Nutrient Solution Temperature Affects Pythium Root Rot of Tobacco in Greenhouse Float Systems

B. A. Fortnum, Professor of Plant Pathology and Physiology, Clemson University, Pee Dee Research and Education Center, Florence, NC 29506-9706; **J. Rideout**, Assistant Professor of Soil Science, North Carolina State University, Mountain Horticultural Crops Research and Extension Center, Fletcher 28732; **S. B. Martin**, Associate Professor of Plant Pathology and Physiology, Clemson University, Pee Dee Research and Education Center; and **D. Gooden**, Professor of Agronomy, Clemson University, Pee Dee Research and Education Center

ABSTRACT

Fortnum, B. A., Rideout, J., Martin, S. B., and Gooden, D. 2000. Nutrient solution temperature affects *Pythium* root rot of tobacco in greenhouse float systems. *Plant Dis.* 84:289-294.

Float nutrient solution, soilless media, and ambient temperatures were monitored in two tobacco greenhouses with different heating regimes. Water temperatures at seeding were 11°C in an unheated greenhouse and 17°C in a heated greenhouse. Water temperature differences between greenhouses continued over the duration of seedling production. Media and air temperatures varied diurnally, but not float solution. In a greenhouse float system where water temperatures were constant at 15, 20, 25, or 30°C, seedling disease caused by *Pythium myriotylum* was correlated with float-water temperature and could be described by quadratic equations ($R^2 = 0.99$) with the lowest level of root necrosis at 15°C. *Pythium* spp.-infected and noninfected seedlings grown in controlled-temperature water baths were transplanted to the field and evaluated for disease development. Previous infection with *P. myriotylum*, regardless of float-temperature regime, failed to alter yield, leaf quality, or root necrosis ($P = 0.05$) in field-grown tobacco.

Greenhouse production of tobacco (*Nicotiana tabacum*) seedlings is replacing the traditional method of seedling production in outdoor seed beds (11). This interest in greenhouse production of transplants is driven by reduced labor requirements and production costs. The direct-seeded float system, where polystyrene seeding trays are filled with a soilless medium and then floated on a common nutrient solution bath (float bay), is the most widespread method of production. The float bay is covered completely by polystyrene trays, which provide an insulating seal between the heated greenhouse air and the water within the float bay. A lack of labeled pesticides to control common seedling diseases in this production system is a growing concern (11,14).

Floating polystyrene seedling trays in a nutrient solution presents some unique challenges to controlling water molds, such as *Pythium* spp. *Pythium* spp. can spread by mycelium within the media or in water by motile, flagellate zoospores (16). Severe

Pythium seedling blight has been observed in float systems in South Carolina and was limited by divisions within the float bays, suggesting that zoospores were the primary method of spread between trays (2).

In ebb-and-flow irrigation systems, *P. aphanidermatum* was more consistently transmitted when inoculum was present in the irrigation solution than when added to the growing medium within the irrigation system (15,17,18). The role of the nutrient solution in the spread of *Pythium* spp. has been confirmed in other hydroponic systems, such as trough culture and the nutrient-film method (13). The role of temperature in the development of *Pythium* root rot is also well documented (1,3,22). In hydroponic culture of spinach, *P. aphanidermatum* and *P. dissotocum* alternated in prominence based on temperature of the nutrient solution and the crop season (5,10). Disease was minimized by management of the temperature of the nutrient solution (5).

The objectives of these studies were to determine the temperature regimes present in commercial greenhouse float-production systems; the role of float nutrient-solution temperature in *Pythium* seedling disease; and the effect of *P. myriotylum* infection of greenhouse-grown tobacco seedlings on *Pythium* root rot in field plantings.

MATERIALS AND METHODS

Greenhouse float-bay microclimate.

The temperature of the greenhouse float water, soilless medium within polystyrene seeding trays, and ambient air 3 cm above

the soil surface was recorded in two tobacco float greenhouses, one each in Florence and Horry Counties, South Carolina. Polystyrene seeding trays containing soilless medium (Carolina's Choice Tobacco Mix, Carolina Soil Company, Kinston, NC) were seeded with pelletized tobacco seed of tobacco cv. K 149. At site 1, float bays were filled with nutrient solution and trays were placed immediately upon the unheated nutrient solution after seeding. At the completion of seeding, the greenhouse was heated according to Clemson Extension recommendations (14). At site 2, nutrient solution was placed within the float bay, the greenhouse ventilation curtains were closed, and the greenhouse was heated 72 h prior to seeding and placement of trays on the nutrient solution. The water within the float bay was heated by convection and solar radiation. Sites 1 and 2 were seeded on 22 January and 2 February 1997, respectively.

