Pythium-Induced Root Dysfunction of Secondary Roots of *Agrostis palustris*

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ABSTRACT

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Pythium aristosporum and *P. arrhenomanes* are reported as new pathogens of the secondary roots of *Agrostis palustris* grown on golf green mixes with a high sand content. *P. vanterpoolii* also was found associated with the roots but was not pathogenic. The total, shoot, and root dry weights of plants root-inoculated with *P. aristosporum* and *P. arrhenomanes* decreased in sand and to a lesser extent in sand-loam media. Both pathogens developed throughout the cortical and vascular tissues of the roots but did not produce lesions or rot. Infected roots occasionally had a light buff coloration. Mycelium was observed in root hairs, and infected root tips were bulbous and ultimately devitalized. Sporangia and oospores were rarely observed in infected roots.

Foliar or cottony blight is caused by several species of *Pythium* and is one of the more serious diseases of creeping bentgrass (*Agrostis palustris* Hud.) golf greens (17,20,22). Various *Pythium* species also are associated with the primary and secondary root systems of *A. palustris* and other perennial grasses (6,7). The pathogenicity of *Pythium* species on primary roots of turfgrass species is documented. *P. aphanidermatum* causes necrosis of primary roots of *A. palustris* (15); *P. aphanidermatum* and *P. graminicola* cause a seedling root rot of *Festuca rubra*, with the latter pathogen the more virulent (21). Varying degrees of primary root rot and decline of *F. rubra* 'Pennlawn' also is attributed to *P. aphanidermatum, P. graminicola*, and *P. myriotylum* under laboratory conditions (21). *Pythium* species are commonly associated with secondary root systems of grasses of temperate and tropical origin (4,6,7), but the pathogenicity of the association has not been elucidated. Some evidence exists for *Pythium*-induced root rots of secondary root systems of tropical origin turfgrasses (5).

A disease of unknown origin destroyed several golf greens in central Iowa during the midsummer heat and high humidity of 1977 (12). The turf had been reestablished the previous season on a high-sand-content growing medium (>60% sand). Entire greens were killed within 7–14 days in a pattern typical of Pythium blight, but *Pythium* was not present in aboveground portions of the plant. Other fungal or bacterial pathogens were not isolated. The root systems appeared normal in size but consistently yielded *Pythium* species and occasionally showed a mild yellow-tan discoloration, which was visible only when comparatively examined with uninfected roots. Since 1977, the disease has been observed in various locations in the northern midwestern and eastern states and in Canada. Almost every occurrence of the disease has been associated with the renovation of old golf greens with high-sand-content mixes. In all instances, the disease occurs during hot weather the first or second growing season after renovation and usually results in complete killing of the grass. Attempts to control the disease by cultural and chemical means are ineffective. The disease may reappear each growing season but usually decreases in severity until, within 3–5 yr, it ceases to be a

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problem or persists at some minimal level of activity.

Between 1977 and 1983, *Pythium* isolates were collected from roots of *A. palustris* afflicted with the disease from midwestern and eastern states and from Ontario, Canada. Six *Pythium* isolates were sent to the Centraalbureau Voor Schimmelcultures, Baarn, Netherlands, for identification. The isolates were identified as *P. aristosporum* Vanterpool, *P. arrhenomanes* Drechs., and *P. vanterpoolii* V. & H. Kouyeas. This study was initiated to evaluate the pathogenicity of these *Pythium* isolates to secondary roots of *A. palustris*.

MATERIALS AND METHODS

Plant materials. *A. palustris* 'Penncross' was used for all studies. Stolons were cut into 2.5-cm lengths; each stolon piece had a node and axillary bud. Plants were vegetatively propagated from the stolon pieces in autoclaved sand. Plants used for root inoculations were grown to the two- to three-leaf stage. This system of propagation provided uniform plants with only secondary roots.

