Inoculum Densities of *Pythium aphanidermatum* in Soils of Irrigated Sugar Beet Fields in Arizona

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ABSTRACT

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Inoculum densities of *Pythium aphanidermatum* in commercial sugar beet fields were estimated by using a species-specific isolation medium. Fields were not uniformly infested with *P. aphanidermatum*; intrafield inoculum densities exhibited a moderately clustered pattern of distribution (for the negative binomial probability distribution, $k = 1.15$) and interfield inoculum densities exhibited a highly clustered pattern of distribution ($k = 0.28$). No fluctuations in inoculum densities were detected in soil samples collected periodically from infested fields prior to the onset of root infection, which occurred about 9 mo after planting. Subsequent to root infection, high population densities of the fungus (from 232 to 5,120 propagules per gram of soil) were detected in rhizosphere soil immediately adjacent to lesions on infected sugar beet tap roots.

In Arizona, sugar beets (*Beta vulgaris* L.), an irrigated crop, are planted in late August through October. Harvest begins in mid-April and is completed by early August. A destructive root rot caused by *Pythium aphanidermatum* Edson (Fitzp.) can be a limiting factor in some commercial sugar beet fields. Root rot, however, occurs about 9 mo after planting (late June, July, and August) and its occurrence coincides with the onset of high soil temperatures (13).

Oospores of *P. aphanidermatum* were identified as the survival structures and source of inocula of the fungus in field soil (3,4,19). Little information is available, however, on the distribution of *P. aphanidermatum* in field soil. Spatial patterns of distribution of soilborne fungal plant pathogens in field soil have been reported as uneven (21), even (16), and clustered (1,2,5,6,8,12). With regard to *P. aphanidermatum*, Burr (3) and Stanghellini and Phillips (20) indicated that the highest inoculum densities occurred in the upper 15 cm of the soil profile and that within that zone the fungus showed a clustered pattern of vertical distribution. Quantitative studies on the horizontal distribution of *P. aphanidermatum* in field soil were not conducted; however, studies on the horizontal distribution of *P. aphanidermatum* in commercial greenhouse soils indicated a clustered pattern of distribution (20). Knowledge of the distribution of *P. aphanidermatum* would be of value in epidemiological studies.

Limited information is also available on the population density stability of *P. aphanidermatum* in field soil. Hall et al (9) recently reported the occurrence of seasonal population density fluctuations of *P. aphanidermatum* in soil and thatch collected from golf course turfs. Highest populations were detected during the cool winter months in the absence of disease activity. Seasonal fluctuations in soil population densities of *P. ultimum* were also reported (10,11,23). Knowledge of seasonal fluctuations in inoculum densities are necessary to establish the time of soil sampling. Such fluctuations, if not taken into consideration, may obscure establishment of a relationship between inoculum density and disease prevalence.

Our objectives were to determine the interfield and intrafield distribution and the occurrence of any population density fluctuations of *P. aphanidermatum* in commercial sugar beet fields during the growing season.

MATERIALS AND METHODS

Estimating inoculum densities of *P. aphanidermatum* in naturally infested soils. A species specific medium was used to estimate inoculum densities in commercial sugar beet fields (4). Unless otherwise specified, all soil samples were air-dried for 24 hr after collection, sieved, and thoroughly mixed before assay. Subsamples of these soils were diluted 1:10 (10 g of soil in 90 ml of 0.1% water agar) and mixed in a Vortex stirrer for 3 min. Ten 1-ml

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aliquots from each subsample were dispensed evenly across the surface of 10 petri plates containing the solidified selective medium and incubated at 36 C for 48 hr. The soil was then washed from the agar surface and colonies were counted. Colony origin was determined microscopically and, unless otherwise specified, all colonies originated from oospores. Estimates of oospore population densities were not affected by air-drying the soil samples.

Soil sampling methods. Since root rot occurs about 9 mo after planting, inoculum density estimates were made from soil samples collected primarily during the latter half of the sugar beet growing season. To estimate the interfield variations in the inoculum density of *P. aphanidermatum*, a composite soil sample was collected from each of 13 and 39 commercial sugar beet fields (~40 ha each) in April 1980 and January 1981, respectively. Soil samples were taken as follows: a soil core, 2.5 cm in diameter and 15 cm long, was collected every 20–25 rows in a diagonal path across each field and pooled into one composite soil sample (consisting of 20–25 cores) per field. Additionally, several fields were selected for estimates of the intrafield variations in the inoculum density of the fungus. Six to seven composite soil samples (each consisting of 20 cores) were collected in a diagonal path across each field. Individual soil cores were also collected and assayed without compositing. All soil cores were collected halfway between adjacent sugar beets from the tops of beds.

