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Occurrence and Pathogenicity of *Pythium* Species Isolated from Leaf Blight Symptoms of Turfgrasses at Golf Courses in Korea

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Eleven species of *Pythium* were isolated from leaf blight symptoms on creeping bentgrass (*Agrostis palustris* Huds.), Kentucky bluegrass (*Poa pratensis* L.) and zoysiagrasses (*Zoysia japonica* Steud., and *Z. matrella* (L.) Merr.) planted on golf courses in Korea. Mycelial growth on potato carrot agar medium under various temperature conditions indicated that *Pythium* species obtained in this study could be divided into four groups based on their responses to temperature conditions. *P. vanterpoolii* was found to favor low temperature conditions with the optimum temperature of 25°C, whereas *P. aphanidermatum* and *P. myriotylum* favored relatively high temperature conditions with the optimum temperature of 35°C. Other species including *P. arrhenomanes*, *P. catenulatum*, *P. graminicola*, *P. oligandrum*, *P. rostratum*, *P. torulosum*, and *P. ultimum* were the intermediate group with the optimum temperature of 25~35°C. *P. periplocum* was similar to the intermediate group but the minimum temperature for its mycelial growth was 15°C, which was approximately 5°C above that for the intermediate *Pythium* spp. group. In the pathogenicity tests conducted in the lab using potted plants, *P. aphanidermatum*, *P. arrhenomanes*, *P. catenulatum*, *P. graminicola*, *P. myriotylum*, *P. periplocum*, *P. rostratum*, *P. torulosum*, *P. ultimum*, and *P. vanterpoolii* were found to be pathogenic to creeping bentgrass and Kentucky bluegrass. *P. aphanidermatum*, *P. catenulatum*, and *P. graminicola* were frequently isolated from leaf blight symptoms of creeping bentgrass and Kentucky bluegrass in golf courses during the warm and humid periods in July-August. On the other hand, *P. vanterpoolii* and *P. torulosum* were frequently isolated during the cool and humid periods in March-May, suggesting both species might be the major causes of leaf blight occuring in the spring time. Zoysiagrass was susceptible to *P. arrhenomanes* and the heterothallic *Pythium* sp. (Ht-F), showing stem and crown rot of turf-grasses at poorly drained areas under cool and humid or rainy conditions. *P. oligandrum* and the heterothallic *Pythium* sp. (Ht-L) isolated from creeping bentgrass were avirulent to all species of turfgrasses tested in this study.

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Pythium spp. are able to infect most turfgrasses, resulting in various types of disease symptoms like leaf blight, damping off, and crown and root rot (Abad et al., 1994; Freeman, 1980; Nelson and Craft, 1991; Saladini et al., 1983). Depending on the types of symptom and the time of their occurrence, the disease is often called 'grease spot', 'cottony blight', 'snow mold', and 'Pythium blight', among which Pythium blight is the most common name for the disease (Smiley, 1992).

Pythium blight is a major disease occurring on turfgrasses in golf courses in Korea (Kim and Park, 1997). Various symptoms of the disease are usually found throughout the growing season of turfgrasses. Damage to creeping bentgrass planted on greens of golf courses is substantial especially during hot and humid periods in summer (Muse, 1974; Saladini, 1980). In Korea, 11 species and two unidentified species of *Pythium* have been reported to occur on turfgrasses in golf courses (Kim and Park, 1997). However, pathogenicity and epidemiological characteristics of the individual species have not been investigated yet. This study was conducted to determine pathogenicity of the species isolated from golf courses in Korea, and their epidemiological characteristics in relation to temperature.

Materials and Methods

Occurrence of *Pythium* species. Leaves with blight symptoms were collected from creeping bentgrasses (*Agrostis palustris* Huds.) planted on greens, Kentucky bluegrass (*Poa pratensis* L.) and Zoysiagrasses (*Zoysia japonica* Steud., and *Z. matrella* (L.) Merr.) planted on tees and fairways of 35 golf courses in Korea from March through September in 1990-1996. One hundred and twenty five isolates of *Pythium* species were obtained from the diseased leaves. Species of the isolates were identified based on the morphological characteristics (Kim and Park, 1997). Monthly occurrence of individual species was determined for each golf course.

