

Survival of *Pythium aphanidermatum* in Golf Course Turfs

T. J. HALL, Graduate Research Associate, P. O. LARSEN, Associate Professor, and A. F. SCHMITTHENNER, Professor, Department of Plant Pathology, The Ohio State University, Columbus 43210, and Ohio Agricultural Research and Development Center, Wooster 44691

ABSTRACT

HALL, T. J., P. O. LARSEN, and A. F. SCHMITTHENNER. 1980. Survival of *Pythium aphanidermatum* in golf course turfs. *Plant Disease* 64:1100-1103.

Pythium aphanidermatum survival in turfs of bentgrass and annual bluegrass was primarily associated with thatch. Depending on the site and time of year, propagule densities in thatch were higher than in soil, on a weight basis and volume basis. *P. aphanidermatum* was isolated frequently from undecayed coarse material in thatch and live roots and occasionally from live shoots. Propagule numbers fluctuated seasonally in both sieved thatch and soil. Numbers were highest from November through January, declined during April and May, and then increased from mid-July until October. Oospores from thatch collected in January germinated and were identified as *P. aphanidermatum*, suggesting that oospores may be an overwintering form of the fungus. Bentgrass plants that were inoculated with thatch and soil fractions became diseased. This viable inoculum from naturally infested turfgrass occurred throughout the 15-mo sampling period.

Additional key words: cottony blight, Pythium blight, turfgrass diseases

A survey of Pythium blight on turfgrasses in Ohio in 1974-1975 (13) showed that *Pythium aphanidermatum* (Edson) Fitzpatrick was the primary incitant. *P. aphanidermatum* survives as oospores in soil, infected host tissue, and organic debris; as a parasite of asymptomatic plants (3,15,17); and in field soils and weed hosts in cultivated and uncultivated areas (3,17,18). However, survival of oospores associated with Pythium blight of turfgrasses has not been examined.

Our objectives were to determine the location, relative amounts, and propagule type of *P. aphanidermatum* inoculum in golf course turfs.

MATERIALS AND METHODS

Sampling. Survival of *P. aphanidermatum* in creeping bentgrass (*Agrostis palustris* Huds. 'Penncross') and annual bluegrass (*Poa annua* L. 'Seaside') turfs was examined on three central Ohio golf courses with a history of Pythium blight (13). Turf was sampled in 2 × 10 m areas at each location throughout the 15-mo study, which began in July 1976.

Samples consisted of 10 soil cores (1.9 cm diameter, 5 cm deep) taken at random from each site and stored in plastic bags at 14 C for 1-3 days until isolations were made. Each core was separated into thatch (a tightly intermingled layer of living and dead stems and roots that developed between the green vegetation and the soil surface [2]), soil immediately

below the thatch, and intact living plants growing in and above the thatch.

Thatch and live plants were placed separately in 250-ml glass jars with 1 ml of Tween 20. Jars were covered with nylon net to prevent loss of material and were washed with cold, running tap water for 20 min. Materials were air-dried at 23 C for 48-72 hr. Drying destroys mycelium and zoospores of *P. aphanidermatum* and other *Pythium* spp. that produce lobate sporangia and would interfere with measurement of oospores as an inoculum source (1,3,7,9).

Thatch was separated into coarse (material that did not pass through the 0.841-mm mesh sieve) and sieved fractions. Soil samples were crushed and sieved. Sieved thatch and soil were dilution-plated to determine surviving propagules. Live plants were separated into root and shoot portions. These and coarse thatch were placed on a selective medium to determine the presence of *P. aphanidermatum*.

Isolation techniques. *Pythium* spp. were isolated from coarse thatch, roots, and shoots on Pythium isolation medium (14). Twenty 1-2 cm pieces of coarse thatch were placed four segments per petri dish on sucrose-asparagine pentachloro-nitrobenzene (SA-PCNB). Agar in the dish was then inverted to create an airtight seal around each piece of thatch material. This allowed rapidly growing mycelium of *P. aphanidermatum* to grow through the agar, facilitated isolation, and minimized contamination from other microorganisms. Plates were incubated at 36 C for 7 days and observed at 24-hr intervals.

