Ecology and Epidemiology of Pythium Species in Field Soil

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ABSTRACT

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The ecology of several species of *Pythium* was studied for 2 years in a field plot at the University of Maryland Vegetable Research Farm, Salisbury. Three components of a *Pythium* disease complex of bean were identified and studied: (i) damping-off and blight of seedlings caused by *P. aphanidermatum;* (ii) damping-off and predominantly seedling blight caused by *P. myriotylum;* and (iii) pre-emergence damping-off or seed rot caused by *P. ultimum* and several other low-temperature *Pythium* spp. *Pythium myriotylum* was most destructive. The population of *P. aphanidermatum* was highest at the beginning of the study in December 1971, and lowest in the spring of 1972, following incorporation of a rye cover crop into the soil. The population remained low thereafter. Populations of the low-temperature *Pythium* spp. also dropped sharply in the spring of 1972; however, they increased again in the fall of 1972 and decreased in the spring of 1973. Increases in populations of these species were attributed to colonization of bean refuse in the fall. The failure of *P. aphanidermatum* to increase after incorporation of bean refuse was thought to reflect its greater sensitivity to a lowered soil pH; oospores of *P. aphanidermatum* did not germinate in soil at pH 5.6, whereas those of *P. ultimum* did. Populations of *P. myriotylum* in soil could not be followed, but greenhouse bioassay for bean blight caused by *P. myriotylum* suggested that this species was uniformly distributed and present in sufficient numbers at each sampling date to cause a constant high rate of plant infection. In contrast, *P. aphanidermatum* was localized and persisted best in a more fertile portion of the field where bean growth was best. The field soil ecological relationships of the three groups of *Pythium* species that make up the bean disease complex were significantly different enough to constitute separate and distinct disease problems.

Additional key words: survival, *Pythium irregulare, P. paroecandrum, P. spinosum, P. mamillatum.*

Several species of *Pythium* cause serious diseases of bean (*Phaseolus vulgaris* L.), including damping-off (8, 10), stem rot or blight (14), root rot (11, 14), and aerial blight (10). Certain aspects of the ecology of *Pythium* species in field soil have been investigated (3, 4, 6, 7, 15, 21, 22, 23, 24, 25), but not in relation to the bean disease complex. In addition, only one brief report (6) related actual populations of *Pythium* in field soil to the incidence of disease in the same fields.

We began a study in 1971 on the ecology and epidemiology of *Rhizoctonia* and *Pythium* species in a field cropped continuously to snapbeans under cultivation practices conventional to this area. We sought to determine some of the biological, climatic, and soil factors that may affect populations of the pathogens, the disease potential, and the incidence of disease in the field. Data on *Rhizoctonia* obtained from that study have been published (18). This paper presents the *Pythium* data obtained from the same study.

MATERIALS AND METHODS

Field preparation and sampling.—In general, monthly soil samples were taken for analysis from areas between plants from 28 standard sites in a 0.2-hectare(ha) (33×83 m) rectangular field at the University of Maryland Vegetable Research Farm, Salisbury, Maryland. The sites were arranged in a checkerboard fashion as follows: sites A1 through A6 were located in a line on the east side of the field with sites B1 through B5 parallel to A but staggered, followed by sites C1 through C6, D1 through D5, and E1 through E6. The sites thus were arranged so that all 1-, 2-, and 3-numbered sites were located in the southern half of the field and 4-, 5-, and 6-numbered sites, in the northern half.

The dates of planting the two bean (*Phaseolus vulgaris* L. 'Topcrop') crops and the one rye (*Secale cereale* L.) cover crop each year, and disking of the field have been reported (18) or are indicated in the text. The soil physical and chemical characteristics, climatological data, and details of the preparation and sampling of the field also are indicated in the text or have been reported (18).

Disease severity in naturally infested soil.—Bean disease severity was determined in the field at each site by evaluating plant stand and the percentage of plants blighted in a 3-m row 2-3 weeks after planting. In addition, 10 Topcrop bean seeds were planted in 1,500 g of soil from each of the 28 sites in 2-liter stainless steel

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beakers in the greenhouse as a bioassay for seedling emergence and blight. The beakers were incubated in a constant-temperature water bath at 21 C for 1 week, at which time the percentage seedling emergence was noted. The temperature was raised to 32 C for 1 more week and the resulting blighted seedlings were counted.