The isolates were stored in sterile deionized water for subsequent uses in the experiments. Culture blocks of individual isolates were made by cutting fresh cultures of the isolates grown on potato carrot agar (PCA) plates with a cork borer of 5 mm diameter. The culture blocks were placed in sterile deionized water in a capped tube, which were then kept at 22~25°C.

Temperature ranges for mycelial growth. Temperature ranges of mycelial growth were determined for 11 species of *Pythium* which were identified previously (Kim and Park, 1997). One isolate representing each species was grown on three plates of PCA medium under temperature conditions of 5~40°C at 5°C intervals. Colony diameters were measured after three days of incubation, and cardinal temperatures such as the maximum, minimum, and optimum temperatures for mycelial growth were determined. In order to differentiate *Pythium* species based on responses to temperature conditions, the principal component analysis was conducted on the cardinal temperatures using the PRINCOM procedure of SAS (SAS Institute Inc., 1988).

Pathogenicity tests. Creeping bentgrass 'Penncross', Kentucky bluegrass 'Glade', and zoysiagrass (*Z. japonica*) were used to evaluate pathogenicity of 76, 35, and 35 isolates of *Pythium* species, respectively. The isolates tested in this study were arbitrarily selected considering their seasonal occurrence and golf courses where they were collected. In order to prepare inoculum for the pathogencity tests, oatmeal-sand medium (oatmeal 1: sand 20: water 4) was infested with culture blocks of individual isolates and incubated for 14 days at 25°C. The sand used to make oatmeal-sand medium was disinfested beforehand with three cycles of dry-heating at 140°C for 2 hr and cooling for a day. Plugs of test plants taken from a nursery by a hole-cutter of 10 cm diameter were placed in the center of plastic pots of 15 cm diameter and the empty spaces of pots were filled with soil from the same nursery where the test plants were taken. After plants were established in the pots, three holes of 13 mm diameter and 30 mm depth were digged in a pot at equilateral triangular positions at a distance of 7 cm between holes using a cork borer. Test plants were inoculated with each isolate by filling the two holes with the oatmeal-sand inoculum. The other hole was filled with sterile oatmeal-sand medium as a check. The inoculated pots were incubated under humid conditions at approximately 25°C for 48 hr. This experiment was replicated three times for each isolate. The surface area of a pot was partitioned into three equal-sized spaces with each hole in the center of the space. Disease severity was rated on a 0-5 scale, where 0 = no disease symptom; 1 = 1~20%; 2 = 21~40%; 3 = 41~60%; 4 = 61~80%; and 5 = 81~100% of area diseased each per space. Diseased plants were confirmed by examining roots and leaves for existence of fungal structures of *Pythium* species under a microscope.

Results

Occurrence of *Pythium* species. Of 125 isolates, 111, 11, and 3 isolates were obtained from blighted leaves of creeping bentgrass, Kentucky bluegrass, and zoysiagrass, respectively (Table 1). The isolates belonged to 11 species including *P. aphanidermatum*, *P. arrhenomanes*, *P. catenulatum*, *P. graminicola*, *P. myriotylum*, *P. oligandrum*, *P. periplocum*, *P. rostratum*, *P. torulosum*, *P. ultimum* var. *ultimum*, and *P. vanderpoolii*, and two heterothallic species which were not identifiable (Kim and Park, 1997). One of the unidentified heterothallic species had filamentous sporangia, and the other had lobate sporangia.

