Effect of Salinity Stress on Development of Pythium Blight in *Agrostis palustris*

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ABSTRACT

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Salinity stress predisposed cultivar Penncross creeping bentgrass to cottony blight caused by *Pythium aphanidermatum* at two temperature regimes. At 25–32 C, complete necrosis of all inoculated plants occurred at electrical conductivity (Ec) levels of 4.3–7.1 decisiemens (ds) per meter in 2 days, whereas at Ec levels of 0.5–2.8 ds/m, death occurred within 3 days. At 25–27 C, complete necrosis of all inoculated plants occurred at Ec levels of 4.3–7.1 ds/m within a period of 5 days; no death was observed in control or inoculated plants at an Ec level of 0.5 ds/m. Increased salinity levels apparently affected the bentgrass rather than *P. aphanidermatum*. Mycelial growth rate of the fungus was increased only slightly by salinity levels up to 7.1 ds/m. Zoospore production of *P. aphanidermatum* and two other species of *Pythium* decreased with increasing salinity levels up to 7.1 ds/m; production was completely inhibited at 14.2 ds/m.

Additional keywords: Pythium catenulatum, Pythium dissotocum.

Penncross creeping bentgrass (*Agrostis palustris* Huds. 'Penncross'), a temperate region grass, is widely used throughout the world for golf course greens, tees, and fairways. Extensive development of resorts and recreational facilities has expanded the use of Penncross bentgrass into tropical, arid, and semi-arid climatic regions where nonpotable and saline water commonly are used for irrigation.

The sensitivity of Penncross bentgrass to salinity has been documented (15); however, no studies have been conducted regarding the effect of increased salinity on the diseases commonly occurring on Penncross. Cottony blight, caused primarily by *Pythium aphanidermatum* (Edson) Fitz., is a particularly devastating disease on Penncross bentgrass. Environmental conditions conducive for disease development include high relative humidity, saturated soils, and daytime temperatures of 30 C or greater (11). The accumulation of salts due to saline irrigation water may impose an environmental stress leading to increased plant susceptibility. Increased disease severity resulting from fungicide and herbicide use (6,14), and susceptibility of bentgrass to *Curvularia lunata* from heat and leaf clipping stress (10), have been reported.

The objective of this study was to determine the in vitro effect of salinity on zoospore production and mycelial growth of *Pythium* spp., and the potential effects of salinity on predisposition of Penncross creeping bentgrass to cottony blight.

MATERIALS AND METHODS

Cultures. A culture of *P. aphanidermatum*, isolated from diseased Penncross bentgrass, was used throughout this study. Additionally, the following *Pythium* spp. were used for comparison purposes in in vitro tests: *P. dissotocum* Drechsler and *P. catenulatum* Matthews isolated from lettuce roots, and *P. aphanidermatum* and *P. catenulatum* isolated from rotted roots of *Salicornia bigelovii* Torr., a halophyte, which was irrigated with sea water (M. E. Stanghellini, unpublished). All cultures were maintained at 25 C on 10% V-8 juice agar (VJA) medium containing 0.1% $CaCO_3$.

Mycelial growth response. A 5-mm-diameter disk was cut from the margin of a 5-day-old VJA culture of each fungus and placed at the perimeter of a 9-cm-diameter petri dish containing VJA amended with a NaCl and $CaCl_2$ solution 1:1 (w/w) to achieve electrical conductivity (Ec) levels of 1.4, 2.8, 4.3, 5.7, 7.1, 14.2, and 28.4 decisiemens (ds) per meter (1 ds/m = 640 ppm). The same medium (0.5 ds/m) without the addition of salt was used as a control. Cultures were incubated at 15, 20, 25, and 30 C, and, after an initial growth period of 12 hr, the radius of each colony was measured after 24 and 48 hr. There were two replicates of each salinity value at each temperature regime, and the experiment was conducted three times.

