Distribution of *Pythium aphanidermatum* in Rhizosphere Soil and Factors Affecting Expression of the Absolute Inoculum Potential

M. E. Stanghellini, L. J. Stowell, W. C. Kronland, and P. von Bretzel

Department of Plant Pathology, University of Arizona, Tucson 85721.
Arizona Agricultural Experiment Station Journal Series Paper 3699.
Accepted for publication 25 May 1983.

ABSTRACT

Stanghellini, M. E., Stowell, L. J., Kronland, W. C., and von Bretzel, P. 1983. Distribution of *Pythium aphanidermatum* in rhizosphere soil and factors affecting expression of the absolute inoculum potential. Phytopathology 73:1463-1466.

The surface area of a mature sugar beet tap root commonly exceeds 500 cm^2. With a direct isolation method, an average of 32% of the 1 cm^2 soil samples from the root-soil interface (RSI) of 10 rhizosphere sections of soil from sugar beet tap roots contained *P. aphanidermatum*. A bioassay with potato tuber tissue also showed that an average of 36% of the surface area of the RSI from 19 rhizosphere sections of soil contained an infective population of *P. aphanidermatum*. Inoculum densities within infested areas ranged from one to five oospores per 0.1 cm^3 of RSI soil. The distribution of inoculum densities within the RSI of individual rhizosphere sections ranged from clustered, to random, to uniform. Inoculum densities effective for infection, as bioassayed with potato tuber tissue, were located primarily within 1 mm of the host surface. Maximum infection occurred at soil temperatures of 27 C or greater under wet soil conditions (0 to −0.1 bar) and time periods of 24–48 hr.

In Arizona, *Pythium aphanidermatum* (Edson) Fitzp. is responsible for a destructive root rot of mature sugar beets (*Beta vulgaris* L.). Although sugar beets, an irrigated crop, are susceptible throughout the growing season, root infection occurs about 9 mo after planting and its occurrence coincides with the onset of high soil temperatures (27 C or greater at the 10-cm soil depth, M. E. Stanghellini [*unpublished*] and [10]). Infection sites, resulting in one to five lesions per tap root (mean 1.6), originate primarily on the smooth surface of the expanded portion of the tap root (Fig. 1). Ingress by the fungus results in complete colonization of the tap root within 7–10 days. The small number of lesions per tap root was surprising in view of the large colonizable surface area of the tap root which often exceeds 500 cm^2 (M. E. Stanghellini, *unpublished*).

A clustered pattern of inoculum, particularly within individual rhizospheres, and/or a low inoculum efficiency (26) may account for the small number of lesions that we observed.

Our objectives were to determine the spatial pattern of distribution of *P. aphanidermatum* in rhizospheres of individual mature sugar beet tap roots, and the environmental factors affecting expression of the absolute inoculum potential (AIP) of *P. aphanidermatum*. The AIP, as defined by Mitchell (12), is a measure of the maximum capacity of a pathogen population to infect fully susceptible plant tissue under optimum conditions for infection. In our study the AIP of *P. aphanidermatum* was bioassayed with potato tuber tissue. A preliminary report has been published (18).

MATERIALS AND METHODS

Rhizosphere studies. A rhizosphere section of soil bearing the impression of the root-soil interface (RSI) from each of 38, 8- to 9-mo-old, healthy sugar beet tap roots (Fig. 2A) was collected from a commercial field that assayed at 9 ± 2 oospores per gram of soil. The oospore population density was estimated by use of a selective medium (3) from a composite soil sample composed of 20 soil cores (2.5-cm diameter) collected from the 0 to 15-cm depth. Rhizosphere sections were placed on a bed of vermiculite in plastic boxes and transported to the laboratory where sufficient tap water was added to the vermiculite to saturate the rhizosphere sections.

