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SEASONAL VARIATION AND TAXONOMIC CLARIFICATION

OF THE DOLLAR SPOT PATHOGEN: SCLEROTINIA HOMOEOCARPA

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SEASONAL VARIATION AND TAXONOMIC CLARIFICATION OF THE DOLLAR SPOT PATHOGEN: *SCLEROTINIA HOMOEOCARPA*

By

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ABSTRACT

SEASONAL VARIATION AND TAXONOMIC CLARIFICATION
OF THE DOLLAR SPOT PATHOGEN: *SCLEROTINIA HOMOEOCARPA*

By

Jon Frederick Powell

Dollar spot (*Sclerotinia homoeocarpa*) of amenity turf in the northern U.S. occurs in two seasonal epidemics; one from May to late-July and a second from mid-August through October. It was not known whether these seasonal epidemics were the result of multiple pathogens or due to seasonal variation within a single species. Isolates were collected from dollar spot lesions from golf courses in Michigan, Illinois, and Wisconsin. Vegetative compatibility reactions between isolates identified six vegetative compatibility groups (VCGs) among over 1300 isolates collected from eight locations. Most vegetative compatibility groups were present throughout the season whereas one was generally recovered only in the late epidemic. Nuclear ribosomal internal transcribed spacer 1 (ITS1) sequences of collected isolates show no variation among VCGs indicating that the identified VCGs represent variation within a species. The results of this study indicate that seasonal dollar spot epidemics are the result of a single pathogen.

The taxonomic status of the dollar spot pathogen, *Sclerotinia homoeocarpa*, has been in question since the 1940's. It has been well documented that this organism does not belong to the genus *Sclerotinia*, but should be placed within the genera *Rutstroemia*, *Lanzia*, or *Moellerodiscus*. ITS1 sequences from *Sclerotinia homoeocarpa* isolates from North America and Australia were compared with those of isolates from Britain, the

original cultures used to describe the species *S. homoeocarpa*, and representative members of the genera of *Rutstroemia*, *Lanzia*, and *Moellerodiscus*. Parsimony analysis identified that *S. homoeocarpa* clustered within the genus *Rutstroemia* indicating that its generic taxa should be *Rutstroemia* rather than *Sclerotinia*. The teleomorphic strain of *S. homoeocarpa* used to describe the species exhibited closer relations to *Rutstroemia cuniculi* and *R. henningsianum* than to isolates responsible for causing dollar spot disease. The species epithet *homoeocarpa* should be applied to the strain previously identified as the teleomorphic strain of *S. homoeocarpa* and not apply to the pathogen responsible for dollar spot symptoms. The remainder of the isolates responsible for dollar spot formed a distinct clade. However, the ITS1 and ITS2 sequences of isolates from North America expressed sequence divergences from isolates from Britain of 16 and 15 bases, respectively. Differences in mycelial morphology, stromatal morphology, and temperature tolerances between these groups also exist. Based on these data and the fact that the species epithet *homoeocarpa* can not be applied to these fungi, new names need to be applied. It is proposed that the dollar spot pathogens of British origin be identified by the epithet *Rutstroemia festucae* to denote the limited host range of this organism in the British Isles. The dollar spot pathogens from North America, including isolates from Australia and Netherlands, are proposed to be identified as *Rutstroemia floccosum* to denote the woolly/fluffy mycelial growth habit of this organism in culture and as part of the infection cycle.

To my wife,
Debbie

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CHAPTER I

VEGETATIVE COMPATIBILITY AND SEASONAL VARIATION AMONG *SCLEROTINIA HOMOEOCARPA* ISOLATES

INTRODUCTION

Dollar spot is one of the most prevalent diseases of turfgrasses throughout the world, occurring on a broad range of warm and cool season grasses (Smiley, 1992). It is also the most economically important disease of turf in the United States and Canada (Goodman and Burpee, 1991; Vargas, 1994). On golf course putting greens and fairways, dollar spots appear as bleached to tan spots up to 5 cm in diameter. Under favorable conditions the spots will coalesce to form larger irregular patches. When occurring on taller turfs (home lawns, athletic turf, etc...) spots may reach 15 cm in diameter. Dollar spot incidence is favored by high humidity, temperatures from 15 to 25° C, and cool nights resulting in dew formation. Following nights of heavy dew formation, white fuzzy "cobwebs" of mycelium may be seen on infected turf. Infected leaves initially appear chlorotic and water-soaked, becoming bleached or straw colored. Lesions are delineated from healthy tissue by the presence of a dark brown to black stroma which occurs as a band across the infected blade. The organism is believed to spread through the movement of mycelial and infected clippings as the causal fungus does not produce conidia or reproduce sexually. Management of dollar spot is commonly attained through the use of fungicides although this organism has developed resistant populations to fungicides including the benzimidazoles (Warren, 1974), dicarboxamide (Detweiler, 1983), and demethylase inhibitors (Golembiewski et al., 1995). Cultural practices employed to manage dollar spot include maintenance of high nitrogen fertility (Markland et al., 1969), removal of dew (Williams et al., 1996), and maintenance of proper irrigation (Couch and Bloom, 1960).

The pathogen responsible for dollar spot is currently identified as *Sclerotinia homoeocarpa* F.T. Bennett (Bennett, 1937). However, inclusion of this pathogen in the genus *Sclerotinia* has been refuted due to apothecial anatomy (Jackson, 1973), stromatal anatomy, stromatal histochemistry (Kohn and Grenville, 1989), and nuclear ribosomal internal transcribed spacer region 1 sequence data (Carbone and Kohn, 1993). The pathogen identified as *Sclerotinia homoeocarpa* is currently believed to belong to the genera of *Lanzia*, *Moellerodiscus*, or *Rutstroemia* (Carbone and Kohn, 1993). There is some belief that dollar spot may not be caused by a single pathogen but by multiple pathogens or by a complex of pathogens (Jackson, 1973; Kohn, 1979; Smith, 1989).

Dollar spot in cool season climates occurs during two seasonal epidemics in most years with one epidemic in the spring (May) to early summer (July) and a later epidemic in the late summer (mid-August) through fall (October) (Smith et al., 1989). Little is understood about the underlying population dynamics of the dollar spot pathogen during these two seasonal epidemics. Questions remain whether the seasonal epidemics are caused by different pathogens, different sub-populations of a single pathogen, or if the same sub-populations are responsible for both epidemics.

In one of the few studies of *S. homoeocarpa* populations, Sonoda (1988) identified 54 vegetative compatibility groups (VCGs) among 119 isolates collected from three locations in central Florida. Vegetative compatibility is the ability of hyphae of two strains of fungi to fuse and form a stable heterokaryon. In order for the strains to form a stable heterokaryon they must share identical alleles at a particular set of loci. Strains that differ at any of these loci will not be able to form a stable heterokaryon and will result in

an incompatible reaction typified by death of the heterokaryotic cells (Leslie 1993). Among asexual fungi, VCGs represent genetically isolated sub-populations and members of the same VCG are generally more similar than members of different VCGs (Jacobson and Gordon, 1991; Gordon and Okamoto, 1992).

The objectives of this study were to determine if seasonal epidemics of *S. homoeocarpa* are caused by multiple pathogens, different sub-populations of a single pathogen, or the same pathogen populations and to examine the broader diversity of VCGs in Michigan.

MATERIALS AND METHODS

Sampling. Investigation into variation of *S. homoeocarpa* populations between early summer and later summer epidemics of dollar spot was conducted by collecting isolates at regular intervals throughout the season at the Hancock Turfgrass Research Center (HTRC; Michigan State University, East Lansing, MI). Isolates were collected every three weeks in 1995 (June 16 through September 20) and 1996 (June 12 through September 21) and every two weeks in 1997 (June 26 through September 18). Daily mean temperature, temperature maximum and minimum, and precipitation data are provided in the appendix. Infected tissue was collected every 3 meters along a transect across the bentgrass (*Agrostis palustris* Huds.) and annual bluegrass (*Poa annua* L.) plots. Samples were recorded according to the order in which they were taken and the host upon which they were collected.

S. homoeocarpa isolates were collected once in the early summer and again in the fall of 1996 and 1997 from golf courses in northern Illinois and Lenawee County in

southern Michigan. Samples collected from the Illinois site were taken from a single fairway at 3 meter intervals whereas isolates taken from the Lenawee County site were collected from three tees located at distal corners on the course. Between 60 and 70 samples were taken at each collection from these courses. Additional *S. homoeocarpa* isolates were collected on single occasions from several sites to gain insight into the variation of vegetative compatibility groups across locations. Samples were taken in 1995 from Oscoda and Lenawee Counties; in 1996 from Ingham, Macomb, and Oakland Counties; and in 1997 from a single site in northern Wisconsin.

Isolate recovery. Dollar spots were sampled by collecting infected blades exhibiting advancing disease margins. Infected blades were plated onto acidified water agar (24 g agar/L with 10 ml lactic acid) and incubated for two days at 26 C. One putative *S. homoeocarpa* isolate from infected turf recovered from each dollar spot was transferred to potato dextrose agar (PDA; Difco, Detroit, MI). Each isolate was placed in long term storage by transferring ten 4 mm plugs into a 1.5 ml microfuge tube with 1 ml of mineral oil and stored at room temperature.

Vegetative Compatibility Testing. Vegetative compatibility testing was performed by transferring 4mm diameter plugs of *S. homoeocarpa* from PDA culture to plates of PDA containing red food color (McCormick Food Color; 10 drops/L PDA) (Kohn, 1990). Plates were incubated at 26 C for one week prior to analysis. Isolates were scored as incompatible if a barrage zone (Newhouse, 1991) was observed upon inspection of the plate from the top or bottom (figure 1). Isolates were also determined to be incompatible if abundant aerial mycelia formed along the border of neighboring colonies.

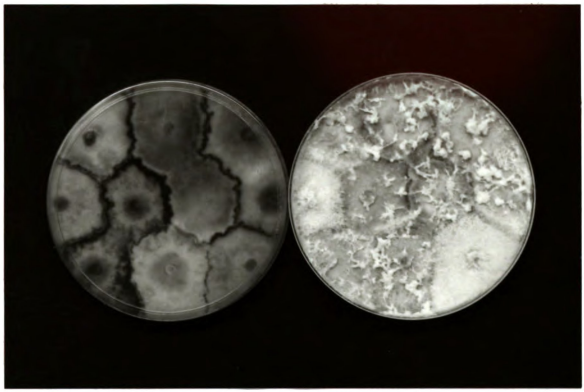

Figure 1. Vegetative interactions among *S. homoeocarpa* isolates on PDA amended with red food color after one week of incubation. The plate on the left shows the bottom of the petri plate and demonstrates the barrage zones typical of incompatible reactions between isolates. The plate on the right shows the sample isolates as viewed from the top of the plate.

Screening of large collections of isolates recovered from a single sampling date for compatibility was conducted by plating ten different isolates on an agar plate. Plugs were placed 3 cm apart from one another on 100 X 15 mm petri plates. Additional plates were prepared until each of the isolates was plated three times so they were neighboring different isolates on each plate. After one week of incubation, plates were scored for compatibility and compatible isolates were pooled into compatibility groups. These groups and isolates not identified as compatible to other isolates were then plated against isolates belonging to different compatibility pools. All the isolates were placed into vegetative compatibility groups or were found to be incompatible with all other isolates collected. Two isolates from each pooled group were then paired against tester isolates representative of common vegetative compatibility groups.

Microscopic examination of compatible and incompatible responses were conducted for select isolates to assure that plate reactions corresponded to hyphal interactions. Slides for microscopic examination were prepared by placing 100 μl of PDA on a sterile glass slide and covering the PDA with a sterile coverslip. *S. homoeocarpa* plugs were placed on either side of the coverslip and the slide was incubated in a sterile petri plate for 48 hours. Slides were stained with lactophenol blue and observed with a compound microscope.

Nuclear Internal Transcribed Spacer Region 1 Sequence Analysis. In order to determine if vegetative compatibility groups represented different species or diversity within a species, Nuclear Internal Transcribed Spacer Region 1 (ITS1) sequences from two isolates (isolated from different locations) from each compatibility group was

amplified and sequenced. *S. homoeocarpa* mycelium was cultivated in 50 ml malt extract broth (10g/L malt extract and 5 g/L glucose) for one week at room temperature. Alternatively, aerial mycelium was harvested directly from PDA plates incubated in an inverted position. DNA was extracted following a modified protocol of Lee and Taylor (1990). Modifications to the protocol were necessary due to the high levels of polysaccharides produced by *S. homoeocarpa*. Enough mycelia were placed into a 1.5 ml microfuge tube to fill up to the 0.25 ml mark along with 500 μl of lysis buffer (50 mM Tris-HCl pH 7.2, 50 mM EDTA, and 3% SDS) and homogenized with a Teflon tissue grinder. Tubes were incubated at 65 C for one hour. Seven hundred μl of chlorofrom:phenol (1:1; v/v) was added and the tubes were vortexed and centrifuged for 5 min. The aqueous phase was transferred to a new microfuge tube along with 700 μl chloroform:isoamyl alcohol (24:1; v/v). Following mixing and centrifugation the aqueous phase was transferred to a clean 1.5 ml tube containing 50 μl 3M sodium acetate. DNA was precipitated with isopropanol and refrigerated overnight. Final DNA recovery consisted of pelleting of DNA by centrifugation for 5 min, washing of the pellet with 1 ml of 95% ethanol, removal of the supernatant and drying under a vacuum. DNA pellets were then resuspended 100 μl TE buffer.

ITS1 sequences were amplified using the ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) primers of White et al. (1990). PCR reactions were carried out following the thermal protocol of Kohn et al. (1991); 1) 93 C, 1 min; 2) 40 C, 1 min; 3) 62 C, 10 sec; 4) increase 9 C at rate of 1 C every 5 sec; 5) 71 C, 1 min; 6) 93 C, 1 min; 7) cycle to step 2, 24 times; 8) 40 C, 1 min; 9) 62 C, 10 sec; 10)

increase 9 C at rate of 1 C every 5 sec; 11) 71 C, 5 min; 12) 4 C, hold. PCR products were purified with the Wizard PCR Purification prep kit (Promega, CA). Sequence reactions were performed using each of the primers used to amplify the ITS1 region. Sequencing of PCR products was performed at the MSU DNA sequencing facility (Michigan State University, East Lansing, MI). Resulting sequences were aligned using the SeqEdit program (Perkin Elmer, 1996) to assure sequence integrity.

RESULTS

All but a few of the infected turf samples collected from dollar spots yielded putative *S. homoeocarpa* isolates on acidified water agar. *S. homoeocarpa* isolates were easily recovered from acidified water agar as a weft of aerial mycelium following two days of incubation. Mycelial growth on PDA was generally rapid, covering the plate within 48 hours, and varied from dense cottony to supinate growth on the agar surface. A few isolates recovered from infected turf grew very slowly on PDA with sparse mycelia growth. These mycelia were brown in color and released a brown pigment into the media. Occasionally a sector formed within these restricted growth isolates which exhibited growth typical of *S. homoeocarpa*.

Vegetative compatibility reactions were easily scored following one week of incubation on food color amended media. Incompatible reactions were observed as barrage zone formation and dense aerial mycelia along colony borders (figure 1). Grouping of isolates from a single collection date into VCGs usually required three rounds of platings. Isolates exhibiting sparse growth were not scored for compatibility because they were rapidly overgrown by typical strains of *S. homoeocarpa* and

compatibility reactions were not clearly identifiable. Compatibility reactions on the food color amended PDA were supported by microscopic observations. Compatible hyphal anastomoses showed similar staining and cellular inclusions as typical cells. Incompatible anastomoses retained less stain and exhibited disorganization of cell inclusions.

Seasonal variation among S. homoeocarpa isolates

S. homoeocarpa vegetative compatibility data from season long samplings at the HTRC for 1995, 1996, and 1997 are listed on table 1. The first two sampling dates in 1995 were taken during the early summer dollar spot epidemic. Isolates collected during these dates belonged to VCGs A and B and were recovered at a ratio of roughly 2:1. At the August 30 sampling, in addition to VCGs A and B, isolates were recovered belonging to VCG C. The ratio of VCG A to B isolates remained similar; VCG C was isolated at a similar frequency as VCG A. Two VCGs, E and F, were not identified until the final sampling date of September 20.