Stock cultures for identification of *Pythium* spp. were maintained on SA medium, which is identical to SA-PCNB

without fungicides and antibiotics (14). To induce sporulation, two to four blades of autoclaved red fescue (*Festuca rubra* L. 'Pennlawn') were added to each petri dish of sporulation medium before seeding with stock cultures of *Pythium*. Isolates were identified after 7 days on the basis of oospore and sporangial morphology (20,21).

Determination of propagule numbers.

The numbers of survival propagules (oospores) of *P. aphanidermatum* in sieved thatch and soil were determined by a dilution plate technique. Five-gram portions of sieved thatch and soil were each suspended in 25 ml of 0.15% water-agar solution. Each suspension was mixed in a Sorvall Omnimixer (Ivan Sorvall, Inc., Newton, CT) at maximum velocity for 30 sec, then serially diluted 5, 10, 50, 100, 1,000, and 10,000 fold with a 0.15% water-agar solution. One-milliliter aliquots of each dilution were pipetted into each of four petri plates containing cornmeal agar-pimaricin-streptomycin sulfate medium (CMAP) (3,10) and spread evenly over the agar surface with a sterile glass rod. This procedure was repeated twice for each sample. Dilution plates were incubated at 36 C in the dark, and fungal colonies were counted after 24 hr. *P. aphanidermatum* was identified by colony morphology and the rapid growth at 36 C.

Oospores in sieved soil and thatch. A 10-fold dilution (0.1 ml) from a January 1977 sample was mixed with 0.1 ml of the following solution: double distilled water, 1 L; cornmeal agar, 1.5 g; pimaricin, 0.01 g; and streptomycin sulfate, 0.3 g. A 0.1-ml portion of this soil or thatch suspension was placed into the well of a hanging-drop slide and a coverslip was placed over the well. Ten hanging-drop slides were prepared in this manner. The slides were placed on U-shaped glass rods in petri dishes with water to saturate the air. Slides were incubated in the dark at 36 C and observed for oospores at 4-hr intervals. After oospores germinated, they were carefully lifted from the slide well by using a standardized root canal reamer No. 10-25 M. M., style D (Kerr Manufacturing Co., Detroit, MI), and transferred to SA-PCNB agar for growth and identification.

Presence of oospores in coarse thatch was determined by clearing tissues in a saturated solution of chloral hydrate, and then immersing in a 0.3% acid fuchsin-

Approved for publication as Journal Article 55-80 of the Ohio Agricultural Research and Development Center, Wooster.

0191-2917/80/12110004/\$03.00/0
©1980 American Phytopathological Society

saturated chloral hydrate solution, and autoclaving for 1 hr at 121 C (19). Specimens were stored in lactophenol until examination.

Pathogenicity. Coarse thatch, sieved thatch, and soil were used as inoculum sources. Inoculum material was from the 26 January, 17 March, 1 and 17 May, 4 July, 1 and 22 August 1977 samplings. Coarse thatch (0.3 g), sieved thatch (1.0 g), and soil (4.0 g) were used to inoculate each pot of creeping bentgrass. Three pots per treatment were used per sample date. Inoculum was placed on the soil surface of pots with 6-wk-old plants. For each sample date, two uninoculated pots of bentgrass were included.

Plants were incubated on a mist bench at 30 C with a 12-hr photoperiod. Plants were observed daily, and when symptoms of *Pythium* blight occurred, isolations from diseased tissues were made on SA-PCNB medium. *Pythium* spp. were identified after sporangia and oospores had formed on SA medium.

