Populations and Biology of Pythium Species Associated with Snap Bean Roots and Soils in New York

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ABSTRACT

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Soil populations of total *Pythium* spp. varied considerably between and within bean fields in New York. Average counts of low- and high-temperature *Pythium* spp. ranged from 133 to 1,560 and 22 to 95 propagules/g oven-dry soil, respectively. *Pythium ultimum, P. irregulare,* and unidentified sporangial-forming *Pythium* sp. isolates were recovered from bean soils at 21 C and accounted for 80, 2, and 16% of the isolates, respectively, obtained from one field. However, the frequency of recovery of these species differed markedly between fields, although *P. ultimum* always was found to be the most abundant pathogenic species. Only *P. oligandrum* was isolated from soil samples incubated at 37 C. These same *Pythium* spp. also were isolated from roots and hypocotyls of naturally infected beans as early as 7 days after planting, and the percent recovery increased with plant age. *Pythium* spp. were recovered more frequently from bean roots than from hypocotyl tissues. Low- and high-temperature *Pythium* spp. were isolated from 55 to 92 and 0 to 28%, respectively, of the root systems of 8-wk-old plants. In pathogenicity tests, *P. ultimum* and *P. irregulare* caused severe pre- and postemergence damping-off and root rot of beans, whereas *P. oligandrum* and the unidentified sporangial isolates produced limited or no damage. Under high moisture conditions, lesions incited by *P. ultimum* on bean tissues below the soil line continued to move upwards until the terminal bud was infected, resulting in plant death. A direct correlation was found between inoculum density of *P. ultimum* and root-rot severity on beans in pasteurized soil. Root-rot severity ratings were increased (1.8 to 4.0) and dry weight per plant was decreased (0.98 to 0.49 g) as inoculum density was increased from 1 to 500 propagules/g oven-dry soil. Four- to 28-day-old bean plants were equally susceptible to root rot incited by *P. ultimum*.

Additional key words: *Phaseolus vulgaris,* blight.

Approximately 25,000 hectares are planted annually to snap beans (*Phaseolus vulgaris* L.) in New York. In 1975, the farm value of snap beans, produced mainly for processing, was about 23 million dollars (20). Over the years, monoculture of beans has increased the prevalence and severity of diseases caused by soilborne pathogens. Presently, root rot is the major disease on snap beans and occurs wherever the crop is grown in New York. This disease is endemic to the bean-growing areas, and considerable yearly variation in the severity of root rot often is observed within- and between fields with a history of the disease. In addition, severe epidemics have occurred repeatedly in recent years, particularly under cool, wet conditions, making the disease a limiting factor in snap bean production.

Fusarium solani (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans., *Rhizoctonia solani* Kuehn, *Thielaviopsis basicola* (Berk. & Br.) Ferr., and several *Pythium* species have been reported to be primary causal agents of bean root rot (31). These pathogens may act independently or as a complex in any possible combination in causing damage to beans. For many years, *F. solani* f. sp. *phaseoli* has been considered the major causal agent of bean root rot in New York (4, 14). However, *P. ultimum* Trow recently has been found to be the major incitant of seed decay and preemergence damping-off of snap beans (7). Furthermore, field observations and greenhouse and field tests with selective fungicides for the control of bean root rot (Abawi and Pieczarka, *unpublished*) suggested that *Pythium* spp. play a major role in the bean root-rot complex. In addition, the incidence of Pythium bean blight was found to be as high as 10% in many bean fields during 1973 (7). Knowledge of the species of *Pythium* involved, their soil population, and the relationship between the different diseases incited by *Pythium* on beans in New York is needed for better understanding and control of these diseases.

The objectives of this study were: (i) to identify *Pythium* spp. associated with bean tissues and soils, (ii) to determine soil populations of *Pythium* spp. in bean fields, (iii) to test the pathogenicity of the different species on bean, and (iv) to investigate the effect of inoculum density and plant age on disease incidence and severity. A portion of this work was published previously (21).

