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BIOLOGY OF *PYTHIUM APHANIDERMATUM*: THE INCITANT OF CUCURBIT ROOT ROT AND DAMPING-OFF IN THE FARS PROVINCE OF IRAN[1]

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Abstract – Biology of *Pythium aphanidermatum* (Edson) Fitz., the cause of damping-off and root rot of cucurbits in Shiraz and vicinity, was studied. The optimum temperature for vegetative growth, germination of zoospores and oospores, and infection of the host was 35°C. Suspension of 100 or more zoospores/ml caused infection in susceptible host seedlings at 35°C. Susceptible cantaloupe plants older than 30 days showed no disease symptoms when inoculated with a suspension of 10^6 zoospores/ml. Different isolates of *P. aphanidermatum* had different maximal growth temperature and varied markedly in virulence in relatively resistant cultivars of cantaloupe. Oospores of *P. aphanidermatum*, aged 3, 4 or 5 weeks, were capable of infecting cantaloupe seedlings at similar rates at 35°C.

Among cucurbits inoculated with cucumber isolate of *P. aphanidermatum*, cucumber was the most susceptible and squash the most resistant host. In addition to the host plants, *P. aphanidermatum* colonized roots of *Amaranthus* spp., predominant weeds in cucurbit fields, under natural conditions.

Total populations of the pathogen in naturally infested soils ranged from 1–28 propagules/g dry soil, of which 1–17.3/g soil were oospores. The population of the pathogen was higher in midsummer and in soil adjacent to cantaloupe plants than away from them. Continuous cropping of the host plant increased population of the pathogen in contrast to the field rotated with non-host.

INTRODUCTION

Pythium aphanidermatum (Edson) Fitz. is a soil-inhabiting plant pathogen, inflicting serious economic losses. It is distributed throughout the world and attacks a large number of economically important crops. Middleton [21] in 1943 listed 80 species of higher plants as hosts of *P. aphanidermatum*. Since then the number of hosts has been increased [2, 6, 12, 13, 14, 16, 20]. The pathogen also causes fruit rot in cucurbits [19, 26] and tomato [27].

The pathogen is reported to attack seedling and mature ryegrass [11]. Isolates of *P. aphanidermatum* from different sources may vary in pathogenicity to a given host [19].

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The pathogen causes serious loss under high soil temperature [15, 19, 25, 32, 33] and high soil moisture [29, 31].

Thick-walled oospores are the major structures of *P. aphanidermatum* capable of long term survival in soil [28]. Burr and Stanghellini [5], using a species-specific isolation medium, found that population of *P. aphanidermatum* in various field soils ranged from 10 to 25 oospores/g soil.

In Iran, *P. aphanidermatum* is an important soil-borne plant pathogen and is isolated from soils collected from various parts of the country [4]. It causes serious loss in cucurbits [3], sugar beet [9], pea [15] and root decay in citrus [10].

The purpose of the present investigation is to study the behavior of *P. aphanidermatum* isolates obtained from various parts of Fars province of Iran under field and controlled laboratory conditions.

MATERIALS AND METHODS

Isolates of *P. aphanidermatum* were obtained from naturally infested soils [4] and infected tissues of different plant species [34] collected from various areas of the Fars province during 1974-1976 (Table 1). Hyphal tip isolates were identified according to

Table 1. Isolates of *Pythium aphanidermatum* obtained from different host and non-host plant species

Plant species	Location	Year
Amaranthus graecizans L. (NH)	Bidzard, Badjgah	1976
Amaranthus retroflexus L. (NH)	Bidzard, Badjgah	1976
Beta vulgaris L. (H)	Marvdasht	1976
Cucumis sativus L. (H)	Maharloo	1975
Cucumis melo L. (H)	Maharloo	1974
Nicotiana tabacum L. (H)	Jahrom	1975
Solanum melongena L. (H)	Bidzard	1976

NH, non-host; H, host.

Middleton [21]. Zoospores were obtained by the following method [23]: the pathogen was grown on V-8 agar (V-8 juice, 200 ml; agar, 17 g; distilled water, 800 ml) incubated under 1200 lx of white fluorescent illumination at 35°C. After 2 days, the culture was cut into 5-mm strips. Half of the strips from each plate were removed and placed in a sterile Petri dish containing 20 ml sterile distilled water (SDW) and incubated at room temperature. Half an hour later water was decanted and replaced with fresh 20 ml SDW and incubated under 1200 lx of white fluorescent illumination at 35°C for 1 day for sporangial formation. Water was changed once more and incubated for 4 hr at 20°C and large numbers of zoospores were obtained.