RESULTS

Isolations. *P. aphanidermatum* was isolated from coarse thatch, roots, and shoots in all samples. The fungus was recovered from 23% of the coarse thatch (359 of 1,560 attempts), from 9.8% of root (153 of 1,560), and from 4.6% of shoot platings (71 of 1,560). Analysis of variance and comparison of means using Duncan's multiple range test (8) indicated that the differences in isolation frequencies between thatch and roots, thatch and shoots, and roots and shoots were significant. Frequency of isolation did not appear to be influenced by sampling dates. The fungus was recovered from coarse thatch on all 26 sample dates and from shoots and roots on 21.

Propagule densities. Propagule densities were considerably lower in soil than in sieved thatch. Figure 1 shows the mean number of propagules per gram of sieved thatch and soil throughout the 15 mo. Propagule densities in sieved thatch and soil differed significantly on a weight or volume basis for 25 of the 26 sample dates.

Seasonal fluctuations in sieved thatch and soil were observed (Fig. 1). Propagule numbers were highest from November 1976 through January 1977 and lowest in May 1977.

Oospore observation. Structures resembling oospores were observed in hanging-drop slides containing suspensions of sieved thatch (Fig. 2). These spores were visible before and after they germinated. The position of ungerminated oospores was noted on the slides so that germination could be observed. Germination usually occurred in 12–16 hr at 36 C. When germinated oospores and attached mycelia were transferred to a selective medium and cultured at 36 C, the fungus was identified as *P. aphanidermatum*. No oospores were observed in

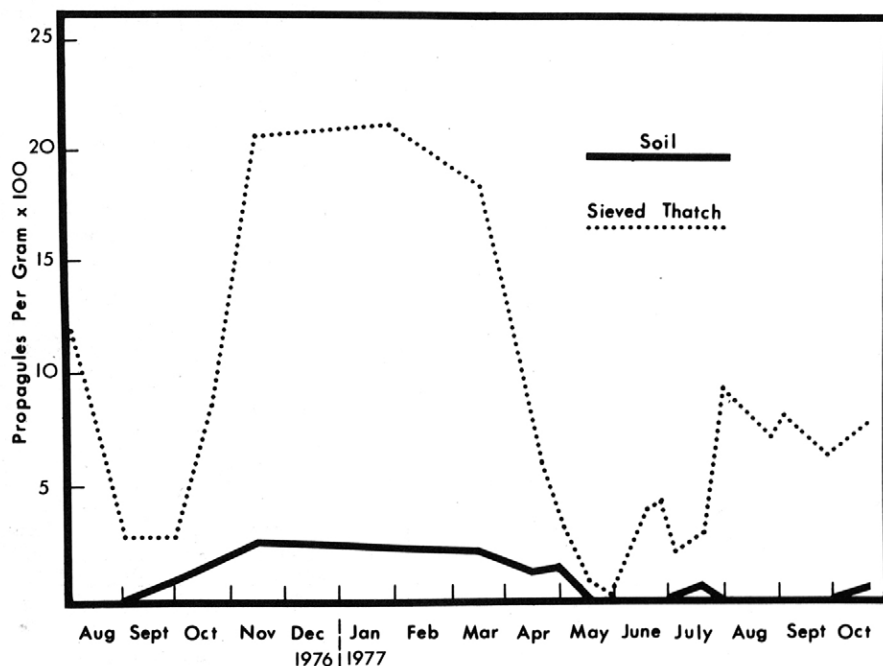


Fig. 1. Number of *Pythium aphanidermatum* propagules in soil and sieved thatch from golf course turfs (*Agrostis palustris* and *Poa annua*) in central Ohio over 15 mo.