MATERIALS AND METHODS

Isolation from soil and bean tissues.—Soil samples were collected from naturally infested bean fields in central and western New York during 1974 and 1975. Soil

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sampling tubes or long-handled trowels were used to collect composite samples from 0-9 and 9-18 cm deep. Eight bean fields were sampled once in 1974 and four fields were sampled five times [4 wk before planting; at planting; and 4, 8 (harvest time), and 16 wk after planting] during 1975. Soil samples also were collected from a cleared woodlot and a field planted continuously to corn for 15 yr on two of the above dates in 1975. Bean fields to be sampled were divided into 60 × 60-m sections, and one composite sample, consisting of 30 randomly collected subsamples, was taken from each section. The soil was transported to the laboratory in plastic bags, stored at 4 C, and assayed for *Pythium* spp. within 10 days. All samples were passed through a 1.68-mm (mesh-size) screen and mixed thoroughly. For determining populations of *Pythium* spp., several selective media were evaluated in preliminary tests for their effectiveness in enumerating the population of *Pythium* spp. in New York soils. The selective medium of Tsao and Ocana (28) was used in 1974; however, a modification of this medium was used exclusively in 1975. The latter medium (PVP) contained pimaricin (Delvocid, Gist-Brocades, Delft, Holland), 5 mg; vancomycin hydrochloride (Eli Lilly and Co., Indianapolis, IN 46206), 300 mg; pentachloronitrobenzene (PCNB) 75% WP, 130 mg; Difco cornmeal agar (CMA), 17 g; and distilled water, 1 liter. Soil dilution series of 1:10, 1:50, and 1:100 (w/v) were made in 0.03% sterile water agar, and three-to-five replicate plates were employed for each sample. The surface-soil-dilution plate technique (19) was used, and 0.5- or 1.0-ml alilquots of the appropriate dilution were added per plate. The plates were incubated in the dark at 20 C for isolation of the low-temperature *Pythium* spp. and at 37 C for the high-temperature species. Colony counts were made 24 and 48 hr later and recorded as the number of propagules/g oven-dry soil.

To determine *Pythium* spp. associated with bean roots and hypocotyls, plants were collected from 10 fields from central and western New York at 1 and 7-8 wk after planting. Root and hypocotyl segments were washed in running tap water for 4 hr, plated on PVP, and incubated at 20 or 37 C. After 24- to 48-hr incubation, the number of tissue segments yielding *Pythium* colonies were identified and counted.

Identity and frequency of isolation from soil and bean tissues.—Hyphal tip transfers to PVP were made from all *Pythium* colonies that appeared on three to five PVP plates incubated at 20 or 37 C from each bean-field soil sampled. The plates were incubated for 24-48 hr at 20-22 C, and then a second hyphal tip or a single spore (sporangium or oospore) transfer was made to hemp seed agar (HSA) (2) for identification of the species. Similarly, transfers to HSA also were made from randomly selected colonies originating from bean root or hypocotyl tissues incubated on PVP plates. The key of Middleton (16) was used for species identification. Stock cultures of identified *Pythium* species were maintained by periodic transfers on CMA at 21 C.

Pathogenicity tests.—The snap bean cultivar Early Gallatin was used throughout this study. Seeds were surface-disinfested for 5 min in 0.25% NaOCl and then rinsed in tap water. Bean-field soil was pasteurized by forced aerated steam (60 C for 30 min) and used in most experiments after 3- to 4-wk storage at 25-27 C. Soil was sterilized by autoclaving at 121 C for 1 hr. Unless otherwise noted, all experiments were conducted in a growth chamber at 21 ± 1 C, 75% relative humidity, with 14 hr of cool-white fluorescent light (11,000 lux) per day. Plants were watered daily and care was taken to keep the soil moist but not saturated. At the termination of each experiment, plants were removed from the soil, and the roots were washed and rated for disease severity. A rating of 0 to 6 was used with 0 indicating no apparent disease, whereas 6 referred to most severe disease (dead plant). Total dry weight was determined by drying plants for 72 hr at 95 C.

Two isolates of each *Pythium* species recovered from bean tissue or soil were used in pathogenicity tests. For low-temperature *Pythium* species, inoculum was prepared by growing each isolate for 2 wk at 25 C in 1-liter flasks containing 300 cc of a sand-cornmeal (10% cornmeal) mixture. Then the inoculum was mixed with moist pasteurized soil at a ratio of 1:9 (v/v). The controls received an equal volume of sterile sand-cornmeal mixture. The infested and control soils were incubated at 25 C for 3 wk before use.