Isolates of *S. homoeocarpa* recovered during the early summer epidemic of 1996 belonged to VCG A, B, C, and E (table 1). VCG A was the most predominate group recovered at the first sampling in June, but was roughly equal to groups B and C in July. The same VCGs were recovered in August and September with the addition of VCG F, and VCG A being the most predominate group present during this season. Vegetative compatibility groups A and B were recovered during all sampling dates of 1997 with group A being the most predominate group. Group F was recovered at a low frequency during samplings in June and July. Through August and September, VCGs C and E were

Table 1. Vegetative compatibility groups from dollar spots recovered from the Hancock Turfgrass Research Center (East Lansing, MI). Diseased tissues were collected every three weeks in 1995 and 1996 and every two weeks in 1997. Data is listed as the number of samples collected and the percent of the samples that was comprised of each VCG.

Collection Date	# of samples[a]	VCG A[b]	VCG B	VCG C	VCG D	VCG E	VCG F
1995							
6-16	47	83%	17%	-	-	-	-
7-21	50	64%	36%	-	-	-	-
8-30	47	43%	19%	38%	-	-	-
9-20	50	36%	32%	22%	-	2%	8%
1996							
6-12	55	68%	15%	13%	-	4%	-
7-19	54	37%	31%	26%	-	6%	-
8-21	54	52%	24%	65%	-	9%	9%
9-21	43	63%	16%	7%	-	9%	5%
1997							
6-26	55	84%	15%	-	-	-	1%
7-10	47	81%	17%	-	-	-	2%
8-7	54	78%	19%	-	-	3%	-
8-21	57	74%	26%	-	-	-	-
9-18	58	71%	21%	5%	-	3%	-

[a] Number of *S. homoeocarpa* isolates collected at the sampling date.

[b] Percentage of the isolates collected at a sampling date that belonged to each VCG.

recovered at low levels with VCGs A and B remaining the most predominate groups present.

Vegetative compatibility groups recovered from seasonal samplings at locations in northern Illinois and Lenawee County, Michigan are listed on table 2. One site in northern-Illinois was sampled during seasonal epidemics in 1996 and 1997. In early summer of 1996 (July 3) the *S. homoeocarpa* isolates recovered belonged to VCGs A, B, and D. The same VCGs were recovered in a second sampling on September 13. However, the early summer (July 9) of 1997 sampling recovered only isolates belonging to VCG's A and B. A marked change in the population recovered was noted into the late summer (September 13) as isolates belonging to VCG A were not recovered. VCG B was now the most common VCG with a minority of the isolates belonging to VCG C which had not be recovered from this site during previous samplings.

A site in Lenawee county MI was sampled during the seasonal epidemics in 1997. The first sampling (July 5) recovered isolates belonging to VCGs A, B, and E. These same VCGs were recovered in the late summer epidemic (August 24) with the addition of VCG F. A second sampling at the same location taken three days later revealed a ratio of VCGs which was not different from the sampling three days earlier when compared by Chi-Square analysis ($p \leq 0.05$), with a Chi-square distribution value of $p = 0.52$.

Several sites were sampled on single occasions to determine if additional VCGs could be identified (table 3). In 1995, VCGs A, B, E, and F were recovered from the Lenawee County site in August. The same VCGs which recovered in the August sampling in 1997 (table 2) at the same site. *S. homoeocarpa* isolates of VCGs C and D

Table 2. Vegetative compatibility groups from dollar spots recovered from seasonal samplings of sites in northern-Illinois and Lenawee county, Michigan. Diseased tissues were collected once during the early and late summer dollar spot epidemics. Data is listed as the number of samples collected and the percent of the samples that was comprised of each VCG.

Collection Date	# of samples[a]	VCG A[b]	VCG B	VCG C	VCG D	VCG E	VCG F
1996							
Illinois							
7-3	47	51%	45%	-	4%	-	-
9-13	57	60%	37%	-	3%	-	-
1997							
Illinois							
7-9	50	60%	40%	-	-	-	-
9-13	33	-	82%	18%	-	-	-
Lenawee							
7-5	57	51%	23%	-	-	26%	-
8-24	60	43%	33%	-	-	13%	11%
8-27	51	35%	31%	-	-	14%	20%

[a] Number of *S. homoeocarpa* isolates collected at the sampling date.

[b] Percentage of the isolates collected at a sampling date that belonged to each VCG.

Table 3. Vegetative compatibility groups from dollar spots recovered at single samplings of sites in Lenawee, Oscoda, Ingham, Macomb, and Oakland counties in Michigan and a site in northern-Wisconsin. Data is listed as the number of samples collected and the percent of the samples that was comprised of each VCG.

Collection Site / Date	# of samples[a]	VCG A[b]	VCG B	VCG C	VCG D	VCG E	VCG F
1995							
Lenawee /8-22	50	58%	14%	-	-	8%	6%
Oscoda /7-6	50	-	-	82%	18%	-	-
1996							
Ingham /8-24	60	41%	25%	-	-	27%	7%
Macomb /8-21	59	54%	-	37%	-	-	8%
Oakland /9-13	44	98%	-	2%	-	-	-
1997							
Wisconsin /8-27	43	100%	-	-	-	-	-

[a] Number of *S. homoeocarpa* isolates collected at the sampling date.
[b] Percentage of the isolates collected at a sampling date that belonged to each VCG.

were recovered in Oscoda County, MI. Three additional sites were sampled in 1996. A site in Ingham County MI yielded isolates representative of VCGs A, B, E, and F. Two sites in Oakland and Macomb counties in MI yielded isolates belonging to VCGs A and C. Of these only one isolate of the 44 isolates collected from the site in Oakland County belonged to VCG C. *S. homoeocarpa* isolates sampled in 1997 from northern Wisconsin all belonged to VCG A.

ITS1 analysis

Amplification of the ITS1 region was conducted on representative isolates of each of the VCGs isolated (A - F). Each of the isolates yielded a fragment of 212 base pairs. Comparison of the resulting sequences identified a single sequence shared by all of the isolates sequenced. This sequence is identical to the ITS1 sequence reported for *S. homoeocarpa* by Carbone et.al. (1993).

DISCUSSION

The results of this study indicate that seasonal dollar spot epidemics are the result of a single pathogen. Examination of isolates throughout the season identified six VCGs that were common to locations sampled in Michigan, Illinois, and Wisconsin. All VCGs identified were recovered during both of the seasonal dollar spot epidemics. Sequencing of the ITS1 region of isolates representative of each VCG yielded a conserved sequence of 212 base pairs, indicating that the VCGs represent diversity with a single species

Five VCGs were recovered from bi- and tri-weekly samplings of dollar spot from the HTRC at Michigan State University. VCGs A and B were recovered at all sampling dates throughout the three years of the study. VCGs C and E were first recovered in the

late summer of 1995 and throughout the year in 1996 and on two dates in 1997, although they may have been present throughout the year at levels beyond detection of the sampling scheme employed. Isolates belonging to VCG F were only recovered during the late summer epidemics of 1995 and 1996 suggesting that it may be specific to the fall epidemic. However, in 1997 VCG F was only recovered during the early summer epidemic.

Similar results were observed when isolates were collected once during the early and late summer epidemics at locations in northern-Illinois and Lenawee county in southern-Michigan. In 1996 VCGs A, B, and D were recovered in early and late summer at the northern-Illinois location. Only VCGs A and B were found in the spring of 1997. VCG A was not recovered in the fall although VCG C was present at a low level. The reason for the large shift in the VCGs recovered is uncertain but may be tied to the low disease pressure as of the fall collection date. The site in Lenawee county was characterized by VCGs A, B, and E in the early summer with the addition of VCG F in the late summer. VCG F was only recovered during the late summer epidemic as was found at the HTRC in previous years, however, in 1997 VCG F was found in the early summer at the HTRC of Michigan State University. The reason for this difference is uncertain, but is likely to be attributed to local environmental differences tied to the usually cool spring in 1997.

Collections of *S. homoeocarpa* isolates from six additional locations supported the notion that there is a limited diversity among VCGs of *S. homoeocarpa* in Michigan. All isolates collected from these additional locations were accommodated within the six

VCGs previously discussed. Two locations of interest include those in Oakland county, MI and northern-Wisconsin. The site sampled in Oakland county has been identified as having *S. homoeocarpa* populations resistant to the demethylase inhibitor fungicides. All but one of the isolates from this site belonged to a single VCG. While fungicide resistance levels were not determined for these isolates it is suggestive of a shift in the population toward that of a clonal line that expresses fungicide resistance. The other site of interest is the site from Wisconsin, of which all of the isolates belonged to VCG A. This golf course was established in 1995 and isolation of only a single VCG suggests that an isolate of this VCG was introduced to this site and is responsible for dollar spot at this location.

The limited number of VCGs recovered in this study is similar to work with the asexual pathogen *Fusarium oxysporum* f.sp. *melonis* (Jacobson and Gordon, 1990) and is suggestive of a clonal population structure. Vegetative compatibility group A was the most commonly recovered VCG, being recovered from 8 of the 9 locations sampled, and was the most frequently recovered VCG at each of these location. Whereas the production of apothecia by *S. homoeocarpa* has been reported to be produced by British isolates (Jackson, 1973, and Baldwin et al, 1993), production of fertile apothecia have not been identified in the U.S. (Bennett, 1937, Fenstermacher, and Jackson, 1973). Limitations on the number of VCGs identified will depend on the rate of migration, loss of VCGs among asexual populations due genetic drift and the lack of sexual recombination of the loci which are responsible for vegetative compatibility (Leslie, 1993).

The limited diversity among VCGs recovered in this study contrasts with the study of Sonoda, who found 16, 20, and 19 VCGs among collections of 35, 37, and 47 isolates respectively, representing three locations in Florida (Sonoda, 1988). The greater diversity among VCGs identified by Sonoda may at attributed to larger populations of *S. homoeocarpa* in the warmer climate, greater diversity of host grasses cultivated, or the possibility of sexual recombination among *S. homoeocarpa* isolates. A direct comparison of isolates collected in this study with the isolates collected by Sonoda from *Paspalum notatum* would yield additional insight into the relationship between these populations.

The identification of a limited number of VCGs raises the potential of using vegetative compatibility for further studies on *S. homoeocarpa*. The limited numbers of VCGs increases the likelihood that biological control strategies making use of hypovirulent strains of *S. homoeocarpa* (Zhou and Boland, 1997) would likely be successful. Population studies may make use of the introduction of VCGs to an area where it has not been previously identified would allow for tracking of the introduced isolate over time. This may include studies tracking the spread of *S. homoeocarpa* isolates at a single location over time, the overwintering capabilities of *S. homoeocarpa*, and whether dollar spots arise in the same location at different seasons from the same inoculum source. Further questions also remain about potential differences among VCGs with respect to virulence, temperature optima, and fungicide sensitivity.

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CHAPTER II

TAXONOMIC CLARIFICATION OF THE DOLLAR SPOT PATHOGEN: *SCLEROTINIA HOMOEOCARPA* BENNETT

INTRODUCTION

Dollar spot is one of the most common and economically important diseases of high maintenance turf grasses (Goodman and Burpee; 1991, Vargas, 1994). Originally described in 1932 by Monteith and Dahl, the name dollar spot was applied to denote the smaller size of brown patches in comparison to the previously described disease "brown patch" which produces larger patches. The causal organism was originally considered to be a *Rhizoctonia* sp. based on the production of cinnamon to reddish-brown aerial mycelia similar to *Rhizoctonia solani* and the lack of spore production in pure culture.

A detailed examination of the pathogen responsible for dollar spot was conducted by F.T. Bennett in 1937. In an examination of isolates from Britain, the United States, and Australia he identified three distinct "strains" of the pathogen; a "perfect" (teleomorph) strain, an "ascigerous" strain, and "non-sporing" (sterile) strains which he considered to be variants of the same species. The teleomorphic strain consisted of a single isolate that produced fertile apothecia and conidia which were borne on a sporophore similar in appearance to the apothecia produced. The "ascigerous" strain produced fertile apothecia, asci, and ascospores similar in appearance to those produced by the teleomorphic strain, although they were found to be larger in size. This strain did not produce conidia or a similar sporocarp; although microconidia were recovered which were not found in the teleomorphic strain. Several isolates were identified as sterile strains; these included two British isolates, as well as isolates from the United States and Australia. These strains were similar to the "ascigerous" strains, however, apothecia produced by these strains were aborted and did not yield asci or ascospores.

Bennett (1937) admitted difficulty in assigning the dollar spot pathogen to a genus but believed the organism to be best accommodated within the genus *Sclerotinia*. The genus *Sclerotinia* is generally limited to members of the Leotiales that produce rounded sclerotia. Bennett considered the plate-like stroma produced by the dollar spot pathogen to be aggregates of microsclerotia because apothecia "sometimes arise from aggregates of sclerotial cells apart from a more extensive stroma" and thus considered the pathogen to be classified as *Sclerotinia*. The species epithet, *"homoeocarpa"*, was based on the similarity between the apothecia (ascocarp) and the sporocarp upon which the conidia were borne in the teleomorphic strain. Bennett's description of the dollar spot pathogen, *Sclerotinia homoeocarpa*, was based on the teleomorphic strain as it produced both ascospores and conidiospores.

Production of fertile apothecia from isolates of *S. homoeocarpa* was not reproduced until 1973 when Jackson was able to generate fertile apothecia from *S. homoeocarpa* isolates from England. Apothecia produced were similar to those of Bennett's ascigerous strain with respect to the production of microconidia, ascus and ascospore size, and lack of production of conidia. Jackson (1973) further suggested that dollar spot symptoms in England may be attributed to more than one pathogen. Examination of apothecia believed to be from *S. homoeocarpa* suggests that the organism(s) belong to the genera *Lanzia* and/or *Moellerodiscus (*Kohn, 1979). Attempts to generate fertile apothecia from American isolates (Fenstermacher, 1970; B. Walsh, personal communication) have resulted in the formation of aborted or sterile apothecia.

Since its inception, inclusion of *S. homoeocarpa* within the genus Sclerotinia has

been in question. Whetzel (1945) did not include *S. homoeocarpa* within the genus *Sclerotinia* based on stromatal morphology. Members of the genus *Sclerotinia* produce determinate, tuberiod sclerotia whereas the stroma of *S. homoeocarpa* is plate-like and more typical of the indeterminate, effuse, substratal stroma. Based on these differences, Whetzel (1945) considered *S. homoeocarpa* to be a member of the genus *Rutstroemia*. Examination of the anatomy and histochemistry of stromatal tissues by Kohn and Grenville (1989) further supported the removal of *S. homoeocarpa* from the genus *Sclerotinia*. Examination of developmentally regulated proteins (Novak and Kohn, 1991) revealed that *S. homoeocarpa* shared characteristics common to indeterminate stromatal members of the Sclerotiniacea as opposed to the determinate stromatal (sclerotial) genera, including the genera *Sclerotinia*.

A phylogenetic analysis of the nuclear ribosomal internal transcribed spacer region 1 (ITS1) sequence from members of the Sclerotiniaceae by Carbone and Kohn (1993) found *S. homoeocarpa* clustered with four species of *Rutstroemia*. However, no isolates of *Lanzia* or *Moellerodiscus* were included in this analysis. A statement of the most appropriate genus of S. homoeocarpa could not be made without reference to each of the genera that S. homoeocarpa may belong. A broader examination of sequences including a portion of the nuclear ribosomal small subunit (18S rDNA), and nuclear ribosomal internal transcribed spacer regions 1 and 2 found *S. homoeocarpa* clustering with the substratal Sclerotiniaceae (Holst-Jensen, et al., 1997) . Of the substratal Sclerotiniaceae, *S. homoeocarpa* was found to have closer relations with members of the genus *Rutstroemia* than the one isolate of *Lanzia* included in the study.