P. aristosporum, P. vanterpoolii, and an Iowa and Canadian isolate of *P. arrhenomanes* were maintained on 20 ml of Bacto agar (3%, v/v) and/or cornmeal agar (25) in plastic petri dishes (100 × 15 mm). All cultures were maintained in an incubator with a 10-hr photoperiod at 20 C. Before use for inoculations, the *Pythium* isolates were transferred to a barley seed medium (5 g of barley seed in 150 ml of distilled water, autoclaved) in 250-ml Erlenmeyer flasks. Cultures were grown for 4 wk before being used for inoculum. Inoculum was prepared by macerating the mycelial mats of two cultures in 100 ml of distilled water for 2 min in a Waring Blendor. Roots of *A. palustris* were inoculated by dipping into the slurry of mycelium and zoospores. (Oospores were not produced on the barley seed medium.)

Treatments. Treatments consisted of inoculating the secondary roots of plants of a uniform size with the respective *Pythium* isolates and planting them in a 100% sand or 50% sand-loam medium. The sand was a river quartz type (pH 6.0–6.5); 50% of the particles were smaller than 0.5 mm in diameter. The sand was steamed for 3 hr before use to reduce or eliminate potential pathogens. The sand was stored in open containers for 4 wk, then placed in 5-cm^2 plastic pots on a greenhouse bench for 2 wk and irrigated daily before inoculated plants were transplanted into the pots. This procedure was followed in an effort not to totally eliminate the microflora of the sand and to help reestablish the microflora after steaming. The 50% sand-loam medium was processed in the same way as the 100% sand medium.

Root-inoculated plants were grown for 8 wk in a greenhouse under natural light at a temperature range of 18–26 C; all plants were fertilized (23-7-7) once each week. Each treatment of a specific *Pythium* isolate and growing medium combination consisted of 10 plants and was replicated three times. Uninoculated control plants were established in each growing medium.

Observations. The effects of *Pythium* root inoculations on plant growth were evaluated by determining the shoot, root, and total dry weights for each plant within each *Pythium* isolate and growing medium combination. Shoot/root ratios also were calculated to determine the specific effects of *Pythium* root infection on shoots and roots.

Root segments (1.5 cm) from inoculated plants were surface-sterilized (10% Clorox) for 5 min, rinsed in sterile distilled water, and placed on Bacto agar (3%, v/v) or incubated within Sykes-Moore tissue culture chambers (Bellco Glass, Vineland, NJ) (11) in sterile distilled water and observed for infection by the *Pythium* isolates. Symptoms and signs of infected roots, location of the *Pythium* species within roots, and reproductive characteristics of the *Pythium* isolates within roots were described. Root segments from inoculated roots were examined 4–8 wk after inoculation. Naturally infected roots from the field were also examined.

RESULTS

Growth of root-inoculated plants. Inoculation of secondary roots of *A. palustris* with *P. aristosporum* and both isolates of *P. arrhenomanes* decreased growth in sand (Fig. 1). No significant differences occurred between the total, shoot, or root weights of control plants or plants inoculated with *P. vanterpoolii* in sand or sand-loam growing media (Fig. 2). Roots of plants inoculated with *P. aristosporum* and both isolates of *P. arrhenomanes* reduced plant growth differentially in the sand and sand-loam media. Total plant weights were decreased by *P. aristosporum* and by both isolates

