Turfgrass and Environmental Research Online

…Using Science to Benefit Golf

Researchers at the University of Maryland are investigating the life cycle of *Ophiosphaerella agrostis*, the causal organism of bentgrass dead spot (BDS), a recently discovered disease of putting greens. During early stages of disease development, the spots are reddish-brown or copper-colored and mimic ball-mark injury. Except in severe cases, the patches do not coalesce, however recovery of BDS patches is slow and spots may not fully recover prior to winter.

Volume 2, Number 2
January 15, 2003

PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 215 projects at a cost of $21 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

Editor

Jeff Nus, Ph.D.
904 Highland Drive
Lawrence, KS 66044
jnus@usga.org
(785) 832-2300
(785) 832-9265 (fax)

Research Director

Michael P. Kenna, Ph.D.
P.O. Box 2227
Stillwater, OK 74076
mkenna@usga.org
(405) 743-3900
(405) 743-3910 (fax)

Understanding Bentgrass Dead Spot

John E. Kaminski and Peter H. Dernoeden

SUMMARY

- Bentgrass dead spot (BDS) is a recently discovered disease of creeping bentgrass (*Agrostis stolonifera*) and bermudagrass (*Cynodon dactylon* X *C. transvalensis*) putting greens that is incited by the fungal pathogen *Ophiosphaerella agrostis.*
- Survey reports and cultivar evaluation trials revealed that creeping, colonial and velvet bentgrasses are susceptible to BDS. Of the 28 different golf courses from which *O. agrostis* was isolated, however, 14 had grown L-93 in monostands or in blends. It is worth noting that only a single isolate was used in this study, and that varying races of the pathogen may exist in nature. Variation among *O. agrostis* isolates could result in varying levels of disease severity among bentgrass cultivars.
- The fungus rapidly produces fruiting bodies in the absence of fungicide use, and the pathogen is rapidly dispersed by ascospores. Under suitable conditions, ascospores can germinate in as little as two hours. The disease was most commonly found on greens within two years following the seeding of new greens or older greens that had been fumigated with methyl bromide.
- Field observations confirm that the disease normally declines dramatically within one to three years. The oldest greens where BDS was found were six years old. However, disease may reappear during periods of prolonged heat stress. Thus far, BDS appears to be restricted to sand-based greens, collars, and tees, and has not been found in bentgrass or bermudagrass grown on native soil.
- Results of the survey and other observations confirmed that the disease is most prevalent in July and August, but may appear in May and can remain active in bentgrass as late as December. In a bermudagrass green in Florida, however, the disease appeared as early as March.

Bentgrass dead spot (BDS) is a relatively new disease of creeping bentgrass (*Agrostis stolonifera*) that is incited by the fungal pathogen *Ophiosphaerella agrostis* (1). In creeping bentgrass grown on golf course putting greens, BDS appears initially as small, dime-sized spots that may increase up to three to four inches in diameter (2).

JOHN E. KAMINSKI is a graduate research assitant, and PETER H. DERNOEDEN, Ph.D., is a Professor of Plant Pathology in the Department of Natural Resource Sciences and Landscape Architecture at the University of Maryland.

During early stages of disease development, the spots are reddish-brown or copper-colored and mimic ball-mark injury. As the disease progresses, grass in the center of the spots becomes tan, while leaves in the outer edge appear reddish-brown. Frog-eye patches occur, but they are uncommon. Patches may be distributed throughout the putting green or localized. Except in severe cases, the patches generally do not coalesce. Sometimes the spots form depressions or pits in the putting surface.

Disease recovery is slow, and in severe cases, BDS spots will not fully recover prior to winter. Foliar mycelium (i.e., microscopic strands of the fungus) is not observed in the field, but when diseased plants are incubated under high humidity for three to five days, a white to pale pink foliar mycelium may develop. Unlike other species of *Ophiosphaerella* that are turf pathogens, pseudothecia (i.e., sexual fruiting bodies of the fungus) often are found in the field on dying leaf, sheath, and stolon tissues.

Need for Research

There is little information regarding the biology of *O. agrostis* or the relative susceptibili-

During the early stage of bentgrass dead spot development, new spots appear reddish-brown or bronze. As diseased spots increase in diameter, the periphery of active spots maintains a reddish-brown appearance, while dead tissue in the center appears tan.