Inoculum for the high-temperature *Pythium* species was produced in petri plates containing 0.5-cm bean-pod segments and 20 ml of distilled water, hereafter referred to as the BPW technique. Sterilized plates were seeded with disks taken from the margins of actively growing colonies on cornmeal agar. After incubation at 23 C for 14 days, oospores and sporangia were separated from the mycelium by fragmenting the culture in a Waring Blendor for 10-15 sec and passing the suspension through a 150-μm sieve. The resulting mixture of oospores and sporangia, essentially free of mycelial fragments, was atomized on and mixed into moist pasteurized soil. Infested soils were placed in closed containers and incubated for 5 mo at 25 C before use. Wooden flats were filled with infested soil, and 40 beans seeds were planted per flat and maintained in growth chambers at 21 or 30 C. Plant-stand counts and root-rot ratings were made 3 and 2 wk after planting at 21 and 30 C, respectively.

Inoculum density, plant age, and disease severity.—Isolation studies and pathogenicity tests showed that *P. ultimum* is the most prevalent and pathogenic of the several *Pythium* spp. associated with bean tissues and soils in New York. Thus, a pathogenic sporangial form of *P. ultimum* was used in these tests because of the uniformity and ease of producing large numbers of readily germinable spores. To determine the relationship between inoculum density and pre- and postemergence damping-off, bean seeds were planted in pasteurized bean-field soil infested with 1, 10, 100, and 500 sporangia/g oven-dry soil. Inoculum was produced by the BPW method and atomized on and mixed into the soil.

The effect of different inoculum densities of *P. ultimum* on the incidence and severity of root rot was evaluated by transplanting 8-day-old bean seedlings into pasteurized soil infested with 1, 10, 100, 500, and 1,000 sporangia/g oven-dry soil. It was necessary to use older seedlings because very low populations of *P. ultimum* caused considerable pre- and postemergence damping-off of direct-seeded beans, resulting in insufficient plant populations. Six surface-disinfested seeds were planted in

10-cm diameter pots containing sterilized sand and incubated at 23 C. After 8 days, the seedlings were removed from the pots by submerging in a tub of water and allowing the sand to fall away from the roots. The seedlings then were transplanted into 12.5-cm diameter plastic pots filled with the infested soils. Each pot received 0.5 g of a complete fertilizer (10-20-10) 1 and 15 days after transplanting. The experiment was terminated and root-rot severity was rated 28 days after transplanting.

Susceptibility of bean seedlings of different ages to the same isolate of *P. ultimum* was determined in 10-cm diameter plastic pots filled with sand sterilized by autoclaving at 121 C for 1 hr. Four surface-disinfested seeds were planted 3.0 cm deep in each pot. Seedlings 6, 14, 21, and 28 days old were inoculated with a sporangial suspension of *P. ultimum* at an equivalent population of 1,000 sporangia/g oven-dry sand. Each pot was fertilized weekly with 40 ml of a complete liquid fertilizer (0.5 g/liter, 20-20-20). Three weeks after inoculation, the plants were harvested and rated for root-rot severity.

All tests were repeated once, and each treatment was replicated four or five times. Data in tables are those of representative experiments. Where appropriate, data were analyzed by the Waller-Duncan's Bayesian K-ratio (LSD) rule (29).

RESULTS

Soil populations.—The total population of low- and high-temperature *Pythium* spp./g oven-dry soil at 0-9 and 9-18 cm deep was similar, and thus only data for the 0-9 cm depth are presented. The data showed that populations of *Pythium* spp. varied considerably between and within bean fields (Tables 1, 2). Soil populations of high-temperature *Pythium* spp. were much lower than those of low-temperature species, but the population trends within- and between fields were similar. Average populations of total low-temperature (20 C) *Pythium* spp. in bean fields sampled in 1974 ranged from 198 to 704 propagules/g of oven-dry soil (Table 1). Populations of *Pythium* spp. in the four bean fields (A to D) assayed in 1975 ranged from 133 to 1,560 and 22 to 95 propagules/g of oven-dry soil for low- and high-temperature species, respectively (Table 2). Populations of low- and high-temperature *Pythium* spp./g of oven-dry soil also differed considerably within each field. For example, counts of low- and high-temperature *Pythium* spp. within field A when sampled at harvest time ranged from 567 to 1,267 and 58 to 103 propagules/g of oven-dry soil, respectively. Furthermore, there was no particular pattern in the seasonal fluctuation of the soil populations except in field

TABLE 1. Soil populations of low-temperature *Pythium* species and root-rot severity of snap beans in eight New York State fields sampled in 1974