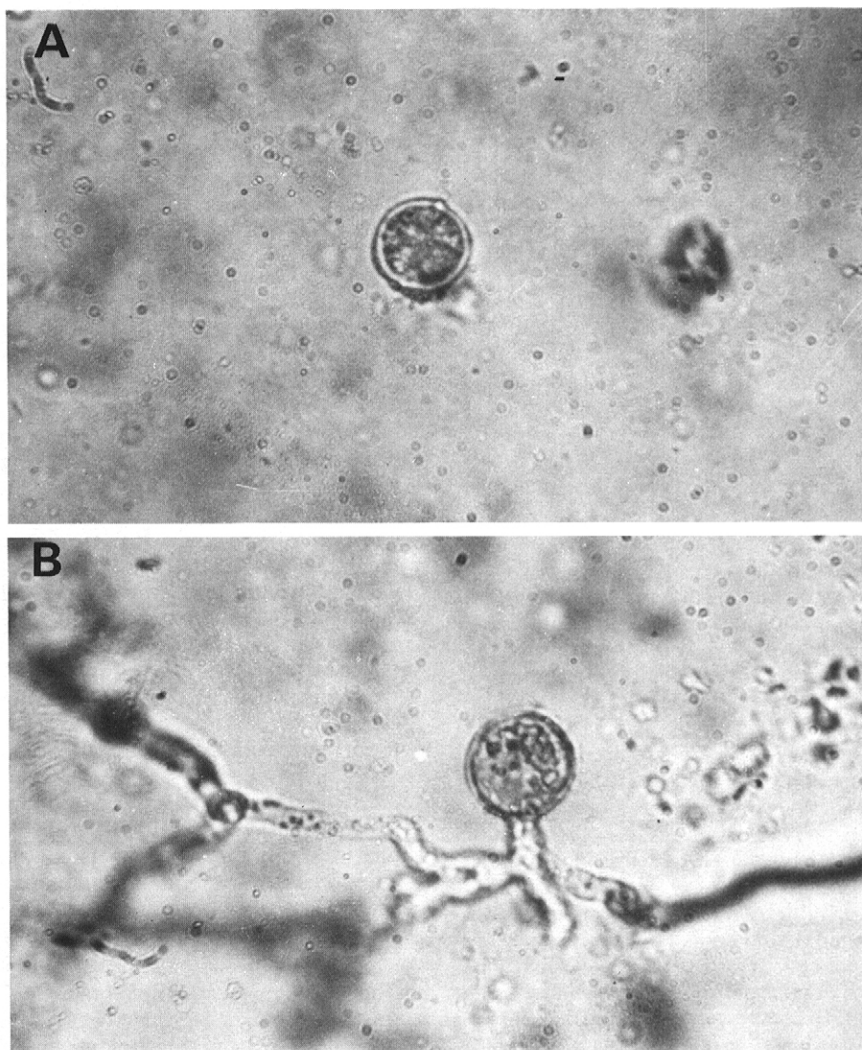


Fig. 2. Ungerminated (A) and germinated (B) oospores of *Pythium aphanidermatum* from a January sample of sieved thatch (0.841-mm mesh) from *Agrostis palustris* and *Poa annua* golf course turf in central Ohio.

Table 1. Development of Pythium blight of bentgrass after inoculation with various sources of *Pythium aphanidermatum*

1977 Sample	No. of pots with blighted plants ^a		
	Sieved thatch (1.0 g/pot)	Coarse thatch (0.3 g/pot)	Soil (4.0 g/pot)
January 26	2	3	3
March 17	2	3	3
May 1	2	3	1
May 17	1	3	... ^b
July 4	3	3	1
August 1	3	3	...
August 22	3	3	...

^a Of three pots per treatment at each sample date. All inocula were air-dried at 22 C 48–72 hr before use.

^b No measurable level of survival propagules.

similar soil preparations, and the fungus could not be isolated.

After clearing and staining, oosporelike structures of 20–23 μ were observed embedded in coarse thatch. They were the size of oospores of *P. aphanidermatum*, but antheridial attachments were not observed, making accurate identification of the fungus impossible. However, *P. aphanidermatum* was isolated from coarse thatch material of the same samples before clearing and staining. Plant shoots and roots were not examined because isolates were infrequently recovered from these tissues and only a small amount of material was available.