USGA Turfgrass and Environmental Research Online 2(2):1-7.
TGIF Record Number: 85062

Cultivar	Bentgrass species	Infection centers per plot[x]					
		2000		2001		2002[y]	
		6 Sept	29 Nov	15 May	16 Aug	18 July	16 Aug
ABT-CRB-1	creeping	27a-dz	11b-e	9bcd	3ab	6abc	22abc
Backspin	creeping	18cde	6fgh	6cde	2bcd	2ef	12c-f
BAR AS 8US3	creeping	21b-e	7d-h	7bcd	2bcd	5a-f	18a-d
BAR CB 8FUS2	creeping	22b-e	10b-e	11ab	2bcd	3c-f	12c-f
Bardot	colonial	32ab	6e-h	4ef	1cd	2def	9def
Bavaria	velvet	8f	4h	2f	0d	2def	4f
Century	creeping	27a-d	14bc	11ab	3ab	6abc	22abc
Crenshaw	creeping	17def	5gh	6de	1bcd	2ef	6ef
Imperial	creeping	33ab	9c-g	8bcd	1cd	4b-f	15b-e
L-93	creeping	37a	11bcd	10abc	2abc	8ab	26a
Penn A-1	creeping	33ab	15bc	11ab	3ab	9a	25ab
Penn A-2	creeping	23b-e	8d-h	8bcd	2bcd	5a-f	17a-d
Penn A-4	creeping	29a-d	15b	12ab	3abc	5a-e	15b-e
Penn G-1	creeping	25a-e	10b-f	8bcd	2abc	3c-f	10def
Penn G-6	creeping	29abc	8d-h	9bcd	1bcd	4b-f	23ab
Penncross	creeping	14ef	6e-h	7bcd	1cd	2ef	7ef
Pennlinks	creeping	17def	7d-h	5de	2bcd	1f	5ef
Providence	creeping	24a-e	11b-e	11ab	3abc	4c-f	9def
SR1119	creeping	22b-e	10b-f	11ab	2bcd	3c-f	18a-d
SR7200	velvet	32ab	24a	14a	5a	6a-d	12c-f

[x] Data were transformed $(y+0.5)^{1/2}$, but pre-transformed means are shown.

[y] Bentgrass dead spot fully recovered in the autumn of 2001 and data from 2002 represent new infection centers.

[z] Means in a column followed by the same letter are not significantly different ($P \leq 0.05$) based on the protected least significant difference multiple mean comparison test.

Table 1. Bentgrass dead spot infection centers for twenty field-grown *Agrostis spp*. selections, College Park, MD between 2000 and 2002.

ty of bentgrass cultivars to the pathogen, geographic distribution of the disease, or cultural factors associated with BDS outbreaks. Hence, the primary objectives of this research were: 1) to determine the susceptibility of various field-grown bentgrass cultivars to *O. agrostis*; 2) elucidate cultural factors associated with BDS outbreaks; 3) determine the distribution of the disease in the U.S.; and 4) investigate pseudothecia production, ascospore release and germination, and other more basic biological properties of the fungal pathogen.

Bentgrass cultivar susceptibility to *O. agrostis* was assessed on a USGA-specified research green between 2000 and 2002 at the University of Maryland Paint Branch Turfgrass Research Facility in College Park, Maryland. Seventeen cultivars and experimental selections of creeping bentgrass, two cultivars of velvet bentgrass, and Bardot colonial bentgrass (Table 1) were seeded on September 20, 1999. The area was subjected to routine cultural practices throughout the study (i.e., fertilization, vertical mowing, aeration, and topdressing). On June 12, 2000, all plots were inoculated with an isolate of *O. agrostis*.

Disease progress at numerous golf courses also was monitored between 1999 and 2001 (Table 2). In addition, a mail survey intended to collect information regarding the soil characteristics, cultivar(s) used, and any chemical or fertilizer applications that may have influenced BDS

incidence and severity was sent to 21 golf courses. A timeline of BDS incidence and severity was developed based on the initial outbreak of BDS and the severity of the disease in consecutive years.

