsii* produces abundant hyphae and sclerotia (Mullen 2001) as asexual resting structures that facilitate pathogen survival in the absence of the host (Adams and Ayers 1979).

Morphological and phenotypic diversity of pathogens may be related to variation in virulence that is important in the selection of pathogen isolates used for the evaluation of disease resistance in crop breeding programs. Variability among *S. rolfsii* isolates and related species such as *S. sclerotiorum* that infect different crops has been investigated in studies relating to sclerotia morphology traits and mycelial growth rates (Lehner et al. 2014; Narayan et al. 2017; Vleugels et al. 2013), pathogenicity in susceptible host plants, and genetics using molecular markers (Jebaraj et al. 2017; Narayan et al. 2017).

Previous work in Uganda showed that common bean yield losses caused by Southern blight in artificially inoculated trials ranged between 4 and 76% over two study seasons (P. Paparu, unpublished data). However, despite the threat of this disease to common bean production (Paparu et al. 2018), knowledge of the morphology and pathogenicity of *S. rolfsii* isolates from common-bean-growing regions in Uganda is lacking. Thus, the objectives of this study were to, first, determine regional differences in *S. rolfsii* growth rate and sclerotia formation and, second, assess the variation in the pathogenicity of selected isolates on common bean.

Materials and Methods

Sample collection. For isolation of *S. rolfsii*, wilting plant samples were collected from seven agroecological zones in Uganda: the

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Eastern highlands, Lake Victoria basin and Mbale farmlands, Northern mixed farming zone, Southwestern highlands, Teso farming zone, Western mixed farming zone, and West Nile mixed farming zone (Fig. 1). Samples were collected over three seasons (March to June and August to November 2013 and March to June 2014) during surveys to determine the prevalence and incidence of root rots of common bean in Uganda (Paparú et al. 2018). The mean annual temperature and rainfall for the agroecological zones (Wortmann and Eledu 1999) and elevation ranges obtained using a GPS during sample collection are detailed in Table 1.

In all, five wilting plants that showed characteristic symptoms of Southern blight disease were collected along a Z-transect in each surveyed field; plants were placed in a paper bag for each field and brought to the pathology laboratories at the National Crops Resources Research Institute and the International Center for Tropical Agriculture-Uganda laboratory at the National Agricultural Research Laboratories in Kawanda, Uganda, for isolation of *S. rolfii*. In total, 2,000 plants were collected, including five peanut plants.

Fig. 1. Agroecological zones in Uganda from which wilting plants were collected for isolation of *Sclerotium rolfii*.

Table 1. Mean annual temperature and rainfall, and altitude range in the seven contrasting agroecological zones in Uganda where *Sclerotium rolfii* isolates were collected

Agroecological zone	Temperature (°C)	Rainfall (mm)	Altitude (masl) ^z
Eastern highlands	20	1,337	1,500–2,200
Lake Victoria basin and Mbale farmlands	22	1,211	1,040–1,433
Northern mixed farming	24	1,258	942–1,182
Southwestern highlands	16	1,177	1,247–2,313
Teso farming	23	795	1,072–1,130
Western mixed farming	20	1,140	1,052–1,501
West Nile mixed farming	23	1,304	918–1,454

^z Altitude in meters above sea level (masl).

Isolation of *S. rolfii* and culture maintenance. Plant roots were washed under running tap water and cut into 5-mm sections. Five root sections per sample were surface sterilized by dipping in 5% sodium hypochlorite for 1 min and then in 70% ethanol for a further minute. They were then rinsed thrice in sterile distilled water, blotted dry on sterile tissue paper, and plated on potato dextrose agar (PDA) (distilled water at 39 g/liter) (Sigma-Aldrich, Steinheim, Germany) in 90-mm Petri dishes, supplemented with rifamycin antibiotic (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at 0.03 g/liter.