The objectives of this study were to examine the taxonomic status of *S. homoeocarpa* isolates from the United States with respect to: i) members of the genera *Rutstroemia*, *Lanzia*, and *Moellerodiscus*; ii) culture collections of Bennett's teleomorphic, ascigerous, and sterile strains of *S. homoeocarpa*; and iii) British isolates of *S. homoeocarpa*.

MATERIALS AND METHODS

Fungal material and DNA extraction

Fungal cultures used for phylogenetic analysis and the sources from which they were obtained are listed in table 4. Cultures were stored for long term use by transferring 8 to 10 4mm plugs of fungal mycelium grown on potato dextrose agar (PDA; Difco, Detroit, MI) into 1.5 ml tubes with 1 ml of mineral oil. Mycelium for DNA extraction was cultivated in 50 ml malt extract broth (10 g/L malt extract and 5 g/L glucose) for one week at room temperature.

DNA extraction followed a modified protocol of Lee and Taylor (1990). Modifications to the protocol were necessary due to the high levels of polysaccharide produced by *S. homoeocarpa*. Enough mycelia were placed into a 1.5 ml microfuge tube to fill up to the 0.25 ml mark along with 500 μl of lysis buffer (50 mM Tris-HCl pH 7.2, 50 mM EDTA, and 3% SDS) and homogenized with a Teflon tissue grinder. Tubes were incubated at 65 C for one hour. Seven hundred μl of chloroform:phenol (1:1) were added and the tubes were vortexed and centrifuged for 5 min. The aqueous phase was transferred to a new microfuge tube along with 700 μl chloroform:isoamyl alcohol (24:1). Following mixing and centrifugation the aqueous phase was transferred to a clean 1.5 ml

Table 4. Fungal cultures used for phylogenetic analysis and morphological comparisons.

Culture	Collection Number	Country	Source
Moellerodiscus lentus (Berk.& Broome) Dumont	DAOM 128588	USA	CCFC[a]
Rutstroemia americana **(Durand) White**	DAOM 152694A	England	CCFC
Rutstroemia conformata **(Karst.) Nannfeldt**	CBS 518.75	---	CBS[b]
Rutstroemia cuniculi **(Boud.) Elliott**	DAOM 109690	England	CCFC
Rutstroemia firma (Pers.:Fr.) Karst.[c]	CBS 341.62	---	CBS
Rutstroemia paludosa **(Cash & Davidson) Groves & Elliott**	DAOM 141378	Canada	CCFC
Rutstroemia petiolorum **(Roberge) White**	DAOM 106852	Canada	CCFC
Sclerotinia homoeocarpa **Bennett** (Teleomorphic)	CBS 309.37	England	CBS
Sclerotinia homoeocarpa **Bennett** (Ascigerous)	CBS 310.37	England	CBS
Sclerotinia homoeocarpa **Bennett** (Sterile)	CBS 311.37	England	CBS
Sclerotinia homoeocarpa **Bennett**	CBS 510.89	Netherlands	CBS
Sclerotinia homoeocarpa **Bennett**	IMI 167641	England	STRI[d]
Sclerotinia homoeocarpa **Bennett**	WA 1547	Australia	WA[e]
Sclerotinia homoeocarpa **Bennett**	WA 1548	Australia	WA
Sclerotinia homoeocarpa **Bennett**	WA 1553	Australia	WA
Sclerotinia homoeocarpa **Bennett**	48 BW	Canada	B. Walsh[f]
Sclerotinia homoeocarpa **Bennett**	103 BW	Canada	B. Walsh
Sclerotinia homoeocarpa **Bennett (VCG A)**	JP 44G	USA	J.F. Powell
Sclerotinia homoeocarpa **Bennett (VCG B)**	JP 44I	USA	J.F. Powell
Sclerotinia homoeocarpa **Bennett (VCG C)**	JP 44J	USA	J.F. Powell
Sclerotinia homoeocarpa **Bennett (VCG D)**	JP 44L	USA	J.F. Powell
Sclerotinia homoeocarpa **Bennett (VCG E)**	JP 44M	USA	J.F. Powell
Sclerotinia homoeocarpa **Bennett (VCG F)**	JP 44P	USA	J.F. Powell
Sclerotinia homoeocarpa **Bennett**	S1-S7	Scotland	J.M Vargas

[a] Canadian Collection of Fungus Cultures, Ottowa, Canada
[b] Centraalbureau voor Schimmelcultures, Baarn, Netherlands
[c] Lectotype culture of the genus *Rutstroemia*
[d] Sports Turf Research Institute, Bingley, England
[e] Western Australia Department of Agriculture, South Perth, Australia
[f] University of Guelph, Guelph, Canada

tube containing 50 μl 3M sodium acetate. DNA was precipitated with isopropanol and refrigerated overnight. Final DNA recovery consisted of pelleting of DNA by centrifugation for 5 min, washing of the pellet with 1 ml of 95% ethanol, removal of the supernatant and drying under a vacuum. DNA pellets were then resuspended in 100 μl TE buffer.

PCR amplification and sequencing of ITS1, ITS2, and 18S regions of rDNA

Nuclear ribosomal internal transcribed spacer region 1 (ITS1) sequences from isolates collected were amplified using the ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) primers of White et al. (1990). PCR reactions for ITS1 amplification were carried out following the protocol of Kohn et al. (1991); 1) 93 C, 1 min; 2) 40 C, 1 min; 3) 62 C, 10 sec; 4) increase 9 C at rate of 1 C every 5 sec; 5) 71 C, 1 min; 6) 93 C, 1 min; 7) cycle to step 2, 24 times; 8) 40 C, 1 min; 9) 62 C, 10 sec; 10) increase 9 C at rate of 1 C every 5 sec; 11) 71 C, 5 min; 12) 4 C, hold. PCR products were run on 1.5% agarose gels and observed by staining with ethidium bromide. A 100 bp ladder was included with each electrophoresis for size comparison.

Additional sequencing data were collected to gain further insight into the relationships among *S. homoeocarpa* isolates including strains from the U.S., England, and Scotland; Bennett's teleomorphic, ascigerous, and sterile strains; and *R. cuniculi*. Nuclear ribosomal internal transcribed spacer region 2 (ITS2) sequences from these isolates was amplified with the ITS1 and ITS4 (TCCTCCGCTTATTGATATGC) primers of White et. al. (1990). The 3' end of the nuclear ribosomal small subunit (18S rDNA) gene was amplified in steps with primer combinations of NS3 (GCAAGTCTGGTGCCA-

GCAGCC) and NS6 (GCATCACAGACCTGTTATTGCCTC), and NS7 (GAGGCAAT-AACAGGTCTGTGATGC) and NS8 (TCCGCAGGTTCACCTACGGA) (White et al, 1990). An additional primer was required for sequencing of the intron region of *S. homoeocarpa* isolates from the U.S., Canada, Australia, and Netherlands. Primer JMV18 (GGAGCCTGCGCTTAATTTCAG) is 3' of an intron in the 18S rDNA. The thermal program used for amplifications started with a 3 min denaturation at 94 C, followed by 35 cycles of 1 min at 94 C, 1 min at 50 C and 3 min at 72 C, and completed with 10 min at 72 C and stored at 5 C. PCR products were examined for quality by electrophoresis on a 1.5% agarose gel followed by staining with ethidium bromide for observation with UV light.

PCR products to be submitted for sequencing were purified with the Wizard PCR Purification prep kit (Promega, CA). Sequence reactions were performed to amplify complementary strands with each of the primers used to amplify the DNA fragment. Sequencing of PCR products was performed at the MSU DNA sequencing facility (Michigan State University, East Lansing, MI). Resulting chromatograms were aligned using the SeqEdit program (Perkin Elmer, 1996) to assure sequence integrity.

Phylogenetic analyses

ITS1 sequences were aligned with ITS1 sequences of *Rutstroemia henningsianum* (Carbone and Kohn, 1993), *Rutstroemia bolaris*, and *Lanzia luteovirescens* (Schumacher et. al. 1997). The sequence from *Sclerotinia sclerotiorum* (Wilmotte et al, 1993) served as the outgroup for phylogenetic analysis for the ITS1 and rDNA 18S small subunit data. *Rutstroemia firma* served as the outgroup of ITS2 data analysis. Sequence analysis of the

ITS1 and ITS2 combined sequences was also performed. Sequences were aligned by the Jotun-Hein method using the DNAstar software and analyzed by maximum parsimony using PAUP (version 3.0). Bootstrap analysis (Felsenstein, 1985) was conducted using 1,000 replications with the Branch-and-Bound algorithm following furthest addition.

Morphological comparisons

Comparison of cultural characteristics between *S. homoeocarpa* isolates from North America and Canada with *S. homoeocarpa* isolates from England and Scotland were conducted by culturing the isolates on PDA in the dark. Comparison of colony morphology of the fungal cultures were recorded after one week of growth. Stromatal tissue comparisons were made after the cultures had been incubated for one month. All comparisons were based on visual examination of tissues.

RESULTS

ITS1 Sequence Analysis

PCR amplification of the ITS1 region yielded a single product ca 200 bp long. Sequence data from sequencing reactions with each of the primers were corroborated to yield a single sequence. Resulting sequence data revealed the exact sequence lengths which ranged from 168 to 203 bp. ITS1 fragments derived from *S. homoeocarpa* isolates from the United States, Canada, the Netherlands, and Australia shared an identical 203 bp sequence, these isolates will be referred to in general as U.S. isolates of *S. homoeocarpa.*

Alignment of ITS1 sequences by the Jotun-Hein method is provided in table 5. Sequences were well conserved over the first 70 bp. The majority of the differences in ITS1 lengths between isolates occurred over the next 30 bp region which was

Table 5. Alignment of ITS1 sequences by Jotun-Hein method.

```
S.h.* Teleomorph   CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATTTATACCATGTT [60]
S.h. Ascigerous    CATTACAGAGTTCACGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACTATGTT [60]
S.h. Sterile       CATTACAGAGTTCACGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACCATGTT [60]
S.h. U.S.          CATTACCGAGTTCACGCCCTCACGGGTAGACCTCCAACCCTTGTGTATCTCTACCATGTT [60]
S.h. England       CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACTATGTT [60]
S.h. Scotland      CATTACAGAGTTCACGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACCATGTT [60]
M. lentus          CAGTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTT [60]
R. americana       CATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTATGTT [60]
R. bolaris         CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATTTATACTTT.TT [60]
R. conformata      CATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTT [60]
R. cuniculi        CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATTTATACCATGTT [60]
R. firma           CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACATTGTT [60]
R. henningsianum   CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATTTATACCGTGTT [60]
R. paludosa        CATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTT [60]
R. petiolorum      CATTACAGAGTTCATGCCCTAACGGGTAGACCTCCCACCCTTGTGTATTTATACTTTGTT [60]
S. sclerotiorum    CATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTT [60]
L. luteovirescens  CATTACAGAGTTCATGCCT-AACGGGTAGACCTCCCACCCTTGTGTAATTATACTTTGTT [59]

S.h. Teleomorph   GCTTTGGCAGGCTGC--TGCCCCCCTCGGGGG-ACAGCCCCAGCGCCTTC--GGGCCTGG [115]
S.h. Ascigerous   GCTTTGGCAGGCTGC--TGGACCCCTCGGGGG-ACAGCCTCGGCGCCCTC--GGGCCTGA [115]
S.h. Sterile      GCTTTGGCAGGCTGC--TGGCCCCCTCGGGGG-ACAGCCTCGGCGCCCTC--GGGCCTGA [115]
S.h. U.S.         GCTTTGGCAGGCTGC--TCGACCCTTCCGGGG-ACAGCCTCAGCGCCCTCCGGGGCCGGA [117]
S.h. England      GCTTTGGCAGGCTGC--TGGACCCCTCGGGGG-ACAGCCTCGGCGCCCTC--GGGCCTGA [115]
S.h. Scotland     GCTTTGGCAGGCTGC--TGGCCCCCTCGGGGG-ACAGCCTCGGCGCCCTC--GGGCCTGG [115]
M. lentus         GCTTTGGCGAG-------------------------------CT-GCCCTT----GGGCCT [85]
R. americana      GCTTTGGCGAG-------------------------------CT-GCCTTC----GGGCCT [85]
R. bolaris        GCTTTGGCGAGCTGCCCTGGGCTTAACTGCCTCA.AGCCTCAA-GCTTTC--GAGCCTGA [117]
R. conformata     GCTTTGGTGAA-------------------------------GA-GCCCCA--GATCTTCT [87]
R. cuniculi       GCTTTGGCAGGCTGC--TGCCCCCCTCGGGGG-ACAGCCCCAGCGCCTTC--GGGCCTGG [115]
R. firma          GCTTTGGCGAGCTGCCTTGGCCTTAACTGCCCCAAGGCCTCAA-GCTTTC--GAGCCTGA [117]
R. henningsianum  GCTTTGGCAGGCTGC--TGCA-CCCTCGGGGG-ACAGCCCCAGCGCCTTC--GGGCCTGG [114]
R. paludosa       GCTTTGGCGAG-------------------------------CT-GCCTTC----GGGCCT [85]
R. petiolorum     GCTTTGGCGAGCTGCCCTGGGCTTAATTGCCC-AGAGCCTCAA-GCTTTC--GAGCCTGA [116]
S. sclerotioum    GCTTTGGCGAG-------------------------------CT-GCTCTT--CGGGGCCT [87]
L. luteovirescens GCTTTGGCGAATTGC--GTGACCTCTCGGGGT-CTCGCCTCGA-GCTTCA--CAGCCTGA [113]

S.h. Teleomorph   GAGTCGCCTGCCGGAGGAAAAACA-AA-CTCTGAATTGTTAGTGTCGTCTGAGTACTATA [173]
S.h. Ascigerous   GAGTCGCCTGCCGGAGGAAAAACA-AA-CTCTGAATTGTTAGTGTCGTCTGAGTACTATA [173]
S.h. Sterile      GAGTCGCCTGCCGGAGGAAAAACA-AA-CTCTGAATTGTTAGTGTCGTCTGAGTACTATA [173]
S.h. U.S.         GAGTCGCCTGCCGGAGGAAAATCACAA-CTCTGAATTGTCAGTGTCGTCTGAGTG--ACT [174]
S.h. England      GAGTCGCCTGCCGGAGGAAAAACA-AA-CTCTGAATTGTTAGTGTCGTCTGAGTACTATA [173]
S.h. Scotland     GAGTCGCCTGCCGGAGGAAAAACA-AA-CTCTGAATTGTTAGTGTCGTCTGAGTACTATA [173]
M. lentus         CGTATGCTCGCCAGAGGTTACCAA-AA-CTCTTTTT-ATTAATGTCGTCTGAGTACTATA [142]
R. americana      AAGCGTCTCGCCAGAGGATATCAA-AA-CTCTTTTT-ATTAATGTCGTCTGAGTACTATA [142]
R. bolaris        GAGTCGCTCGCCGGAGGAAAACTA-AA-CCCTGATA-ATTAATGTCGTCTGAGTACTATA [174]
R. conformata     GGGGCGCCCACCAAAGACTATCAA-AA-CTCTTTTT-ATTAATGTCGTCTGAGTACTATA [144]
R. cuniculi       GAGTCGCCTGCCGGAGGAAAAACA-AA-CTCTGAATTGTTAGTGTCGTCTGAGTACTATA [173]
R. firma          GAGTCGCCCGCCGGAGGAAAAATA-AA-CCCTGATA-ATTAATGTCGTCTGAGTACTATA [174]
R. henningsianum  GAGTCGCCTGCCGGAGGAAAAACA-AA-CTCTGAATTGTTAGTGTCGTCTGAGTACTATA [172]
R. paludosa       TGTATGCTCGCCAGAGAATAATCA-AA-CTCTTTTT-ATTAATGTCGTCTGAGTACTATA [142]
R. petiolorum     GAGTCGTTCGCCGAAGGAAAAATA-AA-CCCTGATA-ATTAGTGTCGTCTGAGTACTATA [173]
S. sclerotiorum   TGTATGCTCGCCAGAGAATATCAA-AA-CTCTTTTT-ATTAATGTCGTCTGAGTACTATA [144]
L. luteovirescens GAGTCGTTCGCCAGAGGATACCAA-AA-CTCTGAAT-ATTAATGTCGTCTGAGTACTATA [170]
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*S.h. = *Sclerotinia homoeocarpa*

Table 5 (cont.). Alignment of ITS1 sequences by Jotun-Hein method.