Oospores of *P. aphanidermatum* were obtained on cleared V-8 broth [8] at 24°C for 3-5 weeks. Mycelial mats with oospores were frozen to kill mycelial fragments [24] and ground in a sterilized mortar with a few ml of SDW and passed through several layers of cheese cloth. Free oospores were collected in the filtrate.

A virgin soil devoid of *P. aphanidermatum*, as determined by soil dilution method [5], was mixed with fine sand (2:1 w/w). The mixture was spread in a thin layer on a piece of paper and the oospore suspension was applied to the soil surface by means of a hand sprayer.

Seeds of cantaloupe (*Cucumis melo* L. Var. *reticulatus* Naud.) cucumber (*C. sativus* L.), watermelon (*Citrullus vulgaris* Schrad.), squash (*Cucurbita pepo* L.), tomato (*Lycopersicum sculentum* Mill.), safflower (*Carthamus tinctorius* L.) and pea (*Pisum sativum* L.) were sown in 10-cm pots (10 seeds/pot) containing a mixture of autoclaved silty clay loam (pH = 8.2) and sand (2:1 w/w) and kept in a greenhouse. After 10–14 days when the seedlings were at the cotyledonary stage, they were thinned to 7 seedlings per pot. Seedlings with soil blocks were then transferred to pots without drainage holes and incubated at specified temperatures in a water bath.

For zoospore inoculation, 20 ml of zoospore suspension (10^2–10^6 zoospores/ml) were added to each pot and remained flooded for at least 2 hr. For oospore inoculation, about 80 g of artificially infested soil (15,000 oospores/g soil) was added to the soil surface in each pot. Pots were irrigated 2–3 times a day. Pots were placed in water baths located on the laboratory bench (room temperature 20–22°C), and incandescent and fluorescent illumination (1000–1400 lx) was provided. Three to five pots were used for each treatment.

Population of the pathogen in naturally infested soil samples obtained from various fields, was determined on the species-specific medium [5] at 35°C.

RESULTS

Effect of soil temperature on infection

More seedlings inoculated with either zoospores or oospores were killed at 35°C than lower soil temperatures (Table 2). The collar region of the symptomless seedlings in

Table 2. Effect of soil temperature on infection of cantaloupe cv. Shahd-e-Shiraz by oospores and zoospores of *Pythium aphanidermatum*

Soil temperature (°C)	Zoospores*	Oospores†
	% Mortality‡	
35	100	70
30	16	50
25	0	28
20	0	14

* 20 ml of 10^6 zoospores/ml per pot.

† 80 g of artificially infested virgin soil with the oospores (15,000/g soil) spread over the surface of each pot.

‡ Per cent mortality seven days after inoculation.

oospore-infested soil was surface sterilized in 0.5% NaOCl and cultured on corn meal agar (CMA). *P. aphanidermatum* was isolated from all seedlings.

In addition to cantaloupe (a warm season plant), 2-week old seedlings of pea cv. Alaska (a cool season plant) were inoculated with 20 ml of zoospore suspension of 10^6/ml) and incubated at 20–35°C. After 1 day of inoculation: 40, 25, 10 and 0%, and after three days: 100, 90, 50 and 20% of seedlings were killed at 35, 30, 25 and 20°C, respectively.

Effect of zoospore concentration

Fourteen-day old cantaloupe seedlings cv. Shahd-e-Shiraz and Gold and Silver (susceptible and resistant to *P. aphanidermatum* respectively [23]) were inoculated with 20 ml of zoospore suspension/pot (10^2–10^6/ml) and incubated at 35°C in a water bath.

Zoospore concentrations of 10^2/ml caused 70% mortality in cv. Shahd-e-Shiraz seedlings, whereas 10^6 zoospores/ml were required to kill 14% of cv. Gold and Silver seedlings and no mortality occurred at lower concentrations (Table 3).

Table 3. Effect of zoospore concentration of *Pythium aphanidermatum* on reaction of two cultivars of *Cucumis melo*

Number of zoospores/ml*	Days after inoculation: cv. Shahd-e-Shiraz 2	4	7	cv. Gold and Silver 2	4	7
	% Mortality					
10^6	0	71	76	0	0	14
10^5	9	57	85	0	0	0
10^4	4	52	61	0	0	0
10^3	4	100	–	0	0	0
10^2	0	50	70	0	0	0
10^1	0	0	0	0	0	0

* 20 ml of zoospore suspension/pot.

