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The Basic Biology and Etiology of *Sclerotinia homoeocarpa*, the Causal Agent of Dollar Spot

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Executive Summary

This research thus far has been designed primarily to focus on the following objective:

> To examine the development, including possible apothetical production, of the pathogen in creeping bentgrass greens when present in leaf tissue, in root tissue, or as isolated stroma and to determine the length of survival of the pathogen in infected tissue or as stroma.

This is the first year of funded research. A summary of observations and tentative conclusions from the first field season are provided below together with action plans for the upcoming months.

1. Small (2 x 4 cm) porous nylon bags were prepared, inoculum of *S. homoeocarpa,* in the form of infected grass or grown on sterile wheat, was placed in the bags, the bags were heat-sealed and they were buried vertically in bentgrass greens. The upper edge of the bags was even with soil line.
2. The bags containing the wheat-based inoculum caused low levels of disease shortly after burial. Conversely, the bags with the turf-based inoculum rarely, if ever, caused disease. Disease was attributed to the bags since the natural epiphytotic had not occurred yet in this area.
3. At the time of the natural epiphytotic in August and September, NO disease from the bags occurred from either inoculum type.
4. Reisolation of *S. homoeocarpa* from the internal region of the bags resulted in slow-growing colonies that were almost overlooked due to the great difference in the growth patterns and morphologies of the laboratory culture.
5. *S. homoeocarpa's* normal growth type is rapid and floccose. This morphotye occurs in laboratory-adapted cultures and is obtained is the pathogen is isolated from infected turf. The slow-growing phase of the organism and the rapid-growing phase are very different.
6. After a week of incubation, the slow-growing *S. homoeocarpa* colonies from the buried inoculum suddenly begin to grow rapidly and become indistinguishable from the rapid-growing phase. The sudden explosive development is the only way that we could recognize the pathogen on the plates. We are still attempting to isolate the pathogen from the dark stromal area on the surface of the bags.

These observations have permitted us to develop some concepts regarding how *S. homoeocarpa* may survive and cause epiphytotics. These are provided below.

- Data suggest that *Sclerotinia homoeocarpa* in soil has a slow-growing near-dormant phase that may not be infective. The lack of infectivity is suggested by the fact that the pathogen in our buried bags, which was in the slow-growing phase, did not cause disease during the time when natural epiphytotics were occurring. It is very difficult to isolate the fungus in this phase; this difficulty has no doubt has interfered with research on the presence, etiology and epidemiology of this disease.

- It may well be that *Sclerotinia homoeocarpa* has two phases - a near-dormant, heretofore undescribed, phase and the expected rapidly growing phase described by other researchers. It is tempting to speculate that the slow-growing (near-dormant) phase may be a survival mechanism and that the rapid-growing phase is the infective one. If so, then the mechanisms that cause the shift between the two phases could be the trigger for the onset of epiphytotics that are typical of the disease.

Upcoming Work

1. Turf Bags - Some of the bags buried in 1998 will be allowed to overwinter and will be recovered in the spring. We will examine the effects of overwintering and determine what, if any, dormancy structures are present in the bags and the surrounding turf. This fall, more bags containing inoculum from our strain, as well as bags containing small cores of naturally diseased turf will be placed in the field similar to last year. Some bags will be recovered in 1999 and some will be left to overwinter to 2000.

2. Genetic Diversity - Genetic assays will be performed during the winter on the isolates obtained this year (Table 1). Assays will include RAPDs (Randomly Amplified Polymorphic DNA) and anastomosis groupings, as well as any new techniques that are applicable. We will compare the similarities and differences between isolates from the same area and host species relative to ones from different geographical areas or hosts.

3. Greenhouse Assays - Flats of creeping bentgrass and possibly other species of turfgrass will be grown in the greenhouse and inoculated with the isolates of *S. homoeocarpa* (Table 1) to assess pathogenicity of the isolates. Dead or infected turf will be inspected for structures related to infection by means of microscopic examination following clearing and trypan blue staining. We will observe how different strains might behave differently.

4. Selective Medium for *S. homoeocarpa* - We will continue to investigate new methods for recovering and enumerating the dollar spot pathogen. Due to competition from *Trichoderma*, *Gliocladium* and *Penicillium* spp., the dollar spot pathogen rarely shows up even when we know it is present. Therefore, we need to eliminate these faster growing species in order to recover and enumerate *S. homoeocarpa*. This may be useful, for instance, for perhaps predicting epiphytotics.