The distribution of *P. aphanidermatum* within the RSI of each rhizosphere section was determined by direct isolation with a selective medium (3) and a modified potato bait method (9,11). First, 1 cm^2 soil samples were individually scraped, to a depth of 0.1 cm, from the RSI of each of 12 rhizosphere sections, placed in 2 ml of sterile distilled water (SDW), agitated, and dispensed on the surface of a selective medium in petri dishes. After 48 hr of incubation at 36 C, soil was washed from the agar surface and colonies were counted. The percentage of the RSI soil samples infested was determined and inoculum density data from RSI soil samples were compared to the negative binomial probability distribution (14,25). Adequacy of fit was determined by the chi-square test. The number of soil samples assayed per RSI was governed by the surface area of the RSI on each rhizosphere section of soil. The soil sampling depth of 0.1 cm from the RSI was chosen because studies reported below indicated that inoculum densities effective for host colonization were located primarily within 0.1 cm of the host surface. Second, pieces of fresh potato tuber tissue, 1 cm^2 and 0.3-cm thick, were placed 1 cm apart on the RSI of each of 26 rhizosphere sections (Fig. 2B). A water agar slice (0.5 cm^2 and 3-mm thick) was then placed on top of the potato tissue and incubated for 48 hr at 27 C. Water agar slices were then removed, placed on the selective medium, and incubated at 36 C. The percentage of the water agar slices colonized by *P. aphanidermatum* was determined after 24 hr. The number of pieces of bait used on each RSI was governed by the surface area of the RSI on each rhizosphere section of soil.

Potato tuber tissue, rather than sugar beet root tissue, was used as bait since it is more readily available, and is a natural host for the fungus which causes leak of potato tubers. Freshly cut potato slices were rinsed in SDW and blotted dry prior to use.

Environmental factors influencing host colonization. The effect of temperature on host colonization was determined. Ten 1-g samples of a naturally infested soil, containing 40 ± 5 oospores per gram of air-dry soil, were dispensed into separate vessels 2.5 cm in diameter and 1.5-cm tall. The soil in each vessel was brought to saturation with SDW and a 1-cm^2 piece of fresh potato tuber tissue, 3-mm thick, was placed on the soil surface. A water agar slice was then placed on top of the bait (Fig. 3). The baited soil samples were then placed in a moist chamber and incubated at various temperatures. Water agar slices were removed at various time intervals and percentage colonization was determined as described above. The experiment was repeated five times.

The effect of soil moisture on host colonization was determined. Büchner funnels (9-cm diameter) with fritted glass plates (Kimax, 600 ml, −90 F) were filled to a depth of 3 cm with a naturally

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

©1983 The American Phytopathological Society

infested soil which contained 28 ± 9 oospores per gram of air-dry soil. Five pieces of fresh potato tuber tissue were then placed 2 cm apart on the surface of the soil. The soil was then brought to saturation and subjected to matric water potentials of 0, −0.1, and −0.2 bars (22). The time required to saturate and reach equilibrium at the desired matric potentials never exceeded 1 hr. Baited soils were then incubated at 27 C for 48 hr. Percentage colonization was determined as described above. The experiment was repeated three times.

The radial extent of the influence of host exudates was estimated by use of the potato bait method. A naturally infested soil containing 28 ± 9 oospores per gram of air-dry soil was dispensed into 9-cm-diameter vessels, brought to saturation with SDW and soil depth adjusted to within 0, 1, 3, and 5 mm from the lip of the vessel. Field soil free of *P. aphanidermatum* was then dispensed on top of the infested soil, leveled with the lip of the container, and brought to saturation with SDW. Five pieces of potato tuber tissue were then placed at 1-cm intervals on the surface of the soil, and incubated in a moist chamber at 27 C for 48 hr. Percentage colonization was determined as described above. The experiment was repeated five times.

