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

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ABSTRACT

SUMMARY AND CONCLUSIONS

The first objective was 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. Our data suggest that the basic biology of *Sclerotinia homoeocarpa*, including the form in which it exists when it is not causing disease, is not known. Further, there may be at least two stages of the life cycle: one is a slow-growing quiescent phase and the other is a pathogenic and aggressive phase. However, even the quiescent phase could not be detected in sites where the pathogen killed the turf. We have not determined where it survives or the form that it is in. Preliminary observational data indicated that the dollar spot lesion itself does not contain living hyphae but rather that the pathogen is located outside of the lesion. An understanding of this life cycle should provide new capabilities of controlling the pathogen, including intercepting the pathogen before significant disease occurs as a consequence of secondary infection. The second objective was and to measure the genotypic variation of the pathogen from similar and diverse geographical locations using RAPD analysis anastomosis groupings. A collection was made that contains a range of isolates from different areas and different hosts. This collection has been sorted to indicate differences in genetic diversity. There is a substantial level of genetic heterogeneity in the strain collection. Resistance to pesticides appears to differ substantially between strains. It may be that variability exists in the resistance to fungicides and that continued application results in selection of resistant phenotypes. Studies on the relationship between genetic diversity and pathogenicity to different cultivars of bentgrass are underway. Differences in pathogenicity must be understood in order for control programs, ranging from resistant varieties to chemical or biological control, to be optimally successful. Our first data suggest that agressiveness of isolates differ between bentgrass cultivars. If so, then the native diversity within Sclerotinia homoeocarpa isolates must be considered in breeding or other efforts to develop disease-resistant bentgrass.

OBJECTIVES

This research was designed to investigate the following objectives:

1. **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.**

2. **To measure the genotypic variation of the pathogen from similar and diverse geographical locations using RAPD analysis and anastomosis groupings.**

OBJECTIVE 1.

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.

EXPERIMENT 1: MESH BAGS

Plant tissue infected with *Sclerotinia homoeocarpa* (S.h.) or S.h. grown on autoclaved wheat kernels were added to mesh bags to provide a very high inoculum potential. The bags were buried in greens soils in the fall. We assessed survival as indicated by plating.

RESULTS FROM EXPERIMENT 1

1. Early in the year, prior to the dollar spot epiphytotic elsewhere, disease was observed from bags containing autoclaved wheat inoculum. However, when natural dollar spot occurred, no disease developed from either type of bag inoculum.

2. Plating experiments were surprising. S.h. could not be isolated from bags containing infected turf.

3. From wheat-grown inoculum, bags became heavily colonized with a dark stromal rind of the pathogen. However, it could not be isolated from this outer layer.

4. From inside the bags we could isolate S.h. However, it was very slow growing and initially was unrecognizable as S.h. After extended growth on a common mycological medium (potato dextrose agar + a colony size restrictor) that colonies sectored and became typical of the pathogen.

5. This data suggest that S.h. enters a 'quiescent phase' where growth is slow or even absent. Does it revert to the rapid-growing phase when epiphytotic occurs?

6. We obtained turf from artificial (greenhouse) inoculations for microscopic observations. Two goals: (1) recognition of S.h. hyphae in tissue and (2) identification of initial site of infection. Primary initial infection was observed to be restricted to the crown and the sheath surrounding the leaf, but that this initial infection did not occur within the leaf tissue

EXPERIMENT 2: WOODEN GOLF TEES

The results found above were discussed with Greg Boland and his colleagues at Guelph in the fall of 1999. They suggested examination of the above factors by following natural infection. Greg's graduate student, Brenda Walsh, had done experiments in which she placed golf tees in

sites where lesions occurred. This allowed sampling of the exact spot where the spots occurred up to a year later.

RESULTS FROM EXPERIMENT 2

1. We attempted to S.h. from isolate the turf from old spots. We attempted to isolate from roots, from crowns, leaves and the entire plants, either with or without grinding and surface sterilization were plated. We were unable to isolate S.h. from any of these.

2. Consequently, we marked areas where many dollar spots coalesced. We could not isolate S.h. from the center of the spot (dead grass) but we could isolate from active lesions on still-green grass, even when dollar spot was still active in plots.