Experimental Plan

Preparation and Burial of Turf Bags

Flats of 'Penncross' creeping bentgrass were grown in the greenhouse and inoculated with our isolate of *Sclerotinia homoeocarpa*. Once disease appeared, infected seedlings were removed from the flats and sealed in nylon mesh bags (4 x 2 cm; 75 micron openings) using an Eurosealer brand bag sealer. Sterile wheat grains were also inoculated with our strain of *S. homoeocarpa* and this inoculum was also sealed in the nylon bags.

Using a small knife, slits were cut into the turf and each bag was buried vertically lengthwise, in a separate slit, with the sealed edge at the soil surface. The bags were buried in three different soils/conditions: a sand research green and a soil research green at the Cornell University Turfgrass Research Facility as well as in a golf course fairway at the Robert Trent Jones Golf Course at Cornell, Ithaca, NY. Fungicides were not applied to these areas.

Enumeration and Recovery of* S. homoeocarpa *from Soil

The dollar spot fungus was in contact with the soil and could grow through the mesh openings and cause disease. The nylon bags do not degrade in the soil, so the bags and their contents could be retrieved for examination.

Methods for selectively isolating and enumerating the dollar spot pathogen from soil and plants are being investigated. Insofar as we are aware, there are no media or specific conditions that permit *S. homoeocarpa* to be specifically isolated and enumerated. We have prepared media containing the fungicide Heritage that effectively eliminate growth of many other fungi but still permit *S. homoeocarpa*, which is resistant to the fungicide, to grow. Unfortunately, naturally occurring *Trichoderma* and *Gliocladium* spp. also are resistant to Heritage so we are still searching for a method to reduce or at least minimize their growth.

***Isolates of* S. homoeocarpa**

Dollar spot disease was evident at other places at the Cornell Turf Facility. Samples of diseased turf were collected for isolation of the pathogen. We also received isolates and/or samples of diseased turf from researchers and golf course managers from across the United States and Canada (see Table 1). These isolates will be used this winter for RAPDs and anastomosis groupings to compare their genetic similarities or differences.

Observations

Recovered Bags

Bags were recovered periodically from the greens throughout the season for macro- and microscopic examination. After a month or so of contact with the soil, the consistency of the wheat-based inoculum inside the bags changed substantially. Originally light and fluffy, the wheat-fungus mixture coalesced into a dense mass. The outside bag surfaces were covered with thick black stromal tissue. When the bags were placed in petri dishes, only the side in contact with the dish continued to form stroma. Under the light microscope, the stroma appeared to be a dense mass of hyphae, tightly interconnected.

The consistency of the turf-based inoculum did not change to any great extent and limited stromal production was seen.

Attempts to reisolate the pathogen were hampered by heavy colonization of *S. homoeocarpa* on either the diseased turf or from wheat-based inoculum by *Trichoderma* and *Gliocladium* spp. These latter naturally occurring biocontrol fungi appear to find *S. homoeocarpa* a favored organism to colonize.

Infected Turf

We recovered turf infected by inoculum in the bags as well as by natural infection. The infected plant tissue was examined under the light microscope. One of the techniques used to show the distribution of fungal hyphae on the plants was trypan blue staining of the cleared turf tissue. The blue stained hyphae of *S. homoeocarpa* can be readily distinguished based on their larger size.

We found that the region most heavily colonized by our strain in these studies was the crown region. The *S. homoeocarpa* hyphae there were extremely melanized and very numerous, forming a dense mass. This was found in naturally infected turf tissue as well as in tissue infected with our strain. The root region was the next most colonized area while the leaf blade region was colonized almost not at all. We were intrigued by this finding since *S. homoeocarpa* is considered a foliar pathogen but does not seem, at least in our findings from this experiment, to colonize the foliage to any great extent.

In August and September a natural epiphytotic occurred as usual. However, as noted elsewhere, no disease developed from our buried bags that contained the pathogen. As expected, natural disease was much more severe in older areas than in newly seeded turf where dollar spot was not established. However, it eventually developed in both areas. Turf naturally infected with dollar spot showed similar colonization of the crown and root regions but the leaves were colonized more heavily than with our strain.