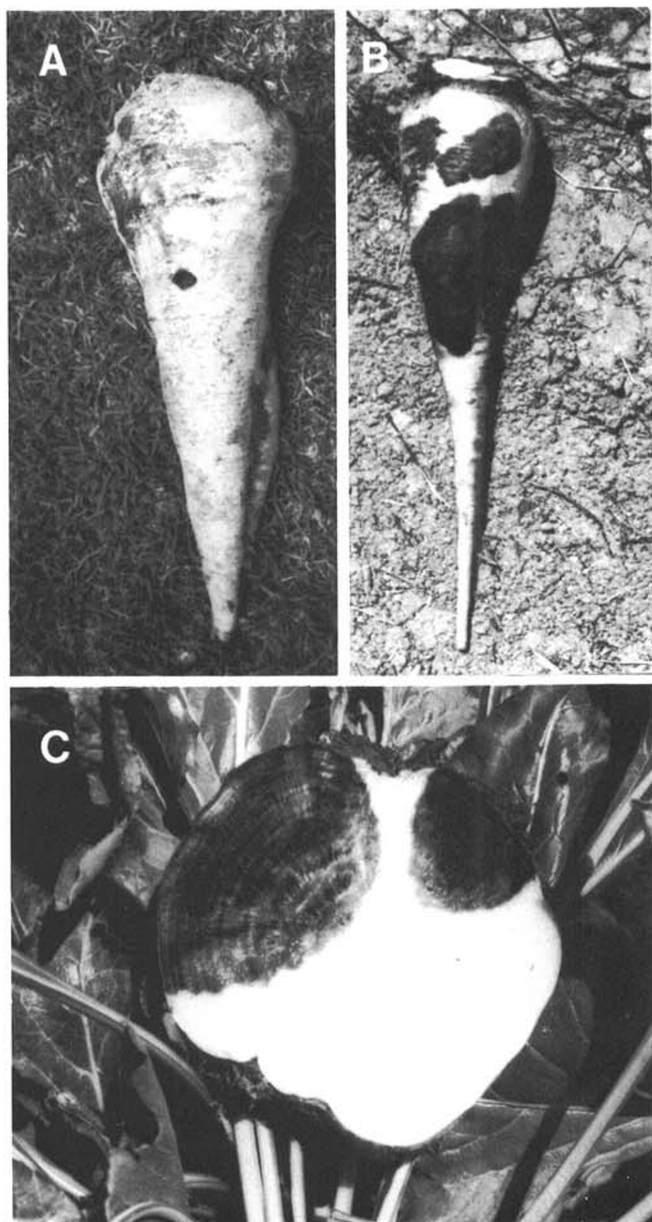

Fig. 1. Root rot caused by *Pythium aphanidermatum* in sugar beets: **A and B,** lesions on smooth surface of tap root, and **C,** cross section of tap root illustrating pathogen ingress from two infections.

RESULTS

Rhizosphere studies. The RSI of 12 rhizosphere sections was assayed by the direct isolation method and 26 by the potato bait method. With the direct isolation method, *P. aphanidermatum* was isolated from 10 of the 12 rhizosphere sections. Of the 10 infested rhizosphere sections, an average of 32% of the soil samples from the RSI contained *P. aphanidermatum* (range, 11 to 75%). The mean number of soil samples assayed per infested RSI was 13.5 (Table 1). Population densities ranged from one to five oospores per infested soil sample. Inoculum density data from soil samples from the 12 rhizosphere sections were compared to the negative binomial probability distribution. The data adequately fit the negative binomial distribution as determined by chi-square (Table 2). The '*k*' parameter was 0.46, indicating that inoculum densities were clustered. However, analysis of the data on the distribution of inoculum densities within the RSI of individual rhizosphere sections showed that oospore population densities were clustered in six ($S^2 / X = <1$, range in '*k*'=0.39 to 7.71), random in one ($S^2 / X = 1$), and uniform in three ($S^2 / X = >1$) of the 10 infested rhizosphere sections collected. With the potato bait method, *P. aphanidermatum* was isolated from 19 of the 26 rhizosphere sections collected. Of the 19 infested rhizosphere sections, an average of 36% of the pieces of