Biology of the Pathogen

Winter-dormant creeping bentgrass field samples showing symptoms of BDS were incubated at temperatures ranging from 59 to 86 F (15 to 30 C). Between 12 and 28 days of incubation, reactivation of BDS symptoms occurred at temperatures 68 F (20 C), but the greatest expansion in BDS patch diameter occurred at 77 F (25 C) and 86 F (30 C). The optimum temperatures for growth of hyphae among ten *O. agrostis* isolates ranged from 77 and 86 F (25 C to 30 C), and growth was suppressed at 95 F (35 C).

Pseudothecia of *O. agrostis* was produced in the lab on a mixture of sterilized tall fescue seed and wheat bran. Pseudothecia developed under constant fluorescent light at 55 to 82 F (13 to 28

Golf course/State	Cultivar(s)/blend	Date Sample received	Planted
P.B. Dye G.C., MD	Penn G-2	Aug. 1998	Apr.-June 1998 (se)[z]
Lowe's Island Club, VA[v]	Pennlinks	Sept. 1998	Autumn 1997 (se)
Beechtree G.C., MD	L-93+Crenshaw	Oct. 1998	Aug.-Sept. 1997 (se)
Ocean City G&YC, MD	Penncross	Oct. 1998	June 1997 (se)
Hayfields C.C., MD	L-93+Crenshaw	Oct. 1998	Autumn 1997 (se)
Sand Ridge C.C., OH	L-93	Oct. 1998	Summer 1997 (se)
Marlton G.C., MD	L-93+Crenshaw	Oct. 1998	Oct. 1997 (se)
Hampshire Greens G.C., MD	Providence	Nov. 1998	Oct. 1996 (se)
Skokie G.C., IL[w,x]	SR 1119	Dec. 1998	Sept. 1996 (se)
Hartefeld National G.C., PA	Crenshaw+Southshore	Dec. 1998	Sept.-Nov. 1994 (se)
Texas A&M University, TX	Champion	June 1999	Summer 1997 (sp)
Trenton C.C., NJ	L-93	June 1999	Nov. 1998 (sd)
Scotch Meadows G.C., NC[w,y]	Penncross	June 1999	Aug. 1998 (se)
Persimmon Woods G.C., MO	Penn G-2	July 1999	Sept. 1997 (se)
Rutgers University, NJ	L-93	July 1999	Nov. 1998 (se)
River Bend G.C., MA	L-93	July 1999	June 1997 (se)
Bulle Rock G.C., MD	L-93	Aug. 1999	June 1997 (se)
The Bridges G.C., PA	Penncross	Aug. 1999	Summer 1994 (se)
Honeybrook G.C., PA	L-93	Nov. 1999	Apr.-June 1999 (se)
Inniscrone C.C., PA	L-93+SR1020+Providence	Mar. 2000	Autumn 1997 (se)
Orchard Creek G.C., NY	L-93	Aug. 2000	Sept. 1998 (se)
Red Hawk G.C., MI	Providence	Sept. 2000	Autumn 1998 (se)
Atlantic City C.C., NJ	Penn A-4	Sept. 2000	Sept. 1999 (se)
Glen View Club, IL[w,x]	SR1119+L-93+Providence	Dec. 2000	Sept. 1999 (se)
Olympia Fields C.C., IL[w,x]	L-93	Dec. 2000	Sept. 1999 (se)
Kelly Plantation G.C., FL	TifDwarf	Apr. 2001	July-Sept. 1998 (sp)
Mountain Branch G.C., MD	L-93	July 2001	Sept. 2000 (se)
Blue Mash G.C., MD	Penn A-4	Aug. 2001	Sept. 2000 (se)

[v] Disease also found on Penncross tees seeded in 1997 and 1998.