The inoculum density of *P. aphanidermatum* in the rhizosphere of healthy and diseased sugar beet tap roots also was determined. Healthy sugar beets were carefully extracted from soil during the growing season. We then used disposable plastic spoons to scrape approximately 10 g of soil from the surface (0–3 mm horizontal profile), which had been in immediate contact with the tap root (Fig. 1). Rhizosphere soil samples from infected sugar beets were collected similarly, but the horizontal soil profiles were sampled at 1-cm intervals from the soil surface adjacent to a root lesion (Fig. 1). All rhizosphere soil samples were individually assayed on the selective medium without air-drying, and unless otherwise specified, all colonies originated from oospores.

Inoculum density data from interfield and intrafield soil samples were compared to the negative binomial and Poisson probability distributions (17). Adequacy of fit was determined by the chi-square test.

Seasonal fluctuations in the inoculum density of *P. aphanidermatum*. Rhizosphere soil samples from naturally infested sugar beet fields were periodically collected, as previously described, during the growing season and assayed on the selective medium to detect fluctuations in the inoculum density of *P. aphanidermatum*. Additionally, seasonal fluctuations in the inoculum density of a known number of oospores of *P. aphanidermatum* were also studied. Twenty-two 2-gram samples of a naturally infested soil collected in August 1979 from the rhizospheres of infected sugar beets in a commercial field were placed in nylon mesh bags (20 μm pore diameter). The soil contained 2,840 ± 180 oospores per gram of air-dry soil. The bags were then buried 10 cm deep between adjacent sugar beets growing in an irrigated sugar beet field. The sugar beets were planted in September and the bags were buried in November. Buried soil samples were recovered monthly (1–2 bags per month) over a 10-mo period and assayed, along with a control soil sample, on the selective medium. Controls consisted of 1-g samples of the naturally infested soil, which was stored air-dry at 24 C.

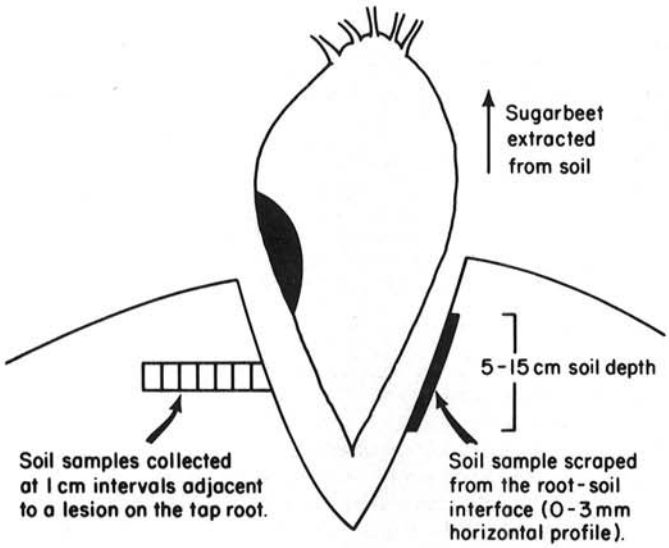

Fig. 1. Methods used to collect soil samples from rhizospheres of healthy and infected sugar beet roots.

TABLE 1. Inoculum densities of *Pythium aphanidermatum* in commercial sugar beet field soil[a]

1980		1981	
Number of fields	Oospores/g air-dry soil	Number of fields	Oospores/g air-dry soil
3	0	26	0
5	1	5	1
1	2	2	2
1	3	2	3
1	9	1	4
1	17	1	7
1	25	1	9
		1	10

[a] One composite soil sample, consisting of 20–25 soil cores, was collected from each field. Inoculum densities were estimated in soil samples by using a species-specific isolation medium (4).