The plates were incubated on a laboratory bench at approximately 25°C for a photoperiod of approximately 12 h (light) and 12 h (dark) for 7 days. All emerging fungal colonies identified as *S. rolfii* using cultural characteristics described by Prasad (2012) were subcultured on PDA for 7 days, after which pure isolate cultures were prepared using single-sclerotia isolation and hyphal tipping. For hyphal tipping, 3-mm-diameter plugs of PDA containing fully grown mycelia were excised from the initial plate and placed on water agar (13.2 g of agar in 1 liter of water) for 24 h in darkness, at approximately 25°C. Hyphal tipping was done using a sterile needle under a stereomicroscope (Leica, Wild M3B; Leica Systems, Germany), and cultures were grown on PDA with rifamycin at 0.03 g/liter. The resulting pure cultures were grown on sterile filter paper (autoclaved twice for 1 h at 121°C and 15 psi) for long-term storage according to the method of Fong et al. (2000), with modifications. Fifteen pieces of triangular-shaped sterile filter papers cut to a size to fit in a 1.5-ml Eppendorf tube were placed in a sterile Petri dish and overlaid with a thin layer of water agar. Then, PDA plugs (3 mm in diameter) containing fully grown mycelia were excised from pure cultures, placed on the filter papers (maximum of three plugs per Petri dish), and incubated for 3 to 4 weeks in darkness at approximately 25°C. During the incubation period, mycelia grew and dried on the filter paper. Under the aseptic conditions of a laminar flow cabinet, the dried filter papers for each isolate were placed in 1.5-ml Eppendorf tubes and kept at –20°C for long-term storage.

Morphological characterization of *S. rolfii* isolates. In total, 348 *S. rolfii* isolates obtained from the seven agroecological zones were assessed for colony growth rate, color and texture of mycelia, and number of sclerotia produced. Three replicates of 3-mm-diameter agar disks from 3-day-old cultures were placed at the center of three separate 90-mm-diameter Petri dishes (replicates 1, 2, and 3) that contained freshly prepared PDA amended with rifamycin at 0.03 g/liter and incubated at 25°C. From the second day of incubation, colony diameter was measured using a ruler along two perpendicular lines drawn on the bottom of each Petri dish. Measurements were completed between 5 and 7 days, when most of the cultures had grown beyond the Petri dish base. Colony texture was examined with the naked eye and recorded at 7 days. The number and color of sclerotia were recorded for 14 to 28 days after inoculation, using the descriptions by Mullen (2001). We assigned the 348 isolates to morphological groups according to mycelial growth rate and number of sclerotia produced (Table 2).

Pathogenicity of *S. rolfii* isolates on common bean. We selected 75 *S. rolfii* isolates based on morphological grouping and agroecological zone for inoculation on five varieties of common bean: MLB49-89A (*Fusarium* root rot resistant) (Ongom et al. 2012), RWR719 (*Pythium* root rot resistant variety) (Otsyula et al.

2003), ALB123 (hybrid of *Pythium coccineus* and *P. vulgare*), NABE15 (commercially available), and K132 (susceptible; CAL96). Pathogenicity tests were conducted using a protocol adapted for *Fusarium solani* (Mukankusi et al. 2011). Inoculum was prepared using 50 g of moistened millet grain in conical flasks that were autoclaved twice on two consecutive days and allowed to cool under sterile conditions in a laminar flow cabinet. Then, the grains were inoculated with four to five agar plugs, 5 mm in diameter, from actively growing cultures and the flasks were plugged with sterile cotton wool, covered with sterile aluminum foil, and incubated on a laboratory bench for 3 to 4 weeks; the grains were mixed weekly under sterile conditions to allow for even growth of the fungus. When the grains had been fully colonized by the fungus, three replicates of 10 g of inoculum for each isolate was thoroughly mixed with approximately 20 kg of steam-sterilized soil (2:1 ratio of loam/sand) in a 70-by-35-by-10-cm wooden tray in which we sowed 16 seeds of each variety along two rows. We used a randomized complete block design, with the replicates as blocks. Isolates were randomized within the replicates and rows of each variety were completely randomized within each tray. Seed from the five varieties were sown in noninoculated soils as controls. The 75 isolates were screened in six separate experiments due to the limited number of trays and limited greenhouse space.

At 14 days after inoculation, we recorded germination (seed emergence) and incidence of Southern blight disease. Nonemerged seed were removed from the soil to assess the presence of preemergence damping-off caused by *S. rolfii*. Positive preemergence damping-off was recorded for seed that were visibly rotten and those that had germinated and failed to emerge above the soil surface and were covered with mycelia or sclerotia. Southern blight severity was assessed at 28 days after inoculation using a modified scale reported by Le et al. (2012) (Table 3; Fig. 2).