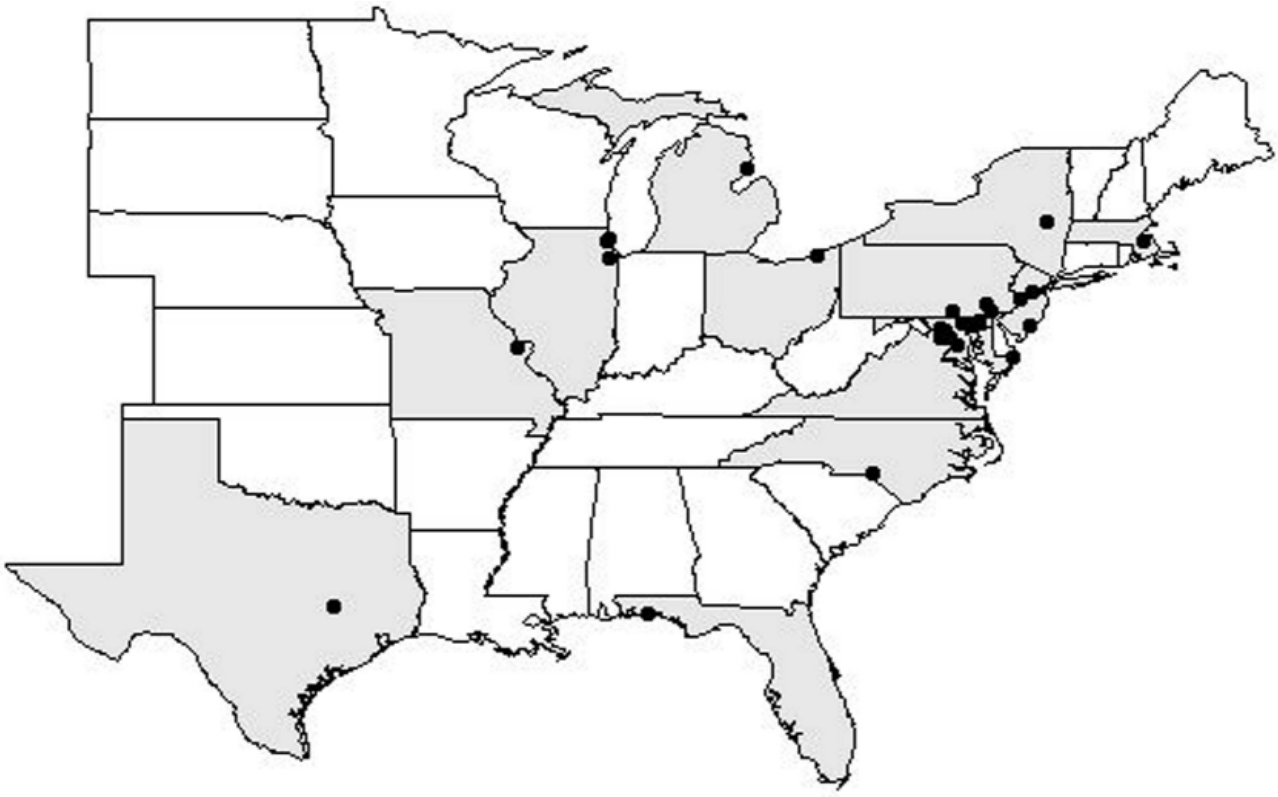
[w] Area fumigated with methyl bromide prior to seeding.

[x] Isolated by Dr. Randy Kane, University of Illinois.

[y] Isolated by Dr. Henry Wetzel, North Carolina State University.

[z] Seeded (se); sodded (sd); sprigged (sp).

Table 2. Location, cultivar, date samples were received and date of planting of nineteen creeping bentgrass and hybrid bermudagrass greens confirmed to be infected by *Ophiosphaerella agrostis*, 1998-2001.



Location of creeping bentgrass and bermudagrass greens confirmed to be infected by *Ophiosphaerella agrostis* in the United States between 1998 and 2002.

C), but no pseudothecia developed in darkness at any temperature. Pseudothecia developed in as few as four days and mature ascospores were forcefully discharged or exuded en masse in the presence of water after a week of incubation.

Ascospores (fungal spores produced in pseudothecia) germination was rapid. Ascospores incubated at 86 F (30 C) germinated in as little as two hours. Germination during the first four hours of incubation was enhanced by both light and the presence of bentgrass leaves or roots. After 18 hours of incubation, however, there were few differences in the percentage of ascospores germinated, regardless of light treatment or presence of plant tissue. Ascospores were observed to either directly penetrate leaves and stems, or to enter leaves through open stomates (i.e., pores in the leaves of all higher plants used for gas exchange and evaporative cooling). Hence, *O. agrostis* can rapidly produce enormous numbers of spores, which are capable of infecting new plants within a few hours.

Cultivar Evaluation

Data from this study revealed that *O. agrostis* attacks all of the common *Agrostis* species and cultivars grown on golf courses. Individual cultivars within a species showed varying levels of susceptibility. The velvet bentgrass cultivars SR7200 and Bavaria generally were the most and least susceptible cultivars, respectively. Bardot colonial bentgrass was highly susceptible to BDS, but exhibited the greatest amount of recovery prior to winter.