RESULTS

Inoculum densities of *Pythium aphanidermatum* in infested soils. Inoculum densities in single composite soil samples collected from 13 and 39 commercial sugar beet fields in 1980 and 1981, respectively, are presented in Table 1. No oospores were recovered from three fields in 1980 and 26 fields in 1981. Inoculum densities in the remaining fields ranged from 1 to 25 oospores per gram air-dry soil. Data from the 39 fields sampled in 1981 were compared to the negative binomial and Poisson probability distributions. The data adequately fit the negative binomial distribution as determined by chi-square (Table 2). The k parameter, which gives an indication of the degree of clumping, was 0.280319.

Data on inoculum density variations of *P. aphanidermatum* within multiple composite and individual soil samples collected from selected fields in 1980 and 1981 are presented in Table 3. Inoculum densities in individual soil samples (cores and

TABLE 2. Observed frequencies of *Pythium aphanidermatum* oospore population densities in soil samples from 39 sugar beet fields, and frequencies expected from the negative binomial probability distribution

Oospores per gram air-dry soil[a]	Number of fields	
	Observed frequency	Expected frequency
0	26	24.67
1	5	5.56
2	2	2.86
3	2	1.75
4	1	1.15
5	1	0.79
6	0	0.56
7	1	0.40
8	0	0.29
9	1	0.22
10	1	0.16

$k = 0.280319$
Calculated $\chi^2 = 10.16$ N.S. ($P<0.05$)

[a] Oospore population densities were estimated by use of a species-specific isolation medium (4).

TABLE 3. Inoculum densities of *Pythium aphanidermatum* assayed in soil samples from naturally infested sugar beet fields by using different sampling methods

Field and soil sampling method	Number of soil samples or cores	Oospores per gram of soil[a] (mean ± SE)	Range	Soil sampling date
Field 1				
One composite[b]	25	17	···	10 Apr 1980
Seven composites	140	14.1 ± 18.3	0–51	6 May 1980
Individual cores[c]	20	13.5 ± 8.1	2–29	20 May 1980
Individual rhizospheres[d]	40	22.1 ± 18.1	2–93	3 Jun 1980
Individual rhizospheres	3	16.3 ± 5.7	10–21	19 Jun 1980
Individual rhizospheres	5	11.6 ± 7.3	1–19	9 July 1980
Individual rhizospheres	6	16.1 ± 10.6	7–30	8 Aug 1980
Field 2				
One composite	25	1	···	10 Apr 1980
Six composites	120	1.5 ± 2.7	0–7	24 Apr 1980
Individual cores	8	0.8 ± 1.1	0–3	20 May 1980
Individual rhizospheres	24	1.6 ± 2.6	0–10	24 Jun 1980
Field 3				
One composite	25	6	···	5 Jan 1981
Seven composites	140	3.1 ± 3.2	0–8	24 Mar 81
Seven composites	140	3.0 ± 3.5	0–9	7 Apr 1981
Individual rhizospheres	3	3.6 ± 2.3	1–5	7 Apr 1981
Individual rhizospheres	10	1.8 ± 2.7	0–8	5 May 1981
Individual rhizospheres	8	4.8 ± 3.6	0–9	9 Jun 1981
Individual cores	10	8.6 ± 10.6	0–35	16 Jun 1981
Field 4				
Seven composites	140	8.8 ± 7.4	0–20	4 Nov 1980
One composite	25	10	···	5 Jan 1981
Six composites	120	2.0 ± 1.5	0–4	4 Nov 1980
One composite	25	3.5	···	5 Jan 1981
One composite	25	2.0	···	16 Jun 1981

[a] Oospore population densities were estimated by use of a species-specific isolation medium (4).
[b] Composite soil samples: soil cores were collected in a diagonal path across the fields.
[c] Individual cores were randomly collected and individually bagged and assayed.
[d] Individual rhizospheres consisted of a 10-g soil sample collected from the soil surface, which was in immediate contact with the sugar beet tap root (Fig. 1).

rhizospheres) exhibited a range in variation similar to multiple composite soil samples from the same field, and the mean inoculum density of multiple composite soil samples approximated those of the single composite soil sample collected from the same field.