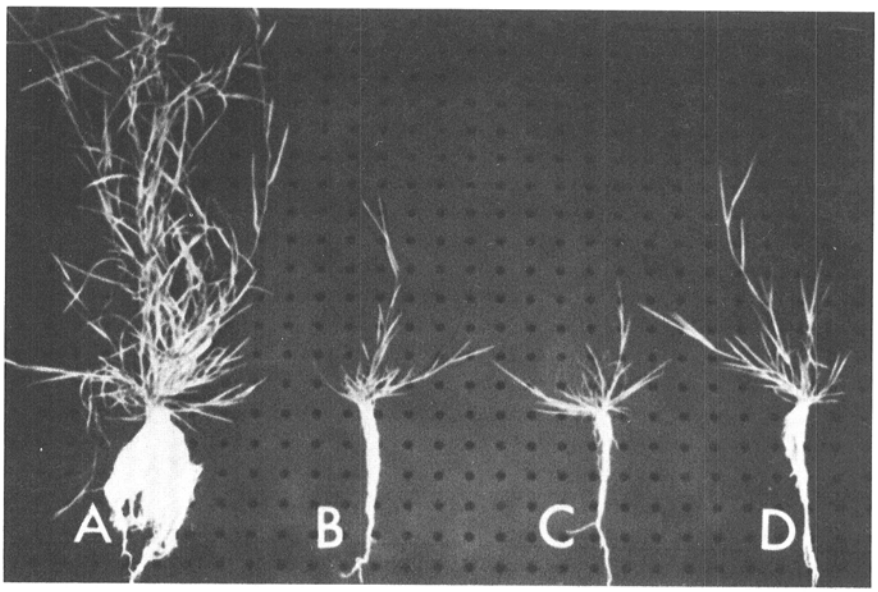

Fig. 1. Growth of *Agrostis palustris* after 8 wk in response to inoculation of roots with *Pythium* isolates. **(A)** Control plant, **(B)** Canadian isolate of *P. arrhenomanes*, **(C)** Iowa isolate of *P. arrhenomanes*, and **(D)** *P. aristosporum*.

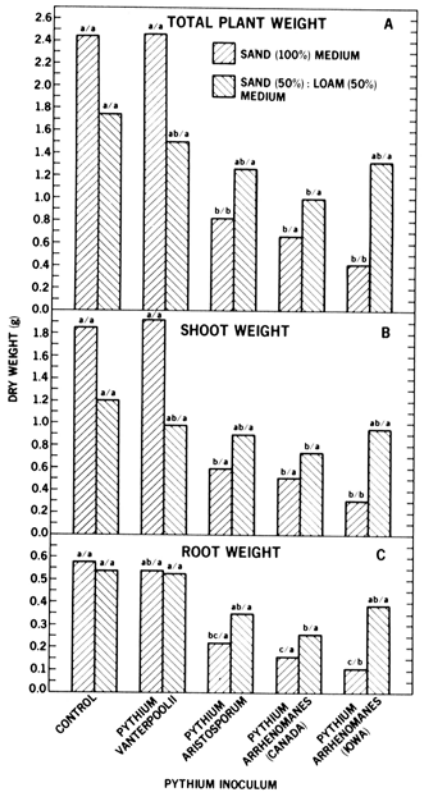

Fig. 2. Growth of *Agrostis palustris* in sand (100%) and sand-loam (50/50%) media after 8 wk of growth after inoculation of roots with *Pythium aristosporum, P. arrhenomanes*, or *P. vanterpoolii*. Differences between *Pythium* isolates (a/) and growing media (/a) followed by the same letter are not significantly different (LSD, $P = 0.05$).