Isolation of Pythium species.—Infected plant stems and seeds collected at intervals from greenhouse and field were washed in vigorously running tap water for 20 minutes, treated for 30 seconds in 0.5% NaOCl solution, rinsed, cut into small pieces, and plated out on water agar or selective media (13). After several days at room temperature, the resultant *Pythium* colonies were examined directly on the water agar plates or were transferred to an autoclaved corn kernel on water agar to facilitate identification, which was based on the key of Middleton (16).

Populations of *Pythium* were estimated with the use of differentially selective media, a most probable number technique or a dilution plate technique (13). The selective media enabled us to differentiate between low- and high-optimum-temperature *Pythium* spp.

Pathogenicity of isolates.—Ninety-four isolates of *Pythium,* mostly from soil, were tested for pathogenicity to bean seeds and seedlings. Isolates were grown for 1 week on autoclaved oat kernels (50 g oats and 50 ml water) and 10 g of the resulting inoculum was thoroughly mixed with 1,000 g of steamed (72 C for 2 hours) soil from

TABLE 1. Correlation coefficients obtained by computer analysis among selected variables studied for 2 years in a bean field infested with *Pythium* species

Variable	No. of times assayed	Correlation coefficients[a] Populations *P. aphanidermatum*	Populations *Pythium* spp.[b]	Pythium blight[c] (%)	Seedling emergence (%)
Rainfall	23	−.0299	−.0153	−.0799	.2840
Maximum temperature	23	−.5157*	−.5885**	.1710	.2084
Minimum temperature	23	−.5249*	−.5989**	.1629	.2320
Rhizoctonia colonization	22	.1674	.0778	.1418	−.2343
Rhizoctonia, greenhouse disease	21	.0121	−.1788	.2538	−.0256
Seedling emergence, greenhouse	21	−.5192*	−.4474++	.1491	...
Pythium blight, greenhouse	21	.3926	.0686	...	−.1491
P. aphanidermatum population	19	...	.7502**	.3926	−.5192*
Pythium spp. population[b]	19	.7502**	...	.0686	−.4474++
Ammonium N	16	−.0185	−.0991	.1836	−.1469
Nitrate N	16	.1361	.2811	−.2253	.5205*
Total inorganic N	16	.0239	.1909	−.1475	.3876
Soil conductivity	9	.0876	−.2518	.2356	.2164
Rhizoctonia, field disease	6	...	...	.2176	−.9023
Plant stand, field	6	.1125	−.3518	.2821	−.2373
Plant weight, field	5	.5375	.5240	−.9769*	−.9949**
Organic matter	5	−.4004	−.7006	.1616	.7534
Plant appearance, field	4	.6299	−.8999	.0688	−.3525

[a]Asterisk (*) indicates correlation significant at $P = 0.05$; two asterisks (**) indicate correlation significant at $P = 0.01$; ++ indicates correlation at $P = 0.06$; and − indicates negative correlation.

[b]*Pythium* spp. identified included predominantly *P. ultimum* and an unidentified *Pythium* sp. (no oogonia), as well as *P. irregulare, P. paroecandrum, P. mamillatum,* and *P. spinosum.*

[c]Pythium blight attributed primarily to *P. myriotylum.*

TABLE 2. Frequency of isolation and the identification of *Pythium* species from diseased bean plants and from Salisbury, Maryland, soil

Location	No. tested	Incubation temperature (C)	Percentage of total isolations/location *P. myriotylum* (%)	*P. aphanidermatum* (%)	*Pythium* spp.[a] (%)	Misc. fungi (%)
Field plants[b]	190	...	79	8	2	11
Greenhouse plants[b]	127	32	99	1	0	0
Greenhouse plants[b]	32	21	45	10	40	5
Greenhouse seed[b]	35	21	35	0	17	48
Soil, GAM[c]	222	20	0	0	100	0
Soil, MPVM[c]	266	38	12	88	0	0

[a]*Pythium* spp. included predominantly *P. ultimum* and *Pythium* sp. (no oogonia), as well as *P. paroecandrum, P. irregulare, P. spinosum,* and *P. mamillatum.*

[b]After incubation at indicated temperature or collection from the field, plant parts were treated with 0.5% NaOCl, for 30 seconds, rinsed, plated on water agar, incubated at room temperature, and resulting colonies identified.