Temperatures in each greenhouse were monitored after completion of seeding with Campbell 21X Microloggers (Campbell Scientific, Logan, UT) with copper-constantan fixed thermocouples. Three thermocouples were wired in parallel to a common lead and dispersed >1 m apart within the float bay. Temperatures were averages for nine readings (three replications $\times$ three thermocouples/replication). Temperature values at seeding for each micrologger were confirmed with an Omega digital thermometer (Model HH-25TC, Omega Engineering, Stamford, CT). Temperatures were recorded every 10 min and averaged for each 60-min period. Fertility and insect control were based on Clemson University Extension recommendations (11,14).

Controlled water-temperature experiments. *Pythium* seedling disease was evaluated in a greenhouse float-seedling production system where float nutrient-solution temperatures were held constant at 15, 20, 25, and 30°C. Float bays (30 cm long by 22 cm wide by 12 cm deep) with a 5-liter capacity were constructed from stainless steel, lined with black polyethylene plastic, and placed within Wisconsin water temperature tanks (WWTT). Water temperature within the WWTT was controlled ($\pm 1^\circ\text{C}$) with digital two-stage controllers (Independent Energy Inc., East

Corresponding author: B. Fortnum
E-mail: bfortnm@clemson.edu

Technical contribution No. 4385 of the South Carolina Agricultural Experiment Station. This research was supported in part by a grant from the RJ Reynolds tobacco company.

Accepted for publication 12 November 1999.

Publication no. D-2000-0120-02R
© 2000 The American Phytopathological Society

Greenwich, RI). Polystyrene 288-cell seedling trays (Speedling, Sun City, FL) were cut with a bandsaw to form miniature trays 22 cm long by 18 cm wide by 8 cm deep containing 48 cells each. Air temperature within the greenhouse was maintained between 20 and 28°C. Water used in the miniature float bays was filtered through a deionizing column (Barnstead Combination Column, Barnstead/Thermolyne, Dubuque, IA) and tested for ionic and bicarbonate content (Clemson University Agricultural Services Laboratory, Clemson, South Carolina).

Soilless medium was covered with a polyethylene tarp and fumigated with methyl bromide (0.1 kg/0.2 m³). The tarp was removed 48 h after fumigation and residual fumigant allowed to escape over a 14-day period. Trays were filled with soilless medium (pH 5.6) and seeded with pelletized tobacco seed (cv. K 326) using a

vacuum seeder. Plants were grown for 4 weeks prior to inoculation with *Pythium* spp. Water temperature within each miniature float bay was within $\pm 1^\circ\text{C}$ of the water within the WWTT. Nutrients (250 mg l⁻¹

of N supplied as 50% Ca(NO₃)₂ and 50% Mg(NO₃)₂, 36 mg l⁻¹ of P supplied as KH₂PO₄, and 208 mg l⁻¹ of K supplied as 33% KH₂PO₄ and 67% K₂SO₄) were added to each float bay 14 days after seeding.

Table 1. Source of variation and probability values for main effects and interactions of temperature and fungal inoculation on disease caused by *Pythium myriotylum* and plant-growth parameters in tobacco seedlings grown in a greenhouse float-production system

Source of variation	<i>P</i> > <i>F</i> values ^a		
	Root-necrosis index	Root-growth index	Shoot weight (g)
Trial 1			
Temperature (Temp)	***	**	*
Fungus inoculation (Inoc)	***	***	***
Temp × Inoc	***	***	***
Trial 2			
Temp	***	ns	**
Inoc	***	***	(0.06)
Temp × Inoc	***	***	**

^a ns = not significant at *P* = 0.05; * at *P* = 0.05; ** at *P* = 0.01; and *** at *P* = 0.001.

Fig. 1. Float nutrient solution, soilless media, and interior and exterior air temperatures in two greenhouse float systems where greenhouse air was unheated (site 1) or heated 72 h prior to seeding (site 2).

