EPIDEMIOLOGY OF SCLEROTINIA HOMOEOCARPA IN MICHIGAN: GEOSTATISTICAL AND POPULATION BIOLOGICAL APPROACHES

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Plant Pathology

2003

ABSTRACT

EPIDEMIOLOGY OF SCLEROTINIA HOMOEOCARPA IN MICHIGAN: GEOSTATISTICAL AND POPULATION BIOLOGICAL APPROACHES

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Dollar spot is a severe turfgrass pathogen in North America, and in particular in Michigan. The disease is caused by the pathogen *Sclerotinia homoeocarpa* F.T. Bennett. This fungus is not known to produce sexual or asexual spores, and therefore, its primary mode of transport is via infected grass clippings on equipment and humans. As fungicide use becomes more restricted, it is important to have a basic understanding of the epidemiology of the major turfgrass pathogens. With this goal in mind, this study used both geostatistical and population biological approaches to better understand the epidemiology of this pathogen. The objectives for this project were: 1) to determine if isolates of *S. homoeocarpa* from golf courses in Michigan could be differentiated using amplified fragment length polymorphism (AFLP) markers and vegetative compatibility groups (VCGs), and 2) to quantify the spatial structure of dollar spot incidence and determine its temporal stability.

Five populations were sampled for this study. Using 32 isolates subsampled from these five populations, AFLPs and VCGs were defined for each isolate. I found no relationship between an isolate's AFLP fingerprint and its VCG. Nor was there any apparent relationship between isolates based on

geographic location since some isolates from opposite parts of the state shared the same fingerprint. A total of 889 isolates were collected from three of the populations for further study of the VCG distributions in populations. While 860 isolates fit into the 6 known VCGs present in Michigan, there were also 29 isolates that did not fit in these 6 groups raising the possibility of the presence of additional groups. Chi-square analysis revealed significant differences between VCG distributions between years and locations. At two of the three locations, differences were also found between fairways within a location indicating that each fairway behaved independently.

The geostatistical study was established on a 9.1 m X 18.3 m area of creeping bentgrass (*Agrostis palustris* Huds.) and annual bluegrass (*Poa annua* L.) at the Robert Hancock Turfgrass Research Center in E. Lansing, MI. The study area was subdivided into 223 areas where dollar spot foci were counted over the entire season. Variograms of disease incidence were constructed for each date and showed clear spatial structuring at relatively small scales (~0-10 m). Closer examination of the variogram model parameters showed that the nugget and sill parameters scaled with each other while the range parameter remained fairly constant within each season. Between 50 and 60 percent of the total population variance in each year was spatially structured. This indicates that the spatial structure of dollar spot remains relatively unchanged regardless of disease severity, suggesting that the factor primarily responsible for the spatial pattern is one that does not move about in space.

To all those along the way who believed that this day would come.

ACKNOWLEDGMENTS

I always read with interest other thesis and dissertation acknowledgements. I find myself wondering where they were and what they were thinking about when they set down for posterity those names of people that they would like to thank. I also find myself thinking about what this will mean to me in 30 years when I open it and read it again but instead of being 30 I will be 60 and in the twilight of my career, not setting off on the beginning of it.

The first group of people I would like to say thank you to is my family. Mom and Dad, you have been there when I needed you most. Through all the rough spots when I felt like I was going it alone you were there. Nate, you're my brother, confidant, and one of the best human beings I know. I am proud of all the accomplishments you have made. You truly have defined who you are as a person. Grandpa, your life ended before I could finish this project, but I was very proud of the fact that I wasn't the first person in our family to pursue an agricultural education and I hope you are proud of my accomplishments. Grandma, not everyone can say they have had the relationship with their grandma that I have had with you. Knowing I was just a phone call away from a hot, home-cooked meal in college will mean more than you'll ever know. Vi, Mim, Mac, and Ash- I can't describe what it has meant to me personally to have such good, close relationships with you all.

I don't have enough room to mention all the people whom I have had the distinct privilege of calling my friends. Jason, you have become a true friend, helping me selflessly when my chips were down, no thank you will ever be enough. Ron and

Cindy Detweiler, if you hadn't opened your home and hearts to me twelve months ago, I don't think I would be writing this today. A thank you doesn't seem like enough. Nancy, thanks for all the little tips and tricks that have helped me navigate through graduate school, I will miss the lunches. Phil, thanks for the game it was fun.

My committee, Dr. Crum, Dr. Robertson, and Dr. Jarosz thank you for helping me to steer a pretty clean course through my Ph.D. I learned a lot about how to do good research from all of you. Dr. Sasha Kravchenko, without your help and instruction I wouldn't understand what geostatistics were let alone be able to explain how to do them. Dr. Kurt Lamour and Dr. Mary Hausbeck, your assistance and collaboration allowed me to complete a section of this dissertation on AFLPs without having to reinvent the wheel. Joe, you have been my mentor and have treated my like a colleague. I plan on being your colleague for a long time to come. I would also be remiss if I didn't mention a few select people that are what I would call "shadow mentors", people that have had an immeasurable impact on your life without being asked. Dr. Jim Miller, your class changed my worldview of science forever and I am eternally grateful for those interactions. Dr. Ken Poff, thank you for taking an interest in me and passing on some of your wisdom, you probably don't realize the impact it has had on my view of science. Doubt, in the sense of not knowing what the future holds is a good thing. Jennifer, I struggled with what to say here that would be worthy of how I feel, and I decided on this: I love you. I don't know what the future holds, but I do hope that you will be in it with me.

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Chapter 1

VARIATION OF *SCLEROTINIA HOMOEOCARPA* WITHIN AND AMONG GOLF COURSES IN MICHIGAN

Introduction

Dollar spot disease of turfgrasses is caused by the pathogen, *Sclerotinia homoeocarpa* F.T. Bennett (3). The disease is common throughout the world and is destructive to both cool and warm season grasses (20, 21, 23). In North America, with the exception of the Pacific Northwest, dollar spot is the most important pathogen of most cultivated fine turfgrass species (20, 21, 23). In Michigan, the disease is a major problem for most golf courses where the epidemic begins in June and can continue into late September causing extensive damage if left untreated. The disease can blight large areas of turf as a result of coalescing disease foci. Diseased turf has a poor aesthetic appearance, impairs the playing surface by creating depressions that affect ball roll, and leaves areas of bare soil where weed species can encroach on the area (20, 23). The pathogen is not known to produce conidia or undergo sexual reproduction in North America (1, 10, 11). Jackson found that while United Kingdom isolates of *S. homoeocarpa* will undergo sexual reproduction, no isolate from the US has been known to develop fertile apothecia (11). However, Hsiang and Mahuku (10) reported that some populations in Southern Ontario had random amplified polymorphic DNA (RAPD) patterns consistent with recombination events within a local population. It is commonly assumed that *S. homoeocarpa* is disseminated via direct transfer of mycelium from infected leaves (7, 20, 21, 23).

Control of dollar spot via fungicide application is generally accomplished using the contact fungicide, chlorothalonil. However, the EPA is expected to restrict the use of this fungicide on golf courses. In the event that a limited amount of fungicide is available to a golf course it is critical that superintendents be able to apply chlorothalonil judiciously. Other single-site mode of action fungicides are available to control dollar spot, but fungicide resistanceis a problem in many dollar spot populations (6, 9, 25).

Vegetative compatibility is the ability of the pathogen to form a stable heterokaryon as a result of a self/nonself genetic recognition event when two individual strains fuse (8, 16). The systems can be allelic or non-allelic in nature. Fungi that have an allelic compatibility system determine whether two strains are compatible via identity of alleles at a particular compatibility locus. In contrast, a non-allelic system usually involves alleles at multiple loci interacting to determine compatibility (16). Studies of compatibility are useful for studying diversity in populations, detecting new lineages in a local area, and observing population dynamics. *Aspergillis flavus* was examined in a cotton field using vegetative compatibility groups (VCGs) as a measure of genetic diversity (2). Large numbers of VCGs were identified and the distribution changed from year to year over the three-year study. The large number of VCGs suggests a large change in the genetic makeup of the population each season. The authors suggested the observed diversity could be a result of the migration of conidia from other locations and/or a seasonal change in the number of strains making up each VCG. Kohn *et al.* (15) studied mycelial compatibility, a specific component of

vegetative compatibility, in *Sclerotinia sclerotiorum*. Using DNA fingerprinting techniques, they found that the mycelial compatibility group (MCG) diversity was high and that MCGs made up genotypically distinct lineages. The observed diversity was attributed to be due to the occasional outbreeding event and the migration of new strains into populations. Powell and Vargas (18) identified 6 VCGs from isolates sampled from creeping bentgrass and annual bluegrass from 8 locations in Michigan and the Midwest. They found that the VCG distributions at a location change over a season. They also reported that isolates from the same VCG could be isolated from both creeping bentgrass and annual bluegrass indicating that host specificity is not associated with particular VCGs. Using the sequence of the nuclear internal transcribed spacer region 1 (ITS1) they also found that all sampled isolates shared the same sequence and were from the same species. Raina *et al.* (19) studied the genetic variability of *S. homoeocarpa* using RAPDs and found that isolates of dollar spot from the midwest and northeastern United States were very similar. Both of these studies support the empirical evidence that *S. homoeocarpa* is a clonal pathogen.

In contrast, Sonoda (22) identified more than 54 VCGs of *S. homoeocarpa* isolated from bermudagrass (*Cynodon dactylon*). One hundred nineteen isolates were collected from three locations; nearly 50% represented VCGs, indicating a significant amount of genetic exchange or migration. Hsiang and Mahuku's (10) study using RAPDs of dollar spot isolates from eight populations in Southern Ontario supported random mating in three of the eight populations sampled.

Many different molecular tools are available for the study of plant pathogens (5, 10, 12, 15, 17, 19). Isozymes are relatively inexpensive, but problems often occur in generating enough polymorphic markers to be of use. RFLPs (restriction fragment length polymorphisms) can often be very informative, however suitable DNA probes must be available. The widely used RAPDs (random amplified polymorphic DNA) suffer most generating reproducible results because of sensitivity to running conditions. AFLPs (amplified fragment length polymorphisms) are noted for their ability to rapidly generate large numbers of reproducible and neutral (not under independent selection) markers at independent loci (17, 24). AFLPs avoid the problems inherent in most other tools used for fungal genetic analysis. The primary drawback to AFLPs is the relatively high startup cost. Cilliers *et al.* (5) used AFLP analysis to differentiate isolates and MCGs of *Sclerotium rolfsii* from South Africa. They identified 9 MCGs in a collection of 73 isolates from 10 locations in South Africa. Isolates were identified with a specific MCG using AFLPs.

The objective of this study was to determine if isolates of *S. homoeocarpa* from golf courses in Michigan could be differentiated using AFLP markers and VCGs.

Materials and Methods

Isolation and Culture. Isolates were collected from symptomatic plants infected with *S. homoeocarpa*. Three different locations in Michigan were sampled in July 2000 and 2001 (Fig. 1). Four fairways at each location were

selected for sampling from which symptomatic leaves of 50 infection centers along a transect running the length of the fairway were individually collected in paper coin envelopes. Two to three small segments of leaf tissue displaying lesions were placed on acidified water agar plates (10mL lactic acid/L) and allowed to grow for 2-3 days at 25°C. Hyphae growing out of the leaf tissue were then isolated onto potato dextrose agar (PDA) plates and allowed to grow for about 5 days at 25°C. Using a modified method of Boesewinkel (4), ten agar plugs were removed from the PDA plates using a sterile coffee stirrer and placed in 1.5 mL microfuge tubes containing 1 mL sterile H_2O for long term storage at room temperature. David Gilstrap provided additional isolates from 2 golf courses (Fig. 1) for the AFLP evaluation of the genetic diversity of *S. homoeocarpa*. They were stored in the same manner as the other isolates.

VCGs. All isolates were paired with six tester isolates representing the six known VCGs in Michigan (18) using a method modified from Kohn (15). Sets of four isolates were paired against all six tester isolates in 24 well culture plates. Each well contained 1 mL of PDA amended with 5 drops/L of McCormick's Red Food Coloring to highlight antagonistic zones. Each isolate was also paired with itself as a control. Isolates that were not classified in the first screening were then paired with all six tester isolates on 100 X 15 mm petri dishes to clarify the interactions between the isolate and tester strains. Chi-square (X^2) analyses were performed on the observed frequency distributions of the three most frequent VCGs (A, B, C) using the null hypothesis that there were

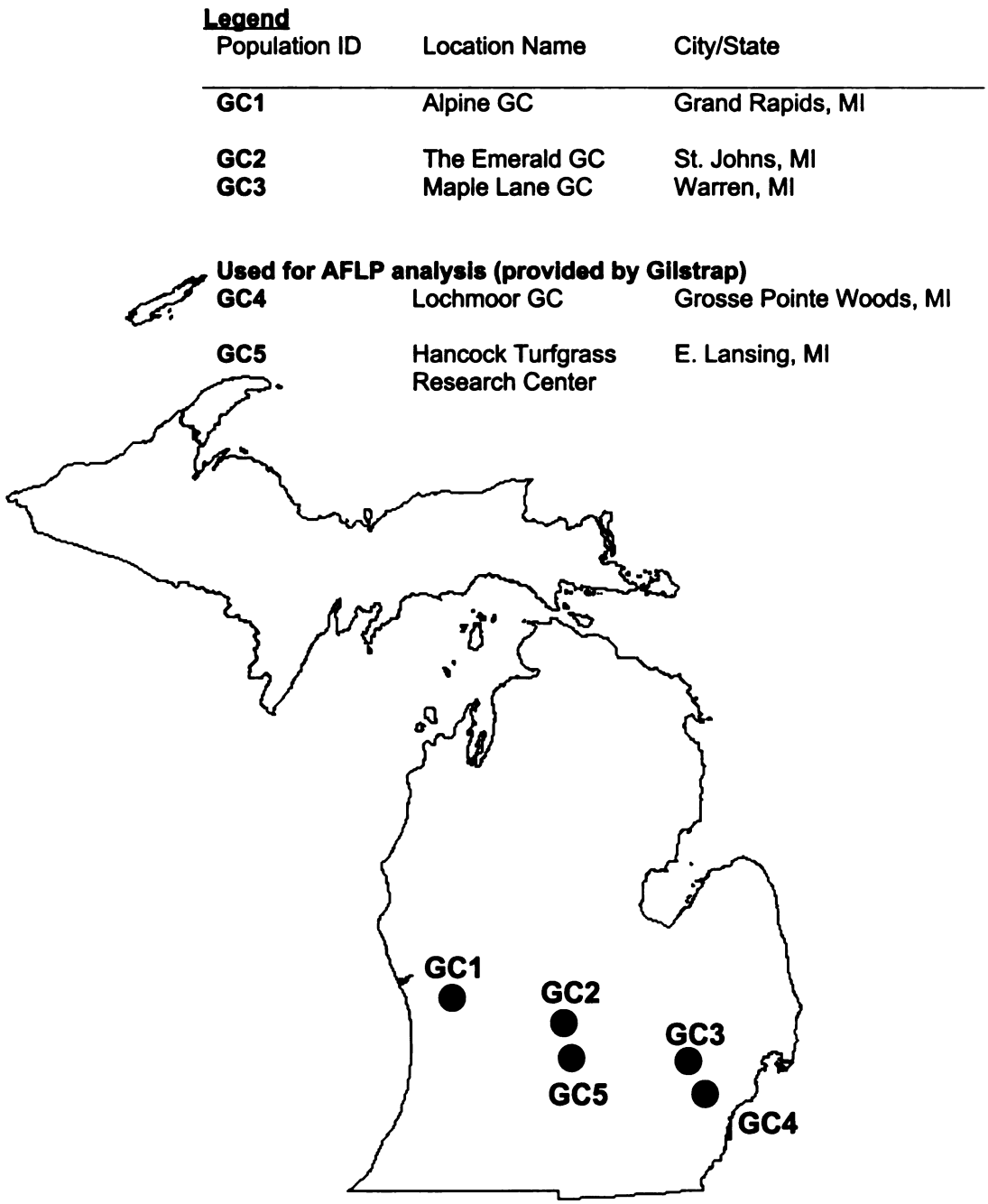

Figure 1. Map depicting geographical location of 5 *S. homoeocarpa* populations sampled for VCG and AFLP analysis. Legend indicates population ID, name of location, and city/state.

no differences in the frequency distributions of these VCGs between fairways within golf courses, within golf courses, or between years. Isolates provided by Gilstrap were also classified into VCGs using the same techniques described above for the other isolates. However, these isolates were not subjected to chi-square analysis due to a different sampling scheme.