3. Microscopic observations: S.h. could be observed on turf leaves OUTSIDE of the lesions but S.h. hyphae were not evident WITHIN typical lesions. Instead, we viewed what appeared to be degraded hyphae in lesion centers.

Taken together, these observations suggest that S.h. may have a quiescent phase from which isolation is difficult. Moreover, the survival structures of the pathogen were not identified based upon isolation from any but active lesions.

QUESTIONS:

1. WHERE DOES THE S.H. GO/HOW DOES IT SURVIVE?
2. WHY CAN'T WE ISOLATE IT?
3. WHERE IS IT THE REST OF THE TIME?
4. WHAT 'PHASE' IS IT IN?

IMPLICATIONS FOR MANAGEMENT OF DOLLAR SPOT

1. We do not understand the biology of S.h. It seems to disappear after infection. Where and how does it survive?

2. Why does it suddenly cause epiphytotics? If the trigger is found (which necessitates discovery of the survival structure(s) and ability to monitor), perhaps the initial steps could be identified. If so, perhaps *S. homoeocarpa* could be controlled before it becomes damaging to turf.

OBJECTIVE 2:

To measure the genotypic variation of the pathogen from similar and diverse geographical locations using RAPD analysis and anastomosis groupings.

The first step was to make a collection of isolates. The final collection contains 37 isolates obtained from different geographical locations and, in a few cases, from different hosts (Table 1).

Anastomosis examinations

We frequently observed hyphal interactions; however, same versus same interactions are qualitatively equal to strain A versus strain B interactions. These interactions were interesting but their significance cannot be assessed at this time.

RAPD analysis

RAPDs clearly demonstrated great variability between strains. ALL strains can be separated from one another on the basis of only two RAPD primers EXCEPT ones that were obtained from the same location (good internal check).

Strains were grouped based on similarity by RAPDs. This is somewhat tentative because different cladistics programs give different cladograms. However, this process at the least provided groupings that are sorted according to genetic diversity. A strain collection that is selected based on strain diversity is valuable for other studies.

What is the importance of all of this genetic diversity?

Pesticide Resistance

Do strains differ in susceptibility to fungicides? What is the potential for simple selection among strains to give resistance to pesticides versus requiring changes (mutation) in the strain? If the former is true, then pesticides would be expected to have shorter life spans. If the latter is true, then resistance should develop more slowly to the pesticides.

We examined, in vitro, resistance or susceptibility to various fungicides by representatives of each grouping from the cladogram. Variability again was found, which suggests that a measure of natural resistance occurs within populations and that mutation or other changes do not have to occur to provide this resistance. Most strains exhibited some resistance to azoxystrin, chlorothalonil and triadimefon and levels of inhibition differed substantially between strains. With iprodione, however, only one strain exhibited any resistance to even low concentrations of the fungicide. This strain was provided to us from Joe Vargas at Michigan State and labeled as being resistant to iprodione (Fig. 1). Of course, these strains were isolated from areas where fungicides were applied, so selection has already occurred. However, since strains are genetically diverse, resistance may occur naturally and may increase with selection.

Pathogenicity

Do strains differ in pathogenicity? Are certain strains more capable than others of causing disease, in general? Does pathogenicity by strains differ for different cultivars of bentgrass?

Tests are underway now. Preliminary data on four cultivars of bentgrass (G2, L93, Putter, and Penncross) indicate that individual strains differ in their aggressiveness towards bentgrass. Some strains caused rapidly expanding spots on all four cultivars while other strains were strongly

aggressive to only one or two cultivars. The cultivars differed in their susceptibility as well; one cultivar was severely damaged by all four strains tested while another variety was more resistant to the strains tested to date.

Data from the pathogenicity studies are important:

1. This information is critical to any program that seeks to produce disease-resistant varieties by conventional, transgenic or other methods.

2. If strains are not pathogenic, they may be useful as biocontrol agents.

3. Strains that differ in pathogenicity may be extremely useful in understanding the bases of pathogenicity of S.h. to turf through genomics-based or other approaches.