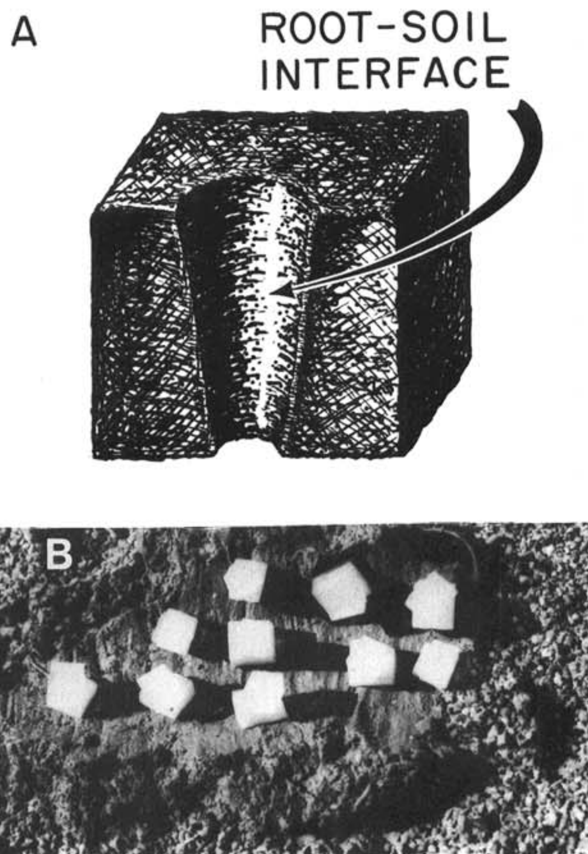

Fig. 2. Root-soil interface. **A,** Schematic drawing illustrating sugar beet root-soil interface and **B,** photograph of an intact root-soil interface assayed with the potato bait method.

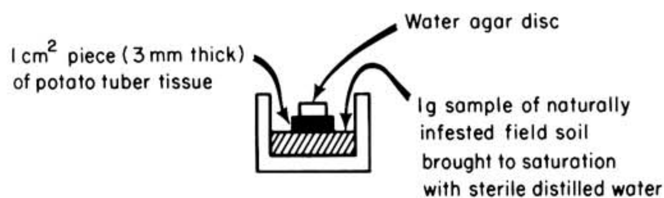

Fig. 3. Method used to estimate the absolute inoculum potential of *Pythium aphanidermatum* in naturally infested soil.

bait placed on the RSI were colonized by *P. aphanidermatum* (range, 10 to 90%) (Table 1). The mean number of pieces of bait used per infested RSI was 8.7.

Environmental factors influencing host colonization. The effects of various incubation temperatures and time on percentage colonization are presented in Fig. 4. Percentage colonization increased dramatically as incubation temperature and time increased. Maximum percentage colonization occurred after 48 hr at temperatures between 27 and 34 C. No colonization occurred at 20 C. Temperature studies were conducted under saturated soil conditions (0 bars). Soil moisture levels below saturation resulted in decreased percentage colonization. After 48 hr at 27 C, 80.3 ± 4.6, 69 ± 12.7, and 36 ± 4.7% colonization occurred at 0, −0.1, and −0.2 bars, respectively. Additionally, percentage colonization decreased rapidly as the distance between the host and infested soil increased. After 48 hr of incubation at 27 C under saturated soil conditions, 68 ± 16, 15 ± 6, 0, and 0% colonization occurred when the host was 0, 1, 3, and 5 mm distant from the infested soil, respectively.