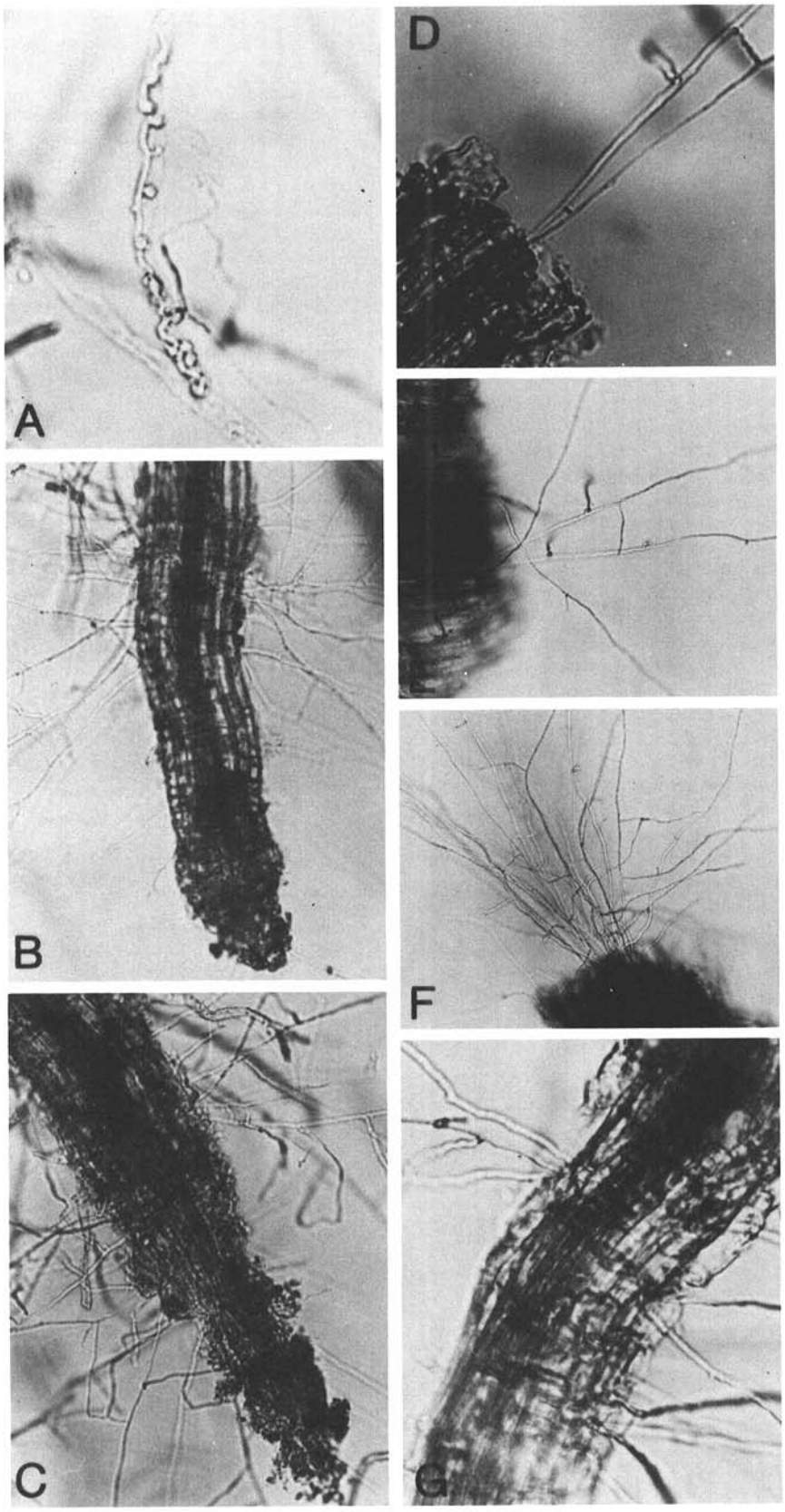

Fig. 3. Symptomatology and histopathology of *Agrostis palustris* infected by *Pythium aristosporum* or *P. arrhenomanes*. **(A)** Mycelium in root hair 2–4 wk after inoculation. **(B)** Bulbous root tip and growth of mycelium from region of elongation. **(C)** Devitalized root tip. **(D)** Growth of mycelium from vascular cylinder within 6 hr of incubation. **(E)** Growth of mycelium from the interface of the cortex and vascular cylinder. **(F)** Massive growth of mycelium from all root tissue after 12-hr incubation. **(G)** Direct growth of mycelium from cortical tissue of root. Note absence of rotted tissue or lesions.

of *P. arrhenomanes* in sand, but only the Canadian isolate of *P. arrhenomanes* decreased total plant weight in the sand-loam medium (Fig. 2A). The decrease in total plant weight in response to *P. aristosporum* and to the Iowa isolate of *P. arrhenomanes* was greater in the sand medium than in the sand-loam medium.