```
S.h. Teleomorph    TTTTAATAGTTAAAACTTTCAACAACGGA [202]
S.h. Ascigerous    TTTTAATAGTTAAAACTTTCAACAACGGA [202]
S.h. Sterile       TTTTAATAGTTAAAACTTTCAACAACGGA [202]
S.h. U.S.          ATCTAATAGTTAAAACTTTCAACAACGGA [203]
S.h. England       TTTTAATAGTTAAAACTTTCAACAACGGA [202]
S.h. Scotland      TTTTAATAGTTAAAACTTTCAACAACGGA [202]
M. lentuseq        T---AATAGTTAAAACTTTCAACAACGGA [168]
R. americana       T---AATAGTTAAAACTTTCAACAACGGA [168]
R. bolarisSEQ'     T---AATAGTTA.AACTTTCAACAACGGA [200]
R. conformata      T---AATAGTTAAAACTTTCAACAACGGA [170]
R. cuniculi        TTTTAATAGTTAAAACTTTCAACAACGGA [202]
R. firma           T---AATAGTTAAAACTTTCAACAACGGA [200]
R. henningsianum   TTTTAATAGTTAAAACTTTCAACAACGGA [201]
R. paludosa        T---AATAGTTAAAACTTTCAACAACGGA [168]
R. petiolorum      T---AATAGTTAAAACTTTCAACAACGGA [199]
S. sclerotiorum    T---AATAGTTAAAACTTTCAACAACGGA [170]
L. luteovirescens  T---AATAGTTAAAACTTTCAACAACGGA [196]
```

*S.h. = *Sclerotinia homoeocarpa*

characterized by the presence of a 30 bp insertion/deletion. Of 209 sites in the alignment, 58 were phylogenetically informative. Sequence similarity data is provided in the appendix. Parsimony analysis yielded 2 most parsimonious trees with tree lengths of 140 steps. The maximum tree length was determined to be 330 steps. The consensus most parsimonious tree with bootstrap values and number of character state changes per branch is provided in figure 2. Consistency index, rescaled consistency index, and retention index values for this tree are 0.761, 0.846, and 0.644, respectively.

ITS2 Sequence Analysis

Amplification of the 5.8S rDNA and ITS2 region yielded fragments of ca 320 bp long. Alignment of the 5.8S and ITS2 sequences is shown in table 6. The first 161 bases correspond to the 5.8S rDNA in which only one base change was found from Bennett's ascigerous strain of *S. homoeocarpa*. The majority of variation occurred in the next 150 bases corresponding to the ITS2 region. Of the characters present, 8 were phylogenetically informative. Sequence similarity data is provided in the appendix. Parsimony analysis revealed 2 most parsimonious trees at a length of 42 steps and the maximum tree length was determined to be 53. The strict consensus of the two most parsimonious tree with bootstrap values and number of character changes per branch is provided in figure 3. The consistency index, rescaled consistency index, and retention index of the most parsimonious tree are 0.98, 0.92, and 0.90 respectively.

ITS1 and ITS2 Sequence Analysis

Alignment of the ITS1, 5.8S, and ITS2 sequences (table 7) from dollar spot isolates, *R. cuniculi*, and *R. firma* yielded sequence data of 495 bp of which 14 were

Figure 2. Most parsimonious tree based on ITS1 sequence data. Bold and underlined numbers represent bootstrap values based on 1000 replications using a Branch-and-Bound search. Boxed numbers correspond to the number of character state changes per branch. (S.h. = *Sclerotina homoeocarpa*)

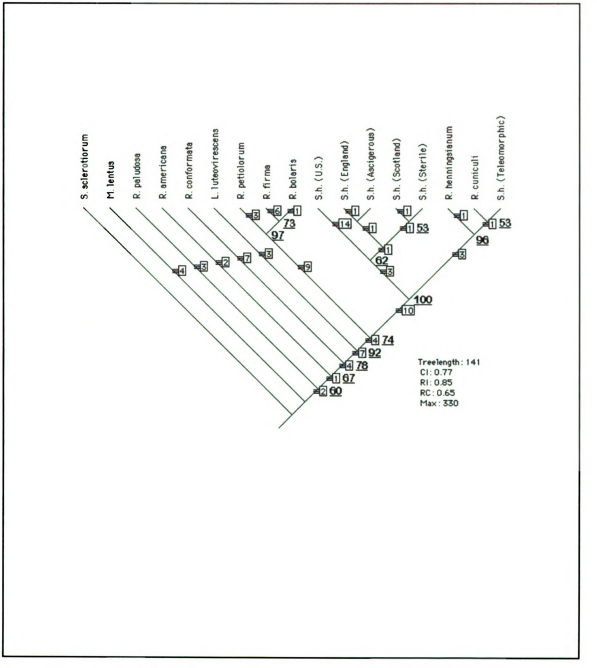

Table 6. 5.8S and ITS2 sequences from Bennett's type cultures, *Rutstroemia cuniculi*, *Rutstroemia firma*, and *Sclerotinia homoeocarpa* isolates from U.S. and Scotland aligned by the Jotun-Hein Method.

```
                          10         20         30         40         50         60
S.h.* (U.S.)          GTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT [59]
R. cuniculi           GTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT [59]
S.h. (Teleomorph)     GTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT [59]
S.h. (Ascigerous)     GTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT [59]
S.h. (Sterile)        GTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT [59]
s.h. (Scotland)       GTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT [59]
R. firma              GTTAAAACTTTCAACAACGGATTCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT [60]

                           70         80         90         100        110        120
S.h. (U.S.)           GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC [119]
R. cuniculi           GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC [119]
S.h. (Teleomorph)     GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC [119]
S.h. (Ascigerous)     GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC [119]
S.h. (Sterile)        GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC [119]
S.h. (Scotland)       GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC [119]
R. firma              GTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATTTTTGAACGCACATTGC [120]

                          130        140        150        160        170        180]
S.h. (U.S.)           GCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCT-CTC [178]
R. cuniculi           GCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCT-C-- [176]
S.h. (Teleomorph)     GCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCT-C-- [176]
S.h. (Ascigerous)     GCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCT-C-- [176]
S.h. (Sterile)        GCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCT-C-- [176]
S.h. (Scotland)       GCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCT-C-- [176]
R. firma              GCCCCTTGGTATTCCGGGGGGCATGCGTGTTCGAGCGTCATTTCAACCCTCAAGTTAA-- [178]

                          190        200        210        220        230        240]
S.h. (U.S.)           TGCTTGGTATTGGGCCTCCGCCGGTCACACGGCGGGCCTTAAAGTCAGTGGCGGCGCCGC [238]
R. cuniculi           TGCTTGGTATTGGGCCTTCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGCGCCGT [236]
S.h. (Teleomorph)     AGCTTGGTATTGGGCCTCCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGCGCCGT [236]
S.h. (Ascigerous)     AGCTTGGTATTGGGCCTCCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGCGCCGT [236]
S.h. (Sterile)        TGCTTGGTATTGGGCCTTCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGCGCCGT [236]
S.h. (Scotland)       TGCTTGGTATTGGGCATTCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGCGCCGT [236]
R.firma               TGGTTGGTATTGGGCATTCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGAGCCGT [238]

[                         250        260        270        280        290        300]
S.h. (U.S.)           TGGGTCCTGAACGTAGTAACACATACCTCTCGTTAC---AGGGTCCCCGCGCGCTCCCGC [295]
R. cuniculi           TGGGTCCTGAACGTAGTAACATACCTCTC--GTTAC---AGGT-CCCCGCGTGCTTCTGC [290]
S.h. (Teleomorph)     TGGGTCCTGAACGTAGTAACATACACCTC--GTTAC---AGGGTCCCCGCGAGCTTCTGC [291]
S.h. (Ascigerous)     TGGGTCCTGAACGTAGTAACATACACCTC--GTTAC---AGGGCCCCCGCGTGCTTCTGC [291]
S.h. (Sterile)        TGGGTCCTGAACGTAGTAACATACCTCTC--GTTAC---AGGGTCCCCGCGTGCTTCTGC [291]
S.h. (Scotland)       TGGGTCCTGAACGTAGTAACATACCTCTC--GTTACCAGGGTG-CCCCGCGTGCTTCTGC [293]
R. firma              TGGGTCCTGAACGTAGTAACATACCTCTC--GTTAC---AGGGTCCCCGCGTGCTTCTGC [293]

                          310        320        330]
S.h. (U.S.)           CGTAAAACCCCCCTCA-TTTTCTCTGGTTGA [325]
R. cuniculi           CATTAAACCCCAA--A-CTTTTTATGGTTGA [318]
S.h. (Teleomorph)     CATTAAACCCCCC--A-CTTTCTATGGTTGA [319]
S.h. (Ascigerous)     CATTAAACCCCCA--A-CTTTCTATGGTTGA [319]
S.h. (Sterile)        CATTAAACCCCAA--A-CTTTTTATGGTTGA [319]
S.h. (Scotland)       CATTAAACCCCAA--AGGTCCTTATGGTTGA [322]
R. firma              CATTAAACCCCAG--A-CTTTTTATGGTTGT [321]
```

*S.h. = *Sclerotinia homoeocarpa*

Figure 3. Most parsimonious tree based on 5.8S and ITS2 sequence data. Bold and underlined numbers represent bootstrap values based on 1000 replications using a Branch and Bound search. Boxed numbers correspond to the number of character state changes per branch. (S.h. = *Sclerotina homoeocarpa*)

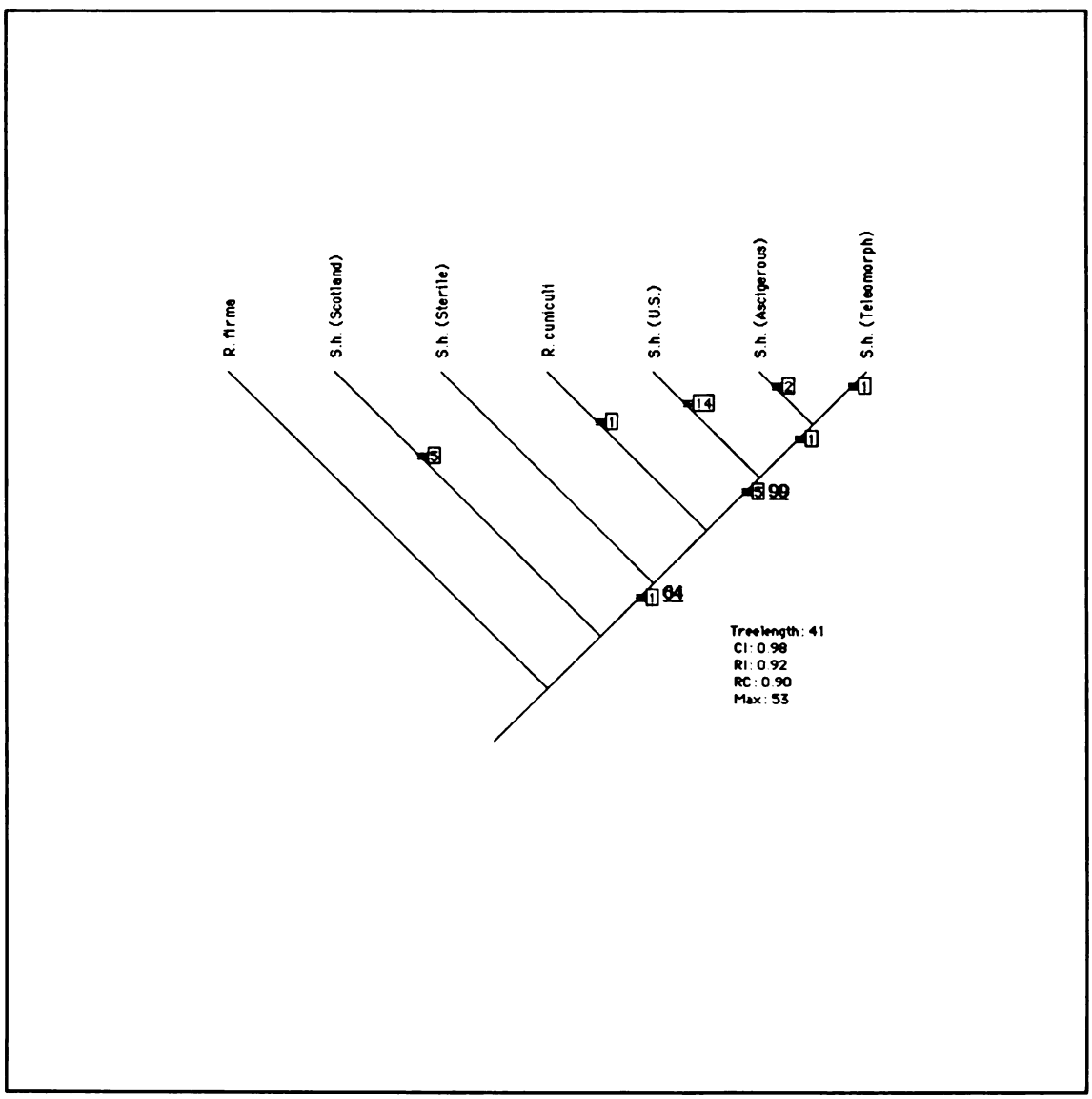

Table 7. ITS1, 5.8S, and ITS2 sequences from Bennett's type cultures, *Rutstroemia cuniculi*, *Rutstroemia firma*, and *Sclerotinia homoeocarpa* isolates from U.S. and Scotland aligned by the Jotun-Hein Method.