Data analysis. The SAS statistical software (version 9.1; SAS Institute, Cary, NC, U.S.A.) was used for data analysis. Differences in isolate growth rate and number of sclerotia across agroecological zones were analyzed using Tukey's Studentized range test. Southern blight disease severity index (DSI) by variety (data pooled across the six experiments) was analyzed using the PROC GLM procedure in SAS.

The DSI was calculated after Chiang et al. (2017): $DSI (\%) = [\sum(\text{class frequency} \times \text{score of rating class}) / (\text{total number of observations} \times \text{maximal disease index})] \times 100$.

In order to determine the effect of *S. rolfii* isolates on seed germination, seed germination percentage, preemergence damping-off, and DSI values were arcsine square root transformed prior to analysis to ensure homogeneity of variance.

Associations between growth rate and number of sclerotia produced and between growth rate–number of sclerotia and Southern

blight disease severity were tested using Spearman's rank correlation in SAS. For all analyses, significance was evaluated at $P \leq 0.05$.

Results

Growth and sclerotia characteristics of *S. rolfii* isolates. Using the convention published by Mahato and Biswas (2017), we characterized the morphology of 348 isolates of *S. rolfii* based on growth, colony texture and color, and number of sclerotia formed 28 days after culture initiation. All isolates formed white cultures with fluffy, fibrous, or compact mycelia (Fig. 3)—fluffy mycelia were most common (84.7%), followed by thin fibrous mycelia (9.2%) and compact mycelia (6.1%). There were differences in growth rate among the agroecological zones ($P = 0.0001$) (Table 4).

Isolates formed small round (Fig. 3A, B, and C) or large irregular-shaped (Fig. 3D) sclerotia that were either light or dark brown. Of the 348 isolates, 69.6% formed light-brown sclerotia, while 30.4% had dark-brown sclerotia, and the number of sclerotia varied among the agroecological zones (Table 4).

Growth rate varied between the agroecological zones, with the highest observed for isolates from Teso farming and the lowest from the Eastern highland (Table 4). Similarly, growth rate for isolates from the same agroecological zone was different ($P < 0.0001$) and this was true for all seven zones studied. Growth rates ranged from 11.6 to 25.7 ($n = 3$), 10.4 to 26.6 ($n = 150$), 10.7 to 26.7 ($n = 36$), 11.4 to 16.7 ($n = 25$), 21.6 to 27.3 ($n = 80$), 19.4 to 29.2 ($n = 43$), and 20.4 to 27.5 ($n = 11$) for Eastern highlands, Lake Victoria basin and Mbale farmlands, Northern mixed farming, Southwestern highlands, West Nile farming, Western mixed farming, and Teso farming, respectively. Growth rate was negatively associated with number of sclerotia produced ($r = -0.33$, $P < 0.0001$).

Morphological grouping of *S. rolfii* isolates. We grouped the 348 *S. rolfii* isolates into nine morphological groups (Table 2) based on growth rate and number of sclerotia produced. We found that no single agroecological zone comprised all the groups. Morphological groups 3 and 6 were the most abundant (28.7 and 27.0%, respectively) and mostly derived from the Lake Victoria basin and Mbale farmlands and Western mixed farming agroecological zones (Table 5). In all, 11 isolates (3.2%) had low growth rates but produced high numbers of sclerotia (morphological group 7) and, of these, 6 were derived from the Southwestern highlands and 5 from the Western mixed farming agroecological zones. Morphological group 8 was represented by a single isolate collected from the Southwestern highlands.

Pathogenicity of *S. rolfii* isolates. We selected 75 *S. rolfii* isolates representing the nine morphological groups to evaluate their pathogenicity on five varieties of common bean. All tested isolates were pathogenic on common bean, including one (HOI356-Peanut) that was isolated from a peanut volunteer in a bean field. The effect of the isolates on seed germination, preemergence damping-off, and

Table 2. Definition of *Sclerotium rolfii* morphological groups based on mycelial and sclerotial characteristics

Morphological group	Mycelial growth rate ^y	Sclerotia number/ Petri dish ^z
1	High	High
2	High	Medium
3	High	Low
4	Medium	High
5	Medium	Medium
6	Medium	Low
7	Low	High
8	Low	Medium
9	Low	Low

^y Mycelia growth was recorded daily until day seven after inoculation, by which time most of the isolates had covered the bottom of the Petri dish. High = slope ≥ 25 , Medium = slope 20 to 24, and Low = slope ≤ 19 .