Data from the 80 soil samples collected from Field 1 in 1980 (Table 3) were compared to the negative binomial probability distribution. The data adequately fit the negative binomial distribution as determined by chi-square (Table 4). The k parameter was 1.51164. Fifty individual soil cores were also collected from Field 1 in 1981. The mean inoculum density was calculated and the expected distribution based on that mean and a k value of 1.51164 was determined. The expected distribution was not significantly different from the observed.

Seasonal fluctuations in the inoculum density of *P. aphanidermatum*. Soil samples buried between sugar beets in an irrigated field plot and recovered monthly over a 10-mo period, with one exception, exhibited no significant change in the inoculum density (Table 5). A significant reduction occurred once in April but did not decrease further in later months. The field in which the soil samples were buried was mistakenly fertilized with double (117 kg/ha) the recommended rate of urea in April. Similarly, we were not able to detect any clear evidence of fluctuations in the inoculum density in soil samples collected from naturally infested fields during the sugar beet growing season before the onset of the disease (Table 3). Inoculum densities in individual soil samples (cores and rhizospheres) exhibited a similar range in variation, which appeared to be independent of sampling date. At harvest in August 1980 (Field 1), several sugar beets were found with lesions. The population density in rhizosphere soil samples collected from nine infected sugar beets ranged from 232 to 5,120 propagules per gram of soil. Highest population densities occurred in soil immediately adjacent to a root lesion and these decreased rapidly with increasing distance from the root surface (Table 6). These propagules consisted of oogonia, oospores, and mycelial fragments as determined by direct microscopic examination of colony origin on the selective medium. Population densities in rhizosphere soil samples from infected sugar beets were diluted 1:10, 1:50, and 1:100 prior to assay on the selective medium.

TABLE 4. Observed frequencies of *Pythium aphanidermatum* oospore population densities in sugar beet field soil samples and frequencies expected from the negative binomial distribution

Oospores per gram of soil[a]	Number of soil samples: Observed frequency	Expected frequency
0	1	1.7
1–5	15	14.4
6–10	16	15.2
11–15	11	12.7
16–20	11	9.9
21–25	6	7.4
26–30	8	5.4
31–35	7	3.9
36–40	2	2.8
>40	3	4.6
Calculated chi-square	$\chi^2 = 6.70$ N.S. df. 9 ($P<0.05$)	
$k = 1.51164$	$N = 80$	

The expected frequencies were calculated by using the following algorithm:

$$f_{(0)} = Np^k \qquad f_{(n)} = f_{(n-1)}\,(k+n-1)\,q/n$$

The parameters were estimated as follows:

$$p = \hat{x}/s^2 \qquad k = \hat{x}p/(1-p)$$

[a] Soil samples were collected from a commercial sugar beet field, and oospore population densities were estimated by use of a species-specific isolation medium (4).

DISCUSSION

Our data indicates that there were no detectable changes in the inoculum density of *P. aphanidermatum* in soil during the sugar beet growing season before the onset of disease. This is in contrast

to the reported occurrence of seasonal fluctuations in the inoculum density of *P. ultimum* and *P. aphanidermatum* (9,10,11,23). Seasonal increases in the inoculum density of *P. ultimum* were attributed, in part, to the saprophytic colonization of organic matter incorporated into soil during the growing season (10,11,23). Cultivation practices in sugar beet fields in Arizona are carried out during the early portion of the growing season when soil temperatures are relatively cool (15–20 C). Such temperatures are apparently limiting to the saprophytic capabilities of *P. aphanidermatum*.

Increases in inoculum density during the growing season were also related to seasonal changes in the type of oospore dormancy. Oospores of *Pythium* spp. are reportedly both endogenously and exogenously dormant (11,15,18). Conversion from the endogenous to the exogenous state reportedly accounts for a portion of the seasonal increase in the inoculum density of *P. ultimum* (11) and probably accounts for the increase in the inoculum density of *P. aphanidermatum* in golf course turf in the cool winter months in Ohio (9). Our results suggest that oospores of *P. aphanidermatum* in naturally infested sugar beet fields are exogenously dormant. Prior to the onset of disease, no increase in the inoculum density was detected either in soil samples collected periodically during the growing season from naturally infested fields or in soil samples that were buried between sugar beets in an irrigated field. The latter soil samples contained a known number of oospores and were recovered monthly throughout the 10-mo sugar beet growing season. If a portion of the oospores in these soils were endogenously dormant, it is unlikely that conversion to the exogenous state and mortality of the existing exogenously dormant populations would have occurred at equal rates capable of maintaining the stable population that we recorded.

