# Growth of *Phytophthora fragariae* on Various Clarified Natural Media and Selected Antibiotics

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**ABSTRACT**

George, S. W., and Milholland, R. D. 1986. Growth of *Phytophthora fragariae* on various clarified natural media and selected antibiotics. Plant Disease 70: 1100-1104.

Clarified frozen pea agar had outstanding clarity and supported excellent mycelial growth for *Phytophthora fragariae* races A-1, A-2, A-4, and A-6, growth that was significantly greater than that on cornmeal agar. Good to excellent mycelial growth of races A-2 and A-6 was recorded on hymexazol, nystatin, and PCNB concentrations through 100, 250, and 500 μg/ml, respectively, and on penicillin and vancomycin through 1,000 μg/ml. These races were very sensitive to benomyl at 100 μg/ml and to streptomycin sulfate and rose bengal at the lowest concentrations tested. Nystatin and rifamycin severely affected zoospore germination at the lowest concentrations tested, but suppression was not as great for germ tube growth. Zoospore germination and growth was very good on hymexazol, vancomycin, and penicillin at concentrations up to 1,000 μg/ml. Gallic acid at 500 μg/ml and rifamycin at 10 μg/ml provided excellent suppression of bacterial contaminants. Maximum recovery of *P. fragariae* was obtained when root segments were surface-disinfected in 70% ethanol, then plated on a selective medium containing benomyl, pimaricin, hymexazol, and rifamycin and incubated for 4–7 days at 20 C.

*Phytophthora fragariae* Hickman, causal agent of the red stele disease of strawberry (*Fragaria* × *ananassa*), is a major factor limiting fruit production in North Carolina and many areas around the world (12). Several methods have been tried, but isolation of *P. fragariae* from diseased tissue is difficult (1,8,10).

Isolations from root tissue that has been infected for 1–2 mo often fail because secondary invaders such as *Pythium* spp. mask the slower growing *P. fragariae*. Thus, a real need exists for investigating alternative isolation techniques that could enhance recovery of *P. fragariae*.

Paper 10262 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695-7601.

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Portion of a thesis submitted by the first author in partial fulfillment of the requirements for the Ph.D. degree, North Carolina State University.

Accepted for publication 21 July 1986 (submitted for electronic processing).

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Information concerning the sensitivity of *P. fragariae* to selected antibiotics is limited (6,7). Recognizing the differential sensitivity of mycelium or zoospores to selected antibiotics is essential to the development of a more effective medium for isolating this pathogen. Also, an easily prepared clarified basal medium that would maximize growth and facilitate aseptic microscopic observation of *P. fragariae* is needed. Information on the performance of individual races of *P. fragariae* on various clarified basal media is also important in the event that certain races might require special media amendments for vigorous mycelial growth.

The present studies were conducted to determine the basal (natural) medium that would result in the optimal combination of medium clarity and maximum mycelial growth of the five major races of *P. fragariae* in the eastern United States, the effects of individual antibiotics on mycelial growth and zoospore germination of *P. fragariae* as well as their ability to suppress bacterial and fungal contaminants, and the influence of different isolation techniques on the recovery of this pathogen.

## MATERIALS AND METHODS

**Isolates of *P. fragariae*.** Cultures of *P. fragariae* representing American races A-1, A-2, A-3, and A-4 were obtained from the American Type Culture Collection, Rockville, MD, as ATCC isolates 13973, 13974, 13977, and 11109, respectively. An isolate of race A-6 was supplied by J. L. Maas, Beltsville, MD. Stock cultures were maintained in the dark on either frozen lima bean agar (FLBA) or oatmeal agar (OMA) at 20 C.

**Basal medium.** The natural media evaluated in this study were FLBA blended or autoclaved, frozen pea agar (FPA) blended or autoclaved, frozen green bean agar (FGBA) blended or autoclaved, frozen strawberry agar (FSA) blended or autoclaved, V-8 juice agar (V-8A), cornmeal agar (CMA), and water agar (WA). FLBA (blended but not clarified) was used as the control. The blended media were prepared by placing 150 g of tissue in 500 ml of deionized water and blending at high speed for 3 min. The mixture was squeezed through eight to 12 layers of cheesecloth, then vacuum-filtered through a piece of coarse filter paper covered by a 2.5-cm layer of Celite 545 (C212, Fisher Scientific Co., Springfield, NJ). The autoclaved media were prepared by placing 150 g of the tissue in 1 L of deionized water and then autoclaved at 20 psi for 2 hr. The resultant liquid was poured through four layers of cheesecloth, then through coarse filter paper and Celite 545 as described. Agar (15 g) was added to each natural medium, then heated in a steamer for 30 min. Each volume was restored to 1 L; 0.5 ml of antifoam spray (Antifoam A Spray, Dow Corning Corp., Midland, MI) was added to each liter and the pH was adjusted to 6.5 with 1 N HCl or NaOH. Last, the media were poured into 200-ml bottles and autoclaved for 30 min at 20 psi.

Fifteen milliliters of each medium was dispensed into plastic petri dishes (100 × 15 mm), and a 4-mm disk cut from the outer margin of 9- to 14-day-old FLBA cultures of race A-1 or A-4 was placed mycelium-down in the center of each plate. Plates were incubated for 8 days in the dark at 20 C. A randomized complete block design with five replicates was used.

An additional study was conducted to determine the influence of clarified and nonclarified basal medium on mycelial growth of five races of *P. fragariae*. Clarified media of FLBA, FPA, and

FGBA were compared with nonclarified