Fields sampled	No. composite samples/field	No. propagules/g oven-dry soil at 20 C[x]		Root-rot severity rating[y]
		Average	Range	
A	8	431 cd[z]	283-615	1.8 f
B	4	533 abc	483-667	3.7 b
C	5	490 bcd	300-783	2.5 e
D	4	616 ab	450-750	0.7 g
E	9	198 e	100-283	3.7 b
F	8	410 cd	250-617	4.6 a
G	5	340 de	217-517	3.1 d
H	4	704 a	433-950	3.4 c

[x]Refers to the total propagules of all *Pythium* spp. that grew on the selective medium of Tsao and Ocana (28) at 20 C.
[y]Ratings were based on scale of 0 to 6 with 0 = no disease and 6 = most severe symptom development (dead plant).
[z]Means in a column followed by the same letter do not differ significantly ($P = 0.05$) by the Waller-Duncan's Bayesian K-ratio (LSD) rule.

TABLE 2. Soil populations of low- and high-temperature *Pythium* spp. in four New York State bean fields; each sampled five times during 1975

Fields sampled[b]	Propagules/g dry soil at various sampling dates[a]									
	I		II		III		IV		V	
	20 C[c]	37 C[d]	20 C	37 C	20 C	37 C	20 C	37 C	20 C	37 C
A	1,320	84	1,560	92	1,103	95	1,020	78	963	76
B	927	79	682	51	1,060	52	783	47	597	69
C	...	...	245	28	456	26	403	21	467	42
D	417	38	387	23	170	22	133	33	600	55

[a]Sampling dates I, II, III, IV, and V correspond to 4 wk before planting, at planting, and 4, 8 (harvest time), and 16 wk after planting, respectively. A total of ten, ten, six, and ten composite samples were obtained from fields A, B, C, and D, respectively.
[b]Root rot was severe in field A and moderate in fields B, C, and D.
[c,d]Refers to populations of low- and high-temperature *Pythium* spp., respectively.

D (Table 2). Soil populations in field D decreased continuously throughout the growing season, then abruptly increased after harvest.

In 1975, root rot was most severe in field A which had the highest population of *Pythium* spp. Root rot was only moderate in fields B, C, and D which had relatively lower soil populations. However, no correlation was found between root-rot severity and populations of low-temperature *Pythium* spp. in 1974 (Table 1).

The populations of low-temperature *Pythium* spp. in a fine-textured woodlot soil and a coarse-textured, corn-field soil averaged 1,266 and 828 propagules/g of oven-dry soil, respectively. Counts of high-temperature *Pythium* spp. averaged 26 and 30 propagules/g of oven-dry soil in the woodlot and corn-field soils, respectively. These populations were within the range detected in the bean soils.

Isolations from bean tissues.—Low-temperature *Pythium* spp. were isolated frequently from root and hypocotyl tissues of field-grown beans (Table 3). *Pythium* spp. were recovered from 26 to 74% of the root segments collected 1 wk after planting. Between 55 to 92% of the roots from 7- to 8-wk-old plants yielded *Pythium* spp. The frequency of isolation of *Pythium* spp. from hypocotyl tissues usually was lower. Furthermore, the percent recovery of high-temperature *Pythium* spp. from root and hypocotyl tissues generally was lower than that observed for low-temperature species.

Identification of Pythium spp. and their frequency of isolation.—*Pythium ultimum, P. irregulare* Buism., and two groups of sporangia-forming isolates that did not produce oogonia and/or oospores were isolated from bean soils at 20 C. One of the two groups of sporangia-forming isolates was found to be identical with *P. ultimum* in all characteristics except that oogonia were not produced. These *P. ultimum* sporangial isolates sometimes were difficult to differentiate from the other unidentified group of isolates that also did not produce oogonia. The latter unidentified species, hereafter referred to as *Pythium* sp., usually produced fewer sporangia on CMA or HSA that were slightly larger and less uniform in shape than those of the sporangial isolates of *P. ultimum*. All of these species also were recovered from bean roots and hypocotyls, but *P. ultimum* was isolated most frequently. *Pythium oligandrum* Drechs. was the only species isolated from bean soils and tissues at 37 C.

The frequency of isolation of each *Pythium* species, especially *P. irregulare* from natural bean soil, varied considerably between fields (Table 4). For example, *P. ultimum, P. irregulare,* and *Pythium* sp. accounted for 80, 2, and 16%, respectively, of the germinable propagules recovered from soil D and 55, 0, and 39%, respectively, of those recovered from soil A.