Table 1. *Pythium* spp. isolated from leaf blight symptoms of creeping bentgrass (*Agrostis palustris*), Kentucky bluegrass (*Poa pratensis*) and zoysiagrasses (*Zoysia japonica* and *Z. matrella*) at thirty-five golf courses in Korea from 1990 to 1996

Pythium species	No. of isolates from host species[a]				No. of golf courses[b]
	Ap	Pp	Zj	Zm	
P. aphanidermatum	21	8	-	-	10
P. arrhenomanes	6	-	1	-	4
P. catenulatum	29	-	1	-	14
P. graminicola	25	-	-	-	16
P. myriotylum	2	-	-	-	1
P. oligandrum	1	-	-	-	1
P. periplocum	1	-	-	-	1
P. rostratum	2	-	-	-	1
P. torulosum	9	-	-	-	4
P. ultimum var. *ultimum*	1	3	-	-	3
P. vanterpoolii	12	-	-	-	6
Ht-F[c]	1	-	-	1	2
Ht-L[d]	1	-	-	-	1
Total	111	11	2	1	-

[a]Abbreviated as follows; Ap = *Agrostis palustris* Huds., Pp = *Poa pratensis* L., Zj = *Zoysia japonica* Steud and Zm = *Z. matrella* (L.) Merr.
[b]Number of golf courses where *Pythium* species were isolated.
[c]Ht-F = Unidentified heterothallic *Pythium* sp. with filamentous sporangia.
[d]Ht-L = Unidentified heterothallic *Pythium* sp. with lobate sporangia.

All of the 11 species and two unidentified species were isolated from creeping bentgrass which was mainly planted on greens of golf courses. However, only *P. aphanidermatum* and *P. ultimum* var. *ultimum* were isolated from Kentucky bluegrass, and *P. arrhenomanes*, *P. catenulatum*, and the unidentified heterothallic species with lobate sporangia were obtained from zoysiagrass (Table 1). It was noted that *P. aphanidermatum*, *P. catenulatum*, and *P. graminicola* were found from most of golf courses surveyed in this study, whereas *P. vanterpoolii* was isolated only from Kyunggi-do, a northern area of South Korea. Of nine isolates of *P. torulosum*, 3 was from Kyunggi-do, and 6 from Gyungsangbuk-do and Chonranam-do which are located at southern areas of South Korea. With regard to seasonal occurrence of individual species, most of species except *P. vanterpoolii* were found from diseased leaf samples collected in summer (Table 2). *P. vanterpoolii* was isolated only in spring. *P. catenulatum* appeared to occur under a wide range of weather conditions from spring to fall. *P. arrhenomanes* and *P. torulosum* were found not only in

Table 2. Number of isolates of *Pythium* spp. obtained in each month from diseased leaves of creeping bentgrass (*Agrostis palustris*), Kentucky bluegrass (*Poa pratensis*) and zoysiagrasses (*Zoysia japonica* and *Z. matrella*) in thirty five golf courses in Korea from 1990 to 1996

Month	Number of isolates of *Pythium* spp.[a]												
	Paph	Parr	Pcat	Pgra	Pmyr	Poli	Pper	Pros	Ptor	Pult	Pvan	Ht-F	Ht-L
March	-	-	-	-	-	-	-	-	-	-	1	-	-
April	-	1	-	-	-	-	-	-	-	-	6	-	-
May	-	-	1	-	-	-	-	-	3	-	5	1	-
June	-	1	4	1	-	-	-	-	3	-	-	-	-
July	27	5	20	21	2	1	-	-	3	4	-	-	1
August	2	-	4	3	-	-	1	2	-	-	-	-	-
September	-	-	1	-	-	-	-	-	-	-	-	1	-

[a]Abbreviated as follows; Paph = *P. aphanidermatum*, Parr = *P. arrhenomanes*, Pcat = *P. catenulatum*, Pgra = *P. graminicola*, Pmyr = *P. myriotylum*, Poli = *P. oligandrum*, Pper = *P. periplocum*, Pros = *P. rostratum*, Ptor = *P. torulosum*, Pult =*P. ultimum*, Pvan = *P. vanterpoolii*, Unidentified heterothallic *Pythium* sp. with filamentous sporangia (Ht-F) and lobate sporangia (Ht-L).

summer but also in spring.