Effect of salinity on zoospore production. A 9-mm-diameter disk was cut from a 5-day-old VJA culture of each fungus and placed in a 9-cm-diameter petri dish containing 20 ml of NaCl and $CaCl_2$ solution 1:1 (w/w) with Ec values of 1.4, 2.8, 4.3, 5.7, 7.1, and 14.2 ds/m. Sterile distilled water was used as a control. After 48 hr incubation at 25 C, all zoospores had encysted and settled to the bottom of the dish. Then 0.1 ml of 10% acid fuchsin was added to each dish to stain the cysts. The total number of zoospore cysts per plate was determined by counting cysts in 10, 0.5 mm^2 arbitrarily selected sites on the bottom of the dish under a compound microscope. The total number of cysts per dish was then calculated by multiplying the mean of the 10 sites by the surface area of the bottom of the dish (6,361 mm^2) and then multiplying by 2. Each treatment was replicated twice, and the experiment was conducted three times.

Salinity effects on Pythium blight. Penncross creeping bentgrass certified seeds were planted in a sterile mixture of 90% sand and 10% peat in 400-ml, 10-cm-diameter pots. Particle size of the sand was consistent with the United States Golf Association specifications for proper root zone mix: 75% of the particles were from 1 mm to 0.25 mm, whereas 25% of the particles ranged from 0.25 mm to 0.05 mm. Plants were fertilized biweekly with a 15-30-15 concentrated water-soluble plant food, (Miracle Gro, Stern's Garden Products, Geneva, NY), clipped to a height of 3 cm biweekly, and irrigated daily with tap water (0.5 ds/m). After 3 mo of growth, plants were irrigated daily with 50 ml of a NaCl and $CaCl_2$ solution 1:1 (w/w) with Ec values of 0.5 (tap water), 2.8, 4.3, 5.7, and 7.1 ds/m. The solution was allowed to drain through the soil, and the Ec of the eluted solution from each treatment was determined. There were four replicates per salinity treatment. Pots were incubated (12-hr light cycles, 5,200 lux) in growth chambers under three temperature regimes. Chamber A had a maximum day temperature of 32 C and a night minimum of 25 C. Chamber B had a maximum day temperature of 27 C and a night minimum of 25 C. Chamber C had a constant temperature of 25 C. In each chamber,

plants were placed in a clear ventilated plastic bag and sealed to retain moisture. After a 7-day incubation period, plants were inoculated by placing one 5-mm-diameter agar plug, from the margin of a 5-day-old culture of *P. aphanidermatum*, into the thatch layer of three of the four pots; one pot in each treatment was the uninoculated check. Plants were monitored for the next 7 days for the development of Pythium blight and rated as having complete tissue necrosis or no disease symptoms. The experiment was repeated three times.

Experimental design and statistics. A completely randomized design was used for all experiments. Data were combined across all trials after homogeneity of error variances was proven by Bartlett's test for homogeneity. Associations between Ec levels and temperature for mycelial growth, and between Ec levels and three species of *Pythium* for zoospore production, were determined by correlation analyses.

RESULTS

Mycelial growth rate. Mycelial growth rate of *P. aphanidermatum*, and all other species of *Pythium* tested, was significantly and negatively correlated ($P = 0.01$) with salinity level at 15, 20, and 25 C (Table 1). A similar trend was observed at 30 C; however, the correlation was not significant ($P = 0.05$). Mycelial growth rate increased slightly up to a salinity level of 7.1 ds/m and then slowly decreased at higher levels at all temperatures; however, changes in growth rates generally were not dramatic.

Zoospore production. Zoospore production of all species was totally inhibited at Ec values of 14.2 ds/m. Sensitivity to salt concentrations varied depending on the species. *P. catenulatum* had the greatest tolerance, whereas *P. aphanidermatum* had the least tolerance throughout the salinity range. Zoospore production was significantly and negatively correlated ($P = 0.01$) with salinity level for *P. catenulatum* and *P. dissotocum* (Table 2). A similar trend occurred for *P. aphanidermatum*, but the correlation was not significant ($P = 0.05$).

Salinity level and incubation temperature effects on the development of cottony blight. At 25 C, no disease occurred in any treatment. At 25–32 C (Fig. 1), all plants died within 3 days after inoculation regardless of treatment. However, the rate of plant death was greatest among treatments irrigated with water at Ec levels >2.8 ds/m. None of the controls died, regardless of treatment. No noticeable differences in color or growth of control plants were observed at any of the salinity levels.