Relation of plant age to infection

Seeds of cantaloupe cv. Shahd-e-Shiraz were sown in 12-cm plastic pots (7 seeds/pot) at 3 successive dates to have a final age of 15, 30 and 60 days at the time of inoculation. Pots were kept in greenhouse and irrigated every 2 weeks with nutrient solution. Pots were watered normally. One day prior to inoculation, pots were placed in water bath at 35°C and thinned to 3 plants/pot. Twenty milliliters of zoospore suspension (10^6/ml) and 30 ml SDW were added to each pot. Controls received only 50 ml SDW. No mortality was observed among 30- and 60-day old plants but 77% of 15-day old plants were killed 7 days after inoculation.

Relation of oospore age to infectivity

Cantaloupe seedlings cv. Shahd-e-Shiraz were inoculated with oospores of *P. aphanidermatum* aged 3, 4 and 5 weeks. Inoculated and non-inoculated (control) pots were incubated in a water bath at 35°C. Disease symptoms were observed 3 days after inoculation. At this time the rate of seedling mortality was higher with 5-week old oospores, but upon longer incubation, no differences were observed among different ages (Table 4). When oospores of different ages were cultured on CMA, and incubated at 35°C for 24 hr, oospore germination was 28, 40 and 35% for 3-, 4- and 5-week old oospores, respectively (Table 4).

Table 4. Infection of cantaloupe seedlings cv. Shahd-e-Shiraz by oospores of *Pythium aphanidermatum* of different ages*

Oospore age (weeks)	Oospore germination†	Days after inoculation		
		3	5	7
	%	% Mortality		
3	28	4	52	57
4	40	4	38	38
5	53	23	42	57

* 80 g of artificially infested autoclaved soil with oospores (15,000/g dry soil) spread on soil in each pot and incubated at 35°C.

† Percentage of oospores germinated on CMA during 24-hr incubation at 35°C.

Reactions of various hosts to P. aphanidermatum

Thirty-three cvs. of melon, 4 cvs. of cucumber, 15 cvs. of watermelon, 3 cvs. of squash, 3 cvs. of safflower and 1 cv. of tomato and pea were inoculated with 20 ml of zoospores of *P. aphanidermatum* (10^4/ml for melon and cucumber and 10^6/ml for others) and incubated at 35°C in a water bath. In general, cucumber, tomato, pea and safflower were the most susceptible and squash was the most resistant hosts to the pathogen. Watermelons were more resistant than melons.

Symptomless carrier of P. aphanidermatum

Three species of weeds and volunteer corn (*Zea mays* L.) were collected from a cantaloupe field naturally infested with *P. aphanidermatum* in Bidzard (30 km from Shiraz). Weeds were identified as *Chenopodium album* L., *Amaranthus retroflexus* L. and *A. graecizans* L. according to Edgecombe [7]. Plant roots were washed, treated in 0.5% NaOCl for 2 min, rinsed with SDW, cut into small pieces, plated on CMA, and incubated at 35°C. The pathogen was isolated only from 7% and 3% of the root segments of *A. retroflexus* and *A. graecizans*, respectively. These two *Amaranthus* species are the

predominant weeds in many cantaloupe, cucumber and sugarbeet fields in Fars. The pathogen was also isolated from the *Amaranthus* species found in Badjgah.

Comparative study of isolates of P. aphanidermatum

Seven isolates of *P. aphanidermatum* from Shiraz and one from the U.S.A. were inoculated to *C. melo* cv. Honey Dew and *C. sativus* cv. Beith Alpha (20 ml of 10^4 zoospores/ml in each pot) and incubated at 35°C in a water bath for 7 days. The former cultivar is moderately resistant, the latter highly susceptible to the pathogen [23]. All isolates killed 100% of cucumber seedlings within 4 days (Table 5). In the resistant melon cultivar, isolates behaved differently. *Amaranthus* isolates were more pathogenic than the other isolates (Table 5).