Infected leaves of creeping bentgrass on greens of golf courses first showed water-soaked symptoms in the early morning or under cloudy weather during the rainy periods in summer (Fig. 1A). Collapsed leaves became covered with fluffy white mass of fungal mycelium under high humid conditions (Fig. 1B). Affected plants rapidly turned light tan to brown, shriveled and matted when leaves became dry under sunlight (Fig. 1C). On closely mowed grasses like greens of golf courses, diseased patches which were initially very small might coalesce to form large and irregular areas of dead zones (Fig. 1D). This large area often became elongated or concentrated in the wet and poorly drained areas of greens (Fig. 1E). Roots of blighted turfgrasses turned brown and a large amount of nematodes were observed on roots and lower leaves. Under hot weather conditions during summer, large areas became reddish in a short period of time (Fig. 1F). Symptoms and signs of the disease under cool and wet conditions in spring or fall were similar to those in summer (Figs. 1G and 1H).

In the case of Kentucky bluegrass planted on tees and fairways of golf courses, disease symptoms were not as apparent as on creeping bentgrass on greens. However, infected leaves eventually died after turning water-soaked and brown. As for zoysiagrasses, Pythium diseases usually occurred on poorly drained areas in spring and fall (Fig. 1I), and infected plants showed soft rot symptoms on the lower part of stems and reddish color on leaves (Fig. 1J).

Temperature ranges for mycelial growth. The temperature range for mycelial growth of *P. aphanidermatum* and *P. myriotylum* was 10~40°C with the optimum temperature of 30~35°C (Fig. 2). *P. vanterpoolii* grew best at 20~25°C and was able to grow at 5°C, but not at 30°C. The optimum temperature for the other species was 25~30°C with the maximum temperature of 35°C. These results suggested that *P. aphanideramtum* and *P. myriotylum* favored high temperature conditions, whereas *P. vanterpoolii* preferred relatively cool conditions.

There was significant correlation between the maximum and optimum temperatures, but the minimum temperature did not appear to correlate with the maximum or optimum temperatures (Table 3). The principal component analysis on the correlation matrix of the cardinal temperatures resulted in three principal components (PRIN1, PRIN2, and PRIN3), accounting for 92.8% of the total variation among *Pythium* species (Table 4). The eigenvectors of the correlation matrix indicated that PRIN1 and PRIN2 represented components contributed mainly by the maximum and optimum temperatures, and by the minimum temperature, respectively. Biological interpretation was difficult for PRIN3, which accounted for only 0.8% of the total variation among *Pythium* species.

Since 92% of the total variation was accounted for by PRIN1 and PRIN2, 11 species of *Pythium* were categorized into 4 groups by PRIN1 and PRIN2 (Fig. 3). The results indicated that *P. vanterpoolii* favored relatively cool conditions, and *P. aphanidermatum* and *P. myriotylum* consisted of a group favoring relatively high temperature conditions. The other species were considered to be the intermediate temperature group which could be divided into two separate subgroups based on the minimum temperature for mycelial growth. Of the 9 species in the intermediate temperature group, *P. periplocum* was not able to grow at 10°C at which the other species could grow.

Pathogenicity tests. Approximately 90% of isolates inoculated in this study showed pathogenicity to creeping bentgrass and Kentucky bluegrass (Table 5). Especially, *P. aphanidermatum*, and *P. graminicola* were highly virulent to both creeping bentgrass and Kentucky bluegrass. In the case of zoysiagrass, only two isolates of *P. arrhenomanes* and the heterothallic species with filamentous sporangia isolated from *Z. japonica* and *Z. matrella*, respectively,