^z Number of sclerotia produced per isolate on a single Petri dish was counted 28 days after inoculation. High = ≥ 200 , Medium = 50 to 199, and Low = ≤ 49 .

Table 3. Definition of Southern blight disease severity scores based on visible symptoms found on common bean plants grown in soils infested with *Sclerotium rolfii*

Severity score	Description
1	No symptoms; plant healthy
2	Gray water-soaked lesions present on stem above soil, but no visible fungal outgrowth
3	Visible fungal outgrowth on stem base, characterized by silky-white mycelia or sclerotia that gradually darken
4	Partial wilting, where younger leaves begin to wilt and stems begin to shrivel
5	Complete wilting, desiccation and browning of leaves and stem; plant collapse and death (rot)
6	Preemergence damping-off; complete seed rot, with no sign of germination, or evidence of germination hampered by fungal colonization

Southern blight disease severity differed (Table 6; Supplementary Table S1).

Preemergence damping-off ranged from 0% (by isolate KYG33) to 100% (by isolate LWR100); isolate KYG33 did not affect seed germination of any of the five common bean varieties, whereas isolate LWR100 completely inhibited seed germination in all varieties (Supplementary Table S1). These two fungal isolates were obtained from the Lake Victoria basin and Mbale farmlands agroecological zones. Disease severity ranged between 4.4 and 100%; the lowest disease severity was caused by isolate SRT110 from the Teso farming agroecological zone.

Susceptibility of common bean varieties tested. There were differences in preemergence damping-off, seed germination, and Southern blight disease severity among the varieties following growth in soils infested with *S. rolfii* (Table 7). Seed germination rates were greater and DSI was lower in MLB49-89A and RWR719 (resistant to *Fusarium* and *Pythium* root rots, respectively) and ALB123 (hybrid) than in the susceptible K132 and commercial NABE15 ($P < 0.0001$). Preemergence damping-off was greater in K132 and NABE15 than in MLB49-89A and RWR719 ($P < 0.0001$). There was a variation in disease severity caused by 10 of the 75 isolates tested on five common bean varieties ($P \leq 0.05$) (Fig. 4). Similarly, preemergence damping-off caused by 22 isolates varied among the five varieties ($P \leq 0.05$) (Fig. 5).

We found weak but positive correlations between the number of sclerotia produced by an isolate and its ability to cause preemergence damping-off ($r = 0.27$, $P = 0.018$), and between sclerotia number and Southern blight DSI ($r = 0.28$, $P = 0.015$). There was no correlation between mycelial growth rate and Southern blight disease severity.

Discussion

Uganda is a major hub for common bean production in sub-Saharan Africa and diseases affecting this crop are of concern. Southern blight caused by *S. rolfii* is an emergent disease of common bean in Uganda and is currently the most important soilborne disease of

common bean in the country (Paparui et al. 2018). Therefore, it is essential to determine its distribution and virulence in order to facilitate development of sustainable management practices. Our study is the first to report on the morphology and pathogenicity of Ugandan *S. rolfii* isolates on common bean.

We found variation in colony morphology, mycelial growth rates, number of sclerotia produced, and morphological groups among *S. rolfii* isolates from the seven Ugandan agroecological zones. For example, we found 3 isolates from the Eastern highland zone and each belonged to a different morphological group, and the 80 isolates obtained from the Western mixed farming agroecological zone

Fig. 3. Phenotypic characteristics of selected *Sclerotium rolfii* isolates from Ugandan common bean agroecological zones 28 days after inoculation on potato dextrose agar. **A**, Fluffy mycelia; **B**, thin fibrous mycelia; **C**, thin compact mycelia with small-sized sclerotia; and **D**, thin compact mycelia with large irregular-shaped sclerotia.