At 25–27 C (Fig. 2), the percentage and rate of plant death differed among the various treatments. No disease occurred in treatments irrigated with tap water (Ec 0.5 ds/m). All of the inoculated plants irrigated with water at Ec levels of 4.3–7.1 ds/m

TABLE 1. Mycelial growth rates of *Pythium aphanidermatum* at various electrical conductivity (salinity) levels and temperatures

	Temperature (C)			
Salinity[a] (ds/m)	15	20	25	30
0.5	12.3[b]	27.5	32.5	41.7
1.3	13.7	27.8	33.3	42.5
2.8	15.0	28.7	32.8	43.2
4.3	15.0	29.2	33.7	43.0
5.7	15.2	29.5	34.3	43.5
7.1	15.7	29.5	34.3	44.2
14.2	10.7	25.0	28.8	43.8
21.2	10.3	23.7	26.7	41.5
28.4	7.7	22.7	25.2	39.5
r[c]	−.84**	−.88**	−.92**	−.62

[a] Varied salinities obtained by amending V-8 juice agar with a 1:1 (w/w) NaCl and $CaCl_2$ solution.
[b] Growth rate in mm/24 hr beginning 12 hr after fungus was transferred to test media. Means of three experiments, two replicates per experiment.
[c] Correlation coefficients showing associations between salinity levels and temperature; ** = significant at $P = 0.01$.

TABLE 2. Zoospore production of three species of *Pythium* at various salinity levels

	Numbers of zoospores produced ($\times 10^3$)		
Salinity[a]	*P. aphanidermatum*	*P. catenulatum*	*P. dissotocum*
0.5	201.6[b]	730.7	617.8
1.3	98.7	579.7	527.0
2.8	14.4	479.4	466.9
4.3	8.7	419.3	412.2
5.7	6.2	349.8	217.8
7.1	5.0	321.6	16.9
14.2	0.0	0.0	0.0
r[c]	−.63	−.98**	−.90**

[a] Varied salinities obtained by amending 20 ml of sterile distilled water with a 1:1 (w/w) NaCl and $CaCl_2$ solution.
[b] Zoospores produced by placing a 9-mm-diameter disk cut from a 5-day-old V-8 juice agar culture into 20 ml of varied salinity solutions. Means of three experiments, two replicates per experiment.
[c] Correlation coefficients showing associations between salinity levels and zoospore production; ** = significant at $P = 0.01$.

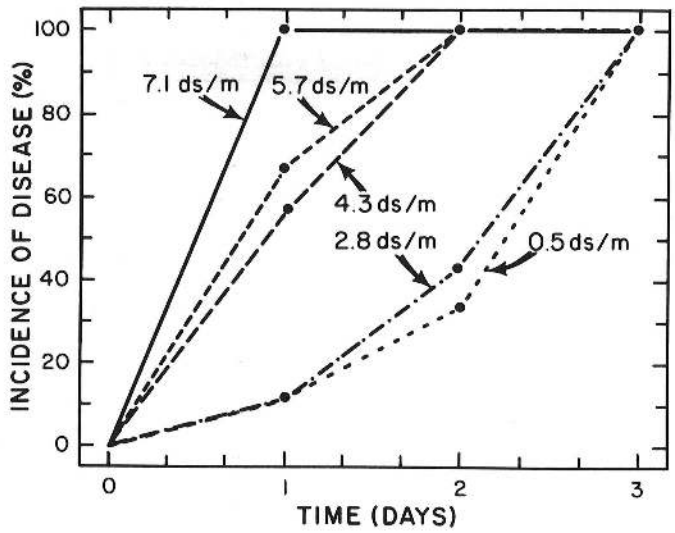

Fig. 1. Incidence of cottony blight on Penncross bentgrass grown under differing salinity levels at the 25–32 C temperature regime. Data presented are from three individual experiments with treatments replicated three times.