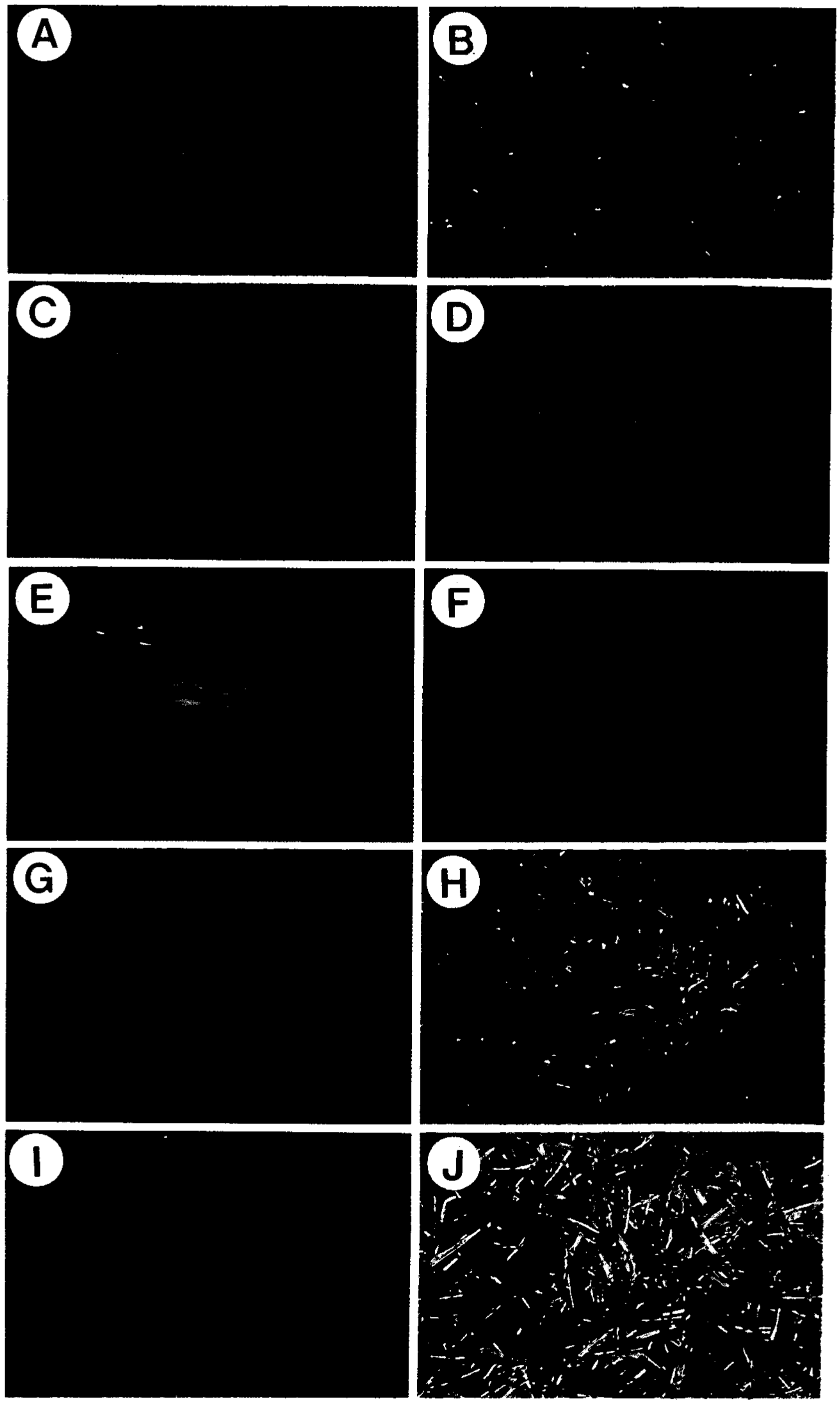

Fig. 1. Symptoms of Pythium blight, associated with warm weather, caused by *Pythium* spp. on creeping bentgrass. In the early morning, infected grass leaves first appeared water-soaked and dark in patches (A), and when humidity remained high, especially at night, the collapsed leaves became covered with a fluffy white mass of fungal mycelium (B). The affected plants rapidly turned light tan to brown, shrivelled and matted under dry and sunny conditions (C). Small patches were coalesced to form large and irregular areas of dead zone (D), and the large areas were often elongated or concentrated in the poorly drained areas on the green (E). Symptoms of Pythium blight, associated with hot summer weather, caused by *P. aphanidermatum* on creeping bentgrass (F). Symptoms of Pythium blight, associated with cool weather, caused by *P. vanterpoolii* on creeping bentgrass (G). Infected leaves appeared water-soak and blighted (H). Symptoms of Pythium blight caused by *P. arrhenomanes* on zoysiagrass fairway (I). Infected stems became soft-rotten and leaves turned red (J).