Fig. 2. Southern blight disease severity index scale (1 to 6): 1 = no disease symptoms, 2 = gray water-soaked lesions on the stem above the soil with no visible fungal outgrowth, 3 = gray water-soaked lesions on the stem above the soil with visible fungal outgrowths such as mycelia and sclerotia, 4 = partial wilting, 5 = complete wilting and necrosis, and 6 = preemergence damping-off. Scale bar = 5 mm.

belonged to eight distinct groups. In related studies, *S. rolfii* characterization using mycelial compatibility groups (MCGs) found great diversity within isolates from limited geographical areas. For example, Harlton et al. (1995) identified seven MCGs for 38 *S. rolfii* isolates from the United States, and it has been suggested that genetically related isolates often occupy the same MCG (Le et al. 2012; Xie et al. 2014).

In our study, we found that mycelial growth rate varied among the agroecological zones, with isolates from the cool Eastern highland zone showing slower growth than those from the other six zones. We speculate that these isolates have adapted to lower temperatures; therefore, culturing them at 25°C in our study may not have been optimal. This observation requires validation, because very few isolates were screened from this region. We found no difference in mycelial growth rates among isolates within an agroecological zone; this may be because, generally, variations in temperature and humidity within a single zone are minimal. The

white fluffy or compact type of *S. rolfii* mycelia observed in this study have been reported previously (Le et al. 2012, Xie et al. 2014). Isolates from peanut in Vietnam had silky-white mycelia (Le et al. 2012), while those from southern United States had thin, compact white mycelia, and those from other diverse crops had fluffy white mycelia (Xie et al. 2014), in contrast to isolates from sweetpotato (*Ipomoea batatas*) in Korea that were characterized by white to pale-brown mycelia (Narayan et al. 2017).

There tended to be no difference in the number of sclerotia produced among isolates, possibly due to high within-isolate variation. For example, isolate KAP415 formed 388, 338, and 0 sclerotia and isolate SIR160-1 produced 43, 50, and 1 sclerotia in replicates 1, 2, and 3, respectively. Although sclerotia formation was not uniform among our isolates, 4 of the 348 isolates consistently failed to produce any sclerotia. Differences in sclerotia number and size among isolates of *S. rolfii* have been reported (Le et al. 2012; Narayan et al. 2017; Xie et al. 2014). In a related study, Lehner et al. (2014)

Table 4. Differences in mean growth rate (millimeters per day) and number of sclerotia for *Sclerotium rolfii* isolates from seven agroecological zones in Uganda^z

Agroecological zone	Mean growth rate (± SE)	Mean number of sclerotia (± SE)	Number of isolates
Eastern highlands	20.7 ± 4.37 b	225.7 ± 113.9 a	3
Lake Victoria basin and Mbale farmlands	24.5 ± 0.18 a	48.6 ± 8.23 b	150
Northern mixed farming	22.8 ± 0.6 ab	79.7 ± 25.0 b	36
Southwestern highlands	22.4 ± 1.32 ab	165.3 ± 39.4 ab	25
Teso farming	25.2 ± 0.67 a	11.3 ± 7.36 b	11
Western mixed farming	23.3 ± 0.34 ab	121.0 ± 15.9 ab	80
West Nile mixed farming	24.1 ± 0.25 a	119.3 ± 7.38 ab	43

^z Different lowercase letters within a column indicate differences among agroecological zones at $P \leq 0.05$ (Tukey's Studentized range test). SE = standard error.

Table 5. Morphological grouping of *Sclerotium rolfii* isolates from seven agroecological zones in Uganda

Agroecological zone	Isolates per morphological group ^z								
	1	2	3	4	5	6	7	8	9
Eastern highlands	1	0	0	1	0	0	0	0	1
Lake Victoria basin and Mbale farmlands	4	13	63	8	7	51	0	0	2
Northern mixed farming	0	1	9	3	10	9	0	0	4
Southwestern highlands	0	8	8	0	1	0	6	1	1
Teso farming	1	5	2	0	0	5	0	0	0
Western mixed farming	6	8	14	10	11	23	5	0	3
West Nile mixed farming	4	10	4	4	15	6	0	0	0
Total	16	45	100	26	44	94	11	1	11
Proportion (%)	4.6	12.9	28.7	7.5	12.6	27.0	3.2	0.3	3.2

^z Morphological group 1 = high growth rate and high number of sclerotia, 2 = high growth rate and medium number of sclerotia, 3 = high growth rate and low number of sclerotia, 4 = medium growth rate and high number of sclerotia, 5 = medium growth rate and medium number of sclerotia, 6 = medium growth rate and low number of sclerotia, 7 = low growth rate and high number of sclerotia, 8 = low growth rate and medium number of sclerotia, and 9 = low growth rate and low number of sclerotia, where high growth rate = >25 mm per day, medium = 20 to 24 mm per day, and low = ≤19 mm per day; and high sclerotia number = >200 per Petri dish, medium = 50 to 99 per Petri dish, and low = ≤49 per Petri dish.