DISCUSSION

Inoculum potential, sensu Garrett (6), is "the energy of growth of a parasite available for infection of a host, at the surface of a host organ to be infected, per unit area of the host surface." Among the factors that affect the inoculum potential are the relative vigor of the pathogen, environmental conditions, and the distribution of inoculum densities in soil, particularly in the rhizosphere. Although quantitative studies have shown that inoculum densities of *Cylindrocladium crotalariae* (8,25), *Sclerotium cepivorum* (2,5), *Rhizoctonia solani* (4), and *Pythium aphanidermatum* (24) had a clustered pattern of distribution in field soil, the in vitro assay methods used in these studies were totally disruptive to the natural spatial distribution and physiological status of these propagules in soil. To our knowledge, our studies provide the first direct evidence of the in situ distribution and physiological status of a soilborne fungus in rhizosphere soil.

In terms of the percentage area infested, a mean of 32% of the 1 $cm^2 \times 0.1$ cm soil samples from the root-soil interface (RSI) of sugar beet tap roots contained a population of *P. aphanidermatum*. Inoculum densities in infested areas of the RSI ranged from one to five oospores. Spatial distribution patterns of inoculum densities within the RSI of individual rhizosphere sections ranged from highly clustered, to random, to uniform. As previously mentioned, the surface area of mature sugar beet tap roots commonly exceeds 500 cm^2. Assuming a 100% efficiency of inoculum, coupled with knowledge of the percentage of the RSI infested, one would expect about 170 lesions per sugar beet tap root. However, we observed that the mean number of lesions per root was 1.6. These results indicate that the distribution of inoculum was not the limiting factor restricting the number of lesions on tap roots.

The AIP, as defined by Mitchell (12), is a measure of the maximum capacity of a pathogen population to infect fully susceptible plant tissue under optimum conditions for infection. We estimated the AIP of *P. aphanidermatum* in the RSI by the use of potato tuber tissue, a natural host, under optimum soil moisture and temperature conditions. Results, in agreement with data from direct isolation studies cited above, showed that a mean of 36% of the surface area of the RSI of infested rhizosphere sections contained an infective population of *P. aphanidermatum*. Since inoculum densities in our direct isolation study were estimated from soil samples collected within 0.1 cm of the sugar beet rhizoplane, effective and sufficient inoculum densities were within striking distance of the tap root. These results indicate that inoculum density per unit of infested area of the RSI was not the factor restricting the number of lesions on the sugar beet tap root.

The limiting factors to expression of the AIP of *P. aphanidermatum*, as bioassayed with potato tuber tissue, were suboptimal soil moisture and/or temperature conditions. Maximum host colonization occurred only at soil temperatures of 27 C or greater under wet soil conditions (0 to −0.01 bar) for test periods of 24–48 hr. Such sustained favorable environmental conditions acting in concert would be, at best, fleeting under irrigated field conditions and the limited number of lesions which we observed on sugar beet tap roots probably reflects the infrequent and localized occurrence of favorable environmental conditions in the RSI for oospore germination. Inopportune oospore germination, in the absence of any capability to form replacement survival structures (20), would result in a decrease in the inoculum density within the rhizosphere. However, no decrease in rhizosphere inoculum densities were detected during the growing season prior to the onset of infection (24). In addition to environmental constraints, any inherent resistance mechanisms or

TABLE 1. The percentage of the surface area of the root-soil interface (RSI) of sugar beet tap roots infested with *Pythium aphanidermatum*

RSI assay method	Rhizosphere sections (no.)	Surface area (cm^2) assayed per RSI (mean ± SE)	Surface area infested per RSI (mean % ± SE)
Potato bait method[a]	19	8.7 ± 2.2	36.4 ± 22.4
Direct isolation from soil on selective medium[b]	10	13.5 ± 4.4	32.3 ± 21.0

[a] Pieces of fresh potato tuber tissue, 1 cm^2 and 0.3 mm thick, were placed 1 cm apart on the RSI of rhizosphere sections of soil from sugar beet tap roots. Percentage colonization was determined after 48 hr incubation at 27 C.

[b] Soil samples, 1 cm^2 and 0.1 cm deep, were scraped from the RSI of rhizosphere sections of soil from sugar beet tap roots and oospore population densities were estimated in each sample by use of a species-specific isolation medium.