P. myriotylum was isolated from a diseased tobacco seedling in a greenhouse float system in Walterboro, South Carolina and cultured on V 8 agar (8). Rye grass leaf blades were placed in 250-ml flasks at a 1:10 (wt/vol) dilution in distilled water, autoclaved for 45 min at 145 kPa and

120°C, and cooled, after which the procedure was repeated. *P. myriotylum* was transferred on a colonized agar block to cooled, sterilized leaf blades and incubated for 1 week at 21 to 23°C before use as inoculum. Float water was infested with 1 g (fresh weight) of rye grass by placing

infested leaf blades directly in the nutrient solution of the miniature float bay. Rye grass is frequently used to stabilize soil around recently constructed greenhouses and as a winter cover, and is a likely source of organic matter within float water. Control plants received rye grass blades free of

Fig. 2. Shoot weight, root-growth index, and root necrosis of tobacco seedlings grown in a greenhouse float system where the float nutrient solution was (A–C) infested with *Pythium myriotylum* or (D–F) noninfested and nutrient solution temperatures were constant at 15, 20, 25, or 30°C Trial 1 = □ and Trial 2 = ◇. Regressions based on means.

Pythium spp. that had been treated in a similar fashion.

Plant growth and disease development were evaluated 7, 14, and 21 days following infestation of the float water. Root necrosis was rated on a 0-to-5 linear scale, where 0 = white, near-translucent root tissue that is turgid, with no brown discoloration (necrosis); 1 = trace to 20%; 2 = 21 to 40%; 3 = 41 to 60%; 4 = 61 to 80%; and 5 = 81 to 100% of the root tissue brown in color, with a loss of turgor. Root vigor was rated on a 0-to-10 linear scale, where 0 = no visible root growth following inoculation and a collapse of existing roots; 1 = 1 to 10%; 2 = 11 to 20%; 3 = 21 to 30%; 4 = 31 to 40%; 5 = 41 to 50%; 6 = 51 to 60%; 7 = 61 to 70%; 8 = 71 to 80%; 9 = 81 to 90%; and 10 = 91 to 100% of seedling roots with healthy, vigorous roots, extensive lateral branching, and root length ≥ 15 cm.

A total of 10 root systems were randomly selected from each tray. Roots were washed free of soilless mix by rinsing in tap water and separated into proximal (within the soilless medium plug) and distal (extending out into the float water) portions for a total of 20 root pieces per tray. Root pieces were surface sterilized in 70% ethanol for 10 to 15 s, rinsed twice in sterile distilled water, blotted on sterile paper towels, and placed on *Pythium* spp.-selective medium P₅ARP (12). Root pieces were incubated for 2 days at 21 to 23°C and the number of proximal and distal infected root pieces recorded.

A randomized complete block factorial design was used with four replications per

trial and the trial was repeated once. Data were analyzed using analysis of variance (ANOVA) and regression techniques (19) by JMP and MACSAS (Statistical Analysis System, SAS Institute, Cary, NC).

Field experiments. Tobacco seedlings (cv. K 326) from the controlled water-temperature experiments were used in these studies. Both test sites were located at the Pee Dee Research and Education Center, Florence, South Carolina, on a Norfolk sandy loam soil (75% sand, 17% silt, 8% clay, 0.8% organic matter, pH 5.9) which had been fallow the preceding year. Disk harrowing and in-row subsoiling 35 cm deep preceded all treatments. A total of 20 seedlings were randomly selected from tray cells within each simulated float bay from the previous experiments and transplanted into test plots consisting of single rows, 12.2 m long with a 1.2-m row spacing, on 24 April and 1 June 1997, respectively. All treatments were replicated four times with main plots as float-water temperature and subplot effects as *Pythium* spp. inoculation. The tobacco was maintained using standard agronomic practices (11), with the exception that no nematicide or fungicide was applied and the plots were not replanted to replace missing plants. The plots received irrigation as needed. Mature leaves from all plots were harvested, cured, and weighed. Cured leaf was rated for quality by United States Department of Agriculture government graders and a quality index calculated (6). At the last harvest, 10 plants from each plot row were excavated at random and the root systems rated for *Meloidogyne incognita*

(Kofoid & White) Chitwood-induced root galling (0-to-10 scale, where 0 = no galls, 1 = 1 to 10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60%, 7 = 61 to 70%, 8 = 71 to 80%, 9 = 81 to 90%, and 10 = 91 to 100% of the root tissue galled) and necrosis (0-to-5 scale, where 0 = no necrosis, 1 = trace to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, and 5 = 81 to 100% of the root tissue brown in color) (4).