Table 5. Comparative virulence of isolates of *Pythium aphanidermatum* on *Cucumis melo** and *Cucumis sativus*†

Source of isolates‡	Days after inoculation§ 2		4		7	
	C. sativus	*C. melo*	*C. sativus*	*C. melo*	*C. sativus*	*C. melo*
	% Mortality					
1. *Amaranthus graecizans*	66	4	100	28	–	42
2. *A. retroflexus*	90	0	100	9	–	38
3. *Cucumis melo*	80	0	100	19	–	28
4. *Nicotiana tabacum*	90	0	100	4	–	28
5. *Solanum melongena*	100	0	–	14	–	19
6. *Beta vulgaris*	42	0	100	0	–	9
7. *C. sativus*	100	0	–	0	–	4
8. Wisconsin	14	0	100	0	–	9

* *C. melo* cv. Honey Dew (resistant).

† *C. sativus* cv. Beith Alpha (susceptible).

‡ Isolates 1–7 were obtained from Shiraz and vicinity, isolate 8 is obtained from Dr. J.E. Mitchell, University of Wisconsin, Madison, WI, U.S.A.

§ Seedling were inoculated with 20 ml of zoospores (10^4 /ml) in each pot and incubated at 35° C.

The isolates were also compared on CMA incubated at different temperatures (35, 40 and 45°C) for 7 days. All isolates failed to grow at 45°C, but all had an optimum at 35°C. Isolates obtained from sugarbeet, eggplant and *A. retroflexus* grew at 40°C, but others isolated from *A. graecizans*, cantaloupe, tobacco, cucumber and one from the U.S.A. did not.

Nature and density of propagules in field soil

Soil samples were collected from 15 sites at 2 depths (0–10 and 10–25 cm) adjacent and away from cantaloupe plants in the fields at 3 locations: Kooshkak, Zarghan and Bidzard. The 15 samples of each location, depth and proximity to cantaloupe plants were mixed and three 1-g aliquots were used and diluted 1:5 or 1:10 with 0.2% water agar [5]. In order to separate oospores from other propagules, diluted soil suspensions from

Bidzard were either frozen at −20°C (to exclude propagules other than oospores) or maintained at 5°C for 24 hr. After thawing, 1 ml of each sample was spread over a selective medium [5]. After 2 days' incubation at 35°C in the dark, colonies of the pathogen were counted. Four plates were used for each replication.

The soil samples taken on 22 June from Kooshkak contained no detectable propagules, and samples from Zarghan had only 1 propagule per g soil. Samples taken from Bidzard contained 3–10 and 1.3–8.3 propagules/g soil for the unfrozen and frozen samples, respectively. The Bidzard soil samples were also taken on 23 July and 23 August. In July samples, the number of propagules were increased up to 12.6–28 and 3.3–17.3/g soil for the unfrozen and frozen samples, respectively. The decrease in propagules was noticed in August soil samples (Table 6). Soils adjacent to the plants had significantly

Table 6. Population density of *Pythium aphanidermatum* in a cantaloupe field located in Bidzard during summer 1976

	Unfrozen soil[a*]				Frozen soil[b†]			
	away‡		adjacent§		away		adjacent	
	depth, cm		depth, cm		depth, cm		depth, cm	
Date of sampling	10	25	10	25	10	25	10	25
	Number of propagules/g dry soil							
22 June	3.0	8.0	9.3	10.0	1.6	8.3	3.3	1.3
23 July	(12.6	28.0)[c]	(25.3	22.0)[d]	(3.3	10.6)[c]	(17.3	14.0)[d]
23 August	(10.0	2.3)[e]	(13.3	12.0)[f]	(1.3	1.3)[e]	(1.6	2.3)[f]

* Field soil used without any treatment.

† Field soil diluted (1/10) in 0.2% water agar and frozen at −20°C for 24 hr.

‡ Away from cantaloupe plants.

§ Adjacent to cantaloupe plants.

Soil samples were taken from 0–10 and 10–25 cm depths adjacent and away from cantaloupes.

Small letters indicate Duncan's multiple range groupings of treatments at 1% level of significancy.

more propagules than soil taken away from the plants on 23 July and 23 August. The number of propagules in 0–10- and 10–25-cm samples were not significantly different (Table 6).

In August 1976, a sugarbeet field (field A) in Marvdasht showed more than 50% damped-off seedlings due to *P. aphanidermatum*. This field had been planted to sugarbeet for 3 successive years. Soil samples were taken from 10 sites in this field and also from two other adjacent fields; one currently cropped with sugarbeet rotated after wheat (field B) and the other with wheat planted after sugarbeet (field C). Number of propagules of *P. aphanidermatum* was 28.3, 13.6 and 0/g soil for field A, B and C, respectively.

