Comparative Pathogenicity of Pythium myriotylum and P. irregulare to the Soybean Cultivar Bragg

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Portion of a thesis submitted by senior author in partial fulfillment of the requirements for the M.S. Degree, University of Florida.

Florida Agricultural Experiment Station Journal Series Paper No. 6036.

The authors wish to acknowledge the technical assistance of Mrs. M. Marie Brennan Southern.

Accepted for publication 26 May 1976.

ABSTRACT

SOUTHERN, J. W., N. C. SCHENCK, and D. J. MITCHELL. 1976. Comparative pathogenicity of Pythium myriotylum and P. irregulare to the soybean cultivar Bragg. Phytopathology 66:1380-1385.

Greenhouse pre- and postemergence tests on the soybean cultivar Bragg were performed with an autoclaved Arredondo fine sand (approximately WHC 8%, pH 6.2) noninfested or infested with oospores or mycelial inoculum of *Pythium irregulare* and *P. myriotylum*. Inoculum densities were 25, 50, 100, 250, 500, and 1,000 oospores/g of soil, except the highest inoculum density with *P. myriotylum* was 5,000 oospores/g of soil. In preemergence tests with mycelial inoculum, *P. myriotylum* significantly reduced the mean height and number of plants, whereas *P. irregulare* significantly reduced the plant height only in one test. In preemergence tests with oospores of *P. irregulare*, a significant plant height reduction occurred at inoculum densities of 500 and 1,000 oospores/g of soil. Root dry weight was significantly reduced in postemergence tests at 250 and 500 oospores/g of soil. With *P. irregulare* at 250 oospores per gram of soil, plant height was significantly reduced at 20, 23, 25, and 33 C. Root weight also was reduced at 20, 30, and 35 C. With *P. myriotylum* at 250 oospores/g of soil, plant height was reduced at 20, 23, 25, and 30 C. Root weight was reduced at 20, 25, 30, and 35 C. Oospores of *P. myriotylum* in pre- and postemergence tests did not affect root weight nor plant height. *Pythium myriotylum* was not recovered from the soil.

Additional key words: oospores, *P. myriotylum*, *P. irregulare*, soybean.

Damage to soybeans [*Glycine max* (L.) Merr.] by *Pythium* spp. in the southeastern USA has been estimated to be $25-$30 million per year (J. Marlowe, *personal communication*).

In 1970, McCarter and Littrell (6) first demonstrated the pathogenicity of *P. myriotylum* to the soybean cultivar Hampton by using mycelial inoculum. The relative pathogenicity of *P. irregulare* Buis. and *P. myriotylum* Drechs. to the soybean cultivar Bragg, using oospore or primarily mycelial inoculum, was compared in this study. The oospore inoculum was at various known densities but the mycelial inoculum was not quantitized. In addition, the effect on disease severity at a single oospore concentration and various soil temperatures was evaluated. This is believed to be the first report establishing the pathogenicity of *P. irregulare* to the soybean cultivar Bragg.

MATERIALS AND METHODS

Cultures and soil.—The cultures of *P. irregulare* and of *P. myriotylum* used in this study were isolated in Florida from a soybean root and a peanut root, respectively. The isolates used for oospore production were reisolated from soybean roots before being prepared for inoculum. Both isolates were stored on V-8 juice agar in test tube slants at 10 C. In separate experiments, mycelial fragments and oospores were evaluated as inoculum. In both experiments, soil was infested either before or after plant emergence and the experiments were referred to as preemergence and postemergence tests, respectively. The soil was an Arredondo fine sand with a maximum water-holding capacity (WHC) of approximately 8%. Soil was adjusted to pH 6.1-6.2 with $CaCO_3$ and then autoclaved twice at 24-hour intervals at 0.68 atmosphere (104 C) for 4 hours. The lime requirement of the soil was determined using a method developed by Woodruff (18). The soil pH was determined using Schofield and Taylor's method (11). In most tests, soil temperature was recorded daily and soil moisture was monitored with one or more tensiometers. All experiments were performed twice. Autoclaved 10-cm diameter clay pots with saucers were used as plant containers.