RESULTS

Greenhouse float-bay microclimate.

Float nutrient-solution temperature at seeding was higher in the heated greenhouse (17°C) than in the unheated greenhouse (11°C). Polystyrene seeding trays formed an insulating barrier between the heated air and the nutrient solution and water temperatures remained lower at site 1 than site 2 throughout the experiment (Fig. 1). Soilless medium and air temperatures varied diurnally, but not the nutrient solution (Fig. 1). The temperatures of the medium within a seeding tray, during periods of peak solar radiation (hour 9 to 18), were similar (15 to 25 February) to the ambient air temperatures 3 cm above the soil surface (Fig. 1). Water temperature at completion of seedling production was 17.7°C for site 1 and 19.5°C for site 2. Seedlings were used for field production from both greenhouse systems.

Controlled water-temperature experiments. When solution temperatures were held constant at 15, 20, 25, or 30°C, *Pythium* seedling blight progressed from no detectable root necrosis 7 days after inoculation to severe root necrosis at 21 days. The root-necrosis index, root-growth index, and shoot weight were altered by float solution temperature (T) and fungus inoculation (F), and $T \times F$ interactions were observed for all plant-growth parameters and root-necrosis index ($P \leq 0.01$; Table 1).

Seedling root necrosis caused by *P. myriotylum* increased with float-solution temperature and could be described by a quadratic equation ($R^2 > 0.99$ at both sites, $P < 0.05$), with the lowest level of root necrosis at 15°C and the highest at 30°C (Fig. 2). Root growth of inoculated plants was negatively correlated with float-solution temperature and could be described by cubic and quadratic relationships ($R^2 > 0.99$ at both sites, respectively ($P \leq 0.05$)). Root growth generally increased in a linear fashion with increasing float-solution temperature in noninoculated float bays ($R^2 = 0.71$ and 0.99, respectively; Fig. 2). Shoot weight of inoculated plants was negatively correlated with float-water temperature and could be described by linear and cubic relationships ($R^2 > 0.99$ at both sites, $P \leq 0.05$), respectively. Shoot weight increased in a linear fashion with increasing float-solution temperature in noninoculated float bays for site 1 ($R^2 = 0.98$) and a cubic re-

Fig. 3. Percent of proximal (□ = within the soilless medium) and distal (◇ = extending out into the float water) roots infected with *Pythium myriotylum* in a greenhouse float system where the float-water temperature was held constant at 15, 20, 25, or 30°C. Regressions based on means from trial 1.

lationship for site 2 ($R^2 = 0.99$). Although higher float-solution temperatures generally increased root and shoot growth, seedlings from all noninoculated float bays were suitable for transplanting irrespective of float-solution temperature.

Root infection by *P. myriotylum* was influenced by float-solution temperature ($P < 0.001$), root location (proximal versus distal; $P < 0.001$), and a temperature $\times$ root location interaction was observed ($P = 0.01$; trial 1, Fig. 3).

Field experiments. Neither float-solution temperature nor infestation with *P. myriotylum* altered cured-leaf yield, leaf quality, leaf value, or root necrosis in field tobacco in trial 1, but cured-leaf yield and value were lower ($P \leq 0.025$) in tobacco grown from seedlings produced in float solution at 15°C than other temperatures in trial 2 (Table 2). Galling caused by root-knot nematodes was lower in plants grown in float solution infested with *P. myriotylum* relative to plants grown in noninfested solution ($P \leq 0.025$; Table 2). Infestation of float solution with *P. myriotylum* did not alter yield, quality, value, or root necrosis in field tobacco grown from these seedlings (trial 2, $P = 0.05$).

DISCUSSION

Many tobacco producers in South Carolina close the ventilation system and heat the interior of the greenhouse prior to seeding in order to heat the float solution. Warmer float solutions generally increased seedling shoot weight and root growth in

our trials, but it may also increase the occurrence of *Pythium* seedling disease. Excellent seedlings also were produced in nonpreheated greenhouses, so preheating float water may not be necessary to produce high-quality transplants.