Shoot and root weights of plants inoculated with *P. aristosporum* and both isolates of *P. arrhenomanes* were significantly decreased when grown in sand (Fig. 2B,C). Only the Canadian isolate of *P. arrhenomanes* decreased shoot and root weights of plants grown in the sand-loam medium (Fig. 2B,C). Only the Iowa isolates of *P. arrhenomanes* reduced shoot and root weights more in the sand medium than in the sand-loam medium. Inoculation of roots with the *Pythium* isolates had little effect on shoot/root ratios; no differences occurred within or between *Pythium* species or growing media.

Root symptoms and histopathology. All *Pythium* species were readily reisolated from inoculated secondary roots of *A. palustris*. Only *P. aristosporum* and *P. arrhenomanes* penetrated roots; *P. vanterpoolii* was superficially associated with roots and was not pathogenic to the roots (Fig. 2). Therefore, no further reference will be made to *P. vanterpoolii* relative to symptoms or histopathology. Roots infected by *P. aristosporum* or *P. arrhenomanes* were white to slightly buff-colored and were without lesions or rot.

Roots collected 2–4 wk after inoculation and incubated in the Sykes-Moore tissue culture chambers showed mycelial development in root hairs (Fig. 3A). Roots collected 4–8 wk after inoculation and roots collected from naturally infected plants in the field showed bulbous root tips (Fig. 3B) as well as disorganized and devitalized tips (Fig. 3C). Diseased root tips incubated 12 hr in Sykes-Moore chambers showed extensive mycelial growth from intact cortical tissue in the region of elongation (Fig. 3B).

Randomly collected 1.5-cm pieces of older roots from inoculated plants and from naturally infected plants from the field incubated in Sykes-Moore chambers showed mycelial growth from the vascular cylinder at the cut ends of the roots within 6 hr (Fig. 3D). Some of the initial growth from the cut ends of the roots seemed to develop from the interface of the cortex and vascular cylinder (Fig. 3E). Within 12 hr, extensive mycelial growth developed from all root tissue at the cut ends (Fig. 3F). Within 12 hr, mycelial growth occurred directly from cortical tissues over the entire length of infected roots (Fig. 3D).

Lobate sporangia typical of *P. aristosporum* and *P. arrhenomanes* were extremely rare in inoculated roots and in naturally infected roots. Oospores were

found occasionally in devitalized root tips and in the cortex of dead, deteriorating roots. Both pathogens readily produced lobate sporangia and oospores on Bacto agar and cornmeal agar, respectively.

DISCUSSION

P. aristosporum and *P. arrhenomanes* are pathogens of secondary roots of *A. palustris* grown in high-sand-content media. *P. arrhenomanes* is widely distributed in North America and is well established as a root pathogen of cereals, corn, and sugarcane (2,19,27); it also is associated with *Festuca* spp., *Agropyron repens*, and *Bromus inermis* (13,26). *P. aristosporum* is found primarily in the cooler regions of North America and Japan and is the cause of snow rot of cereals (10,16). The isolate of *P. aristosporum* examined in this study was from roots of *A. palustris* in Iowa. Of the plants afflicted with the disease, *P. aristosporum* was isolated only once (Iowa); *P. arrhenomanes* has been the primary pathogen responsible for the disease.

The root disease induced by *P. aristosporum* and *P. arrhenomanes* has been observed only on *A. palustris* grown in high-sand-content media (>60%) of renovated golf greens. Few differences occurred in disease severity between the sand and sand-loam media used in our study; therefore, it cannot be concluded that the disease is more severe in sand, but where difference did occur, the disease was more damaging in the sand medium (Fig. 2).

