Sclerophthora macrospora: The Incitant of Yellow Tuft Disease of Turf Grasses

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ABSTRACT

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Infection studies using zoospores of *Sclerophthora macrospora* to inoculate various turf grasses confirm this pathogen as the causal agent of yellow tuft disease.

Additional key words: downy mildew, *Secale cereale*

The downy mildew fungus *Sclerophthora macrospora* (Sacc.) Thirum., Shaw, and Naras. is associated consistently with yellow tuft symptoms of turf grasses and has been proposed as the causal agent of this disease (3,4). Pathogenesis has not been proved in accordance with Koch's postulates because in vitro culture of this fungus is difficult (5). We report here an in vivo method to demonstrate infection by and recovery of the pathogen. A preliminary report has been published (2).

MATERIALS AND METHODS

Inoculum source and preparation. *Poa pratensis* L. and *Agropyron repens* L. were chosen as the sources of zoospore inoculum, the latter because it is a common collateral host for *S. macrospora* in Rhode Island (3). Potted plants of both species with typical yellow tuft symptoms were maintained in the greenhouse to promote good vegetative growth. Twenty 6-cm portions of leaf laminae obtained from these plants were submerged in 25 ml of distilled water for 8–12 hr at 15 C to generate sporangia and zoospore production.

Trial 1. Seeds of five grasses—*Poa pratensis* 'Baron,' *Lolium perenne* L. 'Yorktown,' *Phleum pratense* L. 'Climax,' *Agrostis stolonifera* L. 'Pencross,' and *Festuca rubra* L. ssp. *commutata* Gaud. 'Jamestown'—and one cereal, *Secale cereale* L. (winter rye), were germinated on moist paper towels. When the radicle and plumule were just visible, batches of five uniform seedlings were transferred to 1.5-cm-diameter plastic cylinders 5 cm long and inserted to a depth of 1 cm into soil in shallow dishes. A 50-mesh screened, nonsterile, fine sandy loam soil, pH 5.3, and the same soil amended with calcium hydroxide to raise the pH to 7.6, were used. Two soil pH levels were included because of a report that pH may affect zoospore germination (5).

Cylinders containing five seeds of each species were placed on both soils in sufficient number to allow three replications of each of four inoculum treatments designated 0, X, 2X, and 3X using zoospores from *Agropyron repens*. Inoculum comprising 0.5 ml of zoospore suspension (about 10,000 spores) was withheld (0), applied once (X), twice (2X), or three times (3X) over a 3-day period to the appropriate cylinders. The water level in the soil containers (and hence in the cylinders) was held just above the level of the seeds during this period, and the temperature was maintained at 15 C. After 3 days, excess water was drawn off, and the seedlings were placed under diffuse light on a window bench at prevailing laboratory temperatures.

Infection of the plants was determined after 2–3 wk by inspecting leaf and/or crown tissue with a microscope for the presence of the distinctive *S. macrospora* mycelium (4). Tissues were cleared in boiling 5% potassium hydroxide solution,

Table 1. Infection of seedlings of six grasses grown in soil of pH 5.3 and pH 7.6 after varying exposure to zoospores of *Sclerophthora macrospora* obtained from infected *Agropyron repens* plants

Host	Inoculum level[a]	Percentage of seedlings infected		
		pH 5.3	pH 7.6	Mean
Winter rye	0	0	0	0
(*Secale cereale*)	X	55	78	66
	2X	72	77	74
	3X	83	88	85
	Mean	70	81	
Red fescue	0	0	0	0
(*Festuca rubra* 'Jamestown')	X	20	20	20
	2X	25	35	30
	3X	30	40	35
	Mean	25	32	
Creeping bentgrass	0	0	0	0
(*Agrostis stolonifera* 'Penncross')	X	20	15	17
	2X	30	20	25
	3X	40	30	35
	Mean	30	22	
Kentucky bluegrass	0	0	0	0
(*Poa pratensis* 'Baron')	X	35	25	30
	2X	20	30	25
	3X	45	45	45
	Mean	33	33	
Perennial ryegrass	0	0	0	0
(*Lolium perenne* 'Yorktown')	X	40	40	40
	2X	40	40	40
	3X	50	60	55
	Mean	43	47	
Timothy	0	0	0	0
(*Phleum pratense* 'Climax')	X	35	45	40
	2X	40	25	32
	3X	35	40	37
	Mean	37	37	

[a] Exposure to zoospores for 0, 1, 2, or 3 days.