DISCUSSION

P. aphanidermatum is a high temperature organism and has a great potential activity during summer. The optimum temperature for mycelial growth in culture and for infection by zoospores and oospores by our isolates was 35°C, which confirms other reports [1, 17, 19, 21, 30]. At lower temperatures, growth of the pathogen is reduced and results in lower disease potential.

Plant age apparently influences the reaction to the pathogen. McCarter and Littrell [19] reported no significant damage of rye, oat and wheat when 13–15-cm high plants were inoculated with *P. aphanidermatum*. Our results indicated that the pathogen is of importance on seedlings but not on 4-week old plants or older.

Oospores of *P. aphanidermatum* are the survival structure and are readily able to attack hypocotyl region of seedling resulting in damping-off symptom. Oospores are capable of germination without a long period of dormancy. Oospores produced in the early part of the season, may contribute to inoculum increase in the same season.

Zoospores of *P. aphanidermatum* are capable of infecting root and hypocotyl and functioning as primary infecting units. The production and dissemination of zoospores under field conditions is dependent on the presence of free moisture during submerged irrigation and flooding [29].

P. aphanidermatum has a very wide host range [21]. Among cucurbits, cultivars of *C. sativus* and *C. pepo* used in this study were highly susceptible and resistant to the pathogen respectively. Cultivars of *C. vulgaris* and *C. melo* are both considered to be moderately resistant and susceptible to *P. aphanidermatum.*

Association of *P. aphanidermatum* with roots of *Amaranthus* spp., common weeds in cucurbit and non-cucurbits fields in this area, is an important aspect in the epidemiology of the pathogen in the absence of susceptible hosts.

Isolates obtained from certain host and non-host plants differed in virulence, and results agree with others [22, 19]. Some variations were also noticed in maximum temperature tolerance among isolates [18, 21], which suggest that they might be important under various environmental conditions.

According to our results, population density of *P. aphanidermatum* increases in the root zone of host plants during the growing season. A field cropped with sugarbeet for 3 successive years contained high populations of the pathogen which caused severe damping-off in the seedlings. A sugarbeet field rotated with wheat contained low numbers of propagules of *P. aphanidermatum.* This suggested that rotation is an important practice for reducing inoculum of the pathogen in the field. However, long term experiments should be conducted to confirm our findings.

In addition to viable and infective oospores, sporangia, zoospores and mycelial fragments of the fungus are present during the growing season. Thus, zoospores and mycelium could contribute to infection and disease incidence under field conditions.

The pathogen is associated with some non-hosts such as *Amaranthus* spp. and is not very active on some crops. An effective weed control and a proper rotation program will have a profound result in decreasing the pathogen activity and its population in soil.