DNA extraction and AFLP fingerprinting. A subset of isolates from the three populations sampled for this study and the additional isolates provided by Gilstrap were fingerprinted using the AFLP technique (Table 1). Isolates were grown in approximately 20 mL of potato dextrose broth in 100 x 15 mm petri dishes for seven days at 23 to 25°C. Mycelial mats were washed with distilled water and dried briefly under vacuum before being frozen to -20°C and lyophilized.

Lyophilized mats were ground with a sterile mortar and pestle. Whole genomic DNA from approximately 50 mg of ground mycelium was extracted using a QIAGEN Dneasy Plant Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's directions. DNA was quantified by comparing the intensity of illumination of a 1 uL drop on 1.5% agarose gels amended with ethidium bromide and viewed under UV light to known standards ranging from 10 to 250 ng/uL. Approximately 100 ng of DNA was then subjected to a restriction/ligation reaction, pre-selective amplification, and selective amplifications using the PCR core mix, adaptor sequences, core primer sequences and fluorescence labeled primers provided in the AFLP™ Microbial Fingerprinting Kit (Perkin-Elmer Corp.,

Isolate ID	Year Isolated	Population-VCG ID[a]
A14-8-00	2000	GC1-D
A16-1-01	2001	GC1-D
ML7-29-00	2000	GC3-D
ML8-46-00	2000	GC3-E
ML11-19-01	2001	GC3-D
ML12-40-00	2000	GC3-F
E17-14-01	2001	GC2-A
ML7-7-00	2000	GC3-C
A16-16-01	2001	GC1-E
1-7003-SH-R	1994	GC4-E
7039-SH-S	1998	GC5-E
1-7016-SH-R	1994	GC4-E
ML7-11-01	2001	GC3-A
1-7024-SH-R	1994	GC4-C
1-7018-SH-R	1994	GC4-C
1-7021-SH-R	1994	GC4-C
1-7008-SH-R	1994	GC4-C
1-7005-SH-R	1994	GC4-E
1-7013-SH-R	1994	GC4-E
1-7004-SH-R	1994	GC4-C
7041-SH-S	1998	GC5-C
A9-36-01	2001	GC1-B
7034-SH-S	1998	GC5-A
E4-3-01	2001	GC2-C
E4-1-00	2000	GC2-B
1-7015-SH-R	1994	GC4-E
A9-10-01	2001	GC1-C
ML7-2-01	2001	GC3-B
7043-SH-S	1998	GC5-B
7033-SH-S	1998	GC5-B
7040-SH-S	1998	GC5-B
7036-SH-S	1998	GC5-B

[a] Designation of isolate in Fig. 3 listed by population ID and VCG.

Table 1. Sampled isolates for AFLP fingerprinting. Isolates are listed in the order from top-bottom as they appear in Fig. 3 .

Foster City, CA) and performed exactly as described in the PE/ABI AFLP Microbial Fingerprinting protocol part# 402977 Rev A. All PCR reactions were performed using an MJ Research Minicycler (MJ Research Inc., Waltham, MA) in 0.2 mL tubes according to the cycling parameters outlined in the microbial fingerprinting protocol.

An initial optimization set of reactions was performed using pre-selective products from two randomly chosen isolates. Amplifications with the selective primers EcoRI-AA, AC, AG and AT were performed in all 16 combinations with the MseI-CA, CC, CG and CT selective primers. EcoRI selective primers were labeled at the 5' end with either carboxyfluorescein (FAM), carboxytetramethyrhodamine (TAMRA), or carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE) fluorescent dyes. The fluorescent dyes were excited by laser radiation and visualized by their characteristic absorption-emission frequencies. Only the fragments containing an EcoRI restriction site were resolved.

Selective amplification AFLP products and a carboxy-X-rhodamine (ROX) size standard were loaded into each lane on a denaturing polyacrylamide gel and the fragments resolved in an ABI 3700 DNA Sequencer. Results were prepared for analysis in the form of electropherograms using GeneScan Analysis software (PE/ABI). AFLP fragments were scored manually as present = 1 or absent = 0 using Genotyper software (PE/ABI). Only DNA bands that consistently exhibited unambiguous presence/absence profiles were scored.

Using the program NTSYS-pc (Rohlf, F. J. 1993. NTSYS-pc - Numerical Taxonomy and Multivariate Analysis System, Version 2.02k. Applied Biostatistics Inc.), the combined 0/1 data matrix for isolates was used to construct a genetic similarity matrix of all possible pairwise comparisons of individuals using Jaccard's similarity coefficient: $GS(ij) = a/(a + b + c)$. $GS(ij)$ is the measure of genetic similarity between individuals *i* and *j*, where *a* is the number of polymorphic bands shared by *i* and *j*, *b* is the number of bands present in *i* and absent in *j*, and *c* is the number of bands present in *j* but absent in *i*. Trees were constructed using unweighted pair group with mathematical averaging (UPGMA) cluster analysis to provide a graphical representation of the relationships among isolates.

Results

Isolation and Culture. A total of 1200 samples of *S. homoeocarpa* were collected from three golf courses in Michigan. Of the 1200 samples, 889 isolates were placed in pure culture and stored. The collection efficiency (% success in obtaining an isolate from a sampled spot) was 74.1%. Isolates that were stored in H_2O at room temperature have been routinely recovered over the entire course of our study.

VCGs. 860 isolates were placed into one of the six VCGs (Table 2) described by Powell and Vargas (18). Isolates were scored as compatible when

			VCG Group							
Location	**Year**	**Fairway**	**A**	**B**	**C**	**D**	**E**	**F**	**OTHER**	**Grand Total**
Alpine	2000	9	1	21	25	0	0	0	1	48
		12	0	35	11	0	0	0	0	46
		14	6	26	11	1	0	0	0	44
		16	1	33	12	1	0	1	0	48
	2000 Total		*8*	*115*	*59*	*2*	*0*	*1*	*1*	*186*
	2001	9	14	12	8	0	2	0	0	36
		12	16	20	4	0	2	0	0	42
		14	24	19	0	0	1	0	0	44
		16	19	20	0	2	1	0	0	42
	2001 Total		*73*	*71*	*12*	*2*	*6*	*0*	*0*	*164*
Alpine Total			**81**	**186**	**71**	**4**	**6**	**1**	**1**	**350**
Emerald	2000	4	0	36	5	0	2	0	1	44
		5	0	36	10	0	0	0	0	46
		11	0	37	3	0	0	0	0	40
		17	0	32	13	0	0	0	1	46
	2000 Total		*0*	*141*	*31*	*0*	*2*	*0*	*2*	*176*
	2001	4	3	15	16	0	0	2	6	42
		5	2	33	5	0	2	0	2	44
		11	2	21	7	0	1	1	1	33
		17	4	22	11	0	4	0	0	41
	2001 Total		*11*	*91*	*39*	*0*	*7*	*3*	*9*	*160*
Emerald Total			**11**	**232**	**70**	**0**	**9**	**3**	**11**	**336**
Maple Lane	2000	7	1	11	28	1	7	0	0	48
		8	0	10	3	1	3	1	0	18
		11	0	16	11	1	5	0	0	33
		12	0	21	16	0	3	2	0	42
	2000 Total		*1*	*58*	*58*	*3*	*18*	*3*	*0*	*141*
	2001	7	3	6	0	1	0	0	0	10
		8	3	0	0	2	0	0	4	9
		11	7	5	0	1	0	2	11	26
		12	11	1	2	0	0	1	2	17
	2001 Total		*24*	*12*	*2*	*4*	*0*	*3*	*17*	*62*
Maple Lane Total			**25**	**70**	**60**	**7**	**18**	**6**	**17**	**203**
Grand Total			**117**	**488**	**201**	**11**	**33**	**10**	**29**	**889**

Table 2. Vegetative compatibility group (VCG) distributions of *Sclerotinia homoeocarpa* isolated in 2000 & 2001 from four fairways at each location.

there was no noticeable barrage zone between an isolate and a tester strain. Isolates were scored as incompatible when a barrage zone was formed between an isolate and a tester strain (Fig. 2). Twenty-nine isolates either did not fit in one of these six groups or the incompatibility reaction was indistinguishable, and were classified as "other" (Fig. 2). These isolates were ignored when isolates were sampled for the AFLP analysis and they were not subjected to chi-square analysis. Over the entire course of the study isolates from VCGs A, B, and C were found in all fairways of each golf course. VCGs D, E, and F were either absent completely or present at very low frequencies in each population. Of the three major VCGs, group B was present in the highest frequency over both years, followed by group C, and group A. Over all locations in 2000, group A was much less prevalent than in 2001. The reverse was true for group C where it was more frequent in 2000 than in 2001. Overall, fewer isolates were collected in 2001. Chi-square analysis showed there were significant differences in VCG frequency distributions between fairways within a golf course at the Maple Lane and Alpine locations (Table 3). The analysis also showed there were significant differences between locations and between years.

AFLP Genotyping. The EcoRI + AC/ MseI + CA primer combination resolved the greatest number of clear fragments of the selective primers tested and resulted in more than 80 clearly resolved AFLP fragments in each of the 32 isolates analyzed. In total, 100 AFLP fragments were resolved with 15 being

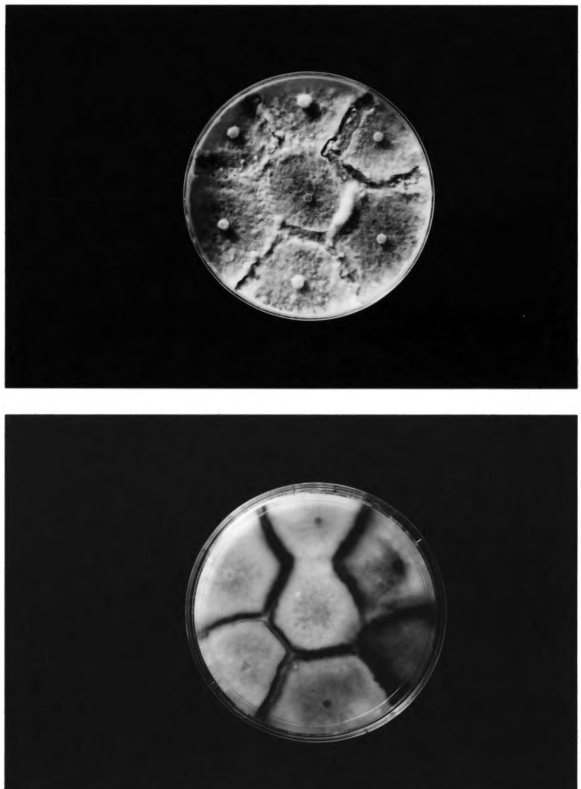

Figure 2. Images showing surface (top) and reverse (bottom) views of mycelial interactions of a *S. homoeocarpa* isolate (center of image) against tester isolates (surrounding center) in a petri dish.

Comparison	Chi-square	d.f.	P-value
Within Maple Lane GC fairways	14.29	6	.0266
Within Emerald GC fairways	8.99	6	.1741
Within Alpine GC fairways	31.73	6	<.0001
Between Locations	79.16	4	<.0001
Between 2000 and 2001	150.16	2	<.0001

Table 3. Results of chi-square analyses of VCG distributions of *Sclerotinia homoeocarpa* from 3 populations in Michigan.

present in some isolates and absent in others (polymorphic). Isolates were from 55 to 100% similar (Fig. 3). Isolates with identical AFLP profiles did not necessarily come from the same location or have the same VCG. Overall, isolates from the same location or with the same VCG were not more similar.

Discussion

Several Our evaluation of as many as 185 isolates from a single sampling at one location represents the largest sample of isolates of *S. homoeocarpa* ever examined for VCG diversity at a location. We found clear evidence that VCG distributions can vary within a golf course, among golf courses, and over time. These data adds to the findings of Powell and Vargas (18) who found that there were differences in the distribution of VCGs over time and location. Other studies have attempted to understand the structure of *S. homoeocarpa* populations (10, 18, 19, 22). It thus appears plausible that VCG distributions on each fairway within a golf course operate as independent populations, each with a unique distribution of VCGs.

One exception in this study was the Emerald location in St. John's, MI. Chi-square analysis for isolates within a fairway at this location revealed no significant differences in the VCG distributions between fairways. This golf course was completely redesigned and renovated in 1996 making it much younger than both Alpine and Maple Lane golf courses that are well established and have been in play for at least 25+ years. The predominance of the VCGs A, B, and C in the populations sampled are similar to the results found

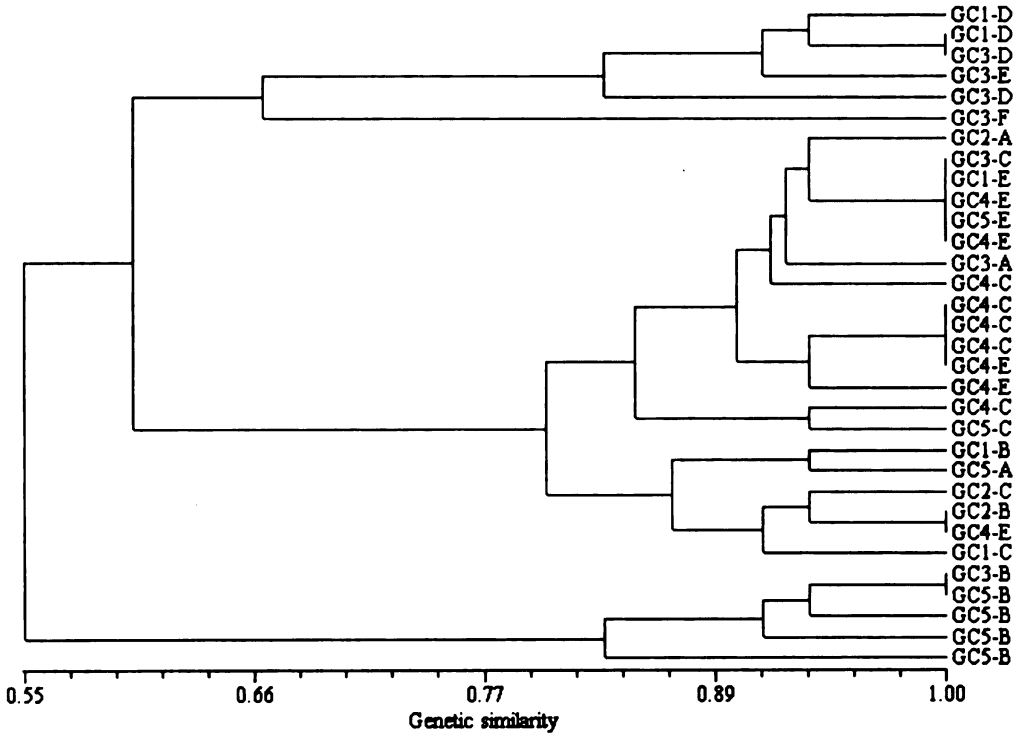

Figure 3. Genetic similarity of 32 *S. homoeocarpa* isolates sampled from five populations in Michigan based on 15 polymorphic AFLP markers. Populations are designated GC1-GC5 followed by the VCG.

for one of the fields sampled by Kohn *et al.*(15) for MCG diversity in *S. sclerotiorum* where they hypothesize that the relative lack of diversity in the field was indicative of the diversity that was initially introduced into the area or as a result of selection of strains from an initially diverse population. The evolutionary forces of drift and migration as well as the putative lack of sexual recombination in *S. homoeocarpa* can limit the number of VCGs found in a population (16, 18). Also, age of the golf courses, cultural practices, fungicide management regimes, and environmental conditions may all be potential factors in the development and distribution of VCGs of *S. homoeocarpa*.