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Table 2. Infection of seedlings of four grasses grown in soil of pH 5.3 and pH 7.6 after varying exposure to zoospores of *Sclerophthora macrospora* obtained from infected *Agropyron repens* or *Poa pratensis* plants

Host	Inoculum level[a]	Percentage of seedlings infected[b]					
		P. pratensis			*A. repens*		
		pH 5.3	pH 7.6	Mean	pH 5.3	pH 7.6	Mean
Timothy	0	0	0	0	0	0	0
(*Phleum pratense* 'Climax')	X	30	20	25	10	10	10
	2X	30	30	30	20	30	25
	3X	30	30	30	30	30	30
	Mean	30	27		20	23	
Red fescue	0	0	0	0	0	0	0
(*Festuca rubra* 'Jamestown')	X	30	30	30	30	40	35
	2X	40	50	45	20	30	25
	3X	30	70	50	40	50	45
	Mean	33	50		30	40	
Perennial ryegrass	0	0	0	0	0	0	0
(*Lolium perenne* 'Yorktown')	X	40	40	40	30	50	40
	2X	30	60	45	50	60	55
	3X	60	60	60	80	90	85
	Mean	43	53		53	66	
Velvet bentgrass	0	0	0	0	0	0	0
(*Agrostis canina* 'Kingstown')	X	30	70	50	10	30	20
	2X	20	60	40	20	70	45
	3X	40	80	60	20	70	45
	Mean	30	70		17	57	

[a] Exposure to zoospores for 0, 1, 2, or 3 days.
[b] Duplicate tubes of five seeds per treatment.

and the mycelium was stained with zinc chloriodide (7).

Trial 2. Seeds of four grasses (cultivars Climax, Jamestown, and Yorktown, previously listed, and *Agrostis canina* L. 'Kingstown') were prepared as in trial 1. The same inoculation procedures and soils were used as in trial 1, but zoospore inoculum from a second source, *Poa pratensis*, was included in trial 2. Data obtained in trials 1 and 2 were subjected to analysis of variance.

Trial 3. Seedlings of winter rye were inoculated with *S. macrospora* zoospores from *Agropyron repens* by the procedure outlined in trial 1. Three weeks after inoculation, leaf laminae detached from these infected rye plants were used to generate more zoospores. A suspension containing approximately 43,750 zoospores/ml was obtained.

Three replicates of 10 seedlings of Yorktown perennial ryegrass were placed in three 60 × 15 mm petri dishes, submerged for 24 hr at 15 C in 10 ml of zoospore suspension, and planted separately in sterile soil. Ten seedlings similarly treated but incubated in distilled water served as controls. The plants were maintained as described for trial 1 and were inspected for the presence of *S. macrospora* mycelium after 20 days.

RESULTS AND DISCUSSION

The percentages of seedlings infected by *S. macrospora* in trials 1 and 2 are presented in Tables 1 and 2, respectively. In trial 3, 73.7% of the plants exposed to zoospores were infected, as opposed to no infection in the controls.

No infection occurred when *S. macrospora* zoospores were withheld. Mycelium was observed in all species exposed to zoospore suspensions, but host type had a significant effect on the percentage of plants infected ($P > 0.0001$ for both trials 1 and 2), as did repeated exposure ($P > 0.0002$ and 0.0007, respectively). In trial 1, percentage infection tended to increase with higher soil pH. Although the relationship was not significant in that trial, it was highly significant in trial 2 ($P > 0.0002$), and there was an indication that the pH effect was species-related.

Zoospores from both donor species, *P. pratensis* and *A. repens*, were equally infective to various other grasses (Table 2). Infected plants in all trials were shown to contain systemic mycelium of *S. macrospora*, and in 4–8 wk developed typical yellow tuft symptoms, ie yellowing of the leaves and proliferation of tillers.

Data presented here and in other studies (1,6) indicate that asexual inocula of *S. macrospora* are unspecialized in host preference. Data from trial 3 support this conclusion by showing that inoculum obtained from one host can be transferred serially through other hosts. Furthermore, our results (Tables 1 and 2) clearly establish *S. macrospora* as the incitant of yellow tuft disease.

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