THE BASIC BIOLOGY AND ETIOLOGY OF *SCLEROTINIA HOMOEOCARPA*, THE CAUSAL AGENT OF DOLLAR SPOT

Gary E. Harman[1], Kristen L. Ondik[1] and Eric B. Nelson[2]

[1] Department of Horticultural Sciences, Cornell University, NYSAES, Geneva, NY 14456;
[2] Department of Plant Pathology, Cornell University, Ithaca, NY 14850

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.

Mesh bags containing an isolate of the fungus were buried in a bentgrass green and then retrieved at various times throughout the year. Upon examination we found that the fungus exhibited two distinct growth phases. One phase grows rapidly and is characterized by copious amounts of hyphae. The other phase is slow growing with smaller colonies for about seven days and then a sudden burst of rapid growth typical of the first phase.

- We hypothesize that *S. homoeocarpa* is present most of the time on turf in a dormant, nonpathogenic state. When directed by environmental or other cues, the fungus suddenly grows rapidly and becomes pathogenic, causing an explosive epiphytotic.

In order to examine the natural infection of dollar spot, colored tees were used to mark certain dollar spots from which turf samples can be taken repeatedly over time. Nonsymptomatic areas were also marked. As expected, samples taken in August (when dollar spot was infective) showed the actively growing phase but not the dormant phase. In November we expect to see the dormant phase of the fungus since the infection phase is finished. Sampling will then resume in the spring, when we once again expect to see the dormant phase, and will continue into late summer when dollar spot epiphytotics usually occur in upstate New York.

Epiphytotics of dollar spot can occur at various times in different areas of the United States and pathogenicity levels can vary depending on turfgrass cultivar. Understanding the genetic diversity of *S. homoeocarpa* is important for controlling and managing the disease. Thus, we collected over 50 isolates of the dollar spot fungus from around the US and Canada to determine their genetic relatedness using RAPDs and anastomosis groupings. Our results are as follows:

- Great diversity exists among collected isolates of *S. homoeocarpa.*
- Only a few isolates share the same overall banding pattern, mostly those from the same location and the same cultivar of turfgrass.
- The isolate sh105ko demonstrates an unusual growth pattern during anastomosis pairings: it coils around the hyphae of the other isolate similar to coiling characteristic of biocontrol interactions.

UPCOMING RESEARCH

1. Mitochondrial DNA (mDNA) assays will be performed to provide a more complete genetic survey of the isolates. Cladistic analyses will be completed to obtain groups with most similar and most dissimilar genotypes.
2. We will determine whether dissimilar genetic types have similar or different modes of infection and levels of pathogenicity to bentgrass.
3. We will continue to isolate and observe the pathogen from bags in soil and from naturally-infected turf.

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

Gary E. Harman[1], Kristen L. Ondik[1], Eric B. Nelson[2]

[1] Department of Horticultural Sciences, Cornell University, NYSAES, Geneva, NY 14456;
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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.

POTENTIAL BENEFITS

Efficient control of plant pests nearly always depends upon knowledge of their life cycles and some knowledge of the variability and differences between different forms of the pathogen. This knowledge is totally lacking in the case of dollar spot caused by *Sclerotinia homoeocarpa*. Even such basic knowledge as whether the pathogen(s) is a single species or how it survives and infects turf plants each year largely is unknown. This study aims to provide practical knowledge of the pathogen and to identify its "Achilles heel(s)." Such basic knowledge can be used to provide more cost-effective control of this serious disease.

IMPORTANCE OF THESE STUDIES

Dollar spot occurs as an explosive epiphytotic. The disease is not present early and then rapidly becomes epidemic on turf. Why does this occur and does it offer methods for control?

1. We must determine when and how dollar spot infects turf.
2. We must determine whether or not apothecia are formed; this could be important as a mechanism of genetic exchange/variability and could explain why explosive epiphytotics occur.
3. These studies can enhance our knowledge of the life cycle of the pathogen/disease, which may be important in control strategies.
4. We have only fragmentary knowledge of the genetics and diversity of *S. homoeocarpa*. We do not know:
   A. Whether or not the pathogen consists of a group of closely related strains or whether they are genetically diverse or even different species or genera.
   B. Whether strains that are genetically different have different properties such as:
      i. Differential resistance to fungicides.
      ii. Different levels of pathogenicity to different species or cultivars of turfgrass.
   C. Whether all are pathogenic to turf, and whether some are nonpathogenic and might actually function as biocontrol agents of other strains.