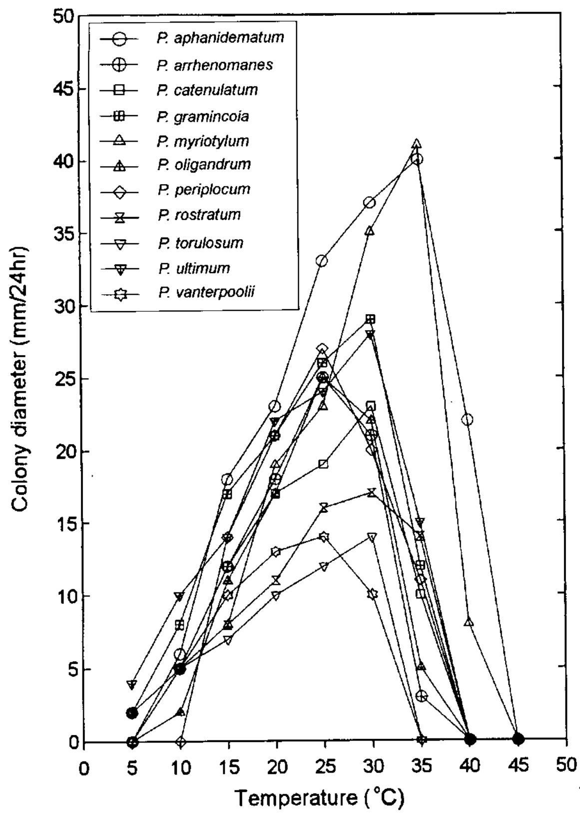

Fig. 2. Effect of incubation temperatures on the mycelial growth of eleven *Pythium* spp. isolates from turfgrasses on PCA.

Table 3. Correlation matrix of the minimum, maximum and optimum temperature for mycelial growth of eleven *Pythium* species

Temperature	Minimum	Maximum	Optimum
Minimum	1.00	0.23	-0.21
Maximum	0.23	1.00	0.63
Optimum	-0.21	0.63	1.00

Table 4. Eigenvectors and Eigenvalues of three principal components of the correlation matrix of the minimum, maximum and optimum temperature for mycelial growth of eleven *Pythium* species

Temperature	Eigenvectors[a]		
	PRIN1	PRIN2	PRIN3
Minimum	0.024	0.925	0.380
Maximum	0.711	0.252	-0.657
Optimum	0.703	-0.286	0.652
Eigenvalue	1.632	1.128	0.240
% of total variance	54.4	37.6	0.8

[a]PRIN1, PRIN2 and PRIN3 indicate three principal components.