The creeping bentgrass cultivars exhibited varying levels of susceptibility and recovery. Among the creeping bentgrass cultivars, L-93 had the greatest number of infection centers during the period of highest disease pressure (September 6, 2000), but the number of infection centers was not significantly different from many other creeping bentgrass cultivars, including Penn A-1, A-4, G-1, G-6, Imperial, ABT-CRB-1 and Providence. Pennlinks, Penncross and Crenshaw had BDS levels that were not significantly different from the least susceptible cultivar (Bavaria) on September

6, 2000, and generally were the least susceptible creeping bentgrass cultivars over the course of the study.

Recovery of BDS patches was slow and did not begin to occur until after September 6, 2000. Once bentgrass growth decreased in late autumn, little recovery occurred and spots remained evident until growth resumed in late spring. Recovery was most apparent during late spring and early summer. Recovery of all cultivars from BDS probably was enhanced by fertilizer applications in September and November. In 2001, BDS levels were considerably less and new infections were minimal. Most infection centers from 2000 continued to recover, but new disease activity was observed in several previously infected spots between June and September, 2001. All bentgrass cultivars fully recovered by November, 2001.

In 2002, the disease reappeared in July following a prolonged period of heat stress. Disease levels were moderately severe and BDS infection centers were greatest in ABT-CRB-1, BAR AS 8US3, Century, L-93, Penn A-1, Penn G-6 and SR1119. The reason for decreased BDS activity by 2001 is unknown. A similar decline occurs with take-all patch (*Gaeumannomyces graminis* var. *avenae*) in *Agrostis* turf in response to a buildup of bacterial antagonists (6,7).

Decline in BDS activity may be attributed to the buildup of antagonistic soil microorganisms, maturation of the turf, variable environmental conditions, or the cultural practices and the chemicals employed. Data collection in 2002, however, revealed that the disease can reactivate in older turf under conditions of high temperature stress.

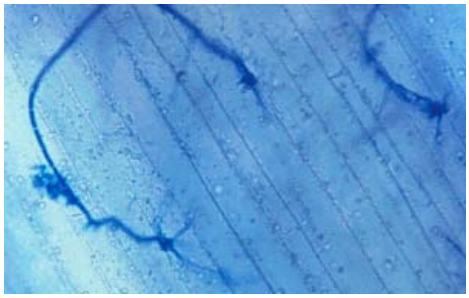
Field Observations and Survey Results

Bentgrass dead spot was found only on newly constructed greens or where older greens were fumigated with methyl bromide. The disease generally developed between one and two years following bentgrass establishment. However, outbreaks also were observed in creeping bentgrass greens that were less than one-year-old and as old as six years of age. With few exceptions, BDS was most severe during the first or second year of symptom expression and declined as the greens aged. The decline phase generally lasted anywhere from one to three years after the first year of disease expression, with the number of infection centers per green normally decreasing in subsequent years.

Unlike other turfgrass pathogens within the genus *Ophiosphaerella*, *O. agrostis* commonly produces flask shaped fruiting bodies known as pseudothecia on necrotic leaf, sheath and stolon tissue.

All newly constructed greens affected by BDS consisted of at least 80% sand as the primary soil medium. In addition, two older golf courses were renovated using methyl bromide, but had a sand-based medium from several years of topdressing. Although BDS was observed primarily on the putting surfaces, occasionally it was found on sand-based bentgrass collars and tees, indicating that *O. agrostis* can attack creeping bentgrass maintained at higher mowing heights. Bentgrass dead spot was not found in fairways or other sites where bentgrass turf was grown on native soil.

Active BDS infection centers generally appeared in areas with full sun and good air circulation and disease severity varied from a few spots to several hundred per green. In addition, *O. agrostis* infection centers occurred predominantly along ridges and on mounds and south-facing slopes of greens. These areas are particularly prone to higher soil temperatures and often are the first to exhibit drought symptoms. These conditions generally result in higher levels of plant stress and may reduce the defense capabilities of bentgrass plants.



Ascospores can germinate in as little as two hours and can enter the plant by directly penetrating leaf and root tissue or through open stomates.