Further research should focus on developing testable theoretical models that seek to explain the variation in VCG distribution that has been observed within sampling locations, between sampling locations, and over time. It would also be worthwhile to investigate the possibility of bias in the sampling scheme used by both this study and Powell and Vargas (18) that is based on selecting a single isolate from a few infected lesions that were cultured from a single dollar spot. An exhaustive sampling scheme that characterizes the presence of all strains of *S. homoeocarpa* growing in a single dollar spot would serve to close this question. Finally, examining the VCG diversity that is present in the less highly maintained areas of a golf course may also aid in our understanding of the factors responsible for the distribution of VCGs present in different populations.

The use of molecular markers for the study of fungal plant pathogen populations is well documented (2, 5, 10, 13, 14, 15, 17, 19). Recently, these techniques have been used with greater frequency for examinations of turfgrass

pathogens. RAPDs were used to examine genetic variation present in a collection of 26 isolates from the northeastern and midwestern areas of the U.S (19). Raina *et al.* found a very high level of genetic similarity between isolates regardless of location, indicating a strong clonal population structure. However, a limitation of their study was the small number of isolates from a single location, making inferences about population structure difficult. Hsiang and Mahuku (10) also used RAPDs to assess variation in *S. homoeocarpa* populations in Southern Ontario. They sampled populations of *S. homoeocarpa* more intensely than Raina *et al.* (19), collecting over 20 isolates per population. They found that 5 of the 8 populations exhibited significant linkage disequilibrium indicating a clonal population structure. The remaining 3 populations had linkage disequilibria consistent with a random mating system. In the populations that they studied, they did not perform any VCG comparisons to corroborate their results of random mating. This could have provided crucial information about the disease cycle of *Sclerotinia homoeocarpa*. Most of the genetic variation was found between populations and very little variation was found within populations. Corroborating the findings of Raina *et al.* (19), our results support a clonal population structure in the *S. homoeocarpa* populations sampled because of the low amount of genetic diversity present.

AFLP fingerprints were not able to resolve isolates based on VCG or geographic location. An isolate from Grand Rapids (Alpine GC) had the same AFLP fingerprint as an isolate from one of the Detroit locations (Maple Lane). Also, isolates from Maple Lane were present in all of the major branches of the

tree. This indicates a significant amount of the genetic variation observed in this study is present within a population. The lack of a pattern between AFLP genotypes and independent measures such as geographic location and VCG is interesting because these results point to a fairly recent introduction of the pathogen into Michigan. The construction and development of golf courses in Michigan is an activity that has taken place over the last century and so the introduction of the pathogen on golf course turf presumably would have occurred at some point over this period. Another possibility that could explain these data is regular migration between populations so that there is no differentiation of the populations. Migration seems to be a less likely scenario because of the large distances between the populations sampled and the lack of any evidence for a spore forming stage that could be aerially disseminated. One other possibility is that selection could be a factor involved in the lack of diversity present at the sampled populations. Kohn *et al.* (15) suggested that diversifying selection (26) was an important driver of diversity because it predicts that a mosaic of pathogen genotypes that are specialized for differing conditions are favored in the absence of other selective factors. Certainly the presence of diverse microclimates, different management practices, and cultivar selections on today's golf courses would provide a similar disturbed environment compared to the environments discussed by Kohn *et al.*(15). This type of selection would also fit well with the data generated by Powell and Vargas (18) who found that the VCGs at a location change over time and hypothesized that the change could be the result of environmental conditions.

Future research should use both molecular as well as VCG characters to test the hypotheses generated by this research. How does dollar spot first appear on a golf course? This question is important to understanding and elucidating the population structure of this pathogen. The question could be approached by monitoring a population over time on a newly established golf course using the techniques applied in this study. Also, research determining the mode and survival of overwintering inoculum of *S. homoeocarpa* would also help to shed light on the recalcitrant population structure of this pathogen.

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Chapter 2

GEOSTATISTICAL ANALYSIS OF DOLLAR SPOT EPIDEMICS IN MICHIGAN

Introduction

Dollar spot disease of turfgrasses is caused by the pathogen, *Sclerotinia homoeocarpa* F.T. Bennett (2). The disease is common throughout the world and is destructive to both cool and warm season grasses (18, 19, 21). In North America, with the exception of the Pacific Northwest, dollar spot is the most important pathogen of most cultivated fine turfgrass species (18, 21). In Michigan, the disease is a major problem for most golf courses where the epidemic begins in June and can continue into late September. The disease can blight large areas of turf as a result of coalescing disease foci and can cause extensive damage if left untreated. Diseased turf impairs the playing surface by creating depressions that affect ball roll and areas of bare soil where weed species can encroach on the area (18, 21).

The biology of dollar spot has not been studied extensively due to the relative ease with which this disease can be controlled with fungicides. Outside of Great Britain, dollar spot has not been reported to form sexual or asexual spores (1, 13, 18, 19). Hsiang and Mahuku (11) reported that a population from Ontario exhibited DNA fingerprints that were consistent with sexual reproduction, but no fruiting bodies or spores were found. Nitrogen fertility is an important factor in the management of dollar spot (4, 14, 18, 19, 21, 25). Most reports support the view that increased fertility leads to a reduction in the severity of the disease (14, 18, 19, 21, 25). However, Couch and Bloom (4) reported that the susceptibility of

Poa pratensis was actually increased in higher fertility treatments in the greenhouse, but that the effect would be masked in the field because symptoms would not appear before the rapidly growing, infected leaf blades were mown off. Spread of the disease is widely believed to be the result of direct movement of the pathogen on infected blades during mowing operations, and human transport of infected clippings on shoes, balls, etc. (7, 18, 19, 21, 25).

Little or no research exists on inoculum sources, but some reports contend that stromatized infected tissue is the primary inoculum source for the pathogen (7, 25). Control of dollar spot via fungicide application is generally accomplished using the contact fungicide, chlorothalonil. However, the EPA has recently banned the use of this fungicide on home lawns, and future restrictions on commercial use of chlorothalonil is expected. Because the amount of fungicide available to an individual golf course is expected to be limited, it will become critical for superintendents to be able to apply chlorothalonil judiciously in areas that require treatment. Other fungicides are available for control, but fungicide resistant dollar spot populations have been reported for most of them including, the demethylation inhibiting (DMI) (8), benzimidazole (5, 23), and dicarboximide (5) fungicides.

Studying the epidemiology of a plant disease traditionally calls for the assessment of the severity or occurrence of disease over some area of interest. These data are generally collected over an area using some sampling scheme. However, traditional statistical techniques require adherence to assumptions such as the independence of samples and normality of data. It makes intuitive

sense that plants located closer in space to a diseased plant have a higher probability of becoming infected than plants located farther away. Recently, using the location of these samples in space as an additional datum has led to the description of disease epidemics over space and time using geostatistical techniques (20, 24, 26, 27).

Important epidemiological questions arise from spatially explicit descriptions of disease: Is the disease appearance clustered in space? If so, what factors contribute to this clustering? What practices might ameliorate the effects of those factors? Can we predict both when *and* where disease is going to occur so that fungicide applications can be targeted? One method available to address these questions is geostatistics. Originally developed for the study of geological phenomena, geostatistics have found wide application in a number of fields including phytopathology (20, 24, 26, 27). The primary goal of these techniques is to explain how a variate of interest (e.g. disease severity) at a location in space is correlated with all the other points where the variate has been measured (9, 10, 12, 15, 17). Further treatments allow for the prediction of the variate at unmeasured locations, and the assessment of prediction confidence. These techniques also allow for the analysis of nominal values such as genotypes or size classes in a similar manner (9, 10). Geostatistics provides a powerful set of tools that can yield insight into the dynamic nature of plant disease.

Research in plant disease epidemiology using geostatistical tools is relatively new. Geostatistics have been used to study the spatial pattern of

disease incidence and severity (20, 26) and inoculum levels (24, 27). These tools have also been used to study physico-chemical properties of soils (10), plant distributions and ecology (15, 17), and microbial distributions (20, 26, 27). Variography was used by Wollum and Cassel (26) to study the spatial variability of *Rhizobium japonicum* in soil planted with soybeans. They concluded that geostatistics were a good tool for studying the dynamics of microbial populations. Stein et. al. (20) examined the spatio-temporal development of *Peronospora parasitica* epidemics in cabbage (*Brassica oleracea*). They were able to determine that spatial variability of the fields was dependent on disease incidence. They also found spatial dependence when fields were recovering from disease. Xiao et. al. (27) studied the spatial patterns of *Verticillium dahliae* microsclerotia in the soil, and verticillium wilt in cauliflower using geostatistics. They found that the spatial structure of microsclerotia in the soil was not very strong. The structure of the microsclerotia did not play a role in the clustered appearance of disease. They attributed this to a very high amount and fairly uniform distribution of microsclerotia. They concluded other factors affect the appearance of wilt. However, they did find that the severity of the wilt was associated positively with the weak spatial pattern for the presence of microsclerotia. The information generated by a geostatistical approach to the study of plant disease epidemics allows one to develop or refine management strategies, and help to determine the contributing factors that deserve further study. Ultimately, this information can be used to design models that can be used

to predict the occurrence of plant disease, helping to minimize pesticide applications, or make cultural practices more effective.

We had 3 objectives: 1) to observe dollar spot epidemics and determine if disease occurrence has a spatial structure, 2) if a spatial structure is present, determine the geostatistical parameters associated with the spatial structuring, 3) Determine if the spatial structure changes during an epidemic, or over seasons.

Materials and Methods

Sampling. The study site was established at the Robert Hancock Turfgrass Research Center on a 9.1 m X 18.3 m area of creeping bentgrass (*Agrostis palustris* Huds.) and annual bluegrass (*Poa annua* L.). The study site received no fungicide applications from 2000-2002. The study site was divided into 200 0.3 m^2 areas on a regular grid at 0.9 m intervals in 2000. In 2001, 23 additional 0.30 m^2 areas were established at random locations within the study site, and all 223 areas were subdivided into four 0.15 m subareas (Fig. 1). These additional locations were added and all areas subdivided to increase the number of data pairs at small lag distances. Each subarea's x,y coordinates were recorded using its center. A 0.3 m^2 wooden frame that was divided into quadrants was used to delineate the subareas. Two points at each sampling location were marked with marking paint in order to place the frame at the same location at each sample time. Isolates were also collected from an arbitrarily selected dollar spot at each location in July 2000 and the vegetative compatibility group (VCG) for each isolate was determined in order to assess if clustering was present in VCGs (Appendix 1).

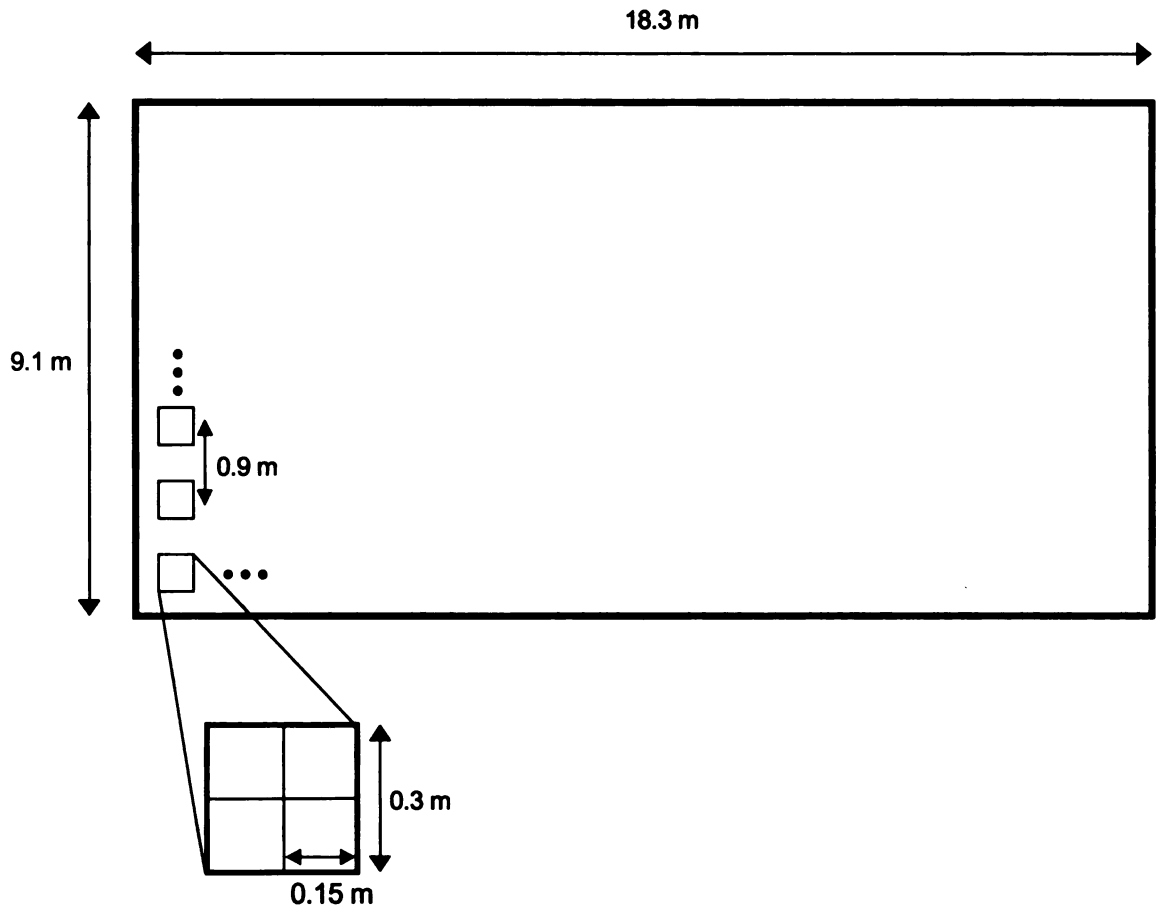

Figure 1. Schematic layout of study area at the Robert Hancock Turfgrass Research Center (E. Lansing, MI) showing overall arrangement of sampling locations.