Pathogen Isolation

We attempted to reisolate *S. homoeocarpa* from the bags in September. This fungus grows rapidly and forms a dense hyphal network when grown on standard laboratory media. The same rapid-growing morphology was obtained also from isolations from infected turf. However, the pathogen obtained from soil, even the same strain, behaved very differently. Instead of the rapid-growing vigorous colonies that researchers recognize as *S. homoeocarpa*, very slow-growing isolates were obtained. These initially did not resemble *S. homoeocarpa* since they were such slow-growing, dense colonies. When the pathogen was inoculated onto plates from a laboratory culture, rapid-growing colonies were evident within 24 hr and reach the edges of petri dishes (9 cm. in diameter) within two to three days. Thus we expected rapidly growing colonies and did not recognize the pathogen upon observation following dilution plating. Seven days after plating, the colonies recovered from the interior of were only about 2 mm in diameter.

Our summaries and conclusions, along with work we expect to do in the upcoming months, are provided in the Executive Summary.

Table 1. *Sclerotinia homoeocarpa* Isolate Inventory - 10/98

Isolate #	ID #	Who	Where	Turf	Area	Date	Comments
1	Sh101ko	Ondik	*CUTF - soil green	"Penncross"	green	Jul-98	
2	Sh102ko	Ondik	CUTF - soil green	"Cobra"	green	Aug-98	no trts
3	Sh103ko	Ondik	CUTF - soil green	"Cobra"	green	Aug-98	biocontrol plot
4	Sh104ko	Ondik	CUTF - near sand green	Agrostis	rough	Aug-98	near bldg off Bluegrass Ln.
5	Sh105ko	Ondik	North Carolina	"Crenshaw"	green?	Sep-98	Lyford/Peacock
6	Sh106ko	Ondik	Weston Golf Club, MA	Agrostis/Poa	16fway	Aug-98	Don Hearn, sample 3
7	16A-Vargas	Vargas	Michigan St. Univ.	Poa annua/ Agrostis	fway	1993	DMI resistant
8	16B-Vargas	Vargas	Michigan St. Univ.	Agrostis	#unkn	1970s	common strain
9	16C-Vargas	Vargas	Michigan St. Univ.	Agrostis	unkn	1984	Benomyl resistant
10	16E-19-Vargas	Vargas	Michigan St. Univ.	Agrostis	green	1981	iprodione resistant
11	SH1-Nebraska-A	Giesler	John Seaton Anderson ->	bluegrass	unkn	1994	
12	SH1-Nebraska-B	Giesler	Turf Research near Ithaca, NE	bluegrass	unkn	1994	
13	Sh107ko	Ondik	Rutgers Univ.; Res. Farm II	"Crenshaw"	fway	Oct-98	Bruce Clarke; sample 1
14	Sh108ko	Ondik	Rutgers Univ.; Res. Farm II	"Crenshaw"	fway	Oct-98	Bruce Clarke; sample 3
15	Sh109ko	Ondik	Nashawtuc CC; Concord, MA	K blue/rye/fescue	fway	Sep-98	sample 2
16	Sh110ko	Ondik	Nashawtuc CC; Concord, MA	K blue/rye/fescue	fway	Sep-98	sample 3
17	Sh111ko	Ondik	CUTF - sand green	Agrostis	green	Sep-98	new sand green
18	Sh112ko	Ondik	CUTF - sand green	Agrostis	green	Sep-98	new sand green
19	Sh113ko	Ondik	CUTF - sand green	Agrostis	green	Sep-98	new sand green
20	S-9-Penn	Uddin	Penn State	unkn	unkn	unkn	
21	S-82-Penn	Uddin	Penn State	unkn	unkn	unkn	
22	S-83-Penn	Uddin	Penn State	unkn	unkn	unkn	
23	Sh48B	Walsh	Cambridge, Ontario	Agrostis	unkn	early 90s	Brenda Walsh
24	Sh101BW	Walsh	Guelph Turfgrass Institute	Agrostis	green	Jul-96	Brenda Walsh
25	Sh105BW	Walsh	Guelph Turfgrass Institute	Agrostis	green	May-97	Brenda Walsh
26	Sh115BW	Walsh	Guelph Turfgrass Institute	Agrostis	green	Jul-97	Brenda Walsh
27	Sh123BW	Walsh	Guelph Turfgrass Institute	Agrostis	green	May-98	Brenda Walsh

*CUTF=Cornell University Turf Facility

#unkn=unknown