Preparation of inoculum.—For tests with mycelial inoculum, 500-ml Erlenmeyer flasks containing 300 ml of V-8 juice broth (16) were autoclaved for 15 minutes at 1.02 atmospheres (121 C), then cooled and seeded with an agar disk from a culture of *P. irregulare* or *P. myriotylum*. After incubation at 30 C for 20-23 days in the dark, the contents of four flasks were decanted separately onto four layers of cheese cloth, allowed to drain, and then washed with 500 ml of sterile tap water. Mycelial mats were examined microscopically and found to contain mycelia and hereafter are referred to as mycelial inoculum. The

mycelia were recovered and comminuted in a Waring Blendor in 500-700 ml of sterile tap water for 5 seconds.

The oospore inoculum was cultured for 21 to 29 days in the dark at 21 to 25 C in 250-ml Erlenmeyer flasks containing 15 ml of a modified Schmitthenner's medium (10). The additional constituents were 55 mg $CaCl_2$ per liter and 0.45 mg of cholesterol dissolved in 1 ml of ether to 15 ml of medium in each flask. After autoclaving, each flask was seeded with a mycelium-agar disk. After incubation, the contents of two flasks were decanted onto a 44-μm (325-mesh) sieve and the mycelial mats were gently rinsed. The mats were ground in a Pyrex tissue grinder containing 10 ml of deionized water until no mycelia were evident. After grinding, the volume of the homogenate was increased with deionized water and 100 ml amounts were sonicated with a Biosonic III Ultrasonic system at 40% of maximum power for 40 seconds. After sonication, the number of oospores/ml was determined with a hemacytometer.

Infestation of soil.—In the mycelial inoculum preemergence tests, mycelium was added to 10 kg of soil (7% moisture) by spraying and then mixed by rolling on a plastic sheet. Approximately 500 g of infested or noninfested soil were placed into each of 20 pots in a greenhouse and watered. After 7 days, a layer of soil 2.5 cm deep was removed from the upper portion of each pot, five seeds were planted, covered with the removed soil, tamped, and then watered. In the postemergence tests, each pot containing three 7-day-old plants received 30 ml of comminuted mycelial inoculum injected with an automatic pipette below the soil surface through six glass tubes (60 × 7 mm) placed in each pot at planting.

In the oospore inoculum tests, various quantities of oospore inoculum and soil were mixed for 15 minutes in the rotating drum of a 114-liter concrete mixer. Inoculum densities were 25, 50, 100, 250, 500, and 1,000 oospores/g of soil, except the highest inoculum density with *P. myriotylum* which was 5,000 oospores per gram of soil. Initial inoculum densities were determined by computation based upon the number of oospores per milliliter obtained by hemacytometer count of the inoculum prior to adding it to the soil. Average soil moisture before infestation was 4.5%. In the preemergence tests, 350 g of soil were added to each pot and tamped. Five seeds were planted and 250 g of soil was placed over the seeds and tamped. In the postemergence tests, three 7-day-old plants were transplanted into 600 g of soil.

Pathogenicity evaluation.—In the preemergence mycelial inoculum tests, the number of emerged plants and their height from the surface of the soil to the middle of the apical meristem were recorded 14 days after planting. In the postemergence tests, roots were washed 14 days after inoculation, oven dried at 70 ± 4 C for 24 hours or longer, allowed to cool in a desiccator, and weighed.