```
                                10        20        30        40        50        60
S.h.* (Teleomorph)    CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATTTATACCATGTT [60]
S.h. (Scotland)       CATTACAGAGTTCACGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACCATGTT [60]
S.h. (Ascigerous)     CATTACAGAGTTCACGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACTATGTT [60]
S.h. (U.S.)           CATTACCGAGTTCACGCCCTCACGGGTAGACCTCCAACCCTTGTGTATCTCTACCATGTT [60]
S.h. (Sterile)        CATTACAGAGTTCACGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACCATGTT [60]
R. cuniculi           CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATTTATACCATGTT [60]
R. firma              CATTACAGAGTTCATGCCCTCACGGGTAGACCTCCCACCCTTGTGTATCTATACATTGTT [60]

                               70        80        90        100       110       120
S.h. (Teleomorph)     GCTTTGGCAGGCTGCTGCCCCCCTCGGGGGACAGCCCCAGCGCC--TTCGGGCCTGGGAG [118]
S.h. (Ascigerous)     GCTTTGGCAGGCTGCTGGACCCCTCGGGGGACAGCCTCGGCGCC--CTCGGGCCTGAGAG [118]
S.h. (Sterile)        GCTTTGGCAGGCTGCTGGCCCCCTCGGGGGACAGCCTCGGCGCC--CTCGGGCCTGAGAG [118]
S.h. (U.S.)           GCTTTGGCAGGCTGCTCGACCCTTCCGGGGACAGCCTCAGCGCCCTCCGGGGCCGGAGAG [120]
S.h. (Scotland)       GCTTTGGCAGGCTGCTGGCCCCCTCGGGGGACAGCCTCGGCGCC--CTCGGGCCTGGGAG [118]
R. cuniculi           GCTTTGGCAGGCTGCTGCCCCCCTCGGGGGACAGCCCCAGCGCC--TTCGGGCCTGGGAG [118]
R. firma              GCTTTGGCGAGCTGCCTTGGCCTTAACTGCCCCAAGGCCTCAAGCTTTCGAGCCTGAGAG [120]

                               130       140       150       160       170       180
S.h. (Teleomorph)     TCGCCTGCCGGAGGAAAA--ACAAACTCTGAATTGTTAGTGTCGTCTGAGT-ACTATATT [175]
S.h. (Ascigerous)     TCGCCTGCCGGAGGAAAA--ACAAACTCTGAATTGTTAGTGTCGTCTGAGT-ACTATATT [175]
S.h. (Sterile)        TCGCCTGCCGGAGGAAAA--ACAAACTCTGAATTGTTAGTGTCGTCTGAGT-ACTATATT [175]
S.h. (U.S.)           TCGCCTGCCGGAGGAAAATCACAACT-CTGAATTGTCAGTGTCGTCTGAGTGACTATCT- [178]
S.h. (Scotland)       TCGCCTGCCGGAGGAAAA--ACAAACTCTGAATTGTTAGTGTCGTCTGAGT-ACTATATT [175]
R. cuniculi           TCGCCTGC.GGAGGAAAA--ACAAACTCTGAATTGTTAGTGTCGTCTGAGT-ACTATATT [175]
R. firma              TCGCCCGCCGGAGGAAAA--ATAAAC-CCTGATAATTAATGTCGTCTGAGT-ACTATAT- [175]

                               190       200       210       220       230       240
S.h. (Teleomorph)     TTAATAGTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAG [234]
S.h. (Ascigerous)     TTAATAGTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAG [234]
S.h. (Sterile)        TTAATAGTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAG [234]
S.h. (U.S.)           --AATAGTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAG [235]
S.h. (Scotland)       TTAATAGTTAAAACTTTCAACAACGGAT--TCTTGGTTCTGGCATCGATGAAGAACGCAG [233]
R. cuniculi           TTAATAGTTAAAACTTTCAACAACGGAT-CTCTTGGTTCTGGCATCGATGAAGAACGCAG [234]
R. firma              --AATAGTTAAAACTTTCAACAACGGATTCTCTTGGTTCTGGCATCGATGAAGAACGCAG [233]

                               250       260       270       280       290       300
S.h. (Teleomorph)     CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA [294]
S.h. (Ascigerous)     CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA [294]
S.h. (Sterile)        CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA [294]
S.h. (U.S.)           CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA [295]
S.h. (Scotland)       CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA [293]
R. cuniculi           CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA [294]
R. firma              CGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATTTTTGAACGCA [293]

                               310       320       330       340       350       360
S.h. (Teleomorph)     CATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAG [354]
S.h. (Ascigerous)     CATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAG [354]
S.h. (Sterile)        CATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAG [354]
S.h. (U.S.)           CATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAG [355]
S.h. (Scotland)       CATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAG [353]
R. cuniculi           CATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAG [354]
R. firma              CATTGCGCCCCTTGGTATTCCGGGGGGCATGCGTGTTCGAGCGTCATTTCAACCCTCAAG [353]
```

*S.h. = *Sclerotinia homoeocarpa*

Table 7 (cont.). ITS1, 5.8S, and ITS2 sequences from Bennett's type cultures, *Rutstroemia cuniculi*, *Rutstroemia firma*, and *Sclerotinia homoeocarpa* isolates from U.S. and Scotland aligned by the Jotun-Hein Method.

```
                          370        380        390        400        410        420
S.h.* (Teleomorph)    CTC-A-GCTTGGTATTGGGCCTCCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGC [412]
S.h. (Ascigerous)     CTC-A-GCTTGGTATTGGGCCTCCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGC [412]
S.h. (Sterile)        CTC-T-GCTTGGTATTGGGCCTTCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGC [412]
S.h. (U.S.)           CTCTCTGCTTGGTATTGGGCCTCCGCCGGTCACACGGCGGGCCTTAAAGTCAGTGGCGGC [415]
S.h. (Scotland)       CTC-T-GCTTGGTATTGGGCATTCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGC [411]
R. cuniculi           CTC-T-GCTTGGTATTGGGCCTTCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGC [412]
R. firma              TTA-ATGGTTGGTATTGGGCATTCGCCGGGCGACCGGCGGGCCTTAAAGTCAGTGGCGGA [412]

                          430        440        450        460        470        480
S.h. (Teleomorph)     GCCGTTGGGTCCTGAACGTAGTAAC--ATACACCTCGTTAC--AGGGTCCCCGCGAGCTT [468]
S.h. (Ascigerous)     GCCGTTGGGTCCTGAACGTAGTAAC--ATACACCTCGTTAC--AGGGCCCCCGCGTGCTT [468]
S.h. (Sterile)        GCCGTTGGGTCCTGAACGTAGTAAC--ATACCTCTCGTTAC--AGGGTCCCCGCGTGCTT [468]
S.h. (U.S.)           GCCGCTGGGTCCTGAACGTAGTAACACATACCTCTCGTTAC--AGGGTCCCCGCGCGCTC [473]
S.h. (Scotland)       GCCGTTGGGTCCTGAACGTAGTAAC--ATACCTCTCGTTACCAGGGTGCCCCGCGTGCTT [469]
R. cuniculi           GCCGTTGGGTCCTGAACGTAGTAAC--ATACCTCTCGTTAC--AGG-TCCCCGCGTGCTT [467]
R. firma              GCCGTTGGGTCCTGAACGTAGTAAC--ATACCTCTCGTTAC--AGGGTCCCCGCGTGCTT [468]

                          490
S.h. (Teleomorph)     CTGCCATTAAACCCC [483]
S.h. (Ascigerous)     CTGCCATTAAACCCC [483]
S.h. (Sterile)        CTGCCATTAAACCCC [483]
S.h. (U.S.)           CCGCCGTAAAACCCC [488]
S.h. (Scotland)       CTGCCATTAAACCCC [484]
R. cuniculi           CTGCCATTAAACCCC [482]
R. firma              CTGCCATTAAACCCC [483]
```

*S.h. = *Sclerotinia homoeocarpa*

phylogenetically informative. Sequence alignment and similarity tables are provided in the appendix. Parsimony analysis yielded a single most parsimonious tree with a length of 93 steps. The maximum tree length was 130 steps. This tree, with bootstrap values and number of character state changes per branch, is shown in figure 4. Consistency index, rescaled consistency index, and retention index of the most parsimonious tree are 0.91, 0.63, and 0.58 respectively.

18S rDNA Sequence Analysis

The 3' end of the 18S rDNA sequence used for analysis aligned with base 959 through base 1600 of the *S. sclerotiorum* 18S rDNA (Wilmotte, 1993); this region included an intron at base 1165 that extended 314 bases. Aligned sequences from *S. homoeocarpa* (U.S.), *S. homoeocarpa* (teleomorphic), *S. homoeocarpa* (ascigerous), *S. homoeocarpa* (sterile), and *S. sclerotiorum* are provided on table 8. There were sequence variations at 17 sites with three of these being phylogenetically informative. Bennett's (1937) sterile strain of *S. homoeocarpa* shared an intron at the same site as that of found in *S. sclerotiorum*. This 327 base intron shared 59% sequence similarity with the *S. sclerotiorum* intron. These introns share the P, Q, R, and S sequences characteristic of group I introns (Cech, 1988).

An insertion element was identified in the sequence from *S. homoeocarpa* from the U.S. This element occurred at the 1520 base of the *S. sclerotiorum* 18S rDNA sequence and extended for 415 bases. A BLASTN (Altschul et al., 1990) search of Genbank (Benson et. al., 1998) revealed three sequences that shared considerable identity for portions of the *S. homoeocarpa* (U.S.) insertion sequence. The *S. homoeocarpa*

Figure 4. Most parsimonious tree based on ITS1, 5.8S, and ITS2 sequence data. Bold and underlined numbers represent bootstrap values based on 1000 replications using a Branch and Bound search. Boxed numbers correspond to the number of character state changes per branch. (S.h. = *Sclerotina homoeocarpa*)

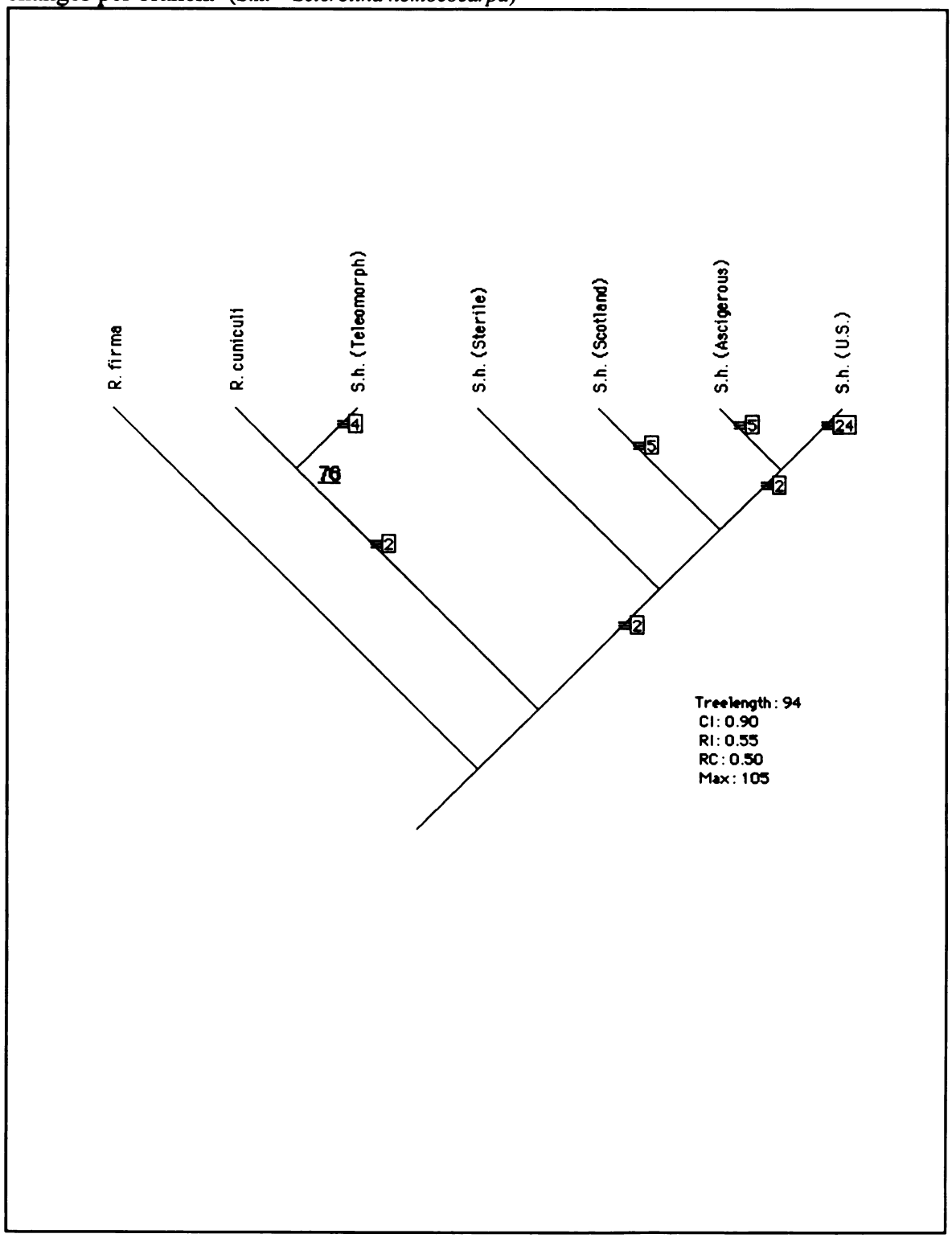

Table 8. 3' end of the 18S rDNA sequences from Bennett's type cultures, *Sclerotinia homoeocarpa* (U.S.), and *Sclerotinia sclerotiorum* aligned by the Jotun-Hein method.

```
            1                                                          60]
Teleomorph  CATTAATCAGTGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAA
Ascigerous  CATTAATCAGTGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAA
Sterile     CATTAGGCGGTGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAA
U.S.        CATTAATCAGTGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGT--TAA
S. scler.   CATTAATCAGTGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAA

            61                                                        120]
Teleomorph  CCATAAACTATGCCGACTAGGGATCGGGCGATGTTATCTTTTTGACTCGCTCGGCACCTC
Ascigerous  CCATAAACTATGCCGACTAGGGATCGGGCGATGTTATCTTTTTGACTCGCTCGGCACCTC
Sterile     CCATAAACTATGCCGACTAGGGATCGGGCGATGTTATCTTTTTGACTCGCTCGGCACCTC
U.S.        CCATAAACTATGCCGACTAGGGATCGGGCGATGTTATCTTTTTGACTCGCTCGGCACCTC
S. scler.   CCATAAACTATGCCGACTAGGGATCGGGCGATGTTATCTTTTTGACTCGCTCGGCACCTC

            121                                                       180]
Teleomorph  ACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAG
Ascigerous  ACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAG
Sterile     ACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAG
U.S.        ACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAG
S. scler.   ACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAG

            181                                                       240]
Teleomorph  AAATTGACGGAAAGGCACCACCAGG----------------------------------
Ascigerous  AAATTGACGGAAAGGCACCACCAGG----------------------------------
Sterile     AAATTGACGGAAAGGCACCACCAGG-GTAAACGCAGTTATTTTGCGC-TGAAAGCAACCC
U.S.        AAATTGACGGAAAGGCACCACCAGG----------------------------------
S. scler.   AAATTGACGGAAAGGCACCACCAGGCGTACAAGCAGTAACTCTGCGCCTAAAAGCAGCTC

            241                                                       300]
Teleomorph  ------------------------------------------------------------
Ascigerous  ------------------------------------------------------------
Sterile     TTAAGCGG-GGTGGTGGTGGCTGCAAACGCTAGTCGAGTTCGCTCGGTACATTTCCCAAC
U.S.        ------------------------------------------------------------
S. scler.   GTAAGAGTTGGTGGTAGTCTTAGGATATGCTAGTTGGAA--ATCAGCTATACCTTCAAAC

            301                                                       360]
Teleomorph  ------------------------------------------------------------
Ascigerous  ------------------------------------------------------------
Sterile     TGCGGGGA-TCCCCTAAAGCTCCAGCTACCAAACTTCGACCGCTGAAAAGCCGGGGTGGC
U.S.        ------------------------------------------------------------
S. scler.   TGCGGGGAACTCCTTAAAAACTCAACTACTAAACCTCAAT---TGAAAGATTGTGGTGGC

            361                                                       420]
Teleomorph  ------------------------------------------------------------
Ascigerous  ------------------------------------------------------------
Sterile     CAGGCTCAACCTGGGTACGGTGATAACGCTGCGAGATGTTACAATGGGCTATCCGCATCC
U.S.        ------------------------------------------------------------
S. scler.   CAG-CTAAATCTGGGTAAAGTAATAACGTTGAGAACT-------TGGACAATCCGCATCC

            421                                                       480]
Teleomorph  ------------------------------------------------------------
Ascigerous  ------------------------------------------------------------
Sterile     AAGCCCTTACGGCCACGCG-TACGGGGAAGGTTCAGAGACTAAACGGAGATGGGTGACAC
U.S.        ------------------------------------------------------------
S. scler.   AATCCTCTAAGGTTCCAAACTATGAGGAAGGTTCAGAGACTAAATGTAGGTAGGTAGCAT
```

Table 8 (cont.). 3' end of the 18S rDNA sequences from Bennett's type cultures, *Sclerotinia homoeocarpa* (U.S.), and *Sclerotinia sclerotiorum* aligned by the Jotun-Hein method.