Bentgrass dead spot activity was observed as early as May, but generally was most active during the summer and early autumn months. Recovery of BDS patches was slow, and active spots often remained evident until the first hard frost. Soil pH at construction and during periods of disease activity ranged from 4.9 to 7.8. Various nitrogen (N) sources were applied at different golf courses throughout the year. Although no association between any single nitrogen source and disease outbreak could be made, applying small amounts of water soluble nitrogen (0.1 to 0.125 lb N 1000 ft^{-2}) with each fungicide application may help to reduce BDS severity and speed bentgrass recovery.

According to Wetzel (9), weekly applications of urea in conjunction with an effective fungicide reduced BDS severity. When applied weekly, however, urea alone did not significantly reduce BDS severity when compared to the untreated control (9). Field fungicide evaluation trials reported by Wetzel (9) and Towers (8) showed that propiconazole (Banner), chlorothalonil (Daconil), thiophanate methyl (Cleary's 3336), fludioxonil (Medallion) and iprodione (Chipco 26GT) effectively controlled BDS.

Bentgrass dead spot can be found in creeping bentgrass as far north as Michigan, as far west as Missouri, and along the eastern seaboard of the United States from Massachusetts to North Carolina. In addition, *O. agrostis* was found in Texas and Florida causing dead spots in hybrid bermudagrass (*Cynodon dactylon* x *C. transvalensis*) greens that had been overseeded with rough-stalk bluegrass (*Poa trivialis*). The occurrence of *O. agrostis* infection of bermudagrass in Texas subsequently was reported by Krausz et al. (5). The role of *Poa trivialis* in the introduction of the pathogen and spread of the disease is unknown.

Acknowledgements

We thank the United States Golf Association for providing financial support for this study, R. Kane and H. Wetzel for providing isolates, and all of the golf course superintendents who contributed information.

Literature Cited

1. Câmara, M. P. S., N. R. O'Neill, P. van Berkum, P.H. Dernoeden, and M.E Palm. 2000. *Ophiosphaerella agrostis sp*. nov. and its relationship to other species of *Ophiosphaerella*. *Mycologia* 92:317-325. (TGIF Record 65820)

2. Dernoeden, P.H., N.R.O'Neill, M.P.S Câmara, and Y. Feng. 1999. A new disease of *Agrostis palustris* incited by an undescribed species of *Ophiosphaerella*. *Plant Disease* 83:397. (TGIF Record 58421)

3. Krausz, J.P., R.H. White, W. Foerster, N.A Tisserat, and P.H. Dernoeden. 2001. Bermudagrass dead spot: A new disease of bermudagrass caused by *Ophiosphaerella agrostis*. *Plant Disease* 85:1286. (TGIF Record 84344)

4. Kaminski, J. E., P. H. Dernoeden., N. R. O'Neill, and B. Momen. 2002. Reactivation of bentgrass dead spot and growth, pseudothecia production and ascospore germination of *Ophiosphaerella agrostis*. *Plant Disease* 86(12):1290-1296. (TGIF Record 84346)

5. Kaminski, J. E. and P. H. Dernoeden. 2002. Geographic distribution, cultivar susceptibility, and field observations on bentgrass dead spot.

Plant Disease 86 (11):1253-1259. (TGIF Record 83302)

6. Smiley, R.W., P. H. Dernoeden, and B. B. Clarke. 1992. Diseases of roots. Pages 57-58. *In:* Compendium of Turfgrass Diseases. The American Phytopathological Society Press, St. Paul, MN. (TGIF Record 21874)

7. Smith, J.D., N. Jackson, and A. R. Woolhouse. 1989. Fungal Diseases of Amenity Turf Grasses. E. & F.N. Spon, New York, NY. (TGIF Record 16673)

8. Towers, G.W., P. R. Majumdar, E. N. Weibel, C. L. Frasier, J. N. Vaiciunas, M. Peacos, and B. Clarke. 2000. Evaluation of chemical and biological fungicides for the control of bentgrass dead spot in creeping bentgrass. 2000 Rutgers Turfgrass Proceedings 32:211-215. (TGIF Record 76402)

9. Wetzel, H.C. 2000. Evaluation of fungicides and urea for the control of bentgrass dead spot in an 'L-93' putting green in Raleigh, NC, 1999. *Fungicide and Nematicide Tests* 55:510. (TGIF Record 70125)