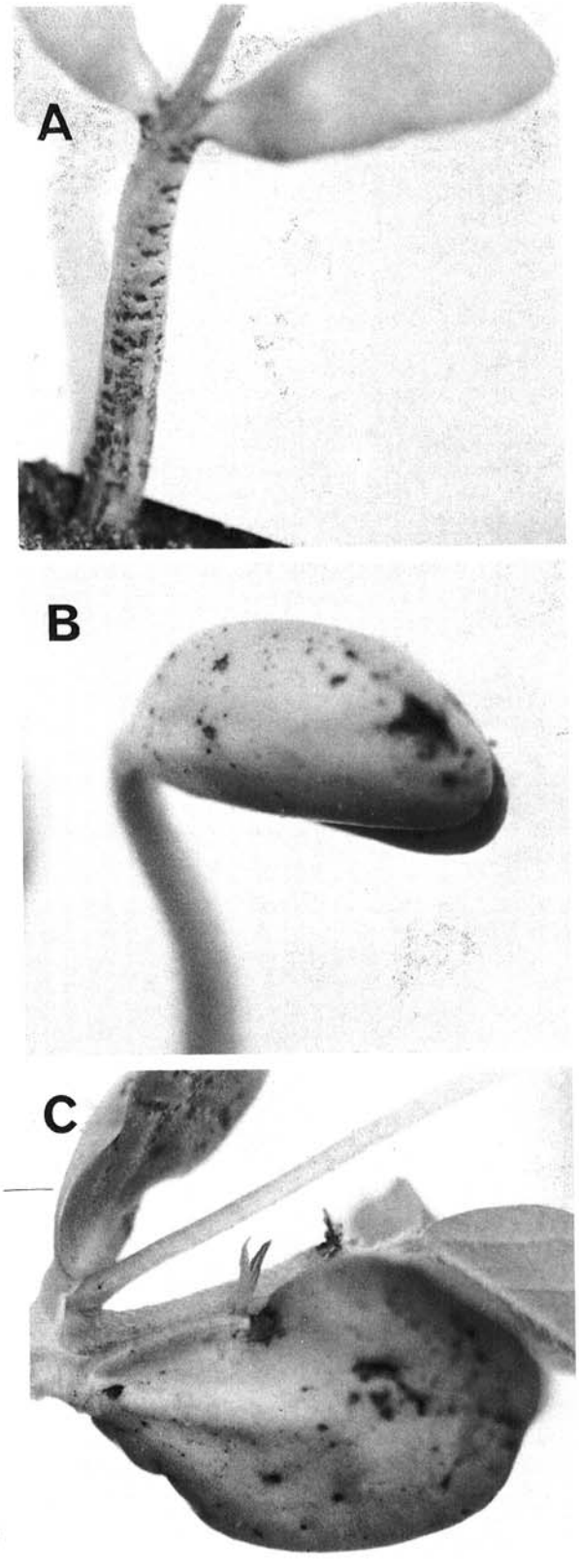

Fig. 1-(A to C). Necrotic lesions on soybean seedlings caused by mycelial inoculum of either *Pythium irregulare* or *P. myriotylum*. **A)** Soybean hypocotyl showing necrotic lesions caused by *P. myriotylum*. **B)** Soybean cotyledon showing necrotic lesions caused by *P. irregulare*. **C)** Lower and upper surface of soybean cotyledon showing necrotic lesions by *P. myriotylum*.

In the oospore inoculum preemergence tests, the number of plants and plant height were recorded 21 days after inoculation in the same manner as above. Dry root weight in the oospore postemergence tests was determined after 7 days. However, prior to being dried, three 5-cm root fragments were removed from each replicate. After surface disinfestation in 70% ethanol for 30 seconds and rinsing in sterile deionized water, sections were plated on modified Tsao and Ocana's medium with PCNB (15). In the noninoculated plants, 10 root systems were sampled. Root fragments obtained from soil infested with *P. myriotylum* were incubated at 30 C; those from *P. irregulare*-infested soil were incubated at 25 C. During a 48-hour incubation period, the number of individual colonies that emerged from the root were recorded. A total of 30 5-cm root fragments were sampled for each oospore inoculum level.

Propagule assay in infested soil.—Upon completion of the preemergence and postemergence tests with *P. irregulare,* soil core samples averaging 47 g were obtained from each oospore inoculum level. Soil samples were taken from each replicate and combined per inoculum level. The number of *P. irregulare* propagules per gram of soil was determined using Tsao and Ocana's modified medium. There were 10 dilution plates per inoculum level. The plates were incubated for 24-72 hours, gently washed, and the number of colonies was recorded.

Controlled soil temperature study.—A water bath, described by Schroder (12) was used. It consisted of four compartments between separate hot and cold reservoirs on opposite ends of the water bath. Each compartment contained 18 plastic containers, containing 500 g of gravel at the bottom overlaid with a 3.8-liter plastic bag containing 2 kg of soil. The treatments consisted of noninfested soil and soil infested with 250 oospores of *P. irregulare* or *P. myriotylum* per gram. The inoculum density was computed prior to infestation. Each treatment was replicated six times and performed twice. The temperatures in the four compartments in test 1 were 20, 25, 30, and 35, and in test 2 were 23, 28, 33, and 39 C. After 14 days, plant height and dry root weight were determined. Root isolations were performed as previously indicated. The number of propagules per gram of soil was determined for *P. irregulare* at each temperature treatment. Upon completion of the test, single soil samples were obtained from each replicate and combined into a treatment sample.