Data Collection. Dollar spot foci were counted three times per week in 2000 and twice per week in 2001 and 2002 from each of the locations in the study area. Foci were counted when they reached a size large enough to observe. This helped to minimize the possibility of accidentally counting an area that was not a true dollar spot. Counting of the study area was stopped in each year when disease became prevalent enough in a location that there were too many disease foci to count accurately.

Geostatistical analysis. Spatial continuity was measured using the variogram, that describes how a variate changes over space. Intrinsic to geostatistical analysis is the hypothesis that the expectation of differences between any pair of points depends solely on the distance (h) between the points (6, 9, 12). Therefore, to estimate the variogram from disease incidence data with the intrinsic hypothesis in mind;

$$\gamma\,(\mathrm{h}) = \frac{1}{2N(h)} \sum_{(i,j)|hij=h} (v_i - v_j)^2$$

where γ is the semivariance, h is the average separation distance between pairs of points, N is the number of data pairs, and v_i and v_j are the ith and jth data values at separation distance h. This results in estimates of semivariance (γ) that are plotted as a function of separation distance (h). Once a variogram is calculated, a model that fits the observed data can be fit to the experimental data. A variogram model defines three key parameters: nugget, sill, and range. The nugget is the result of the discontinuity that occurs when the semivariance, which is defined to be 0 when lag distance, h=0, jumps to some value > 0 at a

very small distance away. The nugget is the result of a combination of sources of variation including experimental error and short-scale variability (9, 12). Generally, as the distance between pairs of points increases, the semivariance will also increase. Therefore as pairs of points are separated by larger distances they become less correlated. However, at some separation distance between pairs the semivariance will reach a plateau where increases in separation distance do not result in a change in the semivariance. This distance where semivariance reaches a plateau is the range, and is also the boundary between spatially dependent and spatially independent variation. The third parameter calculated for a variogram model is the sill. It is defined as the semivariance value reached at the range. If the sampling design has accounted for most of the variation in the system, then the sill value is often very similar to the overall sample variance (s^2). The total variance can be ascribed to three catagories: nugget (Co), structural or spatial variance (C), and sill variance ($Co+C$). The proportion of the total variance that is accounted for by structural variance, or the proportion of structural variance, can be calculated by, $C/Co+C$, and is often expressed as a percentage of the total variance that is spatially structured. When this value approaches 1, a large proportion of the total sample variance is spatially dependent. When the value approaches 0, spatial dependence is low. If the sill value ($Co+C$) is not similar to the total sample variance (s^2), this indicates that there may be further structure at scales larger than those sampled.

Variograms were calculated using the windows interface WinGSLIB (Statios, LLC., San Francisco, CA) for the geostatistical software package,

GSLIB (version 9) (6). The choice of the number of lags, lag interval, and lag tolerance were defined iteratively based on the number, interval and tolerance for lags that yielded a smooth, well-behaved variogram. Ultimately, 15 lags with a 0.61 m lag interval, and 0.30 m lag tolerance were the parameters that gave the most well-behaved result for all three years. The experimental variograms were then modeled using the geostatistical software package, GS+ version 3.0 (Gamma Design Software, Plainwell, MI). The GS+ software package allows one to automatically fit models to the experimental variogram, and then chooses the best fit based on the model with the smallest unweighted least squares value. Any additional changes that were necessary were made by hand to the initial fit provided by the program. The experimental variograms used for modeling were generated in the GS+ program using a 12.2 m lag distance and 1.5 m lag interval as parameters for 2000, and a 6.1 m lag distance and .61 m lag interval for 2001 and 2002. These distances were chosen to avoid over-fitting the models to the increasing semivariance values at larger lag distances seen in the experimental variograms shown in Figure 3.

Results

Sampling and *Data Collection*. Disease was observed and the number of dollar spot foci counted at each location until August 25th in 2000, September 9th in 2001, and September 13th in 2002. Total disease progress curves for each of the three years appear in Figure 2. The epidemic in 2002 was the most severe followed closely by the epidemic in 2000. The epidemic in 2001 was much less severe than either 2000 or 2002. The total disease progress curves for all three

years were similar in shape. Each progress curve had an early season outbreak that was not as severe as the late season outbreak that began in early August and continued into September.

Geostatistical analysis. Variograms were calculated for each date disease counts were taken in 2000-2002. Nine variograms, each representing a variogram from the early, middle, and late phases of the epidemic for each year are shown in Figure 3. The remaining variograms from other sample dates are presented in Appendix 2. Anisotropy was examined at several dates and no anisotropic trends were apparent (Data not shown). The variograms in all three years show clear spatial structuring that occurs at smaller lag distances (<0.9 m), particularly as disease incidence increases over time. Throughout 2001 and 2002 the model that best fit the data was an exponential model (Table 1). In 2000, the first three dates showed a nugget effect indicating no spatial structuring, and then for the remainder of the season both spherical and exponential models were defined. The proportion of structural variance (*C/Co+C*) was about 0.5 for 2000 and about 0.6 for both 2001 and 2002 (Table 2). This value means that about 50% of the total variation in 2000 and about 60% of the total variation in 2001 and 2002 is spatially structured. The range parameter that was calculated for each variogram model in 2000 had larger values and a wider range of variation as compared to the smaller and less variable range parameters calculated in 2001 and 2002.

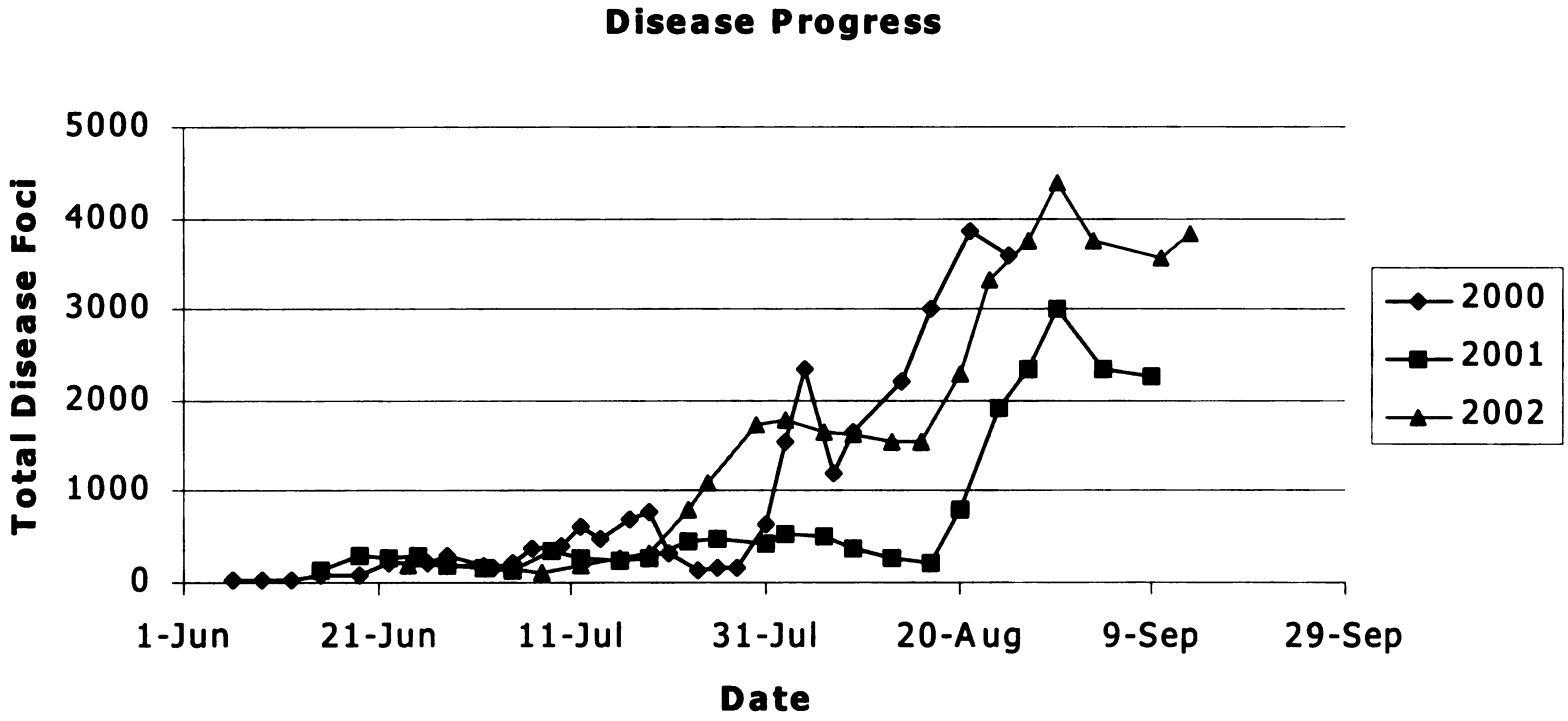

Figure 2- Graph comparing total disease progress from 2000-2002 as measured by counts of total disease foci in all sample locations.

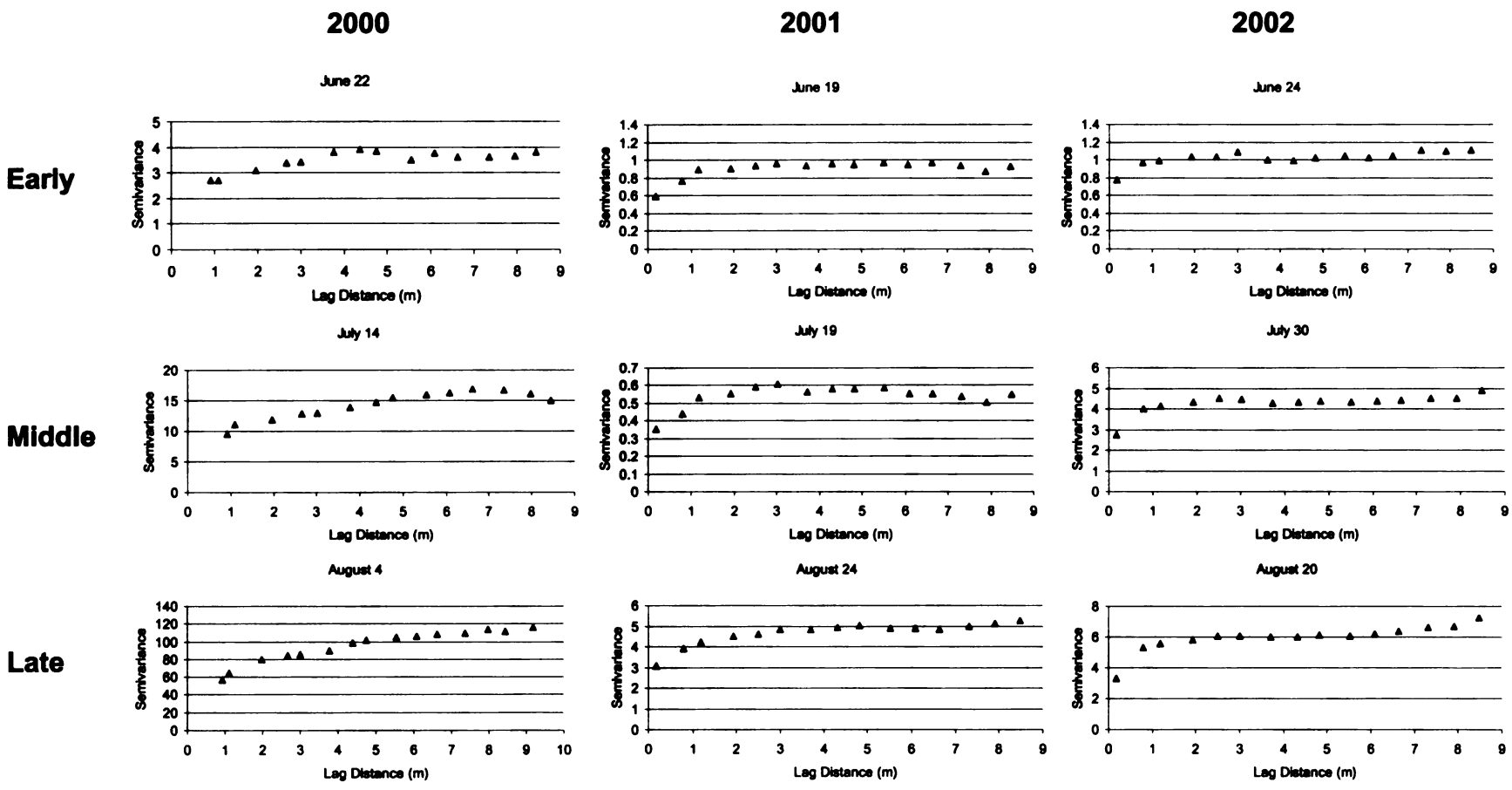

Figure 3. Experimental variograms from 2000-2002 measuring spatial dependence from the early, middle, and late phases of the dollar spot epidemic.

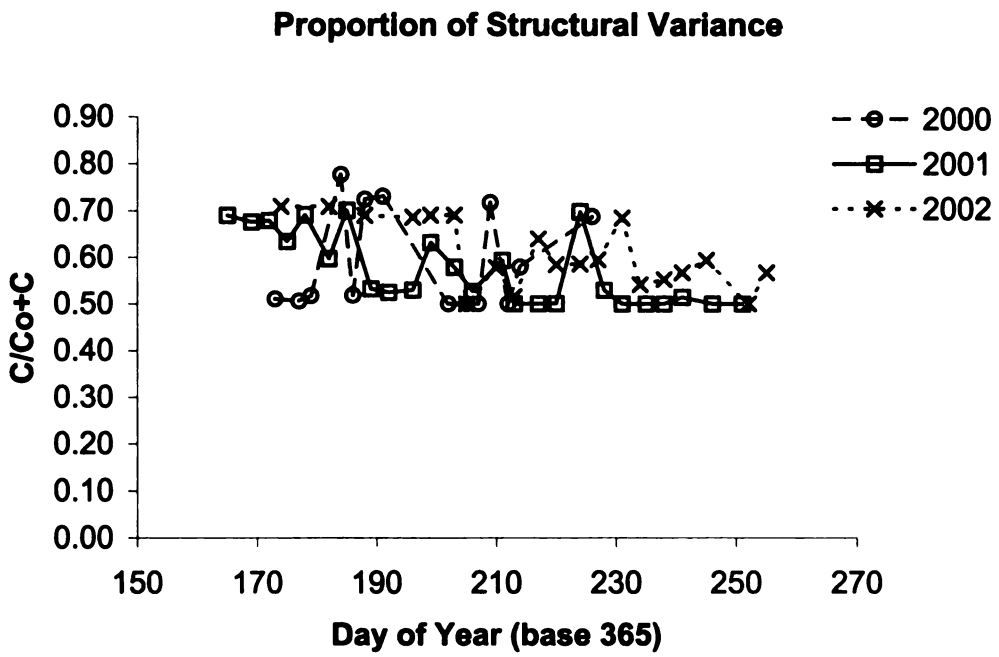

Figure 4. Graph showing the proportion of structural variance (C/Co+C) over time for exponential variogram models from 2000-2002.