4. An understanding of the bases of pathogenicity may provide new concepts for disease control through plant breeding, biocontrol or pesticidal approaches. For example, if a toxin is involved or primarily required for pathogenicity, the goals for control based on resistance or control of toxin production would be very different than the approaches if a toxin is not involved.

Table. 1. Isolates collected

Sclerotinia homoeocarpa Isolate Inventory

Isolate #	ID #	Who	Where	Turf	Area	Comments
1	sh101ko	Ondik	CUT* - soil green	"Penncross"	green	
2	sh102ko	Ondik	CUT - soil green	"Cobra"	green	no trts
3	sh103ko	Ondik	CUT - soil green	"Cobra"	green	biocontrol plot
5	sh105ko	Ondik	North Carolina	"Crenshaw"	green?	Lyford/Peacock
6	sh106ko	Ondik	Weston Golf Club, MA	Agrostis/Poa	16 fway	Don Hearn, sample 3
7	16A-Vargas	Vargas	Michigan St. Univ.	Poa annua/Agrostis	fway	DMI resistant
8	16B-Vargas	Vargas	Michigan St. Univ.	Agrostis	unkn	common strain
9	16C-Vargas	Vargas	Michigan St. Univ.	Agrostis	unkn	Benomyl resistant
10	16E-19-Vargas	Vargas	Michigan St. Univ.	Agrostis	green	iprodione resistant
11	SH1-Nebraska	Giesler	John Seaton Anderson	bluegrass	unkn	
12	SH1-Nebraska	Giesler	Turf Research near Ithaca, NE	bluegrass	unkn	
13	sh107ko	Ondik	Rutgers Univ.; Res. Farm II	"Crenshaw"	fway	Bruce Clarke; sample 1
14	sh108k o	Ondik	Rutgers Univ.; Res. Farm II	"Crenshaw"	fway	Bruce Clarke; sample 3
15	sh109ko	Ondik	Nashawtuc CC; Concord, MA	K blue/rye/fescue	fway	sample 2
16	sh110ko	Ondik	Nashawtuc CC; Concord, MA	K blue/rye/fescue	fway	sample 3
17	sh111ko	Ondik	CUT - sand green	Agrostis	green	new sand green
18	sh112ko	Ondik	CUT - sand green	Agrostis	green	new sand green
19	sh113ko	Ondik	CUT - sand green	Agrostis	green	new sand green
20	S-9-Penn	Uddin	Penn State	unkn	unkn	
21	S-82-Penn	Uddin	Penn State	unkn	unkn	
22	S-83-Penn	Uddin	Penn State	unkn	unkn	
27	Sh123BW	Walsh	Guelph Turfgrass Institute	Agrostis	green	
28	ShVWA3	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	cool season?
29	ShVWC4	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	cool season?
30	ShVWD3	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	cool season?
31	ShVWF8	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	cool season?
32	ShVWK1	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	cool season?
33	UK-1	Vincelli	Univ. Kentucky Turf Center	unkn	unkn	triadimefon tolerable
34	UK-2	Vincelli	Univ. Kentucky Turf Center	unkn	unkn	triadimefon tolerable
35	CB-1	Vincelli	Cabin Brook Golf Course	unkn	unkn	triadimefon tolerable
36	CB-2	Vincelli	Cabin Brook Golf Course	unkn	unkn	triadimefon tolerable
37	sh114ko	Ondik	Smokin' Joe's Golf Course	unkn	unkn	sample 1A

Sclerotinia homoeocarpa Isolate Inventory

Isolate #	ID #	Who	Where	Turf	Area	Comments
38	sh115ko	Ondik	Smokin' Joe's Golf Course	unkn	unkn	sample 2A
39	sh116ko	Ondik	Smokin' Joe's Golf Course	unkn	unkn	sample 3A
40	sh117ko	Ondik	Dutchess Golf & Country Club	unkn	14 fway	difficult to control
41	sh118ko	Ondik	Dutchess Golf & Country Club	unkn	16 fway	A-difficult to control
42	sh119ko	Ondik	Dutchess Golf & Country Club	unkn	16 fway	B-difficult to control

*CUT=Cornell University Turfgrass Research and Education Facility

Fig. 1. Inhibition of growth of S.h. strains by various fungicides.