The only increase in the inoculum density of *P. aphanidermatum* occurred following the onset of root infection about 9 mo after planting. High population densities were detected in rhizosphere soil immediately adjacent to lesions on infected sugar beet tap roots. This localized population increase in the *P. aphanidermatum*, similar to that reported for *Phytophthora* (7,14), may account for the pattern of distribution in infested soils. Our results indicate that *P. aphanidermatum* is not uniformly distributed, but exhibits a moderately clustered pattern of distribution in field soil. The degree of clustering in fields, however, may vary depending on dispersal resulting from tillage practices. Clustered patterns of distribution have recently been documented for *Cylindrocladium crotalariae* (8,12), *Rhizoctonia solani* (5), *Sclerotium rolfsii* (2), and *Sclerotium cepivorum* (1). In addition to a moderately clustered intrafield distribution, interfield inoculum densities exhibited a highly clustered distribution (k = 0.280319). Although the relationships between inoculum density and disease prevalence has not been established at this time, the interfield variation in inoculum densities may account for the occurrence of root rot only in certain fields. In 1969, Hine and Ruppel (13) reported that sugar beet root rot was severe in some fields cropped to sugar beets for the first time following 30 continuous years of cotton production, but they could not find any evidence that the fungus was a pathogen on cotton. Recent studies in California (O. C. Huisman, *personal communication*), however, indicate that *P. aphanidermatum* is frequently isolated from necrotic feeder roots of cotton. In addition to cotton, alfalfa, and watermelon, various grain crops are commonly used in rotation with sugar beets in Arizona. Although no disease losses in these crops have been directly attributed to *P. aphanidermatum* in Arizona, the fungus has been isolated from necrotic feeder roots of alfalfa, watermelon, corn, and oats (M. E. Stanghellini, *unpublished*). Thus, multiplication of *P. aphanidermatum* in rotation crops, in addition to disease prevalence in fields consecutively cropped to sugar beets, may account for the interfield variations in inoculum densities.

Although there was no increase in the inoculum density of *P. aphanidermatum* in soil during the growing season prior to the onset of disease, a decrease was recorded on a single occasion after urea was applied at twice the recommended rate. Enzymatic hydrolysis of urea results in the production of NH_4^+/NH_3, which is reported to be toxic to *Phytophthora* (22). Thus, toxicity may account for the sudden decrease in the inoculum density in buried soil samples that contained a known number of oospores.

Data generated in this study on the inoculum distribution and stability of *P. aphanidermatum* in soil may provide useful information for the development of a practical method for predicting the occurrence of root rot. Such a system, similar to that reported by Adams (1), would enable sugar beet growers to avoid planting in infested fields or to harvest prior to the onset of disease in infested fields.

TABLE 5. Survival of *Pythium aphanidermatum* oospores in air-dry soil samples and samples buried in an irrigated sugar beet field[a]

Soil samples assayed monthly	Number of soil samples assayed	Oospores per gram of soil[b] (mean ± SE)
Air-dried		
November through August	34	2,610 ± 150 a
Buried		
November through March	7	2,829 ± 142 a
April through August	15	1,195 ± 128 b

[a] A naturally infested field soil containing 2,840 ± 130 oospores per gram of soil was used. Air-dried soil samples were stored at 24 C; samples in the field were buried 10 cm deep in tops of beds.

[b] Oospore population densities were estimated by use of a species-specific medium (4). Values followed by a common letter were not significantly different ($P < 0.01$) according to Duncan's multiple range test.

TABLE 6. Horizontal distribution of *Pythium aphanidermatum* propagules in moist soil adjacent to infected sugar beet tap roots

Infected root	Propagules per gram of soil at a horizontal distance (cm) in soil from root lesions						
	0–1	1–2	2–3	3–4	4–5	5–6	6–7
1	2,500[a]	540	12	0	10	3	4
2	890	75	33	20	5	4	...[b]
3	716	20	6	1	0	0	0
4	422	2	0	4	0	...	...

[a] Propagules of *P. aphanidermatum* per g of soil were estimated by use of a species-specific medium (4).

[b] Not tested.

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