Reisolation and identification.—Tsao and Ocana's medium and 1.5% water agar were used to isolate *Pythium* spp. from surface-sterilized root and cotyledon tissue in the mycelial inoculum tests from both inoculated and noninoculated plants. Waterhouse's (17) grass blade pond water culture was used to induce sporulation of the resulting isolates. Middleton's and Waterhouse's (7, 17) monographs were used for identification.

RESULTS

The root symptoms of plants grown in soil infested with mycelium of *P. irregulare* or *P. myriotylum* varied from discrete lesions to a general necrosis. In addition, discrete lesions appeared on the upper and lower surface of the cotyledon and hypocotyl (Fig. 1). Size of lesions on the cotyledons ranged from pin-point to large (2 × 4 mm) irregularly shaped areas. The larger lesions were sunken, up to 3 mm into the tissue. In other instances, the tissue at the cotyledon edge was severely necrotic and had fallen away. All lesions had a reddish-yellow color, 2/2 on Munsell's (8) color chart. Plants inoculated with *P. myriotylum* mycelium had more numerous and deeper lesions on the cotyledon than plants inoculated with *P. irregulare* mycelium. Plants inoculated with *P. myriotylum* or *P. irregulare* oospores had cotyledons with lesions that were shallower and less numerous than those inoculated with mycelium. These symptoms did not appear on plants in soil infested with less than 250 oospores/g of soil. Both *P. irregulare* and *P. myriotylum* were isolated from the respective surface-sterilized root and cotyledon tissue.

Pathogenicity of mycelial inoculum.—Plants in the preemergence test exposed to *P. myriotylum* were significantly shorter ($P = 0.01$) and fewer in number than the noninoculated plants. In the first preemergence test with *P. irregulare,* plant height in infested soil was

TABLE 1. Effect of *Pythium irregulare* oospore inoculation on plant height and root dry weight of Bragg soybean

Inoculum level (oospores per gram of soil)	Plant height (cm)[a] preemergence test		Root dry weight (g)[a] postemergence test	
	Test 1	Test 2	Test 1	Test 2
0 (control)	19.3	30.4	0.160	0.113
25	23.1*[b]	30.9	0.139	0.102
50	22.5*	32.1	0.136	0.100
100	20.6	28.2	0.151	0.096
250	16.6	26.3	0.105**[b]	0.104
500	8.8*	23.5**	0.140	0.085*
1,000	14.7*	21.8**	0.123	0.114

[a]Mean of 20 control pots (no oospores in soil) and 10 pots per oospore inoculum level.

[b]Means of the groups of plants which were inoculated with oospores were statistically different $P = 0.05$ (*) or $P = 0.01$ (**) from the control plants by Dunnett's multiple comparison procedure (2).

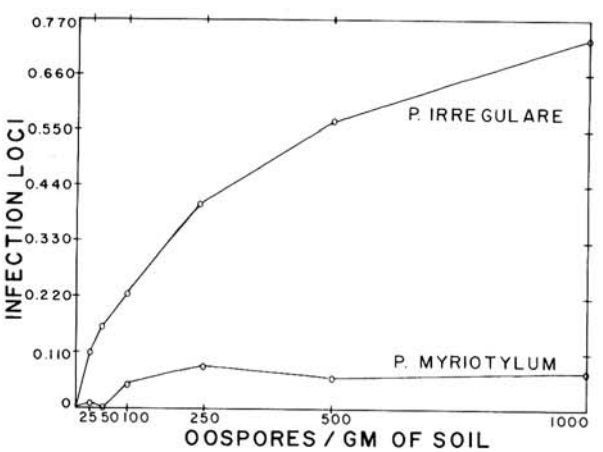

Fig. 2. The number of infection loci per centimeter of Bragg soybean root versus an increasing level of oospore inoculum of *Pythium irregulare* and *P. myriotylum*. Infection loci defined as the number of colonies per centimeter of root tissue.