BACKGROUND

Mesh bags containing an isolate of the fungus were buried in a bentgrass green and then retrieved at various times throughout the year. Upon examination we found that the fungus exhibited two distinct growth phases. One phase grows rapidly and is characterized by copious amounts of hyphae. The other phase is slow growing with smaller colonies for about seven days and then a sudden burst of rapid growth typical of the first phase.

- We hypothesize that *S. homoeocarpa* is present most of the time on turf in a dormant, nonpathogenic state. When directed by environmental or other cues, the fungus suddenly grows rapidly and becomes pathogenic, causing an explosive epiphytotic.

In order to examine the natural infection of dollar spot, colored golf tees were used to mark certain dollar spots from which turf samples can be taken repeatedly over time. Nonsymptomatic areas were also marked. Sampling began in August.

Epiphytotics of dollar spot can occur at various times in different areas of the United States and pathogenicity levels can vary depending on turfgrass cultivar. Understanding the genetic diversity of *S. homoeocarpa* is important for controlling and managing the disease. Thus, we collected over 50 isolates (Table 1) of the dollar spot fungus from around the US and Canada to determine their genetic relatedness using RAPDs (Fig. 1) and anastomosis groupings. RAPDs provide information on differences among genomes by amplification of random pieces of DNA. If RAPDs are highly different, the overall genomes should be different.

RESULTS

Buried bags

1. The bags, when placed into turf plots did not give rise to disease except early in the season, before the normal epiphytotic of dollar spot occurred.

2. The inoculum within bags (we tested one strain only), especially from wheat-based inoculum, formed a heavy black stroma over the outer surface of the bag but no stroma were observed within the bags.

3. The pathogen could be isolated from the interior of the bags on wheat-grown inoculum but not from interiors of bags containing diseased turf.

4. The pathogen obtained from the interior of the bags appeared as very slow-growing colonies that were atypical of *S. homoeocarpa*.

5. Upon further culture, the slow-growing isolates would finally revert to their normal fast-growing phenotype. This indicates that there is a slow-growing, nearly quiescent stage of development of the pathogen and another rapid-growing stage.

6. In other greenhouse studies, the sheath and the root-crown regions of bentgrass seedlings were observed to be the preferred site of infection. However, in the field, the leaf blade was also infected.

Natural infection sites

1. As expected, samples taken in August (when dollar spot was infective) showed the actively growing phase but not the dormant phase. In November we expect to see the dormant phase of the fungus since the infection phase is finished. Sampling will then resume in the spring, when we once again expect to see the dormant phase, and will continue into late summer when dollar spot epiphytotics usually occur in upstate New York.

Genetic analyses

1. We have completed analyses on more than 50 strains using different primers.

2. Most strains provide distinctly different patterns (Fig. 1) from any other strain, indicating a high degree of variability between strains.

3. Strain sh105ko, which was obtained from diseased turf from North Carolina, has unusual properties. It exhibits coiling about hyphae of other strains, which is typical of mycoparasitic biocontrol fungi and not of anastomoses.

SIGNIFICANCE OF FINDINGS TO DATE

The findings to date suggest that there are undefined and unidentified components of the life cycle and infection of turf by *S. homoeocarpa.*

A possible disease cycle for dollar spot

1. The pathogen overwinters as hyphae and stroma. The organism is in a semi-quiescent, survival mode. It is not clear on what tissues or in what form this survival occurs.

2. In response to undefined environmental cues, the pathogen enters its active, rapidly growing and pathogenic phase.

3. Initial infections occur in the leaf sheath and crown of turf.

4. During mowing and in traffic, the newly infected turf plants establish rapidly-growing infections on leaves of new turf plants. This is the point when disease symptoms become obvious and the explosive phase of the disease begins.

5. Eventually, again in response to undefined cues, the pathogen re-enters its survival phase, the epidemic slows and stops and the pathogen probably survives on infected plant tissue.