Table 6. Effects of common bean variety ($N = 5$) and *Sclerotium rolfii* isolate ($N = 75$) on indicators of Southern blight disease development

Factor	Preemergence damping-off		Germination		Disease severity index	
	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value
Variety	26.78	<0.0001	26.85	<0.0001	4.64	0.0010
Isolate	157.32	<0.0001	156.60	<0.0001	24.48	<0.0001
Variety × isolate	3.27	<0.0001	3.24	<0.0001	0.55	1.0000

Table 7. Differences in mean percentage (± standard error) of Southern blight disease parameters among five common bean varieties after sowing in artificially inoculated soils in the greenhouse^z

Variety	Preemergence damping-off	Germination	Disease severity index
ALB123	34.9 ± 0.85 ab	62.8 ± 0.89 bc	48.6 ± 2.47 b
K132	37.7 ± 0.86 a	59.8 ± 0.90 cd	52.4 ± 2.64 a
MLB49-89A	31.6 ± 0.83 c	66.4 ± 0.87 a	46.9 ± 2.54 b
NABE15	38.1 ± 0.86 a	59.2 ± 0.91 d	53.6 ± 2.58 a
RWR719	31.7 ± 0.83 bc	66.1 ± 0.87 ab	47.8 ± 2.52 b

^z Different lowercase letters within a column indicate differences among varieties at $P \leq 0.05$ (Tukey's Studentized range test).

observed variation in the number of sclerotia produced by isolates in two different experiments using a related fungal species (*S. sclerotiorum*). Although Lehner et al. (2014) and Li et al. (2008) found no correlation between mycelial growth rate and the number of sclerotia produced by isolates of *S. sclerotiorum*, we found that these factors were negatively correlated for isolates of *S. rolfisii*.

This study is the first to report the importance of preemergence damping-off caused by *S. rolfisii* in common bean. In total, 8 of the 75 isolates caused between 90 and 100% preemergence damping-off in seed planted in infested soils; other symptoms exhibited by seedlings included blackening and water-soaked lesions on the stem tissue above the soil, wilting, formation of sclerotia at the base of the plant, and necrosis. Similar observations were made by Blum and Rodriguez-Kabana (2004) and Kator et al. (2015). High levels of pre-emergence damping-off and wilting that lead to plant death reduce standing plant density at harvest, thus increasing yield losses (Fery and Dukes 2002; Franke et al. 1998).

Virulence of *S. rolfisii* isolates on the five test varieties varied. Preemergence damping-off and disease severity caused by all isolates (combined effect) was greater in the susceptible K132 variety and the commercially available NABE15. *S. rolfisii* isolates were equally virulent on varieties MLB49-89A (Fusarium root rot resistant) and RWR719 (Pythium root rot resistant), causing pre-emergence damping-off and Southern blight disease (Table 7). It is interesting to know that resistance to *S. rolfisii* has been reported in a range of crops, including cowpea, sweet potato, pepper, tomato (Dukes et al. 1983), and alfalfa (Pratt and Rowe 2002). Xie et al. (2014) reported variation in disease symptoms on pepper and tomato varieties inoculated with *S. rolfisii*. Kator et al. (2015) found differences in the virulence of a single isolate of *S. rolfisii* on three varieties of tomato in Nigeria, where damping-off at 3 weeks in variety Hoozua was 66.6% and in varieties Shase and UTC was 83.3%.