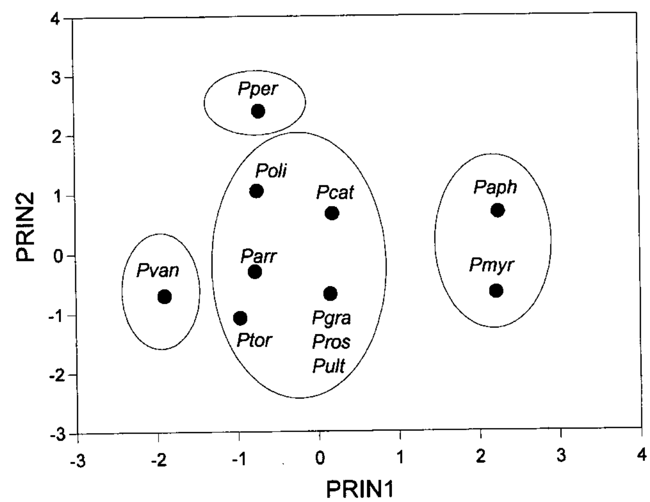

Fig. 3. Grouping of *Pythium* species by the first and second principal components of the minimum, optimum, and maximum temperature data for mycelial growth of eleven *Pythium* species in the principal component analysis. Symbols are names of eleven *Pythium* spp., and abbreviated as follows; Paph = *P. aphanidermatum*, Parr = *P. arrhenomanes*, Pcat = *P. catenulatum*, Pgra = *P. graminicola*, Pmyr = *P. myriotylum*, Poli = *P. oligandrum*, Pper = *P. periplocum*, Pros = *P. rostratum*, Ptor = *P. torulosum*, Pult = *P. ultimum*, Pvan = *P. vanterpoolii*.

resulted in crown and stem rot symptoms. With regard to host specificity, *P. arrhenomanes* isolated from zoysiagrass was pathogenic to creeping bentgrass and Kentucky bluegrass, whereas an isolate from creeping bentgrass was not pathogenic to zoysiagrass. *P. oligandrum* and the heterothallic species with lobate sporangia were not pathogenic to all plants tested in this study.

Discussion

Eleven *Pythium* species obtained in this study have been reported to be often found from trufgrasses and soils of golf courses in other countries (Abad et al., 1994; Hodges and Coleman, 1985; Nelson and Craft, 1991; Saladini et al., 1983). Seasonal occurrence of *Pythium* species indicated that *P. aphanidermatum*, *P. catenulatum*, and *P. graminicola* were the pathogens causing blight in July and August when hot and humid weather conditions occurred often. However, *P. vanterpoolii* and *P. torulosum* were found to be the major causes for Pythium blight on greens of golf courses under cool and humid weather conditions in March-May. Such differences in seasonal occurrence between *Pythium* species appeared to be due to preference of individual species to temperature conditions for mycelial growth, as suggested by the result of principal component analysis on the cardinal temperatures for mycelial growth on PCA medium.

The principal component analysis on the cardinal temperatures for mycelial growth seemed to be a good way of dif-

Table 5. Pathogenicity of *Pythium* spp. to *Agrostis palustris*, *Poa pratensis* and *Zoysia japonica*

Pythium species	No. of pathogenic isolates / No. of isolates tested[a]			Average disease rating (range)[d]		
	Ap[b]	Pp[c]	Zj[c]	Ap[b]	Pp[c]	Zj[c]
P. aphanidermatum	7/7	4/4	0/4	5	5	0
P. arrhenomanes	4/4	3/3	2/3	3(1~4)	3.3(3~4)	0.7(0~1)
P. catenulatum	21/21	2/2	0/2	3.8(1~5)	1.5(1~2)	0
P. graminicola	18/18	9/9	0/9	4.3(2~5)	4.1(2~5)	0
P. myriotylum	2/2	2/2	0/2	1	1.5(1~2)	0
P. oligandrum	0/1	0/1	0/1	0	0	0
P. periplocum	1/1	1/1	0/1	1	2	0
P. rostratum	2/2	3/3	0/3	1	1.7(1~3)	0
P. torulosum	1/5	1/3	0/3	0.2(0~1)	0.3(0~1)	0
P. ultimum	2/2	2/2	0/2	2.5(2~3)	2.5(2~3)	0
P. vanderpoolii	9/10	3/3	0/3	1.5(1~3)	2.3(1~3)	0
Ht-F[e]	1/2	1/1	1/1	1.0(0~3)	2	1
Ht-L[f]	0/1	0/1	0/1	0	0	0
Control	-	-	-	0	0	0
Totals	68/76	31/35	3/35	-	-	-