Year	Date	Model Type	Nugget (*Co*)	Sill (*Co+C*)	Range (3a)	r^2	RSS
2000	22-Jun	Exponential	1.96	4.004	21.72	0.87	0.209
2000	14-Jul	Exponential	7.38	16.29	21.30	0.885	3.121
2000	4-Aug	Exponential	33.9	109.9	20.37	0.916	169.8
2001	19-Jun	Exponential	0.176	0.543	4.39	0.988	6.04E-04
2001	19-Jul	Exponential	0.215	0.584	5.68	0.962	2.05E-03
2001	24-Aug	Exponential	2.474	4.949	8.78	0.977	0.0777
2002	24-Jun	Exponential	0.11	0.378	2.25	0.754	2.52E-03
2002	30-Jul	Exponential	1.841	4.363	4.01	0.964	0.0642
2002	20-Aug	Exponential	1.91	6.051	4.42	0.991	0.0458

Table 1. Variogram model parameters (nugget, sill, range), goodness of fit (r^2), and residual sum of squares (RSS) for the nine experimental variograms shown in Figure 3. (Range for exponential model is defined as 3a due to the asymptotic behavior of the model).

Year	Date	Nugget (Co)	Sill (Co+C)	Proportion of Structural Variance (C/Co+C)
2000	6-Jun	0.082	0.082	0.00
2000	9-Jun	0.135	0.135	0.00
2000	12-Jun	0.111	0.111	0.00
2000	15-Jun	0.633	1.207	0.48
2000	19-Jun	0.629	0.908	0.31
2000	22-Jun	1.96	4.004	0.51
2000	26-Jun	2.182	4.418	0.51
2000	28-Jun	3.41	7.068	0.52
2000	3-Jul	0.812	3.636	0.78
2000	5-Jul	1.873	3.889	0.52
2000	7-Jul	3.6	13.05	0.72
2000	10-Jul	3.56	13.2	0.73
2000	12-Jul	11.96	23.93	0.50
2000	14-Jul	8.08	16.17	0.50
2000	17-Jul	12.33	24.67	0.50
2000	19-Jul	16.86	33.73	0.50
2000	21-Jul	4.04	8.081	0.50
2000	24-Jul	1.766	3.533	0.50
2000	26-Jul	2.097	4.195	0.50
2000	28-Jul	0.913	3.23	0.72
2000	31-Jul	10.62	21.25	0.50
2000	2-Aug	26.9	63.92	0.58
2000	4-Aug	51.9	108.7	0.52
2000	7-Aug	17.92	46.7	0.62
2000	9-Aug	33.7	72.28	0.53
2000	14-Aug	35	112.12	0.69
2000	17-Aug	46.9	130.8	0.64
2000	21-Aug	54.3	171.4	0.68
2000	25-Aug	45.9	161.7	0.72
			Mean	0.51

Year	Date	Nugget (Co)	Sill (Co+C)	Proportion of Structural Variance (C/Co+C)
2001	15-Jun	0.075	0.242	0.69
2001	19-Jun	0.176	0.543	0.68
2001	22-Jun	0.162	0.505	0.68
2001	25-Jun	0.165	0.451	0.63
2001	28-Jun	0.088	0.285	0.69
2001	2-Jul	0.083	0.206	0.60
2001	5-Jul	0.056	0.187	0.70
2001	9-Jul	0.308	0.659	0.53
2001	12-Jul	0.239	0.503	0.52
2001	16-Jul	0.236	0.502	0.53
2001	19-Jul	0.215	0.584	0.63
2001	23-Jul	0.374	0.888	0.58
2001	26-Jul	0.422	0.892	0.53
2001	31-Jul	0.343	0.844	0.59
2001	2-Aug	0.549	1.099	0.50
2001	6-Aug	0.487	0.975	0.50
2001	9-Aug	0.357	0.715	0.50
2001	13-Aug	0.116	0.384	0.70
2001	17-Aug	0.184	0.391	0.53
2001	20-Aug	0.964	1.929	0.50
2001	24-Aug	2.474	4.949	0.50
2001	27-Aug	2.049	4.099	0.50
2001	30-Aug	2.753	5.669	0.51
2001	4-Sep	1.725	3.451	0.50
2001	9-Sep	1.542	3.085	0.50
			Mean	0.57

Year	Date	Nugget (Co)	Sill (Co+C)	Proportion of Structural Variance (C/Co+C)
2002	24-Jun	0.11	0.378	0.71
2002	2-Jul	0.11	0.378	0.71
2002	8-Jul	0.062	0.2	0.69
2002	12-Jul	0.41	0.41	0.00
2002	16-Jul	0.129	0.411	0.69
2002	19-Jul	0.156	0.504	0.69
2002	23-Jul	0.521	1.683	0.69
2002	25-Jul	1.19	2.381	0.50
2002	30-Jul	1.841	4.363	0.58
2002	2-Aug	2.126	4.386	0.52
2002	6-Aug	1.569	4.355	0.64
2002	9-Aug	1.79	4.308	0.58
2002	13-Aug	1.803	4.348	0.59
2002	16-Aug	1.742	4.299	0.59
2002	20-Aug	1.91	6.051	0.68
2002	23-Aug	3.86	8.413	0.54
2002	27-Aug	3.96	8.855	0.55
2002	30-Aug	4.22	9.75	0.57
2002	3-Sep	3.37	8.293	0.59
2002	10-Sep	3.44	6.881	0.50
2002	13-Sep	3.47	8.019	0.57
			Mean	0.58

Table 2- Variogram model parameters for each rating date in 2000-2002 showing nugget, sill, and proportion of structural variance values.

Discussion

Although the total disease severity during the epidemics in each of the three years was different, there were also several similarities between the epidemics. First, the disease progress curves were similar in shape (Fig. 2). All three progress curves showed that dollar spot first appears in early to mid June with a minor outbreak and then becomes much more severe during the months of August and September. Our data agree with those of other researchers that have shown the later season phase of the epidemic is most damaging to a turf area (7, 16, 18, 21). Disease progress was similar in each year despite differences in disease severity indicating that environmental parameters play a large role in the overall severity and timing of dollar spot outbreaks. In all three years dollar spot was observed to decrease in presence during July, presumably because of the hot, humid growing conditions present during that time. These results support the view that environmental parameters are primarily responsible for disease appearance and resulting overall severity.

The experimental variograms that were calculated for each date were also similar over time. Once a variogram is calculated for each date in the study, a model is calculated that fits the observed data. One reason to fit models to the data is so that the key model parameters the nugget, range, and sill, can be compared to observe how they change over time. The sampling design determines the smallest scale at which spatial relationships can be resolved. In 2000 the design consisted of 200 0.3 m^2 areas spaced on 0.9 m centers. The limitations of this design is that the smallest lag interval for the calculated

variogram was 0.9 m, and there was no information about the disease at smaller scales. If spatial dependence exists at a scale smaller than the smallest sampled interval, then it would become a part of the nugget variance and would not be accounted for in the variogram. In 2000, the calculated variograms displayed spatial dependence, and about 50% of the total variation was spatially structured (Table 2). In 2001 and 2002 23 additional areas were added randomly to the study site, and the 0.3 m^2 areas were subdivided into four 0.15 m^2 subareas to protect against the possible problem of the scale of spatial dependence. Increasing the resolution of the sampling design increased the information about small-scale variability. As the smallest sampling interval was 0.15 m, the addition of the random locations was important to be able to evaluate lag distances between 0.15 m and 0.9 m. These changes in the sampling design resulted in a gain of information as reflected by a 10% increase in the proportion of structural variance from 50% in 2000 to 60% in 2001 and 2002 (Table 2). This increase in spatial resolution at the smaller scales is why there is a much stronger spatial dependence observed for the 2001 and 2002 data as compared to the 2000 data. Based on the experimental variograms calculated for all three years we conclude that dollar spot incidence is spatially correlated in our study area, and that the spatial correlation is present on a small scale. Other locations should be included in future studies to determine if dollar spot incidence at other locations is similarly spatially correlated.

Interestingly, the nugget and sill values for variogram models from each date scale with one another indicating that the spatial structure is relatively stable

over time. The stability of this relationship can also be seen in the stability of the proportion of structural variance (*C/Co+C*) over time (Figure 4). The range parameter is also relatively stable further confirming a structure that is stable and relatively constant over time. While the range did fluctuate in 2000, the smallest lag interval was only 0.91 m, and these fluctuations could be a function of the lack of small-scale sampling. The higher resolution in 2001 and 2002 decreased the fluctuations in the range parameter where the overall change in the range from low to high in both years was between two and three meters. Exponential variogram models were defined in all three years. These results clearly show that as disease intensity increases over a season, the spatial structure that is present is stable and doesn't change much over time.

One possible explanation for the observed spatial structure is that areas with more disease increase at the same relative amount as areas with less disease. If this were not the case, then one would expect the spatial structure, as measured by the proportion of structural variance (*C/Co+C*), to change as disease increased over the season. However, the structure that was observed remained stable over the season. The distance between areas with similar disease intensities (i.e. range) also remains relatively stable over time indicating that these areas are not shifting within an epidemic or among epidemics. Because one would expect different locations to behave differently as a result of either micro- or macroclimatic changes that occur over an epidemic, these results support the view that environmental parameters are not a major factor in the spatial structuring at the scales observed.

The literature provides much speculation on the mode of spread for this non-spore forming pathogen (7, 11, 18, 25). These reports range from the movement of mycelial fragments on diseased tissue via human and mechanical transport (7, 18, 25) to the production of an undiscovered spore that is produced (11). Data from this study disagree with both of these possibilities. If the pathogen were transferred via mechanical means, then one would expect the spatial structure of disease incidence to change over time because mycelial fragments would be distributed over the area via regular, uniform mowing practices. If the pathogen was transferred via human means, then the spatial structure should be indicative of a pattern similar to a pattern of movement over the area by people. If this pathogen produced some unknown spore, then one would expect that the dispersal of such a spore would occur such that the spatial structure of the disease would change with the release of spores. However, none of these possible outcomes were observed in this study. Rather, our results indicate that the primary factor governing the spatial structure is one that doesn't move in space and whose spatial structure is relatively constant regardless of the intensity of disease.

One hypothesis that would fit these data is that the host and/or pathogen are important in the spatial structuring. The predominant grasses found on golf course putting surfaces are creeping bentgrass (*A. palustris*) and annual bluegrass (*P. annua*). Both of these grasses are non-uniform in their susceptibilities to *S. homoeocarpa* (3, 22). The breeding strategy employed for creeping bentgrass results in the production of a synthetic cultivar, meaning that

each seed is genetically distinct. This results in a range of variation in susceptibility/resistance to dollar spot. Annual bluegrass is a non-cultivated grass that invades putting surfaces as a weed, and also is known to be genetically variable (21). The area we studied was at least 10 years of age and was a mixed sward of creeping bentgrass and annual bluegrass. Over time the competitiveness of each seedling would govern those genotypes of grasses found in a site. These successful genotypes would then be more or less susceptible to dollar spot, and this would be observed as a mosaic of disease incidence with a spatial structure corresponding to the spatial structure of the grasses. This hypothesis would also predict that the inoculum density of the pathogen would also follow this spatial structure because areas with previous higher disease incidence would produce more infested tissue, which is believed to be the primary inoculum source for dollar spot.

Overall, these data support the view that there is a relatively stable spatial structure governing disease incidence that is unaffected by disease severity. Furthermore, the results support a theoretical model that the host and pathogen are involved in the observed spatial structure over the scales assessed by this study, and that environmental parameters appear to be most important in overall disease severity and timing of disease outbreaks.

Future research in this area should include the evaluation of other locations to determine if the observed spatial structure is ubiquitous, and the testing of the theoretical models posed by this research to confirm or exclude factors associated with the spatial structuring of dollar spot incidence. These

research areas would provide the information that is needed to begin developing predictive models that can predict the incidence and location of dollar spot based on a knowledge of the environmental and geospatial parameters that govern where and when dollar spot occurs. Once predictive models become available it would then be possible to implement precision fungicide applications for the control of this disease.

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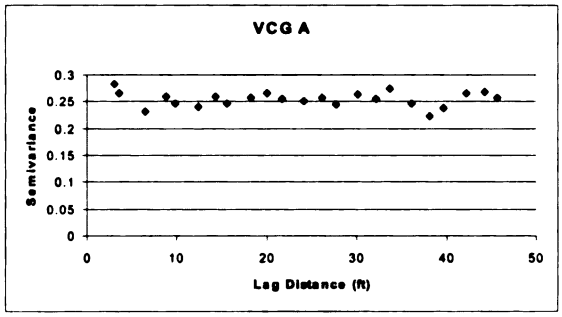
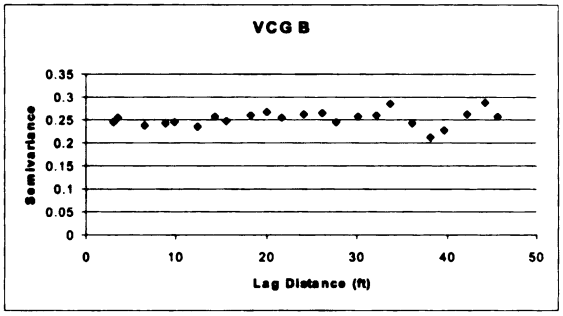
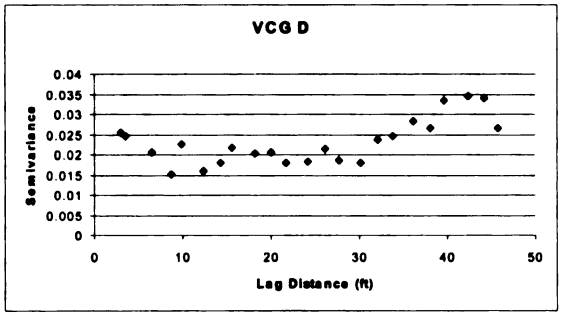
APPENDICES