If this disease cycle does indeed occur, and this is unproven, then detection of steps 2 and 3 might permit control of the disease before step 4 occurs, with less visible disease and requiring less fungicide use.

Challenges:

1. We must determine whether this disease cycle occurs.
2. Even though this cycle occurs with one strain, is this cycle the same for all strains?
3. Regardless, this example indicates that there are gains to be made in control of this important pathogen if we fully understand its life cycle.

Most strains differ at the genetic level as indicated by RAPD analyses, indicating a high level of variability in the pathogen. Given this, several important questions need to be answered:

1. Are strains pathogenic to different species or cultivars of turfgrass pathogenic to other turf species or cultivars? Can this pathogenicity be related to specific genotypes?

2. Do strains resistant to specific fungicides have a similar genotype, i.e., does resistance occur because of selection with a diverse population of pathogens or does resistance occur because of mutations and modifications within strains?

3. In short, do different genotypes have similar disease cycles, pathogenicity levels and resistance to fungicides?

CONTINUING RESEARCH

1. With RAPDs completed, we will begin mitochondrial DNA analyses.

2. Follow up genetic survey with cladistic analyses to obtain groups with most similar and most dissimilar genotypes.

3. Determine whether dissimilar genetic types have similar or different modes of infection and levels of pathogenicity to bentgrass. Differential cultivars??

4. Continue to isolate and observe the pathogen from bags in soil and from naturally-infected turf.

Sclerotinia homoeocarpa Isolate Inventory - 11/99

Isolate #	ID #	Who	Where	Turf	Area	Date	Comments
1	sh101ko	Ondik	CUT* - soil green	"Penncross"	green	7/1/98	
2	sh102ko	Ondik	CUT - soil green	"Cobra"	green	8/25/98	no trts
3	sh103ko	Ondik	CUT - soil green	"Cobra"	green	8/25/98	biocontrol plot
5	sh105ko	Ondik	North Carolina	"Crenshaw"	green?	9/1/98	Lyford/Peacock
6	sh106ko	Ondik	Weston Golf Club, MA	Agrostis/Poa	16 fway	8/24/98	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	10/9/98	Bruce Clarke; sample 1
14	sh108ko	Ondik	Rutgers Univ.; Res. Farm II	"Crenshaw"	fway	10/9/98	Bruce Clarke; sample 3
15	sh109ko	Ondik	Nashawtuc CC; Concord, MA	K blue/rye/fescue	fway	9/29/98	sample 2
16	sh110ko	Ondik	Nashawtuc CC; Concord, MA	K blue/rye/fescue	fway	9/29/98	sample 3
17	sh111ko	Ondik	CUT - sand green	Agrostis	green	9/22/98	new sand green
18	sh112ko	Ondik	CUT - sand green	Agrostis	green	9/22/98	new sand green
19	sh113ko	Ondik	CUT - sand green	Agrostis	green	9/22/98	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	
27	Sh123BW	Walsh	Guelph Turfgrass Institute	Agrostis	green	May-98	
28	ShVWA3	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	Sep-98	cool season?
29	ShVWC4	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	Sep-98	cool season?
30	ShVWD3	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	Sep-98	cool season?
31	ShVWF8	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	Sep-98	cool season?
32	ShVWK1	Walsh	Victoria West GC, Guelph, Ontario	Agrostis/Ann. Blue	tee	Apr-99	cool season?
33	UK-1	Vincelli	Univ. Kentucky Turf Center	unkn	unkn	Jun-99	triadimefon tolerable

*CUT=Cornell University Turfgrass Research and Education Facility

Sclerotinia homoeocarpa Isolate Inventory - 11/99 (Cont.)