[c]Soil dilutions were plated on gallic acid medium (GAM), or pimaricin-vancomycin medium (MPVM), incubated at 20 or 38 C, and colonies transferred for identification.

Salisbury. Bean seeds were planted in these soils and percentage seedling emergence and blighted plants were determined as above for naturally infested soils.

Collection and treatment of data.—Data were collected for 36 climate, soil, plant, and pathogen variables (18). Eighteen of these variables were evaluated repeatedly and analyzed by computer for possible correlations (Table 1). The correlation of each variable of primary concern was determined individually with each of the other variables, with dates of sampling as replications. Duncan's multiple range test was used for analysis with the 28 field sites as replications. In addition, Duncan's multiple range test was applied to data from the individual field sites (with sampling dates as replications) to detect significant differences among variables and field sites.

RESULTS

Isolation and identification of Pythium.—Three major groups of *Pythium* spp. were isolated (Table 2). *Pythium myriotylum* Drechs., was the most prevalent. It was isolated from most of the plants infected in the field and in the greenhouse incubated at 32 C, and from many seeds and seedlings incubated in the greenhouse at 21 C. This species was not detected on gallic acid medium (GAM) and few colonies were observed on pimaricin-vancomycin medium (MPVM) (13). *Pythium aphanidermatum* (Edson) Fitzp. was isolated only occasionally from field plants and from plants grown in field soil in the greenhouse, and then mostly during the first few months of the study. The MPVM selective medium incubated at 38 C, yielded a high percentage of isolates of *P. aphanidermatum,* but GAM at 20 C yielded none. Low-temperature *Pythium* spp. were encountered in seeds and seedlings from the greenhouse tests at 21 C, but not at 32 C; and only occasionally from blighted plants from the field. These species were isolated exclusively and frequently from GAM and were most often identified as *Pythium ultimum* Trow or an unidentified *Pythium* sp. that produced no oogonia in culture. Other species encountered were *P. irregulare* Buis., *P. paroecandrum* Drechs., *P. spinosum* Saw., and *P. mamillatum* Meurs. Individual species could not be recognized macroscopically, therefore, population counts on GAM included all of the low-temperature *Pythium* spp. that grew out on GAM and hereafter are referred to collectively as *Pythium* spp., as distinguished from *P. aphanidermatum* and occasionally *P. myriotylum* that grew out only on MPVM at 38 C.

Pathogenicity of isolates.—All of 21 isolates of *P. aphanidermatum* and 31 isolates of *P. myriotylum* were highly pathogenic to bean seedlings in the greenhouse, and caused pre- or postemergence damping-off. Most isolates of *P. ultimum* (14 of 17) were also pathogenic, and usually caused seed rot or pre-emergence damping-off. Seedlings sometimes were infected as they emerged from the soil, but after that were no longer susceptible to damping-off. Six of 10 of the isolates of *P. paroecandrum, P. irregulare,* and the unidentified *Pythium* sp. were highly pathogenic, whereas none of 10 isolates of *P. spinosum* and *P. mamillatum* was highly pathogenic. Most of the above isolates were obtained from soil; however, some of the *P. myriotylum* isolates came from diseased plants.

Populations, disease severity, and environmental variables.—The average field populations of *P. aphanidermatum* (assayed on MPVM) as well as the *Pythium* spp. (assayed on GAM) were relatively high at the beginning of the study (Table 3, Fig. 1-A). Individual site populations of *P. aphanidermatum* ranged from undetectable to 70 propagules/g soil. The *Pythium* spp. populations ranged from 20 to 1,376 propagules/g dry soil, depending on the location within the field.