```
             481                                                    540]
Teleomorph   ----------------------------------------------------CGTGG
Ascigerous   ----------------------------------------------------CGTGG
Sterile      CTGCTGTCACTTGAGATATAGTCCGGCGTAGCGCCTCAACGGCCTACAGTTTA-GACGGG
U.S.         ----------------------------------------------------CGTGG
S. scler.    ----TGCTACTTAAGATATAGTCCATCTCGAGA--TTAACGTCTCGAGAATAATAATGGG

             541                                                    600]
Teleomorph   AGCCTGCGGCTTAATTTGACTCAACACGGGG-AAACTCACCAGGT--------------
Ascigerous   AGCCTGCGGCTTAATTTGACTCAACACGGGG-AAACTCACCAGGT--------------
Sterlile     AGCCTGCGGCTTAATTTGACTCAACACGGGG-AAACTCACCAGGT--------------
U.S.         AGCCTGCGGCTTAATTTGACTCAACACGGGGGAAACTCACCAGGTTAACCACGGTTGTTA
S. scler.    AGCCTGCG-CTTAATTTGACTCAACACGGG---AACTCACCAGGT--------------

             601                                                    660]
Teleomorph   ------------------------------------------------------------
Ascigerous   ------------------------------------------------------------
Sterile      ------------------------------------------------------------
U.S.         CGACCTCTGGGCCTGGAAAAAGAAAGGGGGTGGCCCACCTCTCTCTAGTGCTTGTCTTTG
S. scler.    ------------------------------------------------------------

             661                                                    720]
Teleomorph   ------------------------------------------------------------
Ascigerous   ------------------------------------------------------------
Sterile      ------------------------------------------------------------
U.S.         TCTGTGTGGGGAAGTCCCCTATTTTGGGCACAGACGCTCCGTAGCGGGAGCGTGACAGGT
S. scler.    ------------------------------------------------------------

             721                                                    780]
Teleomorph   ------------------------------------------------------------
Ascigerous   ------------------------------------------------------------
Sterile      ------------------------------------------------------------
U.S.         GCAACACCAGCTGGAACAGAAGACGCCTCCGTTACATGTAACGAAGCCAATTCTGTGGCG
S. scler.    ------------------------------------------------------------

             781                                                    840]
Teleomorph   ------------------------------------------------------------
Ascigerous   ------------------------------------------------------------
Sterile      ------------------------------------------------------------
U.S.         AGCCTGGGTCACGCCAGGCCGTCGCAACGCGCGCAAAGCGGTGGGTTCACTGAATGCAGT
S. scler.    ------------------------------------------------------------

             841                                                    900]
Teleomorph   ------------------------------------------------------------
Ascigerous   ------------------------------------------------------------
Sterile      ------------------------------------------------------------
U.S.         GGGCTTAAGGTACGTGCTAATCCCGCGAGAAATCGCGCCGCGTGAACAAGGTCCAAAAGC
S. scler.    ------------------------------------------------------------

             901                                                    960]
Teleomorph   ------------------------------------------------------------
Ascigerous   ------------------------------------------------------------
Sterile      ------------------------------------------------------------
U.S.         CAAAGTCACGCGGGCCTATCATCTGATAAGCGGTATTTGCGGGGAATGCCCCAGCACCCT
S. scler.    ------------------------------------------------------------
```

Table 8 (cont.). 3' end of the 18S rDNA sequences from Bennett's type cultures, *Sclerotinia homoeocarpa* (U.S.), and *Sclerotinia sclerotiorum* aligned by the Jotun-Hein method.

```
             961                                                    1020]
Teleomorph   ---------------------------------------CCAGACACAATAAGGATTGA
Ascigerous   ---------------------------------------CCAGACACAATAAGGATTGA
Sterile      ---------------------------------------CCAGACACAATAAGGATTGA
U.S.         CTCTCGATGGAAGGATGATGCGGGGGGGCTCCTCCACATGCCAGACACAATAAGGATTGA
S. scler.    ---------------------------------------CCAGACACAATAAGGATTGA

             1021                                                   1080]
Teleomorph   CAGATTGAGAGCTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTG
Ascigerous   CAGATTGAGAGCTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTG
Sterile      CAGATTGAGAGCTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTG
U.S.         CAGATTGAAAACTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTCTTAATTGGTG
S. scler.    CAGATTGAGAGCTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTG

             1081                                                   1140]
Teleomorph   GAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTTAACCTGCTAAATA-CCAG-
Ascigerous   GAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTTAACCTGCTAAATAGCCAGG
Sterile      GAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTTAACCTGCTAAATAGCCAGG
U.S.         GAATGATTTGTCTGCTTAATTGCGATAACGAACGAAAACTTAACCTGCTAAATA-CCAGG
S. scler.    GAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTTAACCTGCTAAATAGCCCGG

             1141                                  1174]
Teleomorph   CTAACTTTGGCTGGTCGCCG-CTTCTTAAAAGGA
Ascigerous   CTAGCTTTGGCTGGTCGCCGGCTTCTTAGAGGGA
Sterile      CTAGCTTTGGCTGGTCGCCGG-TTCTTAGA-GGA
U.S.         CTAACTTTGGCTGGTCCCCG-CTTCTTAAAAGGA
S. scler.    CTAGCTTTGGCTGGTCGCTGGCTTCTTAGAGGGA
```

(US) insertion from bases 179 to 299 shared 73% identity with a group I intron found in *Cryphonectria parasitica* (bases 1565 to 1685) and *Cryphonectria radicalis* (bases 1625 to 1685) 18S rDNA. Significant homology was also found with an intron from the 18S rDNA of the black yeast *Nadsoniella nigra* sharing regions of 90% (over 33 bases starting at position 133 of the insertion element), 93% (over a 33 base region starting at position 209), and 89% (over 28 bases starting at base 258) homology. Shared sequences were also found with the 26S rDNA of *Gaeumannomyces graminis* var. *tritici* (72% over 65 bases), 18S rDNA of *Plasmodiophora brassicae* (66% over 77 bases), and 18S rDNA of *Rhodosporidium dacryoidum* (67% over 61 bases). No introns were identified in the 18S rDNA sequences amplified from Bennett's teleomorphic and ascigerous strains of *S. homoeocarpa.*

Morphological Comparisons

Following one week of incubation, *S. homoeocarpa* isolates from locations in the northern United States produced a white mycelium that grew from the surface of the agar as a dense cottony mass. *Sclerotinia homoeocarpa* isolates from England and Scotland produced a white mycelium appressed to the agar generating sparse wefts of aerial mycelia (figure 5).

Stromatal production was well developed after one month of incubation with the degree of stromatal production varying among isolates. Stroma produced by U.S. isolates appeared as irregular black plate-like structures on the agar surface. At the edges of the plate-like stroma the rind would cut vertically into the agar to encompass a portion of the media (figure 6). In some cases these vertical growths were produced in irregular

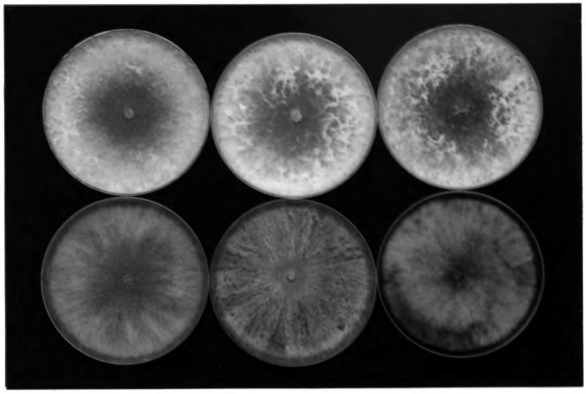

Figure 5. Comparison of mycelial growth characteristic of U.S. and Canadian *Sclerotinia homoeocarpa* isolates (top three plates) with *Sclerotinia homoeocarpa* isolates from England and Scotland (bottom).

Figure 6. Substratal stroma produced by *Sclerotinia homoeocarpa* isolates from the U.S. and Canada on PDA after one month of incubation.

concentric rings. Mycelial growth on the surface of the agar was feltlike and generally white but may include grey, white, yellow, green, or brown regions. Stromatal production by British isolates of *S. homoeocarpa* showed greater variation. Some isolates would produce plate-like stroma similar to those produced by U.S. isolates. All British isolates produced small black rounded stroma (0.5 to 3 mm in diameter) that were embedded within the agar medium (figure 7). This was the only type of stroma produced by some isolates. These rounded stroma could be easily removed from the agar with the a dissecting needle. Mycelial growth on the surface of the media appeared to degenerate to a few aerial wefts with little growth on the agar surface being evident.

DISCUSSION

The results indicate that *S. homoeocarpa* is best accommodated within the genus *Rutstroemia* rather than the genera *Lanzia* and/or *Moellerodiscus* as it is commonly identified (Smiley, 1992). Analysis of ITS1 sequence data (figure 2) revealed that isolates of *S. homoeocarpa* clustered with isolates from the genus *Rutstroemia*, including the lectotype species for the genus, *R. firma* (Korf, 1988). Bootstrap analysis supported a distinction between the genera of *Moellerodiscus* and *Rutstroemia*.

The one species of *Lanzia* (*Lanzia luteovirescens*) also clustered among isolates of the genus *Rutstroemia*. The genera *Lanzia* and *Poculum* were revived by Dumont (1972) to distinguish members previously considered under the genus *Rutstroemia* (deemed unacceptable by Dumont and Korf, 1971). The only character distinguishing the genera *Lanzia* and *Poculum* was the production of a gelatinized ectal excipulum of the apothecia of *Lanzia* versus a non-gelatinized ectal excipulum of the apothecia of

Figure 7. Substratal stromata produced by *Sclerotinia homoeocarpa* isolates from England and Scotland on PDA after one month of incubation.

Poculum. The genus *Poculum* is now considered synonymous with *Rutstroemia* (Korf, 1988). The distinction between these has been called into question (Baral, 1994) as an artificial character that may vary within populations. These results support Baral's (1994) conjecture that the distinction between *Lanzia* and *Rutstroemia* (*Poculum*) is artificial.

Bennett's (1937) teleomorphic strain of *S. homoeocarpa*, the type culture for the species and upon which the descriptive epithet *S. homoeocarpa* is applied, was not included in a clade with the other *S. homoeocarpa* isolates (ascigerous, sterile, U.S., England, and Scotland) based on ITS1 sequence data (figure 2). The teleomorphic strain tightly aligned with two *Rutstroemia* species, *R. cuniculi* and *R. henningsianum*. Furthermore, the teleomorphic strain shared ITS1 identity with *R. cuniculi* suggesting that they may be the same species. Examination of ITS2 sequences among the teleomorphic strain of *S. homoeocarpa* and *R. cuniculi* revealed a 10 base change over the 150 bases of the ITS2 region (93.3% similarity). The ITS2 data conflicts with the ITS1 data as the ITS2 phylogram supports a clade consisting of Bennett's teleomorphic, ascigerous, and U.S. *S. homoeocarpa* (figure 3). However, this phylogeny is based on 8 informative characters as opposed to the 58 informative characters of the ITS1 sequence. Bootstrap analysis of the alignment of the ITS1 and ITS2 sequence data together provided statistical support for a single clade consisting of Bennett's teleomorphic strain of *S. homoeocarpa* and *R. cuniculi*. This association provides further support for a distinction between the teleomorphic strain of *S. homoeocarpa* and *S. homoeocarpa* isolates from the U.S. and the British Isles. That the teleomorphic strain would be aligned closer to other members of the genus *Rutstroemia* than to other isolates of *S. homoeocarpa* indicates that it is not

representative of the pathogen responsible for dollar spot disease.

Bennett (1937) recovered the teleomorphic isolate from diseased tissue received from a colleague. He described this tissue as "brownish or yellowish leaves that were marked with dark blotches but infested with other fungi also" which does not correspond to typical dollar spot symptoms of tan to bleached leaves and raises the concern of isolation of a fungus (identified as the teleomophic strain of *S. homoeocarpa*) that may have been a secondary invader or saprophyte. This strain was selected to represent the species as it was the only isolate to produce asci and conidia. Bennett (1937) described several differences between the teleomorphic and ascigerous strains of *S. homoeocarpa*. The aerial mycelium produced by the teleomorphic strain was white and "abundant wooly to floccose" becoming compact, felted, and cinnamon to reddish brown in color whereas the ascigerous strain (and sterile strain) produced "a sparse, downy, dingy white, mycelium, with sometimes scattered tufts" over time "degenerating later to a sparse, downy residue". Differences in the apothecial anatomy with the ascigerous apothecia being "more globose" and "do not proceed further to disc or funnel forms" with respect to apothecia from the teleomorphic strain. Asci (180-220 * 10.4-12.0μ) and ascospores (18.2-26.0 * 7.8-9.0μ) from the ascigerous strain were larger than those of the teleomorph (asci 140-170 * 10.4-11.5μ; ascospores 16-17 * 5.2-6.5μ) (Bennett, 1937). The apothecia from Bennett's ascigerous strain matched those generated by Jackson (1973) from a *S. homoeocarpa* isolate (IMI167641) from England.

Bennett's ascigerous and sterile strains formed a clade with *S. homoeocarpa* isolates from the U.S., England, and Scotland based on ITS1 analysis (figure 2).

Sequence similarities among the isolates of British origin varied from 98.5 to 99.5%. The sterile strain being most closely related to the Scottish *S. homoeocarpa* isolate, sharing 99.5% sequence similarity.

Mycelial morphology of the isolates from Scotland and England were similar to those described by Bennett of the ascigerous and sterile strains with a "sparse , downy, dingy white mycelium, with... small scattered tufts...degenerating later to a sparse, downy residue". Bennett described the production of "sclerotial dots" and small flakes 1 or 2 mm in diameter which may be similar to the small black rounded stroma (0.5 to 3 mm in diameter) within the agar medium of the England and Scotland *S. homoeocarpa* cultures. The production of these small rounded stroma may have led to Bennetts consideration of microsclerotia which may aggregate to form more extensive sclerotial structures, leading to his generic identification of this organism as a *Sclerotinia.*

The U.S. *S. homoeocarpa* isolates were included within the clade of British *S. homoeocarpa* isolates based on ITS1 data, however, several characteristics distinguish the non-British strains from those of British origin. The non-British ITS1 sequence exhibited a minimum sequence divergence of 16 bases with other members of this clade. Similarly, the ITS2 sequence differed from the other *S. homoeocarpa* isolates by a minimum of 16 bases over the 150 bases of the ITS2 region. These non-British isolates also shared a novel intron in the 3' end of the 18S rDNA. This intron shares sequence homology with introns in *Cryphonectria parasitica*, *Cryphonectria radicalis*, *Nadsoniella nigra*, *Gaeumannomyces graminis* var *tritici*, and *Plamodiophora brassicae*. Mycelial morphology of the non-British strains contrasted with those from England, Scotland, and

Bennett's descriptions of the ascigerous and sterile strains. The morphology was similar to Bennett's description of the mycelial growth by the teleomorphic strain as exhibiting "a well-developed white, woolly growth" with the mycelium becoming a "compact, felted, snow-white layer, whilst the cinnamon floccose growth at the top becomes reddish brown and matted" (Bennett, 1937). Stromatal production also contrasted with those of the British *S. homoeocarpa* isolates with more extensive plate-like stroma and vertical rind stromatal development coupled with the lack of production of the small rounded stroma.

The results of this work suggest that the dollar spot pathogen, identified as *S. homoeocarpa*, should be reclassified within the genus *Rutstroemia* as opposed to *Lanzia* (which appears to be an artificial genera synonymous with *Rutstroemia*) or *Moellerodiscus*. This raises interesting questions about the inclusion of *S. homoeocarpa*, a successful plant pathogen, among a genera of non-pathogenic fungi. This becomes more intriguing as Holst-Jensen et al. (1997) have proposed erecting the family Rutstroemiaceae to accommodate the substratal stromatal taxa of the Sclerotiniaceae. This would identify *S. homoeocarpa* as a pathogen among a family of non-pathogenic fungi.

Bennett's teleomorphic strain of *S. homoeocarpa*, the type species upon which the species is described, shares closer relations with *R. cuniculi* and *R. henningsianum* than to other *S. homoeocarpa* isolates as suggested by ITS1 sequence data. Based on these findings this culture should not be identified as the type species and taxonomic description to represent the pathogen(s) responsible for dollar spot disease. The specific epithet "*homoeocarpa*" is based on the similarity in appearance of sporocarps bearing

conidia and asci produced by the teleomorphic strain, and does not apply to other dollar spot cultures.

Jacksons (1973) description of apothecia and asci generated from dollar spot pathogens is in corroboration of Bennett's description of the ascigerous strain of the dollar spot pathogen. Based on this support and the molecular data presented here, the dollar spot pathogen requires reclassification as a separate species from *S. homoeocarpa* which is restricted to the teleomorphic strain described by Bennett. I propose application of the name *Rutstroemia festucae* to refer to the ascigerous cultures described by Bennett and Jackson which is responsible for dollar spot . The species epithet "*festucae*" is applied to refer to the restricted host range of this organism to *Festuca rubra* L. spp *rubra* in the British Isles (Smith, 1989). Until fertile apothecia are generated and examined in depth, Bennett's (1937) description of the ascigerous strain should serve as the basis identifying the dollar spot pathogen as follows:

Diagnostic characters of *Rutstroemia festucae*.

"Apothecia cupulate 0.4-0.7 mm in diameter, cinnamon to brown in colour, on stalks 4 - 6 mm long, simple. Asci cylindrolavate, inoperculate 180-220 * 10.4-12μ. Ascospores 8, uniseriate, hyaline, oblong elliptical, bi-guttulate, unicellular, a delicate median septum, 18.2-26.0 * 7.8-9.0μ, commonly 18.5-20.8 * 7.8-8.0μ. Paraphyses few, cylindro-clavate, sparsely septate, 80-120 *2.0-2.2μ. Microconidia spherical, hyaline, 1.5-2.0μ in minute cream-coloured pustules; not known to germinate.

> Mycelium abundant, white, faintly tinted blusih green or chalcendony yellow in different strains; cinnamon-coloured floccose hyphae at the tops of slant cultures and cinnamon-coloured hyphae amongst the white as the mycelium ages. Sclerotial structures black, from small flakes to expansive patches, parchment-like, formed by conversion of superficial hyphae of the white mycelium into a mosaic of small thick-walled cells. Ascophores typically erumpent from sclerotial structures, occasionally superficial on the edges or when excessively thin. Habitat. In fine turf on *Festuca*, *Agrostis*, *Poa*, causing "dollar spot" disease, in Britain, America and Australia."

Questions remain as to the nature of the dollar spot pathogen recovered from the U.S., Canada, Australia, and Netherlands. While the ITS1 data support inclusion of these isolates within the proposed new description of the dollar spot pathogen as *Rutstroemia festucae,* significant differences were evident. As previously discussed, ITS1 and ITS2 sequence differences distinguish non-British and British dollar spot isolates. Furthermore, dollar spot isolates from the U.S. are identified as having a broad host range of turf species upon which they may infect, whereas dollar spot in England is generally restricted to *Festuca rubra* L. spp *rubra* (Smith et al., 1989). Cultural morphological differences also differentiate these non-British dollar spot pathogens as discussed. Bennett (1937) documented further differences between British dollar spot isolates and isolates from the U.S. and Australia based on the effect of temperature on growth. The

growth of British isolates was reduced at temperatures greater than 25 C whereas non-British isolates tolerated temperatures up to 30 C. Fertile apothecia have yet to be generated from non-British isolates further raising questions about their lineage. The culmination of these differences in ITS1, ITS2, 18S rDNA, host range and temperature tolerances suggest that the non-British strains represent a unique organism that should be recognized as a different species from the British dollar spot pathogen as defined by *Rutstroemia festucae*.

I propose the adoption of the species epithet *"floccosum"* to identify the non-British dollar spot pathogens. The name *"floccosum"* refers to the woolly mycelial growth of this organism *in vitro* and *in vivo* under favorable conditions. These isolates produce a well developed white floccose mycelium in culture. The mycelium becomes feltlike and may turn cinnamon-brown, olive, yellow, or dark grey in color. These isolates produce a stroma lacking a cortex with abundant protein and lipid storage bodies. Further identity is based on ITS1 and ITS2 sequence data.

Clarification of the taxonomy of these organism will facilitate greater understanding of the biology of these organisms that have unique molecular, physiological, and cultural characteristics.

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CONCLUSIONS and RECOMMENDATIONS

Dollar spot is one of the most persistent and economically important diseases of high maintenance turfs. While there is considerable work dedicated to the improvement OF managing this disease, there are large gaps in our knowledge of the basic biology of the pathogen. The effort of this research was to further our understanding of some of the basic biology of the organism with respect to populations responsible for seasonal variation and clarification of the taxonomy of the pathogen, *Sclerotinia homoeocarpa* Bennett.