Pythium irregulare was not isolated from soil of either the woodlot or the corn fields. However, *P. ultimum* and *Pythium* spp. accounted for approximately 85 and 15%, respectively, of all the *Pythium* isolates that were identified from either of these fields.

Pathogenicity tests.—*Pythium ultimum,* sporangial isolates of *P. ultimum,* and *P. irregulare* caused severe pre- and postemergence damping-off, reducing plant stands 8 to 28, 28 to 49, and 36 to 59%, respectively, compared with the noninoculated controls (Table 5). These pathogens readily attacked germinating seeds and infected the cotyledons, radicle, and hypocotyl tissues prior to emergence (Fig. 1-A, B). Plants that escaped preemergence damping-off frequently developed necrotic lesions on the cotyledons and occasionally the terminal bud became infected, resulting in eventual death of the plant (Fig. 1-C). Isolates of *P. ultimum* and *P. irregulare* also caused softening of the hypocotyl tissues below or above the soil line and collaspse of the plant (Fig. 1-D). Postemergence damping-off was most severe within 3-5 days after emergence. Moreover, these two species damaged the roots of surviving plants as indicated by the high root-rot ratings (Table 5).

Isolates of the unidentified sporangial group of

TABLE 3. Frequency of isolation of low- and high-temperature *Pythium* spp. from segments of bean roots and hypocotyls collected from 10 fields in New York State

Fields sampled	% Isolation from plants of two different ages[a]							
	1 wk[b]				7-8 wk[b]			
	Roots		Hypocotyls		Roots		Hypocotyls	
	21 C[c]	37 C[d]	21 C	37 C	21 C	37 C	21 C	37 C
A	37	9	35	2	73	12	53	10
B	...	...	...	...	66	14	20	4
C	47	7	9	3	64	22	20	28
D	26	0	12	0	55	17	43	18
E	30	17	21	20	91	21	19	1
F	74	50	92	68	92	10	78	9
G	52	40	16	33	73	27	14	1
H	72	13	19	4	69	7	22	4
I	68	22	17	5	67	0	10	0
J	47	33	26	19	63	28	14	1

[a]Data are presented as the percentage of hypocotyls and root systems from which *Pythium* spp. were recovered.
[b]Weeks after planting.
[c,d]Refers to low- and high-temperature *Pythium* spp., respectively.

TABLE 4. Frequency of isolation of the different *Pythium* species from soils of four bean fields in New York State

	Percent of total isolations per field[a]					
	20 C[b]					37 C[c]
Fields sampled	*P. ultimum*	*P. ultimum* (sporangial isolates)	*P. irregulare*	*Pythium* spp.[d]	Others[e]	*P. oligandrum*
A	20	35	0	39	6	100
B	23	46	4	27	0	100
C	37	33	15	15	0	100
D	33	47	2	16	2	100

[a]Data are presented as percentage of the total *Pythium* spp. isolated per field.
[b,c]Species isolated on a selective medium at 20 or 37 C, respectively.
[d]Sporangial isolates that did not produce oogonia and/or oospores.
[e]Refers to fungi with coenocytic mycelium.