significantly less ($P = 0.01$) than that in noninfested soil. In the second test, no significant difference occurred in height or number of plants. The plants in both preemergence tests grown in soil infested with mycelial inoculum of *P. myriotylum* were shorter and fewer in number than plants exposed to *P. irregulare*. No significant difference occurred in root dry weight between inoculated and noninoculated plants in postemergence tests with *P. myriotylum* or *P. irregulare*. The mean low and high soil temperatures were 23.5 and 32.0 C in the first mycelial inoculum test, and 22.0 and 29.0 C in the second test.

Pathogenicity of oospore inoculum.—In preemergence tests with *P. irregulare* oospore inoculum, the first significant reduction in the height of the inoculated plants occurred at initial inoculum levels of 500 and 1,000 oospores per gram of soil (Table 1). There was no reduction in height of the inoculated plants at 25 to 250 oospores per gram of soil. But in test 1 at inoculum levels of 25 and 50 oospores per gram of soil, inoculated plants were significantly taller ($P = 0.05$) than noninoculated plants. With *P. myriotylum* oospore inoculum, no significant reduction in height of the inoculated plants occurred at any of the inoculum densities. In test 1, at 50 oospores per gram of soil, inoculated plants were significantly taller ($P = 0.05$) than the noninoculated plants.

In the postemergence test with *P. irregulare,* a significant decrease in dry root weight of the inoculated plants occurred at 250 oospores per gram of soil in the first test, and at 500 oospores per gram of soil in the second test (Table 1). The number of *P. irregulare* colonies emerging from root segments increased with an increase of oospore inoculum; but this same phenomenon did not occur with *P. myriotylum* (Fig. 2). In both tests with *P. myriotylum,* no significant reduction in root dry weight of the plants occurred.

Pythium irregulare was recovered from soil at all inoculum levels in both pre- and postemergence tests with Tsao and Ocana's modified medium whereas *P. myriotylum* was not recovered. The mean soil temperature of both the pre- and postemergence tests with *P. irregulare* oospore inoculum tests was 28 C.

Controlled soil temperature test.—*Pythium irregulare*

TABLE 2. Effect of temperature and preemergence inoculations with 250 *Pythium irregulare* oospore per gram of soil on plant height and root weight of Bragg soybean and subsequent retrieval of the pathogen

Temperature (C)	Mean plant height (cm) 14 days after emergence		Mean root dry weight (g) 14 days after inoculation		Retrieval from soil (%) 14 days after infestation[a]
	Inoculated	Noninoculated	Inoculated	Noninoculated	
Test 1					
20	7.3**[b]	17.2	0.208**[b]	0.289	412
25	13.9**	16.1	0.216	0.233	310
30	15.8	17.1	0.196**	0.364	242
35	16.3	16.4	0.211*	0.340	50
Test 2					
23	7.3**	10.4	0.266	0.253	236
28	9.2	9.4	0.276	0.291	54
33	9.1*	11.2	0.274	0.254	2
39	7.5	6.6	0.222	0.199	0

[a]Number of propagules per gram of soil recovered in Tsao's medium divided by the number of oospores per gram initially added to the soil (inoculum level) × 100.

[b]Means for inoculated and noninoculated groups were statistically different, $P = 0.05$ (*) and $P = 0.01$ (**) by Dunnett's multiple comparison procedure (2).

TABLE 3. Effect of temperature and preemergence inoculation with 250 *Pythium myriotylum* oospores per gram of soil on plant height and root weight of Bragg soybean

Temperature (C)	Mean plant height (cm) 14 days after emergence		Mean root dry weight (g) 14 days after inoculation	
	Inoculated	Noninoculated	Inoculated	Noninoculated
Test 1				
20	12.8**[a]	17.2	0.229*[a]	0.289
25	12.2**	16.1	0.168*	0.233
30	14.6**	17.1	0.177**	0.364
35	16.4	16.4	0.142**	0.340
Test 2				
23	8.0**	10.4	0.244	0.253
28	9.5	9.4	0.250	0.291
33	10.5	11.2	0.231	0.254
39	6.3	6.6	0.150	0.199