The occurrence of seasonal epidemics of dollar spot is widely recognized, although understanding of the underlying biological principles are poorly understood. The research presented addressed this topic with respect to the population dynamics of the causal organism. The starting hypothesis was that the two seasonal epidemics were caused by different pathogens, and thus, different sets of vegetative compatibility groups (VCGs) would be identified from each of these epidemics. Seasonal samplings at three locations recovered the same VCGs in the spring epidemics as were found in fall epidemics. The one exception to this was VCG F which appeared to be generally found only in the fall epidemics of the year. Among eight sites sampled, only 6 VCGs were identified which represented less diversity in VCGs in northern populations of *S. homoeocarpa* than those found in Florida by Sonoda. Representative isolates of each of these VCGs shared the same ITS1 sequence indicating they belong to the same species. This study did not eliminate the possibility that there are sub-populations of the VCGs which are responsible seasonal epidemics.

The identification of a limited number of VCGs presents the opportunity to use

these VCGs to increase understanding of the epidemiology and management of dollar spot. VCGs may be used as markers to follow populations of the dollar spot pathogen when introduced into areas where that VCG is not found. Such studies can be directed to understanding epidemiological topics including rate of migration of dollar spot, directionality of migration, and whether dollar spots occur at the same point year after year.

The recovery of the same VCGs from each of the seasonal epidemics further suggests that seasonal epidemics are more likely due to environmental or host conditions rather than due to distinct pathogen populations. Future work should be directed to understanding the conditions, environmental or host, that foster the seasonal epidemics. This will allow for the development of accurate predictive models for dollar spot which have yet to be developed. These models can be used to develop site specific management strategies and improve current cultural management strategies.

The level of understanding of the pathogen responsible for dollar spot has extended toward the taxonomy of the pathogen. The generic identification of the pathogen as a member of the genus *Sclerotinia* has long been known to be incorrect, however, a comparison with the taxa it is believed to belong (*Rutstroemia*, *Lanzia*, and *Moellerodiscus*) has not been conducted. The research presented based on nuclear ribosomal internal transcribed spacer region 1 (ITS1) sequence data, indicates inclusion of the dollar spot pathogen in the genus *Rutstroemia* rather than *Lanzia* or *Moellerodiscus* (although only one isolate of each of these genera were included due to availability). Inclusion of ITS1 sequences of cultures from Bennett's original description of *S.*

homoeocarpa in the data analysis revealed that the culture upon which Bennett chose to describe the species (teleomorphic strain) was more closely related to *Rutstroemia* species *R. cuniculi* and *R. henningsianum* than to the isolates responsible for dollar spot. As the species epithet "*homoeocarpa*" is descriptively applied to the "teleomorphic" strain based on the similarity among apothecia and conidia sporocarps, this name should remain with this strain. This name is not applicable to Bennett's ascigerous and sterile strains including recent isolates collected from the U.S., Canada, and Britain as no conidia are produced. These organisms require a new specific identification. As considerable differences exist in the ITS1 sequence data, temperature optima, cultural morphology, and host range between isolates of the dollar spot pathogen from Britain as compared to isolates from the U.S., Canada, Australia, and Netherlands, these two organisms need to be distinguished. I have proposed the name "*Rutstroemia festucae*" for the British dollar spot isolates as dollar spot is identified as only occurring on *Festuce rubra* ssp *rubra* in the British Isles. The name proposed for the dollar spot pathogen from the U.S., Canada, Australia, and Netherlands is "*Rutstroemia floccosum*" based on the woolly/cottony growth habit of this organism *in vitro* and *in vivo* under favorable conditions.

Incorporation of the dollar spot pathogens within the genus *Rutstroemia* raises interesting questions about the occurrence of a vigorous pathogen within a genera typified by saprophytes. This presents the opportunity to investigate the development of pathogenicity. To this extent, work performed to date to understand the dollar spot pathogen(s) has focused on the potential of this organism to produce metabolites in the soil that are toxic to turf root tissues not foliar tissues which are infected by the dollar

spot pathogen(s). Investigations into this pathogenicity should focus on a detailed description of the infection process, coupled with investigations into the production of phytotoxic metabolites. Understanding of these processes will serve as a starting point for the development of resistant cultivars through genetic engineering.

The taxonomic reclassifications proposed here are directed to distinguishing unique organisms with respect to biological and taxonomic considerations. Removal of the dollar spot pathogens from the binomial *Rutstoemia* (*Sclerotinia*) *homoeocarpa* is designed to follow the Code of Botanical Nomenclature in which names are to be descriptively applied. Separation of the dollar spot pathogens from Britain from those of non-British origin will lead to an understanding of each of these organisms without making biological generalizations about one which does not apply to the other. While these changes will result in confusion in the short term, over time they will clarify the distinctions between these organisms.

APPENDICES

APPENDIX A

Figure 8. Daily mean temperature for June and July

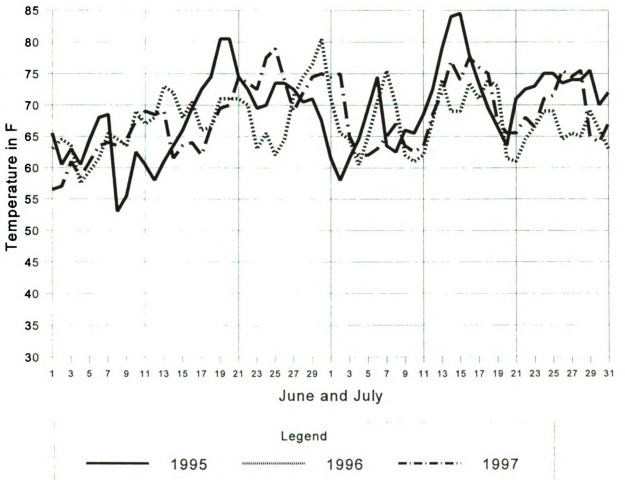

Figure 9. Daily mean temperature for August and September

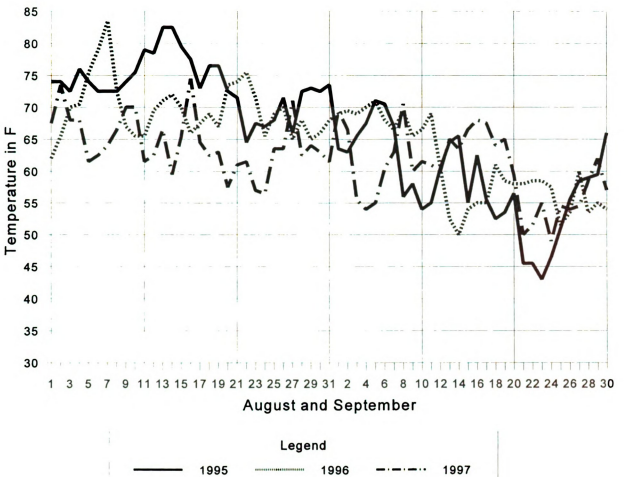

Figure 10. Daily mean temperature for the month of October

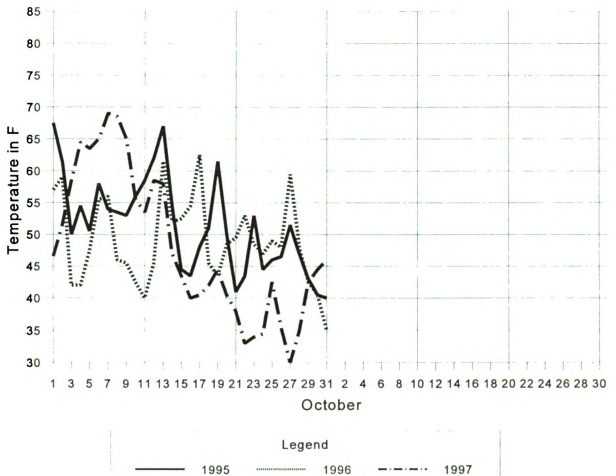

Table 9. Daily mean temperature data for the months of June through October for the years of 1995, 1996, and 1997

June	1995	1996	1997	July	1995	1996	1997	Aug.	1995	1996	1997	Sept.	1995	1996	1997	Oct.	1995	1996	1997
1	65.5	63	56.5	1	61.5	71	74.5	1	74	62	67.5	1	63.5	69	69.5	1	67.5	57	46.5
2	60.5	64.5	57	2	58	65.5	75	2	74	65.5	73.5	2	63	69.5	66.5	2	61.5	59	51.5
3	63	63.5	61	3	61.5	64.5	65	3	72.5	70	68	3	65.5	69	55.5	3	50	42	58.5
4	60.5	57.5	58.5	4	64.5	60.5	62	4	76	70.5	68	4	67.5	70	54	4	54.5	42	64.5
5	64.5	59.5	61	5	69.5	65	62	5	74	75.5	61.5	5	71	71	55	5	50.5	47.5	63.5
6	68	61.5	63.5	6	74.5	70.5	63	6	72.5	79	62.5	6	70.5	68	60.5	6	58	55.5	65
7	68.5	65.5	64	7	63.5	75.5	65	7	72.5	83.5	64	7	67	66.5	63	7	54	56	69
8	53	64.5	63.5	8	62.5	70	67	8	72.5	72.5	66.5	8	56	69.5	70.5	8	53.5	46	68.5
9	55.5	63.5	64.5	9	66	62	63.5	9	74	67	70	9	58	65.5	60	9	53	45.5	65
10	62.5	69	67.5	10	65.5	61	62.5	10	75.5	65.5	70	10	54	66.5	61.5	10	56	42.5	55.5
11	60.5	67	69	11	68.5	62	63.5	11	79	65.5	61.5	11	55	69	61	11	58.5	40	53.5
12	58	68	68.5	12	72.5	67.5	68.5	12	78.5	69.5	62.5	12	60.5	60.5	60	12	62	45.5	58.5
13	61	73	69	13	79	74	72	13	82.5	71	66.5	13	64.5	52.5	65	13	67	61.5	58
14	63.5	72	61.5	14	84	69	77	14	82.5	72	59.5	14	65.5	50	63.5	14	54.5	52	47
15	66	68	63.5	15	84.5	69	74	15	79.5	70	65.5	15	55	54	66.5	15	44.5	52.5	43.5
16	69.5	70.5	64	16	78	73.5	77.5	16	77.5	66	74.5	16	62.5	55	68	16	43.5	54.5	40
17	72.5	66	62	17	73.5	71	76	17	73	67.5	64.5	17	55.5	55	67.5	17	48	62.5	40.5
18	74.5	66.5	66.5	18	69.5	74	75	18	76.5	69	62.5	18	52.5	61	64	18	51	45.5	42
19	80.5	71	69.5	19	66.5	73	67.5	19	76.5	67	63	19	53.5	58.5	65	19	61.5	43.5	44.5
20	80.5	71	70	20	63.5	61.5	65.5	20	72.5	73.5	57.5	20	56.5	58	59.5	20	50	48.5	40.5
21	74.5	71	74.5	21	71	61	65.5	21	71.5	74	61	21	45.5	58	50	21	41	49.5	38
22	72.5	70	73.5	22	72.5	64.5	68	22	64.5	75.5	61.5	22	45.5	58.5	51.5	22	43.5	53	33
23	69.5	63	72.5	23	73	66.5	66.5	23	67.5	71.5	57	23	43	58.5	55	23	53	48.5	34
24	70	65.5	77.5	24	75	69	71	24	67	65.5	56.5	24	46.5	57.5	49	24	44.5	47	34.5
25	73.5	62	79	25	75	69	71.5	25	68	69	63.5	25	51	51.5	54.5	25	46	49	42.5
26	73.5	64.5	74	26	73.5	64.5	75.5	26	71.5	70	63.5	26	55.5	53.5	54	26	46.5	48	35.5
27	72.5	71.5	69	27	74	65.5	74.5	27	66	65	71	27	58.5	60	54.5	27	51.5	59.5	30
28	70.5	74.5	72	28	74	65	75.5	28	72.5	68	62.5	28	59	53.5	58.5	28	47	48	35
29	71	76.5	74.5	29	75.5	69	65	29	73	65	64	29	59.5	55	62	29	43	42.5	42.5
30	67.5	80.5	75	30	70	66	64	30	72.5	66	63	30	66	54	57	30	40.5	40.5	44.5
				31	72	63	67	31	73.5	68	61.5					31	40	35	46
Avg.	67.4	67.5	67.4		70.7	67.2	69		73.6	69.7	64.3		58.2	60.6	60.1		51.5	49	48.1

Source: Michigan State University Dept. of Horticulture Teaching and Research Center, East Lansing, MI

Table 10. Daily maximum and minimum temperature data for the months of June to October for the years 1995, 1996, and 1997

	1995		1996		1997			1995		1996		1997			1995		1996		1997			1995		1996		1997			1995		1996		1997	
June	Max	Min	Max	Min	Max	Min	July	Max	Min	Max	Min	Max	Min	Aug	Max	Min	Max	Min	Max	Min	Sept	Max	Min	Max	Min	Max	Min	Oct	Max	Min	Max	Min	Max	Min
1	80	51	80	46	63	50	1	73	50	82	60	82	67	1	87	61	72	52	81	54	1	75	52	85	53	78	61	1	76	59	78	36	57	36
2	71	50	75	54	63	51	2	74	42	76	55	88	62	2	83	65	79	52	88	59	2	79	47	86	53	76	57	2	74	49	73	45	70	33
3	67	59	73	54	73	49	3	78	45	73	56	68	62	3	80	65	84	56	76	60	3	81	50	86	52	66	45	3	53	47	53	31	80	37
4	73	48	65	50	76	41	4	82	47	77	44	70	54	4	86	66	86	55	78	58	4	85	50	87	53	71	37	4	60	49	58	26	76	53
5	82	47	72	47	78	44	5	80	59	83	47	79	45	5	85	63	88	63	72	51	5	88	54	87	55	74	36	5	57	44	66	29	82	45
6	86	50	73	50	78	49	6	81	68	88	53	79	47	6	82	63	94	64	79	46	6	86	55	81	55	84	37	6	65	51	75	36	84	46
7	81	56	77	54	73	55	7	67	60	87	64	76	54	7	82	63	95	72	80	48	7	73	61	72	61	74	52	7	58	50	64	48	84	54
8	62	44	73	56	77	50	8	75	50	85	55	79	55	8	82	63	79	66	82	51	8	65	47	85	54	75	66	8	60	47	50	42	83	54
9	67	44	70	57	82	47	9	78	54	71	53	73	54	9	83	65	79	55	86	54	9	72	44	75	56	64	56	9	70	36	53	38	71	59
10	78	47	79	59	85	50	10	81	50	75	47	78	47	10	87	64	77	54	80	60	10	68	40	78	55	65	58	10	68	44	47	38	71	40
11	66	55	78	56	84	54	11	83	54	80	44	78	49	11	92	66	75	56	63	60	11	74	36	83	55	65	57	11	76	41	55	25	69	38
12	70	46	77	59	78	59	12	89	56	84	51	85	52	12	90	67	83	56	70	55	12	81	40	68	53	65	55	12	82	42	65	26	79	38
13	79	43	86	60	82	56	13	97	61	86	62	90	54	13	94	71	85	57	75	58	13	79	50	58	47	75	55	13	80	54	74	49	72	44
14	82	45	85	59	73	50	14	97	71	82	56	95	59	14	94	71	85	59	75	44	14	77	54	54	46	73	54	14	57	52	62	42	52	42
15	84	48	83	53	80	47	15	96	73	80	58	85	63	15	91	68	78	62	85	46	15	70	40	61	47	81	52	15	51	38	71	34	54	33
16	88	51	87	54	75	53	16	89	67	87	60	92	63	16	90	65	75	57	86	63	16	82	43	67	43	82	54	16	52	35	70	39	51	29
17	90	55	69	63	74	50	17	85	62	80	62	85	67	17	81	65	83	52	67	62	17	60	51	67	43	76	59	17	69	27	76	49	52	29
18	91	58	73	60	80	53	18	79	60	84	64	88	62	18	87	66	87	51	73	52	18	68	37	74	48	80	48	18	69	33	48	43	58	26
19	95	66	80	62	83	56	19	81	52	78	68	77	58	19	90	63	80	54	75	51	19	68	39	75	42	82	48	19	77	46	50	37	62	27
20	94	67	78	64	81	59	20	71	56	74	49	83	48	20	81	64	86	61	63	52	20	65	48	77	39	65	54	20	50	50	61	36	50	31
21	90	59	81	61	85	64	21	82	60	76	46	74	57	21	85	58	85	63	68	54	21	47	44	66	50	63	37	21	42	40	58	41	48	28
22	88	57	77	63	86	61	22	86	59	83	46	80	56	22	78	51	88	63	70	53	22	52	39	68	49	65	38	22	52	35	60	46	40	26
23	89	50	78	48	88	57	23	83	63	83	50	77	56	23	83	52	79	64	74	40	23	58	28	69	48	66	44	23	72	34	50	47	44	24
24	85	55	78	53	91	64	24	87	63	83	55	83	59	24	78	56	81	50	67	46	24	63	30	67	48	65	33	24	45	44	56	38	45	24
25	84	63	75	49	85	73	25	86	64	80	58	85	58	25	79	57	83	55	72	55	25	66	36	64	39	75	34	25	58	34	66	32	47	38
26	84	63	79	50	82	66	26	85	62	75	54	91	60	26	87	56	84	56	76	51	26	71	40	68	39	65	43	26	60	33	63	33	40	31
27	83	62	86	57	86	52	27	87	61	81	50	89	60	27	76	56	72	58	85	57	27	76	41	69	51	70	39	27	63	40	70	49	32	28
28	78	63	89	60	88	56	28	85	63	79	51	84	67	28	81	64	78	58	70	55	28	79	39	60	47	77	40	28	50	44	55	41	46	24
29	79	63	91	62	89	60	29	87	64	77	61	78	52	29	82	64	80	50	73	55	29	80	39	64	46	73	51	29	51	35	58	27	57	28
30	76	59	89	72	85	65	30	90	50	76	56	80	48	30	84	61	83	49	76	50	30	80	52	76	32	60	54	30	47	34	49	32	61	28
							31	93	51	72	54	84	50	31	86	61	85	51	72	51								31	44	36	41	29	60	32
June	1995		1996		1997		July	1995		1996		1997		Aug	1995		1996		1997		Sept	1995		1996		1997		Oct	1995		1996		1997	
Avg	81	54	79	56	80	55		83	58	80	54	82	56		85	63	82	57	75	53		72	44	73	49	72	48		61	42	60	38	61	36

Source Michigan State University Dept of Horticulture Teaching and Research Center, East Lansing, MI

Table 11. Daily precipitation data for the months of June through October for the years of 1995, 1996, and 1997

June	1995	1996	1997	July	1995	1996	1997	Aug.	1995	1996	1997	Sept.	1995	1996	1997	Oct.	1995	1996	1997
1	0	0.18	0.1	1	0	0	0	1	0.1	0	0	1	0	0	0	1	0	0	0
2	0.11	0.09	0.12	2	0	0	0.01	2	0.87	0	0	2	0	0	0	2	0	0.01	0
3	0.03	0	0	3	0	0	0.01	3	0.57	0	0.91	3	0	0	0	3	0.39	0	0
4	0	0.2	0	4	0.6	0	0	4	0.18	0	0	4	0	0	0	4	0	0	0.11
5	0	0.17	0	5	0.17	0	0	5	0	0	0	5	0.02	0	0.04	5	0.52	0	0
6	0	0.35	0	6	0	0	0.06	6	0	0	0.22	6	0.19	0.23	0.21	6	0.15	0	0
7	0	0	0	7	0	0	0	7	0	1.1	0	7	0	0.02	0	7	0.03	0.15	0
8	0	0.31	0	8	0	0	0.22	8	0	0	0	8	0	0.24	0	8	0	0.2	0
9	0.06	0.12	0	9	0.01	0.02	0	9	0	0	0	9	0	0.08	2.55	9	0	0.02	0.2
10	0.15	0	0	10	0	0	0	10	0	0	0.1	10	0	0	0.47	10	0	0	0
11	0	0	0	11	0	0	0	11	0	0	0.33	11	0	0.09	0.02	11	0	0	0
12	0	0.11	0.23	12	0	0	0	12	0	0	0.43	12	0	0.02	0.02	12	0	0	0
13	0	0	0.22	13	0	0	0.19	13	0	0	0	13	0	0.01	0.03	13	0	0	0.39
14	0	0	0	14	0	0.2	0.07	14	0.26	0.01	0	14	0	0.31	0.01	14	0.05	0	0
15	0	0	0.03	15	1.01	0.01	0	15	0.05	0	0.41	15	0	0	0	15	0	0	0
16	0	0	0.3	16	0.23	0	0.11	16	0.57	0	0.29	16	0.27	0	0.75	16	0	0	0
17	0	2.86	0	17	0	0.04	0.9	17	1.62	0	0.21	17	0	0	0.6	17	0	0.84	0
18	0	0.71	0	18	0	0.03	0	18	0.01	0	0	18	0.01	0	0	18	0	0	0
19	0	0.16	0.21	19	0	0	0	19	0	0.45	0.06	19	0.13	0	0.16	19	0	0	0
20	0	0	0.35	20	0.8	0	0	20	0.32	0	0.09	20	0.05	0	0	20	0.56	0	0
21	0	0.01	0.37	21	0	0	0.7	21	0	0	0.22	21	0.57	0.26	0	21	0.15	0	0.01
22	0	0	0	22	0.23	0	0	22	0	0.26	0	22	0.03	0	0.06	22	0	0.26	0
23	0	0.26	0.2	23	0	0	0	23	0	0	0	23	0	0.25	0	23	0.01	0.21	0
24	0.01	0	0	24	0	0	0	24	0	0	0	24	0	0	0	24	0.01	0	0.07
25	0.2	0	0	25	0	0	0.21	25	0	0	0	25	0	0	0	25	0	0	0
26	0.81	0	0	26	0.03	0	0	26	0	0	0	26	0	0.49	0	26	0.62	0	1
27	0.03	0	0	27	0	0	0	27	0	0	0	27	0	0.05	0	27	0.17	0	0
28	0.17	0	0	28	0.01	0.03	0	28	0	0	0	28	0	0	0.21	28	0.01	0	0
29	0.09	0	0	29	0	0.48	0	29	0	0	0	29	0	0	0	29	0	1.09	0
30	0.87	0	0.15	30	0	0.35	0	30	0.02	0	0.05	30	0	0	0.02	30	0.05	0.02	0.01
				31	0	0.01	0	31	0	0	0					31	0.09	0	0.01
Avg.	0.08	0.18	0.08		0.1	0.04	0.08		0.15	0.06	0.11		0.04	0.07	0.17		0.09	0.09	0.06

Source: Michigan State University Dept. of Horticulture Teaching and Research Center, East Lansing, MI

APPENDIX B

Table 12. ITS1 sequence similarity (top) and sequence difference (bottom). (*S.h.* = *Sclerotinia homoeocarpa*)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
***S. h.* (Teleomorph)**	**1**	--	95.6	96.6	89.6	96.1	97.0	78.1	81.7	84.8	77.8	100	85.9	99.0	81.1	84.3	77.8	81.2	**1**
***S. h.* (Ascigerous)**	**2**	4.6	--	99.0	92.0	99.5	98.5	78.1	80.5	84.8	78.4	95.6	85.9	95.5	79.9	84.3	77.8	82.7	**2**
***S. h.* (Sterile)**	**3**	3.6	1.0	--	92.0	99.5	99.5	77.5	79.9	84.3	77.8	96.6	86.4	95.5	79.3	83.8	77.2	81.7	**3**
***S. h.* (U.S.)**	**4**	11.3	8.4	8.4	--	91.5	91.5	73.1	75.4	79.6	72.2	89.6	80.6	89.5	75.4	78.6	72.8	75.9	**4**
***S. h.* (England)**	**5**	4.1	0.5	1.5	9.0	--	98.0	78.7	81.1	85.4	78.9	96.1	86.4	96.0	80.5	84.8	78.4	83.2	**5**
***S. h.* (Scotland)**	**6**	3.0	1.5	0.5	9.0	2.0	--	77.5	79.9	83.8	77.8	97.0	85.9	96.0	79.3	83.3	77.2	81.2	**6**
M. lentus	**7**	25.9	25.9	26.7	33.4	25.1	26.7	--	92.9	81.7	87.6	78.1	79.9	78.1	94.7	80.5	96.4	83.3	**7**
R. americana	**8**	21.0	22.6	23.5	29.8	21.8	23.5	7.5	--	84.0	87.0	81.7	82.2	81.1	93.5	82.8	93.5	84.5	**8**
R. bolaris	**9**	17.1	17.2	17.8	24.2	16.5	18.5	21.0	18.0	--	81.3	84.8	95.5	84.8	83.4	95.5	81.9	84.8	**9**
R. conformata	**10**	26.6	25.7	26.6	35.3	24.8	16.6	13.7	14.3	21.6	--	77.8	80.7	77.8	87.0	81.3	87.7	85.3	**10**
R. cuniculi	**11**	0.0	4.6	3.6	11.3	4.1	3.0	25.9	21.0	17.1	26.6	--	85.9	99.0	81.1	84.3	77.8	81.2	**11**
R. firma	**12**	15.8	15.8	15.2	22.6	15.2	15.8	23.4	20.3	4.6	22.4	15.8	--	85.3	82.2	94.0	80.1	82.7	**12**
R. henningsianum	**13**	1.0	4.6	4.6	11.4	4.1	4.1	25.9	21.8	17.2	26.6	1.0	16.5	--	81.1	84.3	77.8	81.6	**13**
R. paludosa	**14**	21.8	23.5	24.3	29.8	22.6	24.3	5.5	6.8	18.7	14.3	22.8	20.3	21.8	--	82.8	96.4	82.7	**14**
R. petiolorum	**15**	17.7	17.8	18.5	25.6	17.2	16.2	22.6	19.5	4.7	21.6	17.7	6.3	17.9	19.5	--	80.7	83.8	**15**
S. sclerotiorum	**16**	26.4	26.4	27.2	34.0	25.5	27.2	3.6	6.8	20.8	13.5	26.4	23.1	26.4	3.6	22.3	--	83.5	**16**
L luteovirescens	**17**	21.9	19.9	21.2	29.7	19.2	21.9	18.8	17.3	17.0	16.4	21.9	19.6	21.3	19.6	18.3	18.6	--	**17**
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	

Table 13. 5.8S rDNA and ITS2 sequence similarity (top) and difference (bottom)

		1	2	3	4	5	6	7	
S. homoeocarpa (Teleomorph)	1	---	97.5	95.6	95.3	95.6	97.2	94.7	1
S. homoeocarpa (Ascigerous)	2	1.3	---	97.5	94.4	95.9	97.5	94.4	2
S. homoeocarpa (Sterile)	3	2.5	2.5	---	93.8	98.1	99.7	96.9	3
S. homoeocarpa (U.S.)	4	4.8	5.9	6.6	---	92.2	93.4	90.9	4
S. homoeocarpa (Scotland)	5	4.5	4.5	1.9	8.3	---	97.8	95.6	5
R. cuniculi	6	2.9	2.6	0.3	6.9	2.2	---	96.6	6
R. firma	7	5.5	5.9	3.2	9.7	4.5	3.5	---	7
		1	2	3	4	5	6	7	