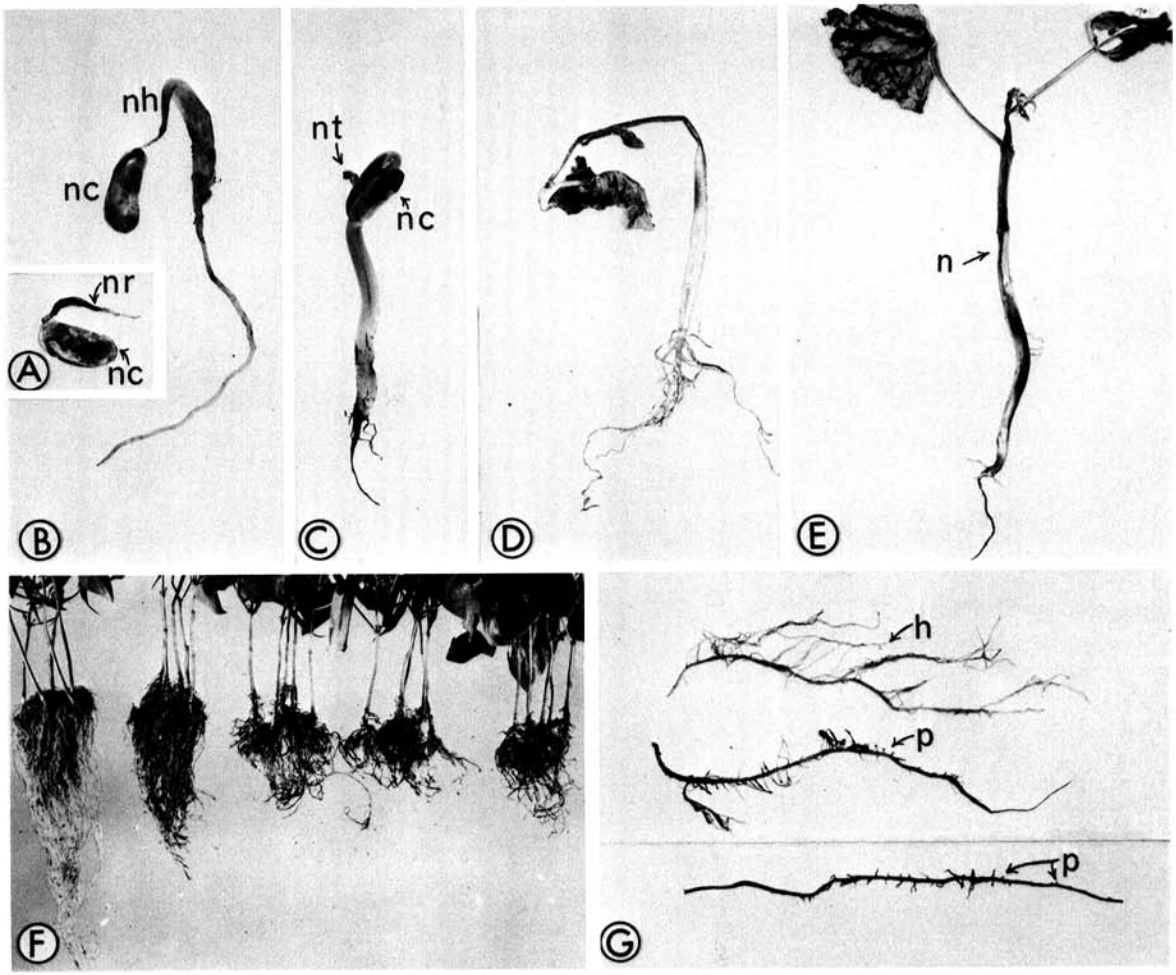

Fig. 1-(A to G). Disease symptoms on snap beans grown in pasteurized soil infested with *Pythium ultimum*. **A, B)** Preemergence damping-off showing necrotic radicle (nr), cotyledons (nc), and hypocotyl (nh). **C)** Necrotic cotyledons (nc) and terminal bud (nt) on a seedling that was infected but survived preemergence damping-off. **D)** Postemergence damping-off. **E)** Dead 3-wk-old plant as a result of necrosis (n) extending above the soil line to the terminal bud. **F)** Roots of plants grown in pasteurized soil infested with (left to right) 0, 1, 10, 100, and 500 sporangia of *P. ultimum*/g oven-dry soil. **G)** A root segment from a check plant (top) showing healthy fibrous roots (h), and a root segment infected with *P. ultimum* showing extensive pruning (p) of fibrous roots.

Pythium species were less damaging to beans (Table 5). These isolates caused slight root discoloration and minor root pruning. All *Pythium* spp. tested at 21 C were reisolated from necrotic roots of plants grown in the artificially infested soils.

Pythium oligandrum was not pathogenic to beans at 21 C. However, it reduced emergence counts slightly at 30 C. No visible symptoms developed on roots of surviving plants, although minor superficial hypocotyl necrosis was observed on a few plants. Despite the lack of root necrosis, *P. oligandrum* was reisolated from roots of plants tested at 21 and 30 C.

Inoculum density, plant age, and disease development.—*Pythium ultimum* and an isolate of its sporangial form reduced stand counts by as much as 85% at inoculum densities as low as one viable propagule/g oven-dry soil when tested under very moist soil conditions. In addition, the extent and type of damage caused by these isolates were similar.

The influence of inoculum density on root-rot severity

TABLE 5. Percent emergence and root-rot severity of beans grown in soil infested with various *Pythium* spp.