[a]Abbreviated as follows; Ap = *Agrostis palustris* Huds. (creeping bentgrass), Pp = *Poa pratensis* L. (Kentucky bluegrass), Zj = *Zoysia japonica* Steud (zoysiagrass)
[b]Of 76 isolates tested, 69 isolates were obtained from creeping bentgrass, 4 from Kentucky bluegrass and 3 from zoysiagrasses.
[c]Of 35 isolates tested, 29 isolates were obtained from creeping bentgrass, 3 from Kentucky bluegrass and 3 from zoysiagrasses.
[d]Disease rating; 0 = non-pathogenic, 1 = 1~20%, 2 = 21~40%, 3 = 41~60%, 4 = 61~80%, and 5 = 81~100%.
[e]Ht-F = Unidentified heterothallic *Pythium* sp. with filamentous sporangia isolated from zoysiagrass (*Z. matrella*) and creeping bentgrass.
[f]Ht-L = Unidentified heterothallic *Pythium* sp. with lobate sporangia.

ferentiating *Pythium* species based on their responses to temperature conditions. Principal components are independent of one another and each component can be considered to be the response of a stimulus, which can be considered to be a genetic or environmental feature (Pimentel, 1979). The total response, the component, could be subdivided into individual variable responses that are measured by the coefficient corresponding to each variable. In this study, PRIN1 and PRIN2 were uncorrelated with each other and provided collective information on growth behavior of *Pythium* species at the cardinal temperatures. In biological terms pertinent to the mycelial growth of *Pythium* species, PRIN1 and PRIN2 represented growth behavior at warm (25°C or above) and cool (10°C or below) conditions, respectively. The cardinal temperatures determined for individual species in this study was similar to the report by Yu and Ma (1989).

'Spring dead spot' syndrome on zoysiagrasses has been known to be caused by binucleated *Rhizoctonia cerealis* in Korea (Kim et al., 1992), and by a complex of binucleated *Rhizoctonia* spp., *Pythium* spp., and *Fusarium* spp. in Japan. The implicated *Pythium* species were found to be *P. graminicola*, *P. periplocum*, and *P. vanterpoolii* in Japan (Tani, 1991). However, only *P. arrhenomanes* was isolated in this study from blighted leaves of zoysiagrasses growing at poorly drained areas on tees and fairways of golf courses. In Korea, binucleated *R. cerealis* was reported to occur on well-drained slopes or sandy loam of tees and surrounding areas of greens (Kim et al., 1992). In order to control 'spring dead spot' syndrome, appropriate control strategies for *Pythium* species have to be established as well as for *Rhizoctonia* spp.

Although all species except *P. oligandrum* were pathogenic to creeping bentgrass and Kentucky bluegrass, differences in virulence were observed between *Pythium* species. Relatively low virulence of *P. vanterpoolii*, *P. rostratum* and *P. torulosum* might have been due to their slow growth as compared with other fast growing species. However, growth rate may not be the only reason for the difference in virulence because *P. myriotylum* and *P. periplocum* which grew as fast as *P. aphanidermatum* and *P. graminicola*, respectively, showed substantially low virulence. Genetic interactions between *Pythium* species and turfgrasses need to be further studied in the future. In this study, genetic specificity between *P. arrhenomanes* and species of turfgrasses was noted.

Use of non-pathogenic *Pythium* species to control Pythium diseases has been reported in the literature (Paulitz and Baker, 1987). Martin and Hancock (1987) found that suppression of *P. ultimum* by non-pathogenic *P. oligandrum* was due to strong saprophytic ability of *P. oligandrum* as compared with that of *P. ultimum*. *P. oligandrum* and the unidentified heterothallic species with lobate sporangia obtained in this study was not pathogenic to any plants tested. It needs to evaluate their saprophytic ability in the thatch layers of greens and antagonistic effects on pathogenic *Pythium* species.

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