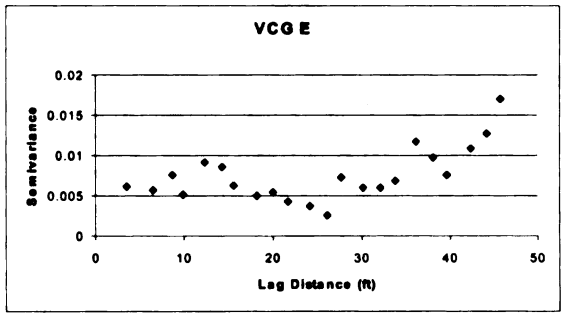
APPENDIX 1

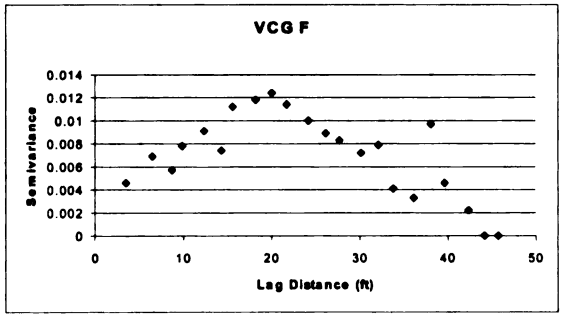
RAW INDICATOR SEMIVARIOGRAMS OF ISOLATE VCGS





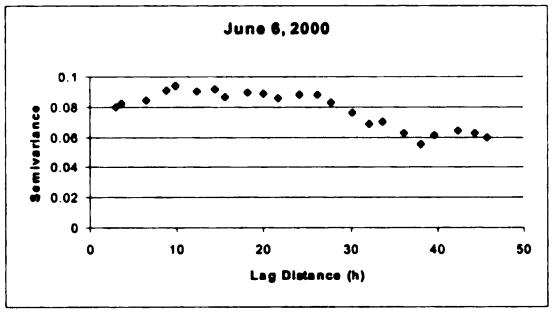


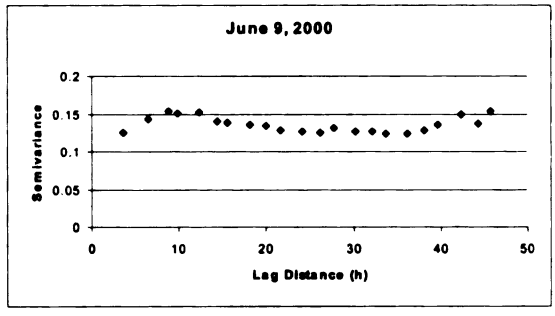


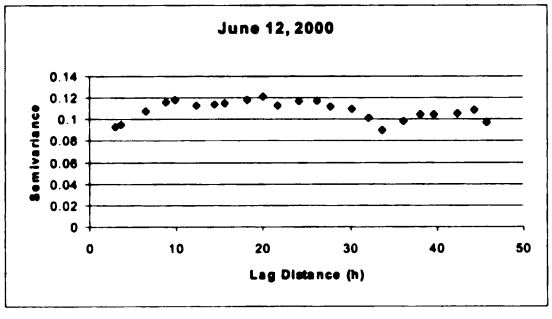


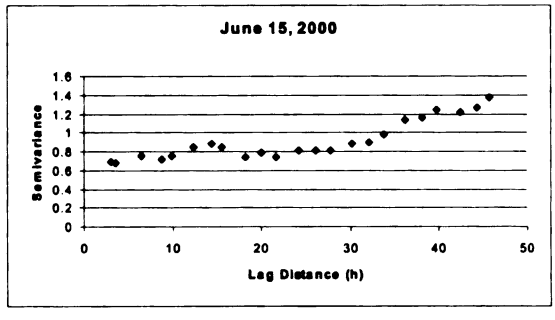
APPENDIX 2

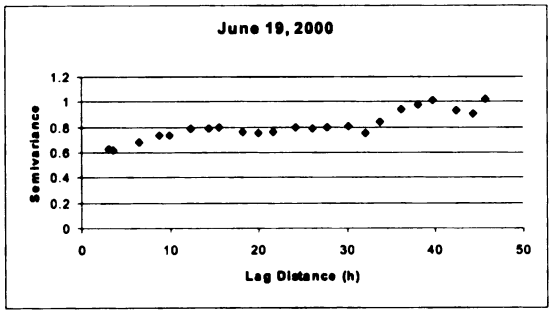
RAW SEMIVARIOGRAMS FOR ALL DATES FROM 2000-2002

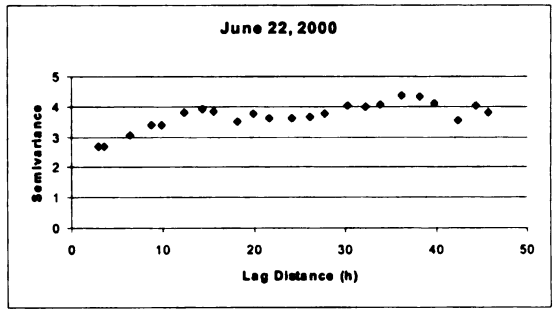


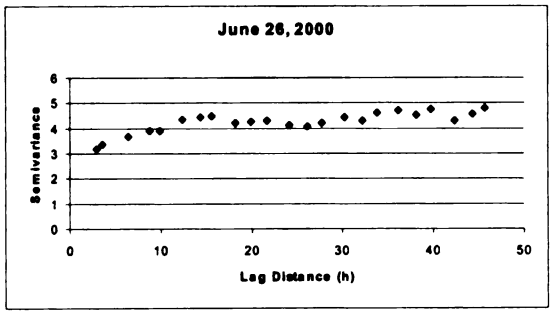


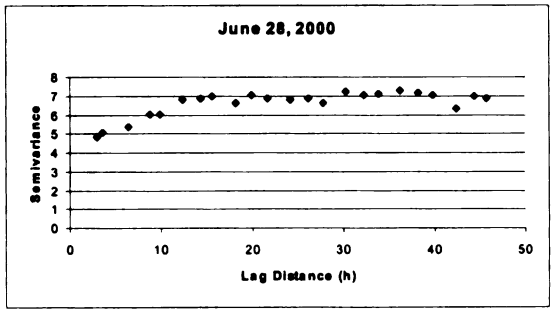


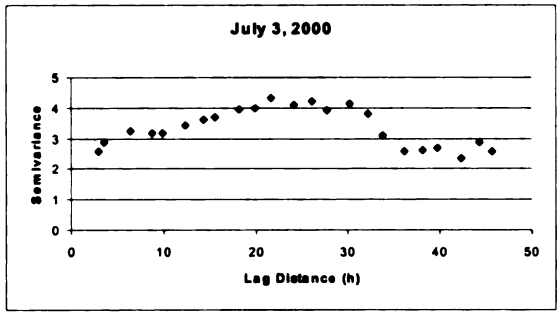








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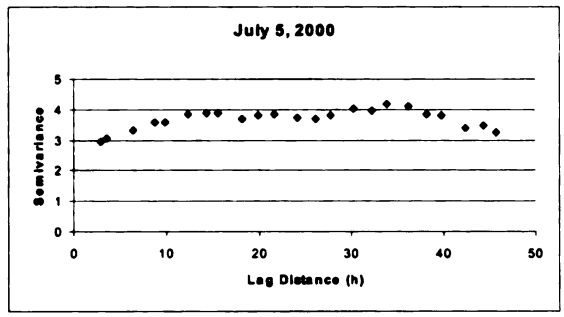
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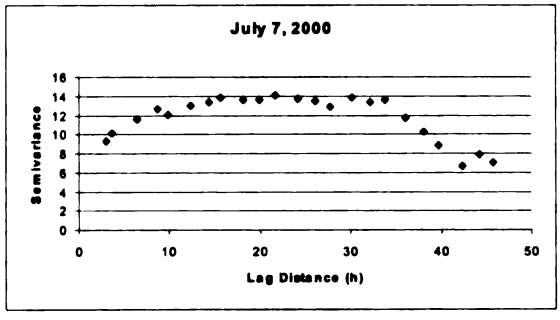
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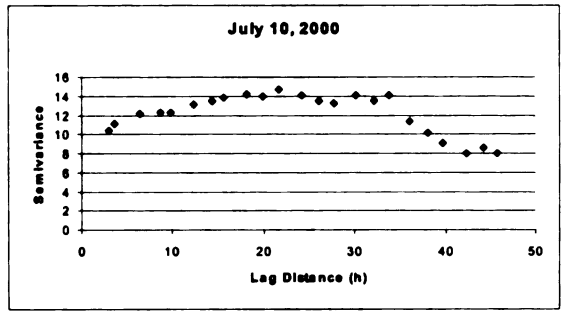
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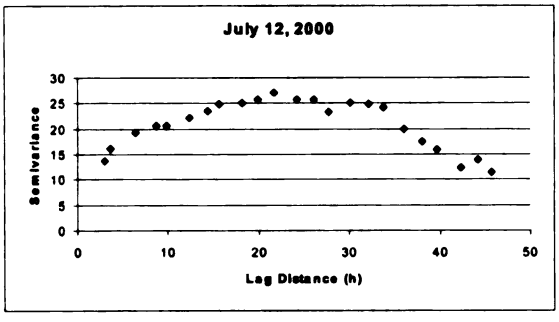
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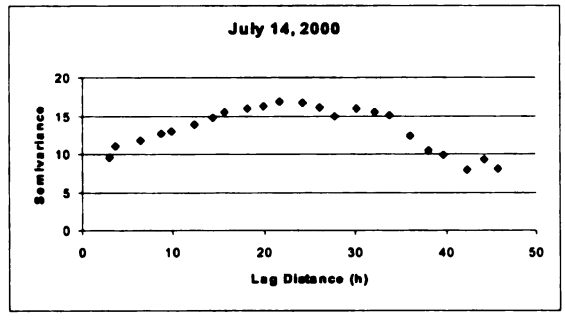
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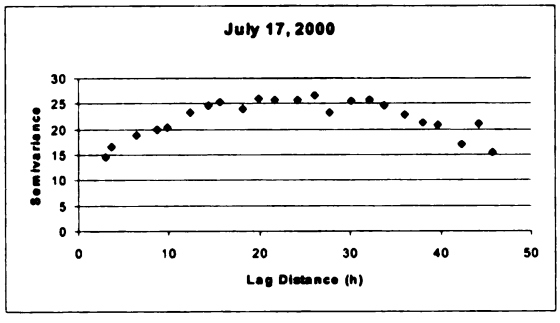
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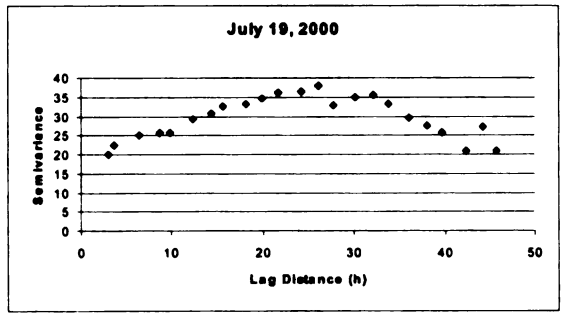
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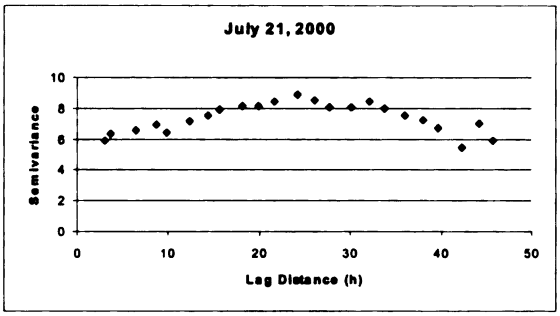
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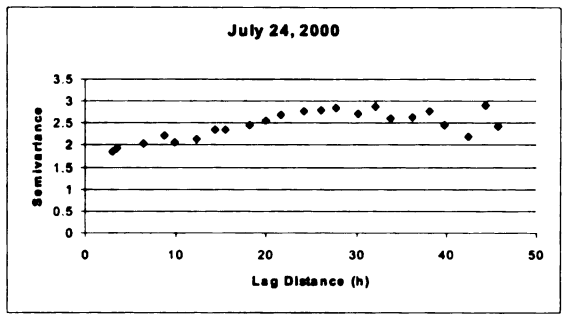
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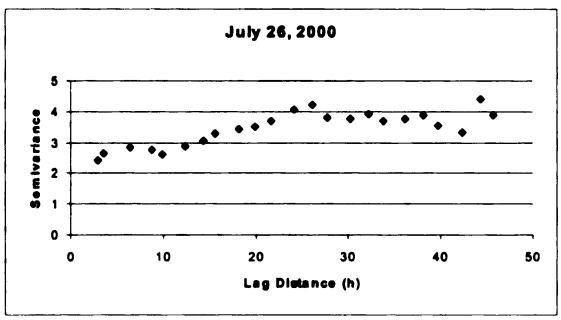
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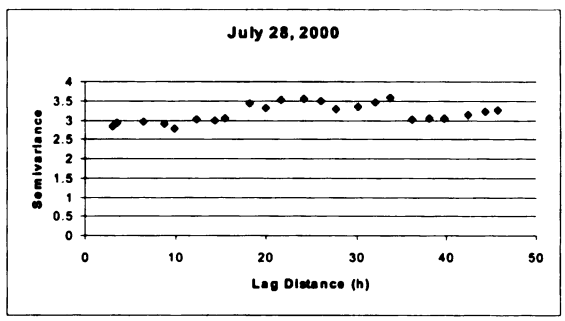
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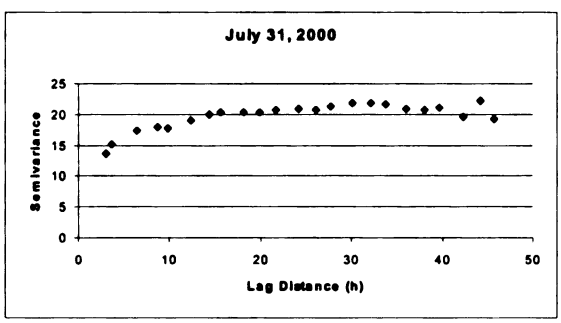
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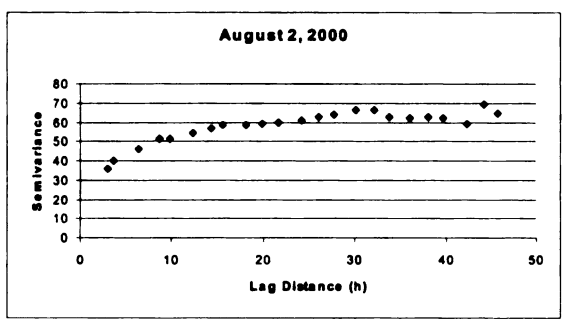
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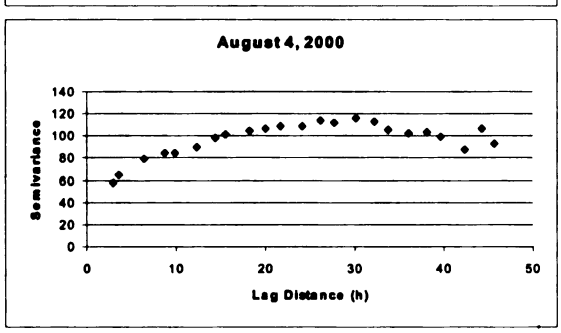
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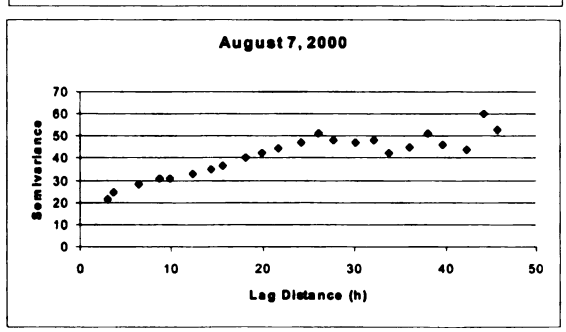
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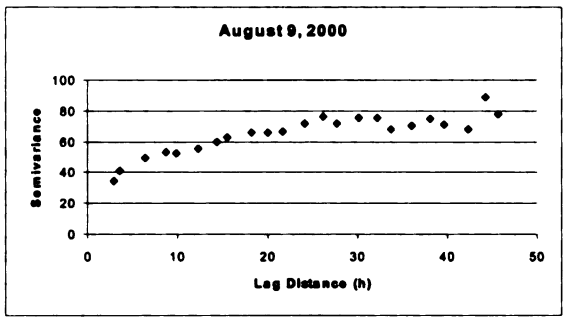
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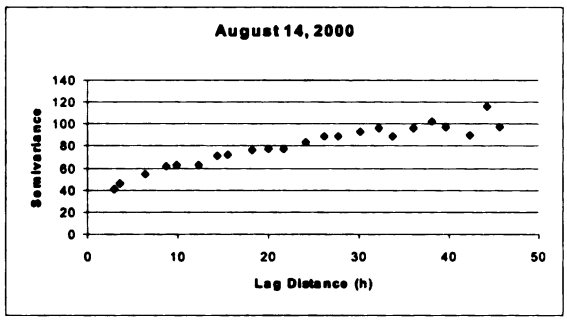
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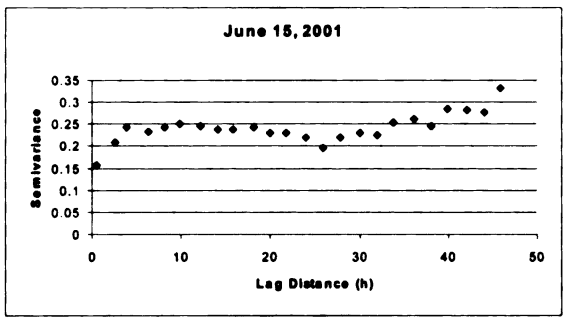
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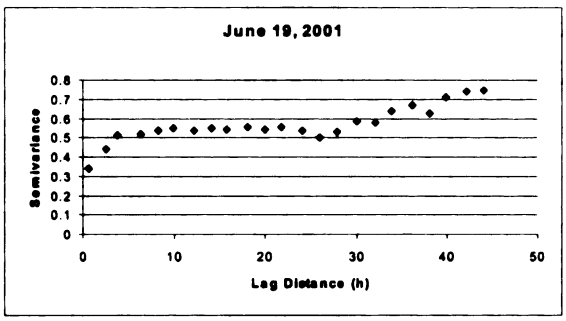
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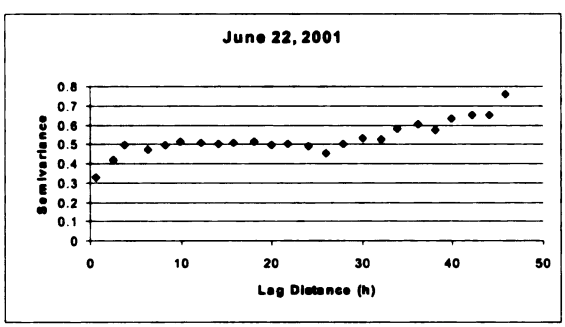
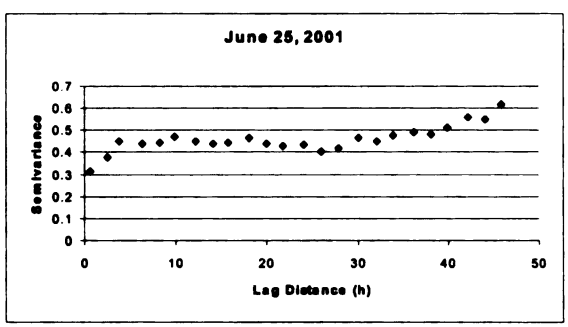
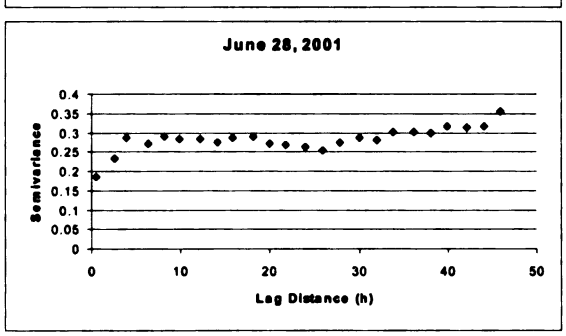
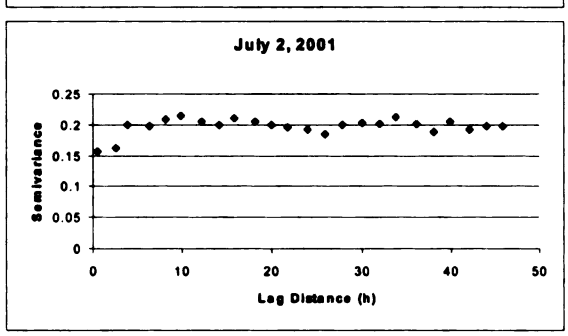
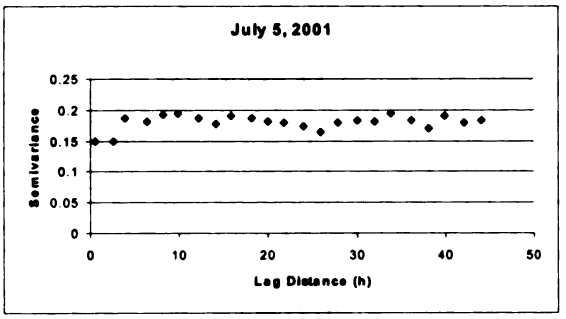
Semivariance
Lag Distance (h)