The severity of *Pythium* seedling disease increased as float solution temperatures increased above 15°C. The first report of *Pythium* seedling disease in South Carolina (2) occurred in a greenhouse where the producer was attempting to grow a second crop of seedlings late in the spring. Ground and float water temperatures likely were higher later in the spring; therefore, an environment conducive for *P. myriotylum* may have favored development of the epidemic. *Pythium* disease is common in float bays late in the spring when greenhouses are partially emptied and uncovered bays gain heat from radiant energy.

Seedlings infected with *P. myriotylum* showed reduced shoot weight and were yellow and partially wilted. Although the seedlings recovered when planted in the field with no detectable loss in cured-leaf yields, the unthrifty appearance of the infected seedlings within the float bay would have reduced the value of the seedling in a commercial seedling production system.

Pythium spp. with lower cardinal temperatures, such as *P. ultimum*, have been found in commercial potting medium used for tobacco transplant production (7). *P. ultimum* causes disease in traditional seed-bed production (16) and has been observed in other greenhouse production systems

(20). Although not previously observed in South Carolina float bays, *Pythium* spp. have been reported in other greenhouse systems (7,10,15) and may cause damage in float systems when solution temperatures are less than 20°C. *Pythium* spp. were recovered from roots in infested and noninfested float bays in trial 2. Fungus gnats (*Bradysia impatiens*) observed in trial 2 are a likely source of contamination with nonpathogenic *Pythium* spp. (9). Normally, greenhouse media for tobacco seedling production will have a resident microflora (7). Although the medium from trial 2 had a resident microflora of nonpathogenic *Pythium* spp., disease development in bays inoculated with *P. myriotylum* was identical in both trials. This suggests that the temperature of the float water is a key element in the development of *Pythium* seedling disease, with or without a resident microflora.

Greenhouse float-bed production of tobacco seedlings is common in the southeastern United States and is becoming more common in other tobacco-production areas of the world. Greenhouse float-seedling technologies in more tropical locations might increase the occurrence and severity of *Pythium* disease.

LITERATURE CITED

1. Adams, P. B. 1971. *Pythium aphanidermatum* oospore germination as affected by time, temperature, and pH. *Phytopathology* 61:1149-1150.
2. Anderson, M. G., Fortnum, B. A., and Martin, S. B. 1997. First report of *Pythium myriotylum*

Table 2. Yield, value, and quality of flue-cured tobacco grown from seedlings produced in a greenhouse float-production system where float water temperatures were controlled and float water was infested with *Pythium myriotylum*^a

Solution temperature (°C)	Inoculation	Yield (kg/ha)	Quality index ^b	Value (\$/ha)	Root gall index ^c	Root necrosis index ^d
Trial 1						
15	—	3,360	44	12,627	1.2	1.1
15	+	3,721	43	13,983	1.4	1.0
20	—	3,368	43	12,523	1.2	1.0
20	+	3,438	44	12,933	1.4	0.9
25	—	3,312	46	12,508	1.4	1.0
25	+	3,448	42	12,938	1.2	1.2
30	—	3,284	43	12,281	0.8	0.9
30	+	3,246	41	12,133	1.3	1.0
$P > F$						
Temperature (Temp)	...	ns	ns	ns	ns	ns
<i>P. myriotylum</i>	...	ns	ns	ns	ns	ns
Temp $\times$ <i>P. myriotylum</i>	...	ns	ns	ns	ns	ns
Trial 2						
15	—	1,332	28	4,456	2.0	1.1
15	+	1,032	26	3,572	1.1	1.1
20	—	1,832	29	6,237	2.5	1.2
20	+	1,931	30	6,570	2.4	1.2
25	—	1,576	30	5,417	2.6	1.1
25	+	1,612	27	5,441	2.0	1.2
30	—	1,612	30	5,577	3.3	1.2
30	+	1,556	24	5,254	1.8	1.1
$P > F$						
Temp	...	**	ns	**	ns	ns
<i>P. myriotylum</i>	...	ns	ns	ns	**	ns
Temp $\times$ <i>P. myriotylum</i>	...	ns	ns	ns	ns	ns

^a Significance indicated by * at $P = 0.05$, ** at $P \leq 0.025$, and *** at $P \leq 0.01$.

^b Quality index based on government grade on a scale of 1 to 100, where 100 is best.