[a]Means for inoculated and noninoculated groups were statistically different, $P = 0.05$ (*) and $P = 0.01$ (**) by Dunnett's multiple comparison procedure (2).

oospore inoculum of 250 oospores per gram of soil significantly reduced plant height at temperatures of 20, 23, 25, and 33 C, and significantly reduced root weight at temperatures of 20, 30, and 35 C (Table 2). *Pythium myriotylum* oospore inoculum at 250 oospores per gram of soil significantly reduced plant height at soil temperatures of 20, 23, 25, and 30 C, and significantly reduced root weight at temperatures of 20, 25, 30, and 35 C (Table 3). The percent retrieval of *P. irregulare* propagules from the soil was the greatest at 20 C and generally decreased with an increase in temperature (Table 2). *Pythium myriotylum* was not retrieved from soil.

DISCUSSION

The dark necrotic lesions that appeared on the cotyledons of plants grown in infested soil in this study appeared to be comparable to the lesions noted by Strissel and Dunleavy (14) with *P. debaryanum* on soybean. The significant reduction in plant height and emergence obtained with mycelial inoculum of *P. myriotylum* substantiates the work of McCarter and Littrell (6) who applied mycelial inoculum to plants of the cultivar Hampton and demonstrated *P. myriotylum* to be a pathogen of soybean.

Preliminary results with mycelium suggested that inoculum of *P. myriotylum* was more pathogenic than that of *P. irregulare* to the soybean cultivar Bragg. However, in the oospore inoculum tests, the results obtained were the reverse of the results of the preemergence mycelial inoculum tests. Although both pathogens were isolated from the roots, no significant reduction in dry root weight was obtained in the postemergence tests with soil infested with *P. myriotylum* or *P. irregulare* mycelium. This may have occurred because the plants became pot-bound after 14 days. Hendrix and Campbell (4) noted that both noninoculated and inoculated root systems eventually became equal when grown in restricted containers.

In general, an increase in the initial oospore inoculum density of *P. irregulare* resulted in an increased reduction of plant height and root dry weight (Table 1).

In the controlled soil temperature experiment, oospores of *P. irregulare* caused the greatest reduction in plant height of 20 C. This would indicate that disease severity was the greatest at the lowest temperature (Table 2) and agrees with the results obtained by others that *P. irregulare* is more pathogenic at low temperatures (1, 9). In soil infested with oospores of *P. myriotylum,* the greatest reduction in root weight occurred at 30 to 35 C (Table 3). Both pathogens caused a significant reduction in either root or shoot growth over a temperature range of 20 to 35 C (Tables 2 and 3). Both Gay (3) and Littrell and McCarter (5) reported that *P. myriotylum* caused the greatest disease severity at 35 C but was also virulent at lower temperatures.

The highest number of *P. irregulare* propagules retrieved from the soil occurred at 20 C and corresponded with the greatest reduction in plant height (Table 2). A decrease in the number of propagules of *P. irregulare* retrieved from the soil from 20 to 39 C was inversely proportional to mean plant height.

A higher level of disease severity following inoculation of plants with oospores of *P. myriotylum* occurred in the plastic containers in the temperature tank than in the clay pots. The chief differences between these two studies were (i) volume of soil (the clay pot contained 600 g of soil and the plastic container contained 2 kg of soil); (ii) the soil moisture content (soil in the clay pots varied from 30-100% WHC and in the plastic container it fluctuated from 75-100% WHC); and (iii) soil temperature (the clay pots had a mean diurnal fluctuation of 3 C and in the plastic pots the diurnal change was 0.5 C). Although several factors could have influenced this difference, the most probable cause was the lack of proper soil moisture. Stanghellini and Burr (13) stated that soil moisture acts as a solubilizer and carrier of exogenous nutrients and root exudates. They reported a maximum oospore germination of 90% in soils at 95 and 96% of WHC. However, the rate and percentage of oospore germination was reduced when both amended soils were reduced to 10 and 28% of their WHC (13). Thus, the fluctuating soil moisture levels in the clay pots may have prevented oospore germination and subsequent infection of the host root tissue.

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