Pathogenicity. Potted bentgrass had symptoms of Pythium blight 7–14 days after inoculation with sieved thatch, coarse thatch, and soil fractions from which the fungus had been isolated previously (Table 1). *P. aphanidermatum* was isolated from all inoculated plants with blight symptoms but from none of the uninoculated controls. Thatch was a consistent inoculum source, particularly the coarse fraction. Pots of bentgrass inoculated with soil samples from the survey areas that did not have measurable numbers of propagules remained healthy.

DISCUSSION

Coarse thatch material appears to be the most consistent source of viable oospores of *P. aphanidermatum* in the turfgrass environment. The fungus was isolated from all coarse thatch samples from turfgrass areas with a history of Pythium blight. Coarse thatch also was consistently an effective inoculum source for pathogenicity studies (Table 1).

Survival propagules overwinter in association with host debris of numerous plant species (12, 15–17). Apparently, propagule survival in dead nodes, stem material, and roots (ie, coarse thatch) is more consistent than in smaller particulate organic debris (ie, sieved thatch) and soil. Decreased survival in soil and sieved thatch may be related to various biotic and abiotic selection pressures resulting in propagule inactivation or death (1,4,5,7,10,11,15,16).

Propagule numbers fluctuated in both sieved thatch and soil at similar times. Maximum numbers occurred from November through January and declined during spring months. Differences in numbers of propagules among sites were probably associated with the variations in disease activity. During this study, Pythium blight was severe at one site, moderate at another, and slight at the third. This variation in disease activity probably affected survival of propagules but did not appear to affect the seasonal fluctuations at the three areas.

The sites with moderate and slight disease activity did not have detectable levels of survival propagules in sieved thatch or soil from mid-May to mid-July, but *P. aphanidermatum* was isolated from coarse thatch during these times at all sites. Levels in these less active sites were again detectable in sieved thatch and soil 2–4 wk after severe cottony blight epiphytotics (6). Root and shoot recovery was more frequent during the summer months when epiphytotics were prevalent but was significantly lower than recovery from coarse thatch.

Oospores of *P. aphanidermatum* appear to be important as overwintering inoculum since they were observed in sieved and coarse thatch during January. Pathogenicity studies showed viable inoculum in soil and thatch material. And levels of inoculum were high in dried soil and thatch material. Mycelium, lobate sporangia, and zoospores are not thought to be involved with long-term survival of this particular species (7,15). The drying of samples facilitated measurement of oospore numbers and probably caused lysis of mycelium, lobate sporangia, and zoospores that might be present (9,17). Encysted zoospores and dormant mycelium in asymptomatic hosts could be involved as overwintering inoculum but were not examined in this study.

The spring decline of propagules may be due to death of survival propagules before or during germination, or death of germ tubes before saprophytic colonization of substrate or infection of a host. If pathogenic *Pythium* spp. are unable to compete saprophytically with other microorganisms for a food source during

the mycelial state, they die, infect a susceptible host, or form survival propagules (5,7,15). The lignified organic debris in the coarse thatch fraction might protect survival propagules embedded in it until the encapsulating organic material decays and leaves the survival structures exposed. These protected positions may help to ensure inoculum survival from one blight season to the next.

Pythium blight on turfgrasses is difficult to control. Control measures involve fungicide application when the cottony white mycelium is visible on turf during hot, humid weather. Because currently used fungicides do not appear to reduce inoculum levels, the disease recurs when environmental conditions are suitable. An integrated control program for Pythium blight should aim at reducing inoculum levels. Inoculum reduction might be achieved by mechanical removal of thatch material and enhancing the microbial deterioration of thatch.