^c Root-knot nematode galling was rated from 0 to 10 on a linear scale, where 0 = no galling and 10 = 100% of the root tissue galled.

^d Root necrosis was rated on a 0 to 5 linear scale, where 0 = no necrosis and 5 = 100% of the root tissue necrotic.

- lum* in a tobacco seedling float system in South Carolina. *Plant Dis.* 81:227.
3. Ayers, W. A., and Lumsden, R. D. 1975. Factors affecting production and germination of oospores of three *Pythium* species. *Phytopathology* 65:1094-1100.
 4. Barker, K. R., Townshend, J. L., Byrd, G. W., Thomason, I. J., and Dickson, D. W. 1986. Determining nematode population responses to control agents. Pages 283-296 in: *Methods for Evaluating Pesticides for Control of Plant Pathogens*. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
 5. Bates, M. L., and Stanghellini, M. E. 1982. Root rot of hydroponic-grown spinach caused by *Pythium aphanidermatum* and *Pythium dissotocum*. *Plant Dis.* 68:989-991.
 6. Bowman, D. T., Tart, A. G., Wernesman, E. A., and Corbin, T. C. 1988. Revised North Carolina grade index for flue-cured tobacco. *Tob. Sci.* 32:39-40.
 7. Cartwright, D. K., Spurr, H. W., Jr., and Shew, H. D. 1995. Commercial potting medium as a source of *Pythium* causing disease on tobacco transplants. *Plant Dis.* 79:538.
 8. Dhingra, O., and Sinclair, J. B. 1985. *Basic Plant Pathology Methods*. CRC Press, Inc. Boca Raton, FL.
 9. Gardiner, R. B., Jarvis, W. R., and Shipp, J. L. 1990. Ingestion of *Pythium* spp. by larvae of the fungus gnat *Bradysia impatiens* (Diptera: Sciaridae). *Ann. Appl. Biol.* 116:205-212.
 10. Gold, S. E., and Stanghellini, M. E. 1985. Effects of temperature on *Pythium* root rot of spinach grown under hydroponic conditions. *Phytopathology* 75:333-337.
 11. Gooden, D. T., Rideout, J. W., Christenbury, G. D., Loyd, M. I., Manley, D. G., Martin, S. B., and Stanton, L. A. 1992. *South Carolina Tobacco Grower's Guide—1993*. Coop. Ext. Circ. EC 569.
 12. Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Dis.* 70:1038-1043.
 13. Jenkins, S. F., Jr., and Averre, C. W. 1983. Root diseases of vegetables in hydroponic culture systems in North Carolina greenhouses. *Plant Dis.* 67:968-970.
 14. Rideout, J. W., Gooden, D. T., Christenbury, G. D., Fortnum, B. A., Manley, D. G., and Suttin, R. 1994. Tobacco transplant production in greenhouses. *Clemson Univ. Coop. Ext. Circ.* 682.
 15. Sanogo, S., and Moorman, G. W. 1993. Transmission and control of *Pythium aphanidermatum* in an ebb-and-flow subirrigation system. *Plant Dis.* 77:287-290.
 16. Shew, H. D. 1991. Pages 20-21 in: *Compendium of Tobacco Diseases*. G. Lucas and H. D. Shew, eds. APS Press, St. Paul, MN.
 17. Stanghellini, M. E., and Rasmussen, S. L. 1994. Hydroponics a solution for zoospore pathogens. *Plant Dis.* 78:1129-1138.
 18. Stanghellini, M. E., White, J. G., Tomlinson, J. A., and Clay, C. 1988. Root rot of hydroponically grown cucumbers caused by zoospore-producing isolates of *Pythium intermedium*. *Plant Dis.* 72:358-359.
 19. Steel, R. G. D., and Torrie, J. H. 1960. *Principles and Procedures of Statistics*. McGraw-Hill, New York.
 20. Stephens, C. T., and Powell, C. C. 1982. *Pythium* species causing damping-off of seedling plants in Ohio greenhouses. *Plant Dis.* 66:731-733.
 21. Thomson, T. V.B., Athow, K. L., and Laviolette, F. A. 1971. The effect of temperature on the pathogenicity of *Pythium aphanidermatum*, *P. debaryanum*, and *P. ultimum* on soybean. *Phytopathology* 61:933-935.