Isolate #	ID #	Who	Where	Turf	Area	Date	Comments
34	UK-2	Vincelli	Univ. Kentucky Turf Center	unkn	unkn	Jun-99	triadimefon tolerable
35	CB-1	Vincelli	Cabin Brook Golf Course, KY	unkn	unkn	Jun-99	triadimefon tolerable
36	CB-2	Vincelli	Cabin Brook Golf Course, KY	unkn	unkn	Jun-99	triadimefon tolerable
37	sh114ko	Ondik	Smokin' Joe's GC, Lewiston, NY	unkn	unkn	Sep-99	sample 1A
38	sh115ko	Ondik	Smokin' Joe's GC, Lewiston, NY	unkn	unkn	Sep-99	sample 2A
39	sh116ko	Ondik	Smokin' Joe's GC, Lewiston, NY	unkn	unkn	Sep-99	sample 3A
40	sh117ko	Ondik	Dutchess Golf & CC, Poughkeepsie, NY	unkn	14 fway	Sep-99	difficult to control
41	sh118ko	Ondik	Dutchess Golf & CC, Poughkeepsie, NY	unkn	16 fway	Sep-99	A-difficult to control
42	sh119ko	Ondik	Dutchess Golf & CC, Poughkeepsie, NY	unkn	16 fway	Sep-99	B-difficult to control
43	sh120ko	Ondik	Bellport Country Club, Bellport, NY	unkn	11 grn	Oct-99	A-DMI tolerable
44	sh121ko	Ondik	Bellport Country Club, Bellport, NY	unkn	12 grn	Oct-99	A-DMI tolerable
45	sh122ko	Ondik	Bellport Country Club, Bellport, NY	unkn	14 grn	Oct-99	A-DMI tolerable
46	sh123ko	Ondik	Bellport Country Club, Bellport, NY	unkn	16 grn	Oct-99	A-DMI tolerable
47	sh124ko	Ondik	Smokin' Joe's GC, Lewiston, NY	unkn	unkn	Sep-99	sample 1B
48	sh125ko	Ondik	Smokin' Joe's GC, Lewiston, NY	unkn	unkn	Sep-99	sample 2B
49	sh126ko	Ondik	Smokin' Joe's GC, Lewiston, NY	unkn	unkn	Sep-99	sample 2C
50	sh127ko	Ondik	Smokin' Joe's GC, Lewiston, NY	unkn	unkn	Sep-99	sample 3B
51	sh128ko	Ondik	Bellport Country Club, Bellport, NY	unkn	12 grn	Oct-99	B-DMI tolerable
52	sh129ko	Ondik	Bellport Country Club, Bellport, NY	unkn	14 grn	Oct-99	B-DMI tolerable
53	sh130ko	Ondik	Bellport Country Club, Bellport, NY	unkn	16 grn	Oct-99	B-DMI tolerable

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Figure 1

Sclerotinia homoeocarpa
Primer BC335

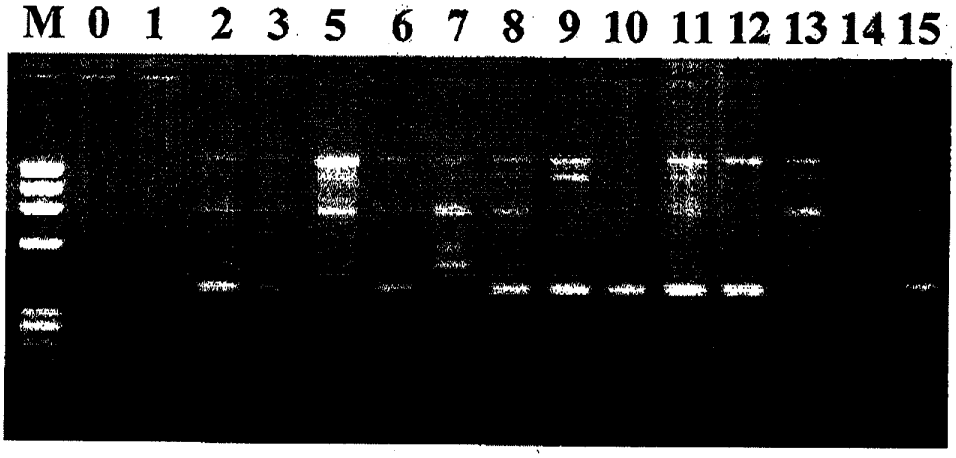

Fig. 1. Example of a gel depicting the RAPDs banding patterns of *Sclerotinia homoeocarpa* DNA from several different isolates. Each number above the picture identifies the isolate used in the corresponding lane below the number. M represents the DNA marker. 0 is an empty lane and isolate 4 is absent from this gel.

Although some isolates have some bands in common, most of the overall banding patterns are different indicating genetic diversity.

Table 1 on the preceding pages provides a complete list of isolates.