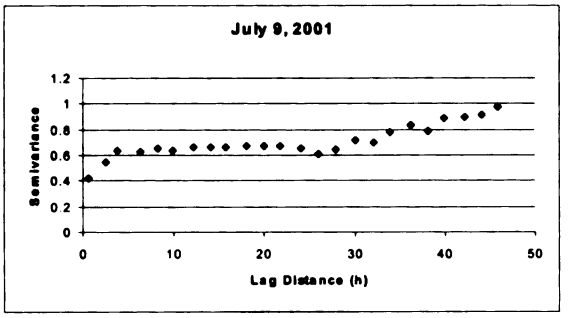

June 22, 2001
Semivariance
Lag Distance (h)

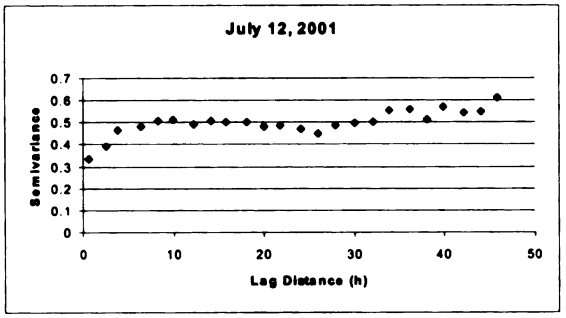

June 25, 2001
Semivariance
Lag Distance (h)

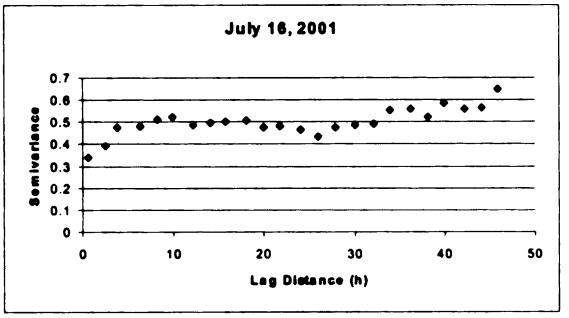

June 28, 2001
Semivariance
Lag Distance (h)

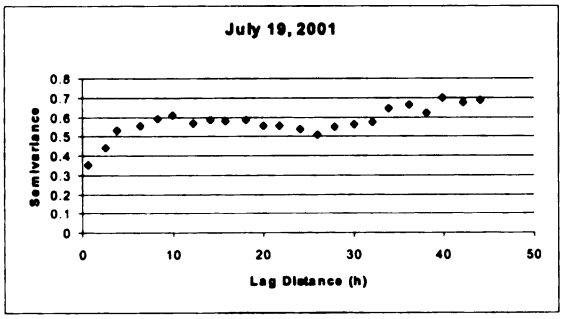

July 2, 2001
Semivariance
Lag Distance (h)

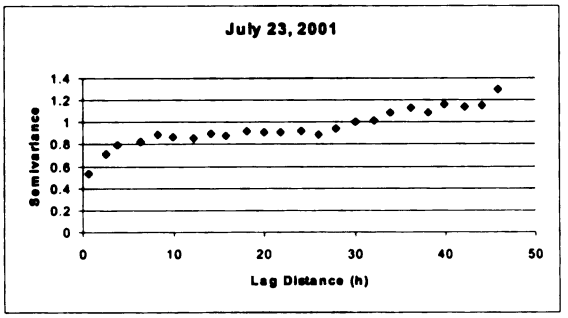

July 5, 2001
Semivariance
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
Lag Distance (h)

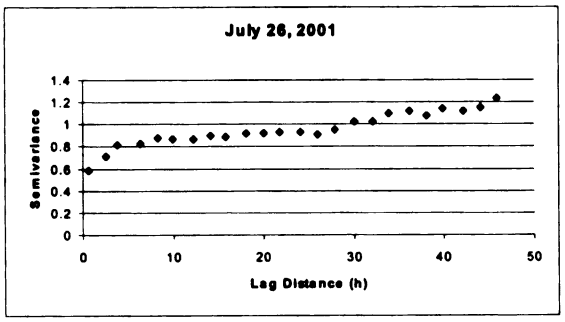

July 9, 2001
Semivariance
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
Lag Distance (h)

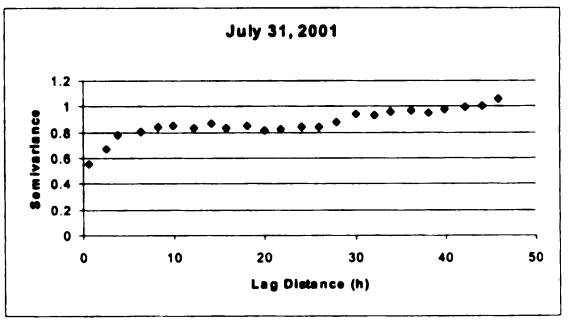

July 12, 2001
Semivariance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
Lag Distance (h)

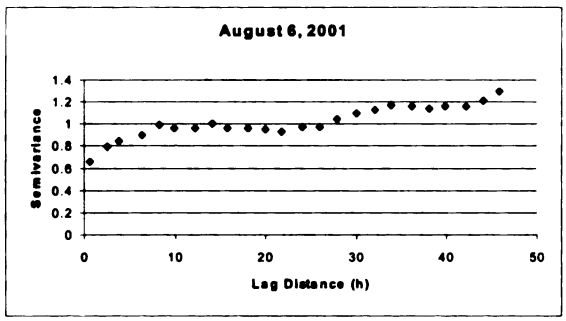

July 16, 2001
Semivariance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
Lag Distance (h)

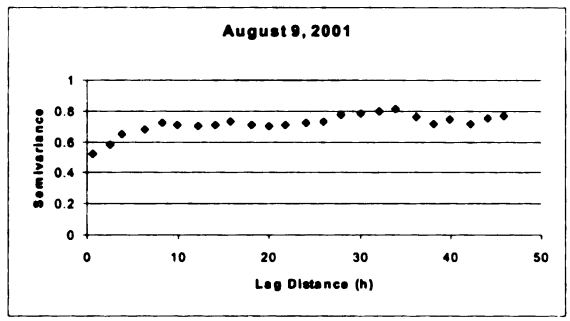

July 19, 2001
Semivariance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
Lag Distance (h)

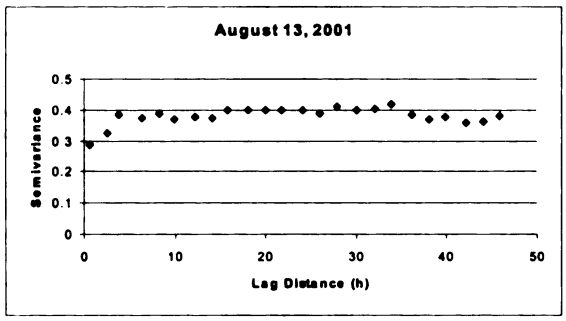

July 23, 2001
Semivariance
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
Lag Distance (h)

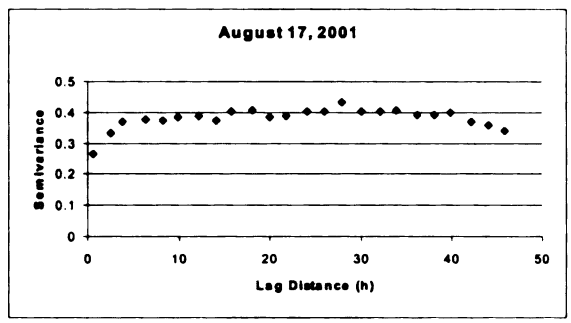

July 26, 2001
Semivariance
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
Lag Distance (h)

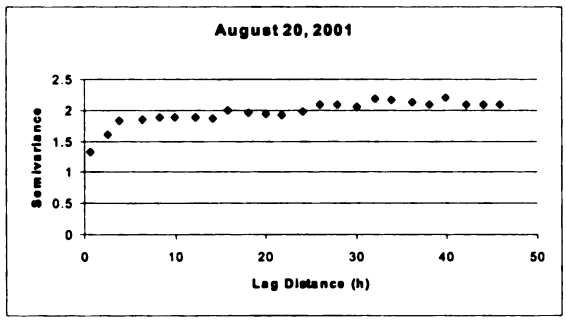

July 31, 2001
Semivariance
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
Lag Distance (h)

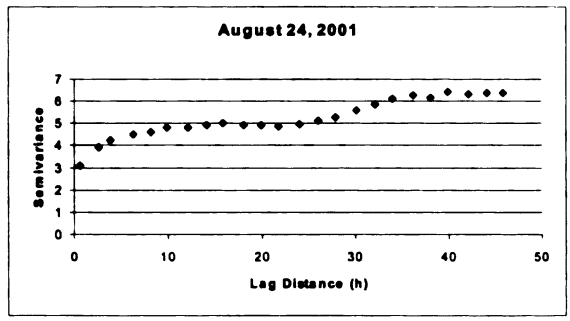

August 6, 2001
Semivariance
Lag Distance (h)

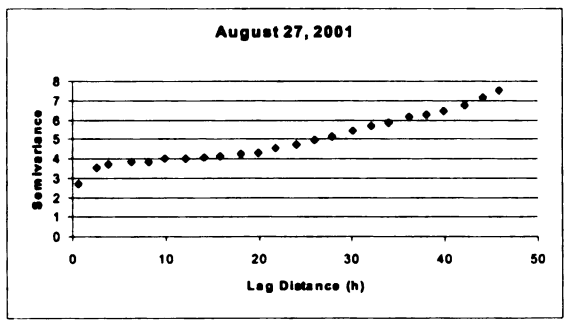

August 9, 2001
Semivariance
Lag Distance (h)

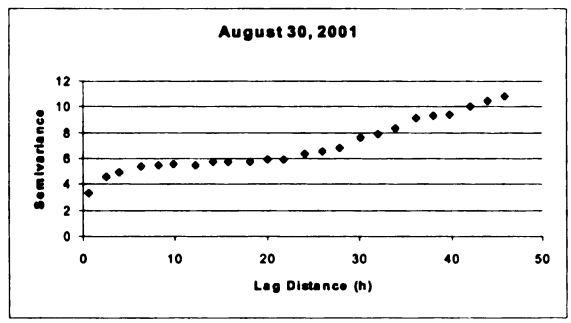

August 13, 2001
Semivariance
Lag Distance (h)

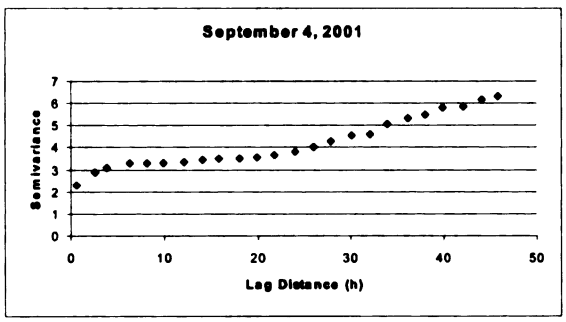

August 17, 2001
Semivariance
Lag Distance (h)