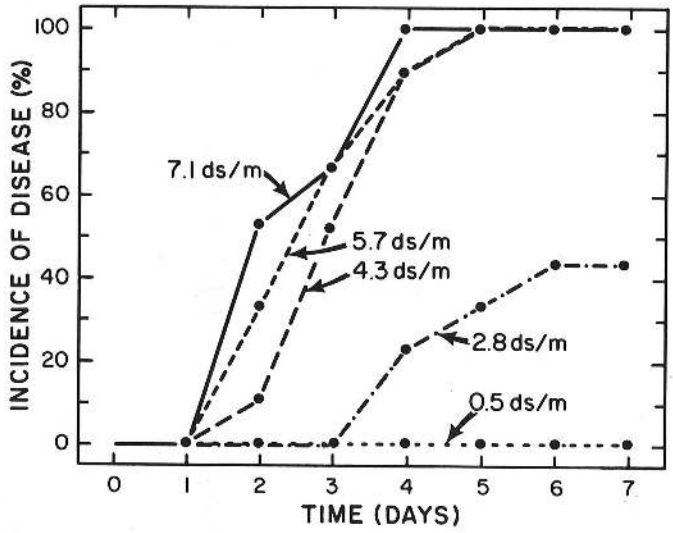

Fig. 2. Incidence of cottony blight on Penncross bentgrass grown under differing salinity levels at the 25–27 C temperature regime. Data presented are from three individual experiments with treatments replicated three times.

died within 4 to 5 days, whereas 40% of the plants died at an Ec level of 2.8 in the same period. None of the controls died, regardless of treatment.

DISCUSSION

Results indicate that increased salinity levels predispose Penncross bentgrass to cottony blight caused by *P. aphanidermatum*. Salinity accelerated the onset and development of disease. Additionally, and perhaps more importantly, salinity broadened the temperature range over which severe disease has been reported to be a problem (4,12,14). Salinity stress has been implicated in the development of Phytophthora root rot of citrus and chrysanthemum (2,7,8). Orange seedlings exposed briefly to high salinity (22 ds/m) or grown under a continual salinity stress (3–4 ds/m) were predisposed to root rot caused by *Phytophthora parasitica* (2). Chrysanthemum was predisposed to *Phytophthora cryptogea* following pre- and postinoculation exposures to high levels of salinity (22 ds/m) (7,8). Single-pulse exposures of high salinity of this type may not be typical of field conditions and raise questions as to whether the predisposition was from the salinity stress or other osmotic shocks. To eliminate these questions, we exposed plants to continual low levels of salinity over a period of time, much like the previously mentioned work with citrus. We exposed the fungus to the same salinity level as the plant, thereby eliminating the extreme shocks that can occur following pulse exposures to high salinity.

The primary effect of increased salinity levels was apparently on the plant, because salinity had little effect on mycelial growth rate of the fungus. Similar studies have been conducted on salinity and osmotic effects on the various stages of the life cycle of the Oomycetes (5), with much information concerning terrestrial species of *Phytophthora* spp. and *Pythium* spp. (1,3,9,13). These studies showed that Oomycetes are tolerant of high salinity levels. In fact, an increase in mycelial growth in our study accompanied increased salinity levels up to 7.1 ds/m at all temperature regimes. Thus, mycelial growth rate of *P. aphanidermatum* and other species of *Pythium* apparently is stimulated by relatively high salinity levels. In contrast to mycelial growth, zoospore production was inhibited at an Ec level of 14.2 ds/m. Our results with *P. aphanidermatum*, and other *Pythium* spp., are consistent with the known salinity sensitivity of zoospores in other Oomycetes (1,5,9).

Our results indicate that salinity stress may increase significantly the time that the pathogen is active. Golf course superintendents normally apply preventive fungicides for control of *Pythium* on the basis of forecasting systems (11). Seasonal time frames, based on temperature and relative humidity, are established, and preventive fungicide applications are then made weekly or biweekly. Our data indicate that these periods of susceptibility would be increased by the stress imposed on the plant by salinity. Salinity levels in turfgrass situations rarely are static and may fluctuate considerably over the course of a year, depending on irrigation variables. Irrigation frequency, duration, water quality, soil permeability, and syringing cycles contribute to these fluctuations. Fluctuations in salinity levels may account for the sporadic occurrence of cottony blight on golf courses in Arizona during periods not normally conducive for *P. aphanidermatum* (S. L. Rasmussen and M. E. Stanghellini, unpublished).

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