After the rye cover crop was plowed under in April 1972, the populations of both *P. aphanidermatum* and *Pythium* spp. dropped markedly to relatively stable low levels during the spring, summer, and fall of 1972 (Table

TABLE 3. Field and greenhouse evaluation of average bean seedling emergence, Pythium blight and *Pythium* populations obtained at various intervals from 28 locations in a Salisbury, Maryland, field

Month and year of sampling	Population (propagules/g soil)[a]		Seedling emergence		Pythium blight[b]	
	P. aphanidermatum (no.)	*Pythium* spp. (no.)	Greenhouse (%)	Field (plants/3m row) (no.)	Greenhouse (%)	Field (%)
December 1971	8.6 A[c]	410.9 A	47.7 D	...	88.8 ABC	...
January 1972	8.2 A	422.7 A	56.1 D	...	96.6 ABC	...
February 1972	9.4 A	372.9 A	52.5 D	...	100.0 A	...
April 1972	0.8 B	49.4 CD	55.7 D	...	96.8 AB	...
June 1972	0.7 B	16.8 D	47.0 D	54.9 A	75.5 EF	1.8 C
August 1972	1.5 B	17.2 D	80.5 AB	50.9 B	73.4 F	25.3 A
October 1972	1.2 B	8.8 D	73.2 BC	...	80.4 DEF	...
November 1972	0.8 B	474.3 A	77.5 AB	...	78.4 DEF	...
February 1973	0.6 B	199.1 BC	66.7 C	...	26.9 G	...
June 1973	1.6 B	19.9 D	66.1 C	43.7 C	90.0 ABCD	13.2 B
August 1973	0.6 B	8.2 D	82.1 AB	26.3 D	79.5 DEF	90+[d]
October 1973	1.3 B	12.4 D	67.4 C	...	86.6 BCDE	...
January 1974	0.3 B	2.1 D	83.9 A	...	90.5 ABCD	...

[a]Estimated by soil dilution assay on selective media. The *Pythium* spp. included *P. ultimum, Pythium* sp. (no oogonia), *P. irregulare, P. paroecandrum, P. spinosum,* and *P. mamillatum.*

[b]Attributed primarily to infection by *P. myriotylum.*

[c]Numbers followed by the same letter do not differ significantly ($P = 0.05$) by Duncan's multiple range test.

[d]Data not analyzed statistically. Entire field destroyed by *P. myriotylum* after plant emergence.

3, Fig. 1-A). In October 1972, at the time the bean residue was incorporated and rye was planted, the population of *Pythium* spp. increased markedly, whereas that of *P. aphanidermatum* did not. The subsequent decrease in populations of *Pythium* spp. with incorporation of rye in the spring was similar to that observed in 1972. These population fluctuations were statistically significant (Table 4). For the remainder of the study, however, the populations of *Pythium* spp., as well as those of *P. aphanidermatum,* did not fluctuate appreciably.

The incidence of pre-emergence damping-off, based on the percentage of bean seedling emergence at 21 C in sample soils in the greenhouse, was relatively stable (Fig. 1-B). Emergence or plant stand in the field (expressed as plants/3 m of row) was determined four times. These data reflect relatively high levels of emergence in the first two plantings (June and August) and a less-dense stand in the two plantings in the 2nd year.

Pythium blight at 32 C in the greenhouse also was relatively consistent over the study period (73% in August 1972 to 100% in February 1972). An exceptionally low value of 27% in February 1973 could be attributed to experimental error. The Pythium blight in the field was consistently much lower in June (when temperatures were relatively mild) than later in the season. On the other hand, disease incidence was much greater in August than in June, especially in 1973. Climatic conditions were ideal (hot, damp weather) during that time for maximum Pythium blight and consequently the entire field of beans was destroyed.

Greenhouse experiments confirmed that incorporation of bean tissue resulted in a marked increase in *Pythium* spp. populations. Populations increased several hundred-fold when chopped bean plants were added to the Salisbury soil, but not when rye plants were added to the same soils several weeks later. Populations of *P. aphanidermatum* did not change during these treatments.