TABLE 2. Observed frequencies of oospore populations of *Pythium aphanidermatum* in soil samples from the root-soil interface of 12 sugar beet tap root rhizospheres and frequencies expected from the negative binomial probability distribution

Oospores per soil sample[a]	Number of soil samples	
	Observed	Expected
0	103	101.9
1	25	25.2
2	8	9.8
3	4	4.3
4	2	2.0
5	3	1.0
$K = 0.46$	$N = 145$	
Calculated $\chi^2 = 4.361$		

[a] Oospore population densities were estimated by use of a species-specific isolation medium. Soil samples, 1 $cm^2 \times 0.1$ cm deep, were scraped from the root-soil interface of rhizosphere sections of soil.

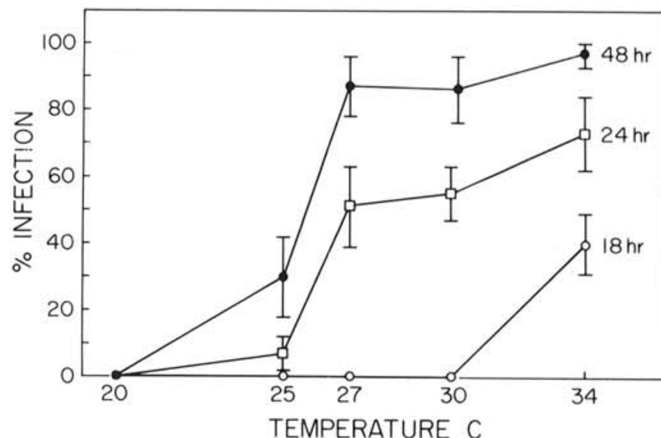

Fig. 4. Effect of temperature and incubation time on infection of potato tuber tissue by *Pythium aphanidermatum*.

differences in the quality or quantity of exudates of sugar beet roots would, in effect, place additional constraints upon expression of the AIP of *P. aphanidermatum*.

Our results on environmental factors affecting the AIP are in general accord with data from in vitro studies of others on environmental factors affecting oospore germination. Exogenously dormant oospores of *P. aphanidermatum* germinate maximally with 24 hr (1,15,19,20,23) at temperatures between 27 and 35 C (1,15,19,20) at matric water potentials between 0 and −0.1 bars (21). These high matric water potentials increase the availability and favors the diffusion of nutrients, primarily host exudates, which are required for oospore germination (19,21). Although exudates, in sufficient quantities to stimulate propagule germination in *Pythium ultimum* (21) as well as other fungi (7,13,16,17), can diffuse into soil up to 20 mm from a host surface, highest percentage germination occurs within 0 to 1 mm of the host surface and decreases rapidly with increasing distance. Our results extend these findings. Specifically, inoculum densities effective for infection, even under optimum soil temperature and moisture conditions, must be located within 1 mm of the host surface. Infection of potato tuber tissue was reduced from 68 to 15% when the inoculum was 1 mm distant from the host surface.