LITERATURE CITED

1. AYERS, W. A., and R. D. LUMSDEN. 1975. Factors affecting production and germination of oospores of three *Pythium* species. *Phytopathology* 65:1084-1100.
2. BEARD, J. B. 1973. Turfgrass. Science and Culture. Prentice-Hall, Inc., Englewood Cliffs, NJ. 658 pp.
3. BURR, T. J., and M. E. STANGHELLINI. 1973. Propagule nature and density of *Pythium aphanidermatum* in field soils. *Phytopathology* 63:1499-1501.
4. FREEMAN, T. E. 1963. Age of ryegrass in relation to damage by *Pythium aphanidermatum*. (Abstr.) *Plant Dis. Rep.* 47:844.
5. GARRETT, S. D. 1970. Pathogenic root-infecting fungi. Cambridge Univ. Press, London. 294 pp.
6. HALL, T. J. 1978. Survival of *Pythium aphanidermatum* on three naturally infested *Agrostis palustris-Poa annua* golf course turfs and the influence of air temperature and relative humidity on naturally occurring Pythium blight epiphytotics. M. S. thesis, The Ohio State Univ., Columbus. 122 pp.
7. HENDRIX, F. F., and W. A. CAMPBELL. 1973. Pythiums as plant pathogens. *Annu. Rev. Phytopathol.* 11:77-98.
8. LITTLE, T. M., and F. J. HILLS. 1972. Statistical Methods in Agricultural Research. Univ. of California, Davis. 242 pp.
9. LOYD, A. B., and J. L. LOCKWOOD. 1966. Lysis of fungal hyphae in soil and its possible relation to autolysis. *Phytopathology* 56:595-599.
10. LUNA, L. V., and R. B. HINE. 1964. Factors influencing saprophytic growth of *Pythium aphanidermatum* in soil. *Phytopathology* 54:955-959.
11. MOORE, L. D., H. B. COUCH, and J. R. BLOOM. 1963. Influence of environment on diseases of turfgrasses. III. Effect of nutrition, pH, soil temperature, air temperature, and soil moisture on Pythium blight of Highland bentgrass. *Phytopathology* 53:53-57.
12. MUSE, R. R., A. F. SCHMITTHENNER, and R. E. PARTYKA. 1974. *Pythium* spp. associated with foliar blighting of creeping bentgrass. *Phytopathology* 64:252-253.
13. SALADINI, J. L. 1976. A study of *Pythium* species associated with turfgrasses in Ohio: Their prevalence and pathogenicity. Ph.D. thesis, The Ohio State Univ., Columbus. 94 pp.
14. SCHMITTHENNER, A. F. 1973. Isolation and identification methods for *Phytophthora* and *Pythium*. Pages 94-110 in: *Proc. First Woody Ornamentals Dis. Workshop*, Univ. of Missouri,

- Columbia.
15. STANGHELLINI, M. E. 1974. Spore germination, growth, and survival of *Pythium* in soil. Proc. Am. Phytopathol. Soc. 1:211-214.
 16. STANGHELLINI, M. E., and T. J. BURR. 1973. Effect of soil water potential on disease incidence and oospore germination of *Pythium aphanidermatum*. Phytopathology 63:1496-1498.
 17. STANGHELLINI, M. E., and E. L. NIGH. 1972. Occurrence and survival of *Pythium aphanidermatum* under arid soil conditions in Arizona. Plant Dis. Rep. 56:507-510.
 18. STANGHELLINI, M. E., and J. D. RUSSELL. 1973. Germination in vitro of *Pythium aphanidermatum* oospores. Phytopathology 63:133-137.
 19. TUIITE, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess Publ. Co., Minneapolis, MN. 239 pp.
 20. WATERHOUSE, G. M. 1967. Key to *Pythium* Pringsheim. Mycol. Pap. 109. Commonw. Mycol. Inst., Kew, Surrey, England. 15 pp.
 21. WATERHOUSE, G. M. 1968. The genus *Pythium* Pringsheim. Mycol. Pap. 110. Commonw. Mycol. Inst., Kew, Surrey, England. 121 pp.