An explanation was sought for the failure of populations of *P. aphanidermatum* to increase as did those of *Pythium* spp. Because germination of *P. aphanidermatum* oospores is inhibited under acid conditions (1, 13), the soil pH was suspected of being a

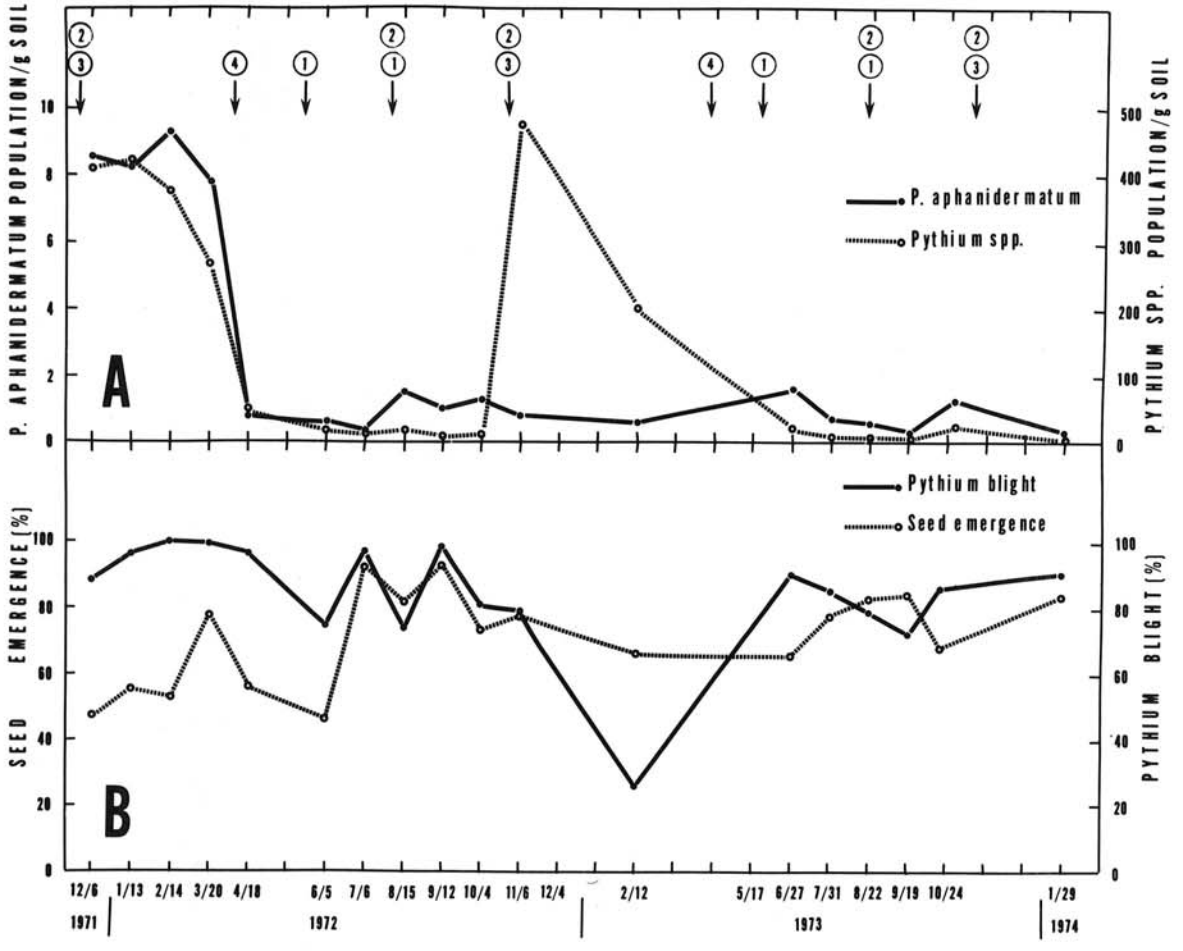

Fig. 1-(A, B). A) *Pythium aphanidermatum* and other *Pythium* spp. populations in soil collected at intervals over 2 years from 28 field locations. The two populations were significantly correlated ($P = 0.01$). **B)** Percentage Pythium blight and bean seedling emergence in field soil brought to the greenhouse. Bean seedling emergence was correlated with *P. aphanidermatum* and *Pythium* spp. populations in A above. Encircled numbers indicate the following: 1, beans planted; 2, bean plant refuse disked into soil; 3, rye planted; and 4, rye cover crop disked into soil.