LITERATURE CITED

1. Adams, P. B. 1971. *Pythium aphanidermatum* oospore germination as affected by time, temperature, and pH. Phytopathology 61:1149-1150.
2. Adams, P. B. 1981. Forecasting onion white rot disease. Phytopathology 71:1178-1181.
3. Burr, T. J., and Stanghellini, M. E. 1973. Propagule nature and density of *Pythium aphanidermatum* in field soil. Phytopathology 63:1499-1501.
4. Campbell, C. L., and Pennypacker, S. P. 1980. Distribution of hypocotyl rot caused in snapbean by *Rhizoctonia solani*. Phytopathology 70:521-525.
5. Crowder, F. J., Hall, D. H., Greathead, A. S., and Baghott, K. G. 1980. Inoculum density of *Sclerotium cepivorum* and the incidence of rot of onion and garlic. Phytopathology 70:64-69.
6. Garrett, S. D. 1970. Pathogenic root infecting fungi. Cambridge University Press, Cambridge, England. 294 pp.
7. Griffin, G. J. 1969. *Fusarium oxysporum* and *Aspergillus flavus* spore germination in the rhizosphere of peanut. Phytopathology 59:1214-1218.
8. Hau, F. C., Campbell, C. L., and Beute, M. K. 1982. Inoculum distribution and sampling methods for *Cylindrocladium crotalariae* in a peanut field. Plant Dis. 66:568-571.
9. Hine, R. B., and Luna, L. V. 1963. A technique for isolating *Pythium aphanidermatum* from soil. Phytopathology 53:727-728.
10. Hine, R. B., and Ruppel, E. 1969. Relationship of soil temperature and moisture to sugarbeet root rot caused by *Pythium aphanidermatum* in Arizona. Plant Dis. Rep. 53:989-991.
11. Khan, S., and Baker, R. 1968. Residual activity of Dexon. Phytopathology 58:1693-1696.
12. Mitchell, J. E. 1979. The dynamics of the inoculum potential of populations of soil-borne Plant Pathogens in the soil ecosystem. Pages 3-20 in: Soil-borne Plant Pathogens. B. Schippers and W. Gams, eds. Academic Press, New York. 686 pp.
13. Papavizas, G. C., and Davey, C. B. 1961. Extent and nature of the rhizosphere of Lupinus. Plant Soil 14:215-236.
14. Pollard, J. H. 1977. Handbook of Numerical and Statistical Techniques. Cambridge University Press, Cambridge, England. 349 pp.
15. Ruben, D. M., Frank, Z. R., and Chet, I. 1980. Factors affecting behavior and developmental synchrony of germinating oospores of *Pythium aphanidermatum*. Phytopathology 70:54-59.
16. Short, G. E., and Lacy, M. L. 1974. Germination of *Fusarium solani* f. sp. *pisi* chlamydospores in the spermosphere of pea. Phytopathology 64:558-562.
17. Short, G. E., and Wyllie, T. D. 1978. Inoculum potential of *Macrophomina phaseolina*. Phytopathology 68:742-746.
18. Stanghellini, M. E. 1982. Distribution of *Pythium aphanidermatum* in the rhizoplane of field-grown sugarbeets. (Abstr.) Phytopathology 78:996.
19. Stanghellini, M. E., and Burr, T. J. 1973. Germination in vitro of *Pythium aphanidermatum* oospores. Phytopathology 63:133-137.
20. Stanghellini, M. E., and Burr, T. J. 1973. Germination in vivo of *Pythium aphanidermatum* oospores and sporangia. Phytopathology 63:1493-1496.
21. Stanghellini, M. E., and Burr, T. J. 1973. Effect of soil water potential on disease incidence and oospore germination of *Pythium aphanidermatum*. Phytopathology 63:1496-1498.
22. Stanghellini, M. E., and Hancock, J. G. 1971. Radial extent of the bean spermosphere and its relation to the behavior of *Pythium ultimum*. Phytopathology 61:165-168.
23. Stanghellini, M. E., and Nigh, E. L. 1972. Occurrence and survival of *Pythium aphanidermatum* under arid soil conditions in Arizona. Plant Dis. Rep. 56:507-510.
24. Stanghellini, M. E., von Bretzel, P., Kronland, W. C., and Jenkins, A. D. 1982. Inoculum densities of *Pythium aphanidermatum* in soils of irrigated sugar beet fields in Arizona. Phytopathology 72:1481-1485.
25. Taylor, J. D., Griffin, G. J., and Garren, K. H. 1981. Inoculum pattern, inoculum density-disease incidence relationships, and population fluctuations of *Cylindrocladium crotalariae* microsclerotia in peanut field soil. Phytopathology 71:1297-1302.
26. Tomimatsu, G. S., and Griffin, G. J. 1982. Inoculum potential of *Cylindrocladium crotalariae* infection rates and microsclerotial density-root infection relationships on peanut. Phytopathology 72:511-517.