There is no immediate explanation for the source of the pathogens in the renovated greens or why the disease has been observed only in high-sand-content media. The pathogens may be introduced with the sand or peat, or they may be present in the collar-apron soil that is commonly left during renovation to the sand medium. The severity of the disease in sand media may be related to the microbiology of sand, which may be different from that of soil, or the microbial population may be poorly established. In either instance, the ability of the *Pythium* species to function in sand may be related to inadequate competition from other microbes. There is evidence that *P. aristosporum* and *P. arrhenomanes* are more destructive on cereals, grasses, tomatoes, and beans on light and sandy soils (14,24,26). The observation that the severity of the disease decreases over a period of 3–5 yr may relate to a more competitive microflora becoming established in the sand.

We have termed the root disease induced by *P. aristosporum* and *P. arrhenomanes* "*Pythium*-induced root dysfunction." Both *Pythium* species thoroughly colonize infected roots, but both fail to produce root rot. The decrease in growth of plants infected by either pathogen was extensive (Fig. 1), but infected plants were not killed. This suggests that under optimal growing conditions, the roots may become extensively infected and the host and pathogen may coexist without evidence of the disease. This may explain why the infected plants are killed very rapidly during periods of high temperature stress. Rapid death of root-infected plants in the absence of rotting suggests that under stress, infected roots dysfunction relative to water uptake and translocation. Both *Pythium* species become associated with the vascular cylinder (Fig. 3D,E) and with the root tip (Fig. 3B,C). There is no evidence for physical blockage of vascular tissue; however, the water content of wheat seedlings is reduced when roots are infected by *P. arrhenomanes* (23), and culture filtrates of *P. arrhenomanes* also inhibit water uptake by wheat seedlings (29).

The reduced growth and absence of rot among *A. palustris* roots infected by *P. arrhenomanes* or *P. aristosporum* is similar to nonparasitic pathogenesis induced by some *Pythium* species (9). *P. myriotylum* produces a toxin(s) that inhibits root growth and causes necrosis on seedling tomato roots (3). *P. sylvaticum* produces 3-indoleacetic acid (IAA) and results in swelling of the root directly behind the root tip (1,18). Both reduced root growth and root tissue swelling directly behind the root tip are primary responses of *A. palustris* roots to *P. arrhenomanes* and *P. aristosporum*. The responses are not present, however, without infection. Root hairs (Fig. 3A) and root tips (Fig. 3B) are clearly infected by the pathogens, and the exclusive outward growth of mycelium from the cut end of vascular cylinders of infected roots within 6 hr establishes the endogenous presence of the pathogens. The response of *A. palustris* roots to *P. arrhenomanes* and *P. aristosporum* suggests that both species infect the roots and probably produce toxins and/or growth regulators as a means of facilitating pathogenesis without causing rotting.

Sporangia and oospores are rare in *A. palustris* roots infected by *P. aristosporum* or *P. arrhenomanes*. Transferring either pathogen to Bacto agar (3%, v/v) or cornmeal agar (25) results in abundant production of lobate sporangia and oospores on the respective media. Inoculation of roots of *Dactylis glomerata* (orchard grass) also results in production of sporangia and oospores in cortical and vascular tissues (*unpublished*). These responses suggest that *A. palustris* may not be an ideal host for either pathogen. These developmental characteristics also may relate to the persistence of the disease for 3–5 yr. If the *Pythium* species survive primarily in a vegetative state within *A. palustris* roots, they could be vulnerable to the potential development of microbial competitors in the sand.

The taxonomic status of the *Pythium* species presented in this study is not well defined. The most recent monograph on the taxonomy of *Pythium* species recognizes *P. aristosporum, P. arrhenomanes*, and *P. graminicola* as separate species (25), but the similarities of the species are recognized. The morphological and physiological variability of *P. arrhenomanes* is extensive (19), and the literature contains numerous studies in which *P. aristosporum, P. arrhenomanes*, and *P. graminicola* are viewed as the same pathogen (2). Some researchers recognize *P. graminicola-P. arrhenomanes* as a species complex (8). Immunofluorescence studies have separated *P. graminicola* from 18 other species of *Pythium* but not from *P. aristosporum* (28). Thus, the taxonomy of the *Pythium* species examined is not clearly established.

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