Pythium spp.[a]	Emergence[b] (% control)	Disease rating[d]	
		Hypocotyl	Root
Control (21 C)	100	0	0
P. ultimum	18	2.6	5.2
P. ultimum (sporangial)	39	2.4	4.2
P. irregulare	48	1.1	4.3
Pythium sp.	88	0.7	0.9
P. oligandrum	100	0	0
	82	0.2	0
Control (30 C)	100[c]		

[a]Two isolates of each species were tested.
[b]Forty seeds were planted per flat. Stand count was recorded 3 wk after planting. Data presented are the average of the two isolates evaluated.
[c]Incubated at 30 C; all other tests were conducted at 21 C.
[d]Disease rating was based on a scale of 0 to 6 with 0 = no symptoms and 6 = most severe symptoms (dead plant).

TABLE 6. Effect of inoculum density of *Pythium ultimum* in pasteurized bean-field soil on root-rot severity in New York State

Inoculum density (sporangia/g dry soil)	Disease rating[y]		Dry weight	
	Hypocotyl	Root	g/Plant	Percent of control
0	0 d[z]	0 c	0.98 a	...
1	0.9 cd	1.8 b	0.68 bc	69
10	2.8 a	3.9 a	0.59 cde	60
100	1.7 bc	3.6 a	0.64 cd	65
500	2.6 ab	4.0 a	0.49 e	50

[y]Disease rating was based on a scale of 0 to 6 with 0 = no symptoms and 6 = most severe symptoms (dead plant).
[z]Means in a column followed by the same letter do not differ significantly ($P = 0.05$) by the Waller-Duncan's Bayesian K-ratio (LSD) rule.

TABLE 7. Effect of plant age on the severity of bean root rot incited by *Pythium ultimum* in pasteurized soil infested with 500 propagules/g of oven-dry soil

Plant age (days)	Disease rating[x]		Dry weight per plant		
	Hypocotyl	Root	Control (g)	Infested (g)	Percent of control
6	1.2[y]	3.0[y]	0.34	0.30	88
14	1.7	2.8	0.90	0.69[z]	77
21	1.6	2.3	0.96	0.61[z]	63
28	1.7	2.2	1.50	1.14[z]	76

[x]Disease rating was based on a scale of 0 to 6 with 0 = no symptoms and 6 = most severe symptoms (dead plant). No disease developed on the controls.
[y]There were no significant differences ($P = 0.05$) in ratings when analyzed by the Waller-Duncan's Bayesian K-ratio (LSD) rule.
[z]Values are significantly different from the control.

was determined by transplanting 8-day-old beans into *P. ultimum*-infested soil. Root-rot-severity ratings were increased from 1.8 to 4.0, and dry weight per plant was decreased from 0.98 to 0.49 g as the inoculum level was increased from 1 to 500 viable sporangia/g of oven-dry soil (Table 6, Fig. 1-F). No increase in hypocotyl necrosis occurred at inoculum levels greater than 10 propagules/g oven-dry soil. The relation between inoculum density and disease severity was similar in sterilized and pasteurized soils; however, root-rot ratings at lower inoculum levels tended to be higher in sterilized soils. Roots infected with *Pythium ultimum* were brown, stunted, and had extensive pruning (Fig. 1-F, G). Foliar symptoms on plants grown in pasteurized soil infested with 10 propagules/g oven-dry soil or higher were stunting, yellowing, and loss of lower leaves. In addition, when 3- to 4-wk-old plants growing in *P. ultimum*-infested soils were maintained under high moisture conditions, hypocotyl necrosis extended above the soil line (Fig. 1-E) without causing the stem to collapse. Often, the pathogen continued to move upward and infected the terminal bud, causing plant death.

Bean plants, 4- to 28-days-old, were about equally susceptible to Pythium root rot; no significant differences were observed in disease ratings and reduction in dry weight between the different age groups (Table 7). Seedlings less than 4 days old usually failed to emerge because of pre-emergence damping-off.

DISCUSSION

Pythium spp. are common in cultivated and virgin soils (9). Populations in agricultural soils have been reported to range from less than 100 to over 2,000 propagules/g of soil (12, 13, 18, 21, 26, 27). However, the percentage of pathogenic species is usually less (12, 13, 18) and changes in their populations do not necessarily follow fluctuations of the total count of *Pythium* spp. Kraft and Burke (12) reported that total populations of *Pythium* spp. were low in noncultivated soils in Washington State. Furthermore, none of the *Pythium* spp. they obtained from these soils was pathogenic to beans or peas. In contrast, 85% of the *Pythium* isolates obtained from a woodlot within the bean-growing area in western New York were identified as *P. ultimum*. Differences in environmental conditions, vegetation, soil characteristic, etc. between the two states may account for the contrasting results obtained.