In our study, the virulence of 10 isolates varied among the five tested bean varieties, ranging from high (DSI $\geq$ 50%) to low (DSI $\leq$ 50%) (Fig. 4). Similarly, preemergence damping-off among the five varieties varied for 22 *S. rolfisii* isolates. Several studies on the

pathogenicity of *S. rolfisii* have similarly showed isolate differences in virulence. For example, Xie et al. (2014) observed differences in virulence among *S. rolfisii* isolates inoculated on pepper and tomato, and Mahato and Biswas (2017) found variation in the virulence of 10 *S. rolfisii* isolates on a single variety of tomato. In a related study, Jebaraj et al. (2017) showed differences in virulence of *S. rolfisii* isolates from peanut obtained from different regions of India.

We found no correlation between mycelial growth rate and disease severity, in contrast to results of Punja et al. (1985), who observed that *S. rolfisii* isolates with high growth rates also had high virulence (characterized by symptoms of water soaking, root colonization, and seedling infection). Mullen (2001) reported that the production of large quantities of oxalic acid by fast-growing isolates causes tissue degradation during the *S. rolfisii* infection process. Similarly, contrasting effects of the closely related *S. sclerotiorum* have been reported; for example, Vleugels et al. (2013) found a positive correlation between mycelial growth rate and isolate aggressiveness but Lehner et al. (2016) reported no such association. These contradictory findings indicate that mycelial growth rate may not be a reliable marker for aggressiveness of *S. rolfisii* isolates.

Unlike our results for mycelial growth rate, we noted weak positive correlations between sclerotia number and preemergence damping-off and between sclerotia number and Southern blight disease severity. Vleugels et al. (2013) suggested that a negative correlation between sclerotia number and isolate aggressiveness in *S. sclerotiorum* may be due to the production of sclerotia at the expense of enzyme production for host tissue invasion. However, we did not find published reports on the association between number of sclerotia produced and aggressiveness of *S. rolfisii* isolates. In our study, some isolates exhibited a high degree of aggressiveness and produced high numbers of sclerotia on PDA media. We hypothesize that this was due to the ability of these isolates to produce large numbers of sclerotia in the millet inoculum which, in turn, increased the chances of host colonization. Sclerotia are known to play an important role in disease cycles by germinating vegetatively for local colonization (Bolton et al. 2006) and acting as inoculum; they are the primary

Fig. 4. Southern blight disease severity caused by selected *Sclerotium rolfisii* isolates that varied in their effect on common bean test varieties ALB123, K132, MLB49-89A, NABE15, and RWR719. For each isolate, different letters indicate differences in disease severity index at $P \leq 0.05$ (Tukey's Studentized range test).

Fig. 5. Preemergence damping-off caused by selected *Sclerotium rolfii* isolates that varied in their effect on common bean test varieties ALB123, K132, MLB49-89A, NABE15, and RWR719. Different letters indicate differences at $P \leq 0.05$ (Tukey's studentized range test).

source of long-term survival structures in soils. Therefore, we consider that sclerotia density relates directly to the severity of Southern blight disease. However, further studies on the relationship between sclerotia number and Southern blight disease are required before sclerotia number can be considered as an indicator of isolate aggressiveness.

The Southern blight pathogen *S. rolfii* on common bean in Uganda is morphologically and pathogenically diverse. This indicates the organism's adaptability to diverse agroecological conditions, a factor that has probably resulted in recent increases in the disease in all Ugandan bean-growing regions (Paparu et al. 2018). The threat of Southern blight to common bean was confirmed in our study, because all varieties tested were highly susceptible to *S. rolfii*, including those known to be resistant to *Fusarium* root rot (MLB49-89A) and Pythium root rot (RWR719). Although our study provides information that was previously unavailable, additional work on *S. rolfii* is required to include molecular evaluations of the isolates. This will give a fuller characterization of this organism and is the subject of ongoing work in our laboratory. Because the challenge to common bean plant breeders worldwide is to identify varieties that are tolerant or resistant to pathogens, a genotypic study would help to ensure that any variety developed for resistance to Southern blight is resistant to all known pathogen genotypes.

There is currently no common bean variety that is resistant to Southern blight in Uganda. Therefore, we advise farmers to manage Southern blight disease using effective and affordable methods such as removal and destruction of infected plants that act as sources of

inoculum, treatment of seed with fungicides (e.g., Metalaxyl), inclusion of nonhost plants in crop rotations, and use of inorganic and organic soil fertility amendments.

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