LITERATURE CITED

1. Adams P.B. 1971. *Pythium aphanidermatum* oospore germination as affected by time, temperature, and pH. *Phytopathology* **61**, 1149–1150.
2. Apple J.L. & Crossan D.F. 1954. Pythium stem rot and wilt of pepper. *Pl. Dis. Reptr* **38**, 555–556.
3. Banihashemi Z. 1969. Cucurbit wilt and root rot diseases in Iran. *Proc. of the 2nd Plant Med. Congr. of Iran*, pp. 97–98.
4. Banihashemi Z. 1970. A new technique for isolation of *Phytophthora* and *Pythium* species from soil. *Plant Dis. Reptr* **54**, 261–262.
5. Burr T.J. & Stanghellini M.E. 1973. Propagule nature and density of *Pythium aphanidermatum* in field soil. *Phytopathology* **63**, 1499–1501.
6. Dewolfe T.A., Calvan E.C. & Sufficool J.R. 1955. A new damping-off disease of citrus. *Citrus Leaves* **33**, 16.
7. Edgecombe W.S. 1970. *Weeds of Lebanon.* American University of Beirut, Beirut, Lebanon.
8. Erwin D.C. & McCormick W.H. 1971. Germination of oospores produced by *Phytophthora megasperma* var. *sojae. Mycologia* **63**, 972–977.
9. Fatemi J. 1971. *Phytophthora* and *Pythium* root rot of sugarbeet in Iran. *Phytopath. Z.* **71**, 25–28.
10. Fatemi J. 1972. Pythiaceous fungi associated with citrus decline in Iran. *Phytopath. Z.* **74**, 153–160.
11. Freeman T.E. 1963. Age of ryegrass in relation to damage by *Pythium aphanidermatum. Pl. Dis. Reptr* **47**, 844.
12. Freeman T.E., Luke H.H. & Sechler D.T. 1966. Pathogenicity of *Pythium aphanidermatum* on grain crops in Florida. *Pl. Dis. Reptr* **50**, 292–294.
13. Gemawat D.D. & Prasad N. 1965. Pre-emergence blight of *Sesamum* caused by *Pythium aphanidermatum. Sci. Cult.* **31**, 315–316.
14. Govindo R.P. & Gopalaraju D. 1955. Wilt of *Talinum Triangular* Wild, caused by *Pythium aphanidermatum. Sci. Cult.* **20**, 502–503.
15. Kaizer W.J., Okhovat N. & Mossahebi G.H. 1971. *Pythium aphanidermatum*, an important pathogen of peas (*Pisum sativum*) in Khuzestan, Iran. *Pl. Dis. Reptr* **7**, 1–2.
16. Kilpatrick R.A. 1968. Seedling reaction of barley, oats, and wheat to *Pythium* species. *Pl. Dis. Reptr* **52**, 209–212.
17. Lumsden R.D., Ayers W.A., Adams P.B., Dow R.L., Lewis J.A., Papanizas G.C. & Kantzes J.G. 1975. Ecology and epidemiology of *Pythium* species in field soil. *Phytopathology* **66**, 1203–1209.
18. Luna L.V. & Hine R.B. 1964. Factors influencing saprophytic growth of *Pythium aphanidermatum* in soil. *Phytopathology* **54**, 955–956.
19. McCarter S.M. & Littrell R.H. 1970. Comparative pathogenicity of *Pythium aphanidermatum* and *P. myriotylum* to twelve plant species and interspecific variation in virulence. *Phytopathology* **60**, 264–268.
20. Mehrotra B.S. 1954. The cottony leak of *Citrullus fistulosus. Sci. Cult.* **19**, 564–565.
21. Middleton J.T. 1943. The taxonomy, host range, and geographic distribution of the

genus *Pythium*. *Mem. Torrey bot. Club* **20**, 1-171.

22. Morgan F.L. & Hartung E.E. 1964. *Pythium aphanidermatum* a virulent soybean pathogen. *Phytopathology* **54**, 901 (abstract).
23. Rahimian M.K. 1977. Biology and control of *Pythium aphanidermatum* (Edson) Fitz. in cucurbits. M.S. thesis, Shiraz University, Shiraz, Iran.
24. Satour M.M. & Butler E.E. 1968. Comparative morphological and physiological studies of the progenies from interspecific matings of *Phytophthora capsici*. *Phytopathology* **58**, 183-192.
25. Sechler D.T. & Luke H.H. 1967. Stand loss of small grains in Florida. *Pl. Dis. Reptr* **51**, 919-922.
26. Singh B. & Singh K. 1953. Fruit rot of cucurbits. *Sci. Cult.* **18**, 489-491.
27. Sonoda R.M. 1973. *Pythium* fruit rot of tomato. *Pl. Dis. Reptr* **57**, 638-639.
28. Stanghellini M.E. & Nigh E.L. 1972. Occurrence and survival of *Pythium aphanidermatum* under arid soil conditions in Arizona. *Pl. Dis. Reptr* **56**, 507-510.
29. Stanghellini M.E. & Burr T.J. 1973. Germination *in vivo* of *Pythium aphanidermatum* oospores and sporangia. *Phytopathology* **63**, 1493-1496.
30. Stanghellini M.E. & Russell J.D. 1973. Germination *in vitro* of *Pythium aphanidermatum* oospores. *Phytopathology* **63**, 133-137.
31. Stanghellini M.E. 1974. Symposium on the genus *Pythium*. Spore germination, growth and survival of *Pythium* in soil. *Proc. Am. phytopath. Soc.* **1**, 200-223.
32. Stanghellini M.E. & Phillips J.M. 1975. *Pythium aphanidermatum:* Its occurrence and control with pyroxychlor in the Arabian Desert at Abu Dhabi. *Pl. Dis. Reptr* **59**, 559-563.
33. Thomson T.B., Athow K.L. & Laviolette F.A. 1971. Effect of temperature on the pathogenicity of *Pythium aphanidermatum, P. debaryanum*, and *P. ultimum* on soybean. *Phytopathology* **61**, 933-935.
34. Tsao P.H. 1970. Selective media for isolation of pathogenic fungi. *A. Rev. Phytopathol.* **8**, 157-186.