In this study, populations of *Pythium* spp. varied considerably between and within bean fields throughout the growing season and appeared independent of the fluctuation in root-rot severity. This indicates that either low populations of *Pythium* spp. can cause considerable damage or that soil populations above 10 propagules/g of oven-dry soil are independent of disease severity. This agrees with reports in the literature concerning diseases caused by *Pythium* species on other crops (1, 5, 17, 25). Host susceptibility, environmental conditions (9, 25), pathogen interactions (23), and particularly soil mositure (22) play a more important role in determining disease severity and losses due to *Pythium* species. In addition, soil moisture (30) and organic matter content (24) influence the population levels of *Pythium* spp. This may explain the reason for the higher populations of pathogenic and total *Pythium* spp. found in fine-textured and poorly drained soils in New York.

Several *Pythium* species are known to cause seed decay, damping-off, root rot, and wilt (blight) of beans (7, 10, 12, 13, 31). However, *P. ultimum* is considered to be the most important species for causing damage to beans in cooler climates, whereas *P. aphanidermatum* (Edson) Fitz. and *P. myriotylum* Drechs. are more important under warmer conditions (9, 31). This investigation showed that *P. ultimum* and its sporangial isolates (3, 6) are the most important *Pythium* spp. causing damage to snap beans in New York. *Pythium irregulare,* although it was pathogenic on bean, appears to be of little importance; it was not consistently found in bean soils and/or was present only at low levels. Kendrick and Wilbur (11) showed that inoculum densities of *P. irregulare* greater than 500 propagules/g soil were needed to significantly reduce lima-bean stands. High populations of this species also may be required to cause appreciable damage to snap beans. The unidentified *Pythium* sp. recovered in this study is probably of little importance in reducing emergence or plant vigor of snap beans. Similar isolates also were reported to be very weakly pathogenic on muskmelon (15). Likewise, *P. oligandrum* caused only minor discoloration of hypocotyl tissues and thus is not considered a major pathogen on beans in New York. This species is commonly isolated from roots of a variety of plants, including beans, and is considered a secondary invader after other *Pythium* spp. (8). However, isolates of *Pythium* sp. and *P. oligandrum* may be involved with other soilborne microorganisms in a disease complex that may play a significant role in bean root-rot development.

Only limited information is available concerning the influence of inoculum density of *Pythium* species on disease incidence and severity. Under the conditions employed in this study, *P. ultimum* at one propagule/g of oven-dry soil, reduced stand count of beans by 85% as a result of seed decay and pre- and postemergence damping-off. On older plants, maximum root stunting, pruning, and considerable reduction in plant dry weight occurred at 500 propagules/g soil, and the disease also was severe at 10 and 100 propagules/g oven-dry soil. Pythium blight developed when humid conditions were provided and was independent of inoculum level. In this study, infection of the terminal bud occurred as a result of the upward extension of *P. ultimum* lesions established on bean tissues below the soil line and not from splashing of infested soil onto the above-ground parts as was previously reported (1).

Pythium spp. generally are associated with seedling diseases (9). However, in this study, no correlation was found between plant age and susceptibility to root rot and blight of beans incited by *P. ultimum*. In preliminary studies, wounding the roots of 4- to 28-day-old plants, prior to inoculation, resulted in an increase of root-rot severity as compared to nonwounded plants. This suggests that root damage by cultural practices or soilborne microorganisms such as nematodes could enhance Pythium root-rot incidence and development.

Pythium ultimum is capable of causing seed decay, pre- and postemergence damping-off, root rot, and blight on snap beans. These disease symptoms can be considered as sequential stages of a disease complex. The different

stages of *P. ultimum* damage observed on beans under controlled conditions also occur in bean fields with a history of severe root rot. In these fields, populations of *P. ultimum* are greater than 100 propagules/g oven-dry soil. At this inoculum density, *P. ultimum* is capable of causing severe damage to beans, particularly in cool, wet soils (22).

In addition, the bean cultivars, Black Turtle Soup, and Spartan Arrow, the Plant Introduction accession 203958, and the breeding line 1273 (7), all of which are resistant to seed decay and preemergence damping-off incited by *P. ultimum,* were evaluated for resistance to the root-rot stage of *P. ultimum* and found susceptible (Pieczarka, *unpublished*). This suggests that resistance to the different stages of infection by *P. ultimum* may be inherited independently. Thus, bean germplasm should be evaluated separately for the different stages of attack by *P. ultimum.*

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