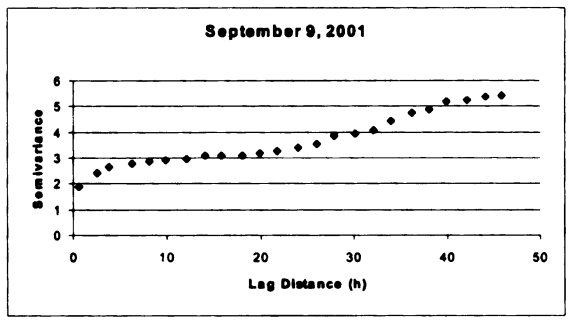

August 20, 2001
Semivariance
Lag Distance (h)

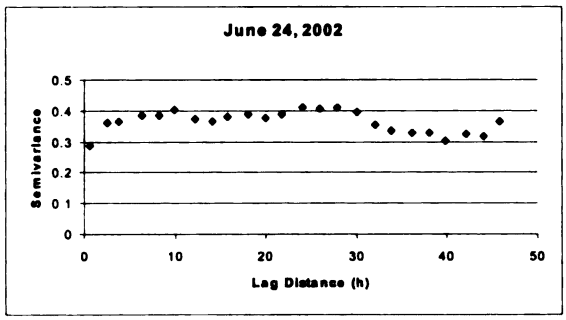

August 24, 2001
Semivariance
Lag Distance (h)

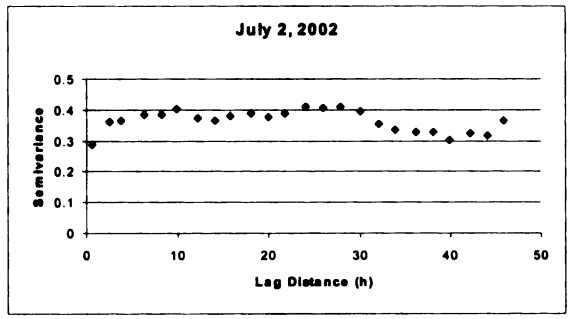

August 27, 2001
Semivariance
Lag Distance (h)

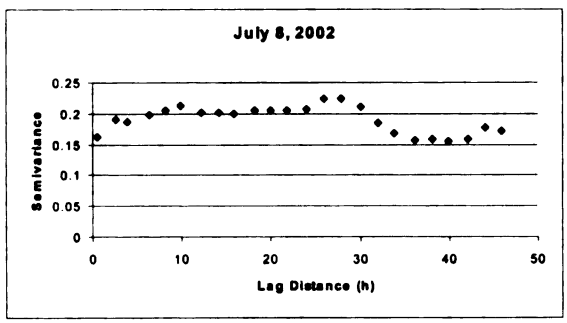

August 30, 2001
Semivariance
Lag Distance (h)

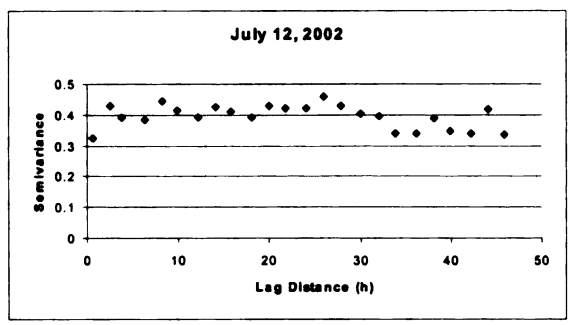

September 4, 2001
Semivariance
Lag Distance (h)

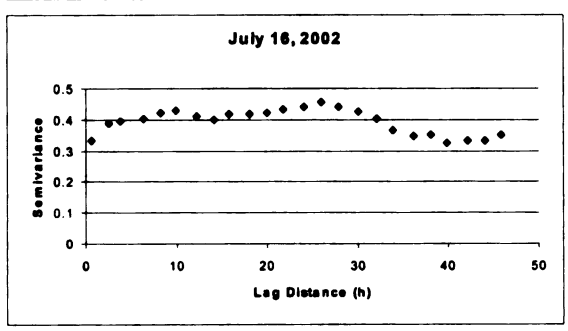

September 9, 2001
Semivariance
Lag Distance (h)

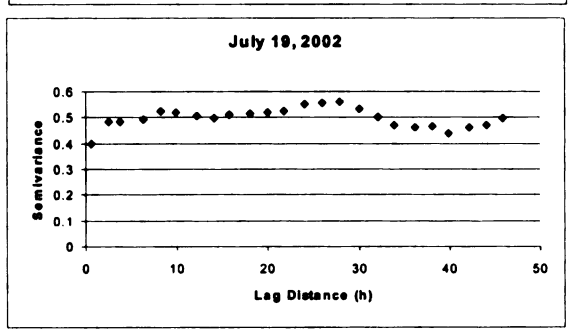

June 24, 2002
Semivariance
Lag Distance (h)

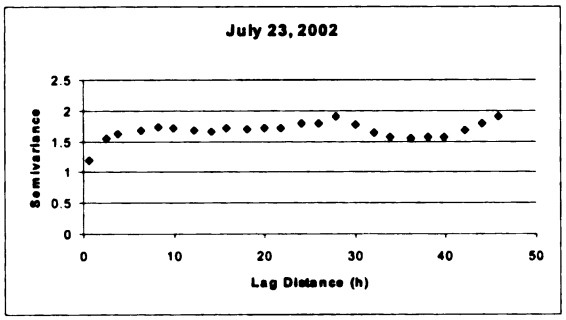

July 2, 2002
Semivariance
Lag Distance (h)

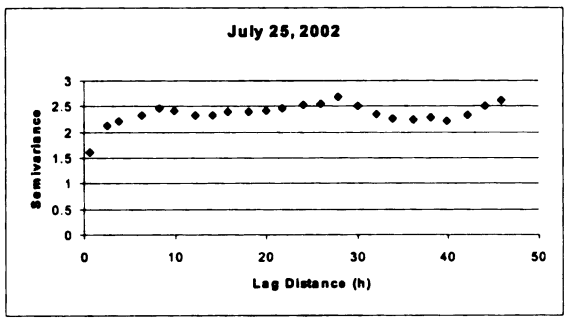

July 8, 2002
Semivariance
Lag Distance (h)

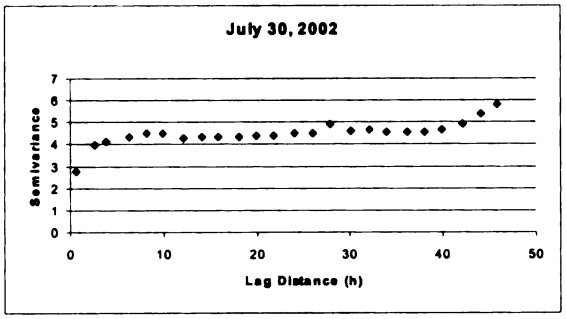

July 12, 2002
Semivariance
Lag Distance (h)

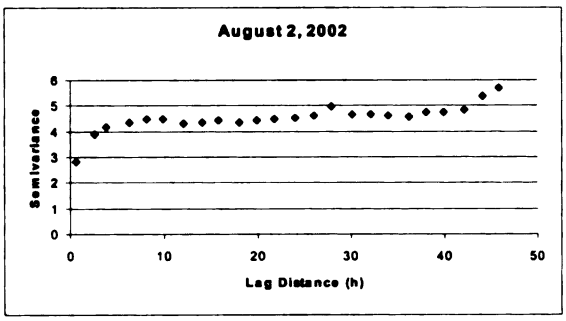

July 16, 2002
Semivariance
Lag Distance (h)

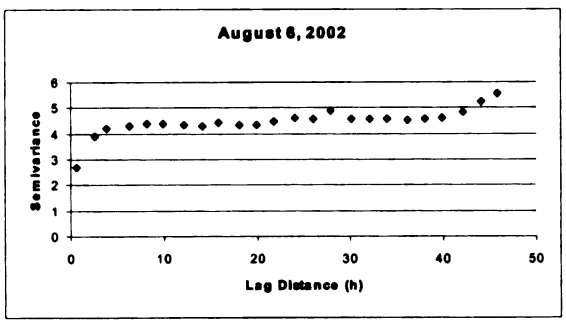

July 19, 2002
Semivariance
Lag Distance (h)

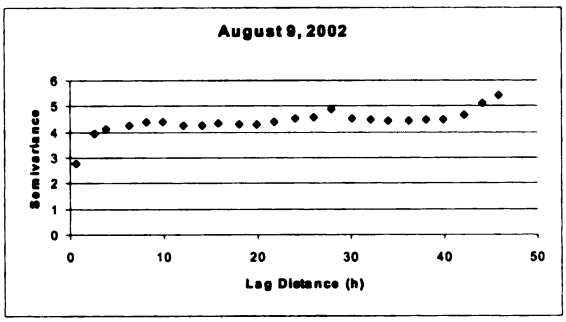

July 23, 2002
Semivariance
Lag Distance (h)

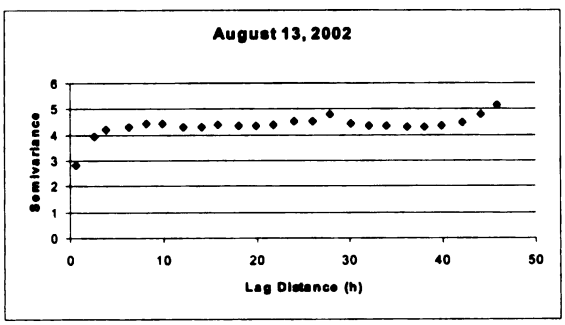

July 25, 2002
Semivariance
Lag Distance (h)

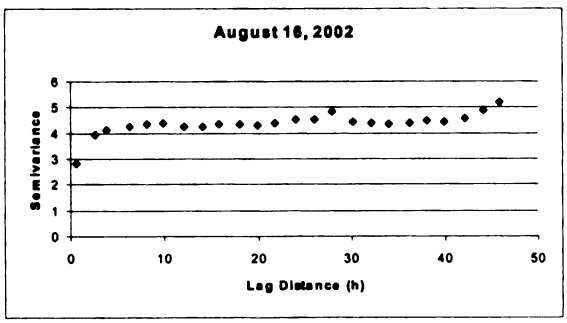

July 30, 2002
Semivariance
Lag Distance (h)

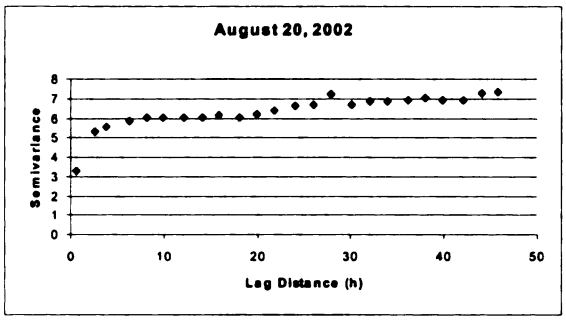

August 2, 2002
Semivariance
Lag Distance (h)

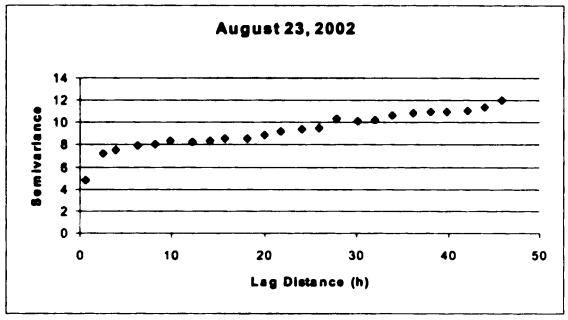

August 6, 2002
Semivariance
Lag Distance (h)

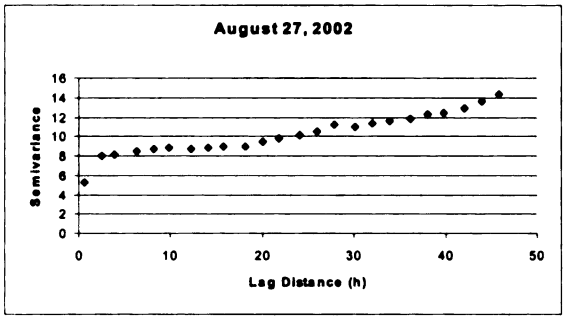

August 9, 2002
Semivariance
Lag Distance (h)

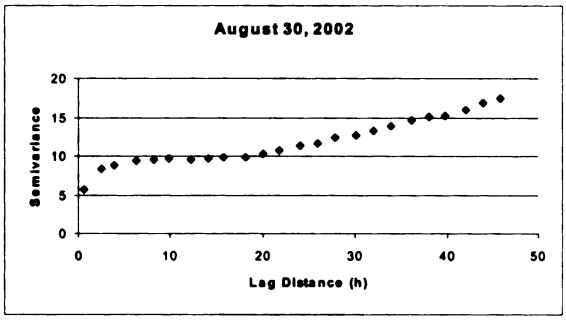

August 13, 2002
Semivariance
Lag Distance (h)

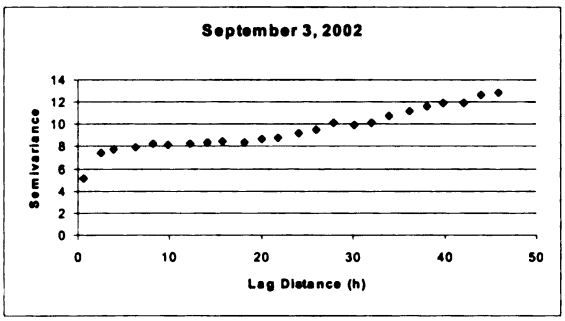

August 16, 2002
Semivariance
Lag Distance (h)

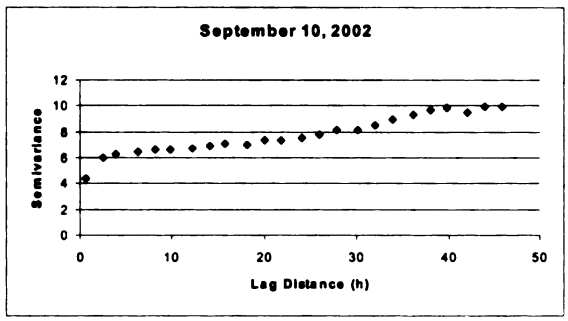

August 20, 2002
Semivariance
Lag Distance (h)

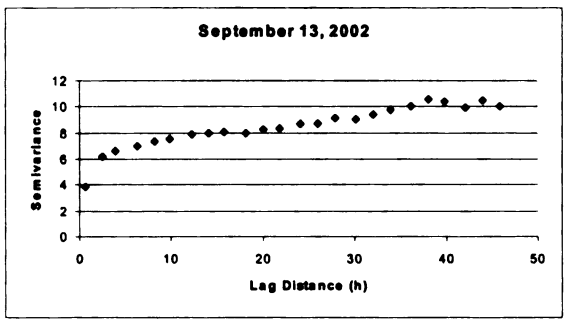

August 23, 2002
Semivariance
Lag Distance (h)


August 27, 2002
Semivariance
Lag Distance (h)


August 30, 2002
Semivariance
Lag Distance (h)


September 3, 2002
Semivariance
Lag Distance (h)


September 10, 2002
Semivariance
Lag Distance (h)


September 13, 2002
Semivariance
Lag Distance (h)