contributing factor to this behavior. Air-dry samples of soil were stored during the study. The pH of samples of these stored soils from 19 sample dates and two sites from each end of the field was measured in a soil:water (1:1, v/v) paste. The average pH of the soils was 6.1 at the beginning of the study (18), and thereafter, the pH decreased over the 2-year period to an average low of 5.1 in October 1973. Ground dolomitic limestone had been applied in 1972, but the amount was not sufficient to raise the pH of the soils above 5.7. There were no differences in pH between the northern and southern ends of the field.

The effect of pH on germination of *P. aphanidermatum* and *P. ultimum* in pH-adjusted soil was studied as previously described for *P. aphanidermatum* (1). In autoclaved soils adjusted to pH 4.8, 5.6, and 6.2, *P. ultimum* oospores germinated very well at pH 5.6, but poorly at 4.8 over a 10-hour incubation period at 30 C. *P. aphanidermatum* with a similar incubation period germinated well at pH 6.2, but no germination occurred at pH 5.6.

Correlation between parameters.—Maximum and minimum field air temperatures were negatively correlated with populations of *P. aphanidermatum* and *Pythium* spp. (Table 2); i.e., when temperatures were high, populations were low. Greenhouse bean seedling emergence was also negatively correlated ($P = 0.05$) with *P. aphanidermatum* populations. Populations of *Pythium* spp. and *P. aphanidermatum* were highly positively correlated with each other. Field plant weight was negatively correlated with *Pythium* blight and seedling emergence in the greenhouse. Nitrate-N was correlated positively ($P = 0.05$) with greenhouse seedling emergence.

Distribution of Pythium species and disease within the Salisbury field.—We selected the Salisbury field for study because differences were evident in disease incidence and appearance of plants within the field. There were two areas in the field that generally differed in overall appearance of plants. These differences were statistically verified when the 28 sites within the field were rated on a scale from 1 to 4 based on overall vigor of plants (Table 4), in which 1 = poor plants and 4 = healthy, vigorous plants. Sites in the northern half of the field, including eight contiguous sites numbered either 4, 5, or 6 were significantly different ($P = 0.05$) from nine sites numbered 1, 2, or 3 in the southern half. In addition, the northern half of the field had higher values than the southern half for: *Pythium aphanidermatum* population; greenhouse seedling emergence; *Rhizoctonia* sp. colonization of beet seed; NO_3-N, NH_4-N, and total N; field yield and plant weight; organic matter and soil color; and soil conductivity. Pythium blight and *Pythium* spp. populations did not differ significantly between the northern and southern portions, whether observed in situ in the field or tested in the greenhouse.

Populations of plant parasitic nematodes, determined with the Baermann funnel technique with soils from selected sites in the northern and southern ends of the field, ranged from 0 to 3,634 nematodes/100 g soil, depending on the time of year the soil was sampled. From four different sites, two at each end of the field, the following average populations were obtained from four sampling times: *Pratylenchus penetrans* (Cobb) Filip. & Schuurm.-Stekh. averaged 427 nematodes/100 g of soil in the northern end of the field and 776/100 g soil in the southern end. *Trichodorus christiei* Allen averaged 105

TABLE 4. Sites in a Salisbury, Maryland, bean field with significantly different values obtained for 20 parameters

Parameter	Unit of measure[a]	Sites significantly different[b]	
		High values[c]	Low values[c]
Field appearance	good = 4, poor = 1	D4,D5,A5,C5,B4,E4,E5,E6	A2,A3,B2,B3,C2,D1,D2,E1,E2
Greenhouse seedlings emerged	% emerged	C4,C5,E5,B5	C3,B2
Greenhouse Pythium blight	% blight	D3	E5
Rhizoctonia colonization	% beet seed colonized	E6,E4,E5,B4	E1,E2
Pythium aphanidermatum	Propagules/g soil	E5,E4,D4	B2,C1,C2,E2,D2,B3,B1,D1,C3,A3,D3
Pythium spp.	Propagules/g soil	B4	E1,D2,A5,A6
NO_3 nitrogen	μg/g dry soil	D5,E6,E5	A2,D2,A5,A3
NH_4 nitrogen	μg/g dry soil	E5,D5,E6	C2,D1,C4,E1
Total inorganic nitrogen	μg/g dry soil	E5,D5,E6,D4	E1,A1,B3,C2,A5
Field *Rhizoctonia*	Disease, 1 = low, 5 = high	D4,C4,B3	E1,E2,C6
Plant stand	No./3 m row	C5	D3,D2
Field plant yield	g of pods/plant	D4,E4,E5	B3,E2,E3,D2,C3,C2
Field plant weight	g/plant	D4,E4,E5	A2,C2,E2,B2,D2,B3,D1,E3,C3
Field Pythium blight	% infected plants	C4	C1
Soil texture	Sandy loam vs. loamy sand	E5,E6,D4,D5,C5	E1,E2,C1,D2,B3,A2,B1,C2
Organic matter	mg/g dry soil	D4,D5,E5	D1,D2,E2
Soil color	lightest = 0, darkest = 5	E5,D4,B4,C5,E6,D5,E4	E1,C1,E2,C3,D2,C2
Sulfur	μg/g dry soil	D1	E2,C1,B4,E3,C2,E5,E6
Phosphorus	μg/g dry soil	E5,B4	E1
Conductivity	μmho's	E6,D5,D4	D2,C2

[a]Measured by standard means.

[b]Twenty-eight standard sites located as follows: sites A1 through A6 located in a line on the east side of the field with sites B1-B5 parallel to A but staggered, followed by sites C1-C6, sites D1-D5, and sites E1-E6. The sites were thus arranged so that all 4-, 5-, and 6-numbered sites were located in the north half and 1-, 2-, and 3-numbered sites in the south half of the field.

[c]Data were collected over the study period with dates as replicates. Significant difference between high- and low-valued sites was determined by Duncan's multiple range test.

nematodes/100 g soil in the northern end and 116/100 g in the southern end. These differences were not significant.

DISCUSSION

The Salisbury field represents a situation that is probably quite common in agricultural fields in which several *Pythium* species combined may interplay to cause one or more plant diseases. The *Pythium* populations in the Salisbury field conveniently may be divided into three groups: (i) *P. myriotylum* which can cause pre- and postemergence damping-off (blight); (ii) *P. aphanidermatum,* which can cause similar diseases, but which differs significantly in its oospore germinability, cultural properties, and ecological behavior (2, 13) from *P. myriotylum;* [these two species therefore probably differ taxonomically although they are morphologically similar (9)]; (iii) *P. ultimum, P. paroecandrum, P. mamillatum, P. spinosum, P. irregulare,* and the unidentified *Pythium* sp., all of which can cause pre-emergence damping-off. Species of the latter group produce spherical sporangia (or conidia) and sometimes are very difficult to differentiate morphologically and to identify with certainty.

Pythium myriotylum was the most important of the *Pythium* species encountered in the Salisbury field, both as a pre- and postemergence damping-off pathogen (Table 2). It was important enough to destroy completely the fall bean crop in 1973 (Table 3). However, because oospores of this fungus do not germinate readily (2, 13), we were unable to assess populations in the field soil and, therefore, to relate populations to disease incidence. Our only means of monitoring activity was by estimating bean blight caused by *P. myriotylum* in the greenhouse. These data suggested that populations of *P. myriotylum* were distributed widely over the entire field (Table 4), and apparently did not fluctuate in any recognizably significant manner during the study (Table 3). Of course, with the use of the greenhouse blight test, a striking pattern of increase or decrease in disease may not have been detectable. A similar test described by Mitchell (17) indicated that soil artificially infested with 25 oospores of *P. myriotylum*/g of soil caused 50% infection of rye plants and 150 oospores/g caused 100% infection. A large change in propagule numbers was required to greatly affect disease incidence.

These observations perhaps reflect the recalcitrant germination of oospores of *P. myriotylum*. Oospores that do not germinate readily even over long periods of time (2) would be expected to survive well with consequent little change in their total numbers. However, the true nature of the dormancy and importance of these survival structures will need to be understood in order to evaluate their role in survival.

In contrast to the seemingly stable population of *P. myriotylum,* the population of *P. aphanidermatum* changed significantly during the course of our study; i.e., from relatively high numbers to nearly undetectable levels in some sample sites. Early in the study, *P. aphanidermatum* was probably a potentially significant cause of disease in the field. This is suggested by the negative correlation between *P. aphanidermatum* populations and greenhouse bean seedling emergence (Table 1); i.e., when *P. aphanidermatum* populations were high, seedling emergence was low. Overall, however, the importance of *P. aphanidermatum* in the field was minimal. We wanted to determine why this was so. The high populations early in the study probably resulted from appreciable disease levels the season before or the extensive colonization of fresh bean tissue the previous fall. *Pythium aphanidermatum* is capable of rapidly colonizing fresh tissue (19, 23), and with the formation of sporangia and oospores in the colonized tissue, would have significantly increased the total numbers of propagules. The reason for the decrease in *P. aphanidermatum* populations in the spring of 1972 is not clear, but could be attributed to a germination-lysis phenomenon. Oospore populations of *P. aphanidermatum* decreased significantly after cyclic wetting and drying or after amendment with asparagine (20). Perhaps rye tissue incorporated into the soil in the spring of 1972 supplied nutrients for rapid germination of the oospores of *P. aphanidermatum* (2, 4). The resultant germ tubes were perhaps unable to colonize the rye tissue or to form resistant survival structures. Temperature was correlated negatively with the population of *P. aphanidermatum,* but a moderate increase in temperature in the spring should not adversely affect this high temperature *Pythium*. Elevated temperature has been suggested as the factor responsible for decreases in population of certain *Pythium* species during summer months (6, 15).

Pythium aphanidermatum was localized in the northern end of the field, especially at site E5, and was associated with high levels of NO_3-N, NH_4-N, and total N, more loamy soil, higher soil organic matter, darker soil color, and greater soil conductivity, than in the southern end of the field. These higher values are associated with greater fertility and therefore increased vigor and yield of the bean plants. The higher fertility and more desirable soil tilth and structure in these areas also may encourage greater microbial activity than in the less fertile sites. Increased microbial activity may have enhanced the fungistatic properties of the soil, thus inhibiting germination of oospores and favoring prolonged survival. The decreased pH of soil also may have suppressed germination and indirectly enhanced survival. Low pH prevented germination of oospores of *P. aphanidermatum* in soil in this study, similar to the suppression observed elsewhere (1, 13). The soil pH, however, would not account for differences in propagule survivability between the northern and southern portions of the field, since the pH did not differ significantly between these areas.

Populations of *P. ultimum* and other low temperature *Pythium* spp., associated with pre-emergence damping-off in the Salisbury field, also fluctuated significantly during the year, and correlated with populations of *P. aphanidermatum* (Table 1). This indicates that the two groups have similar behavior. Fresh tissue added to soil increases populations of *P. ultimum* (6) and *P. mamillatum* (3), and thus accounts for the increased populations of *Pythium* spp. in the fall when the bean tissue was incorporated into the soil. However, bean blight caused by *P. myriotylum* was so severe during August 1973, that virtually no bean plants remained in the field at the end of the season; thus, there was no tissue

present to be saprophytically colonized. The populations of *Pythium* spp. did not increase, therefore, as they did the previous fall.

The decrease in population of *Pythium* spp. in the spring, as with *P. aphanidermatum,* is puzzling. The decrease also may have been caused by a germination-lysis mechanism in which sporangia and oospores are stimulated to germinate (12, 21) but are unable to produce survival structures on the incorporated rye tissue.

Disease caused by *Pythium* in the Salisbury field, especially *P. myriotylum,* does not account totally for the poor vigor of plants in one area of the field in contrast to the greater vigor of plants in the other area (Table 4). The differences in appearance cannot be explained by this study. However, interactions among plant nutrition, soil texture, and a possible role of plant parasitic nematodes, previously implicated in disease caused by *P. myriotylum* (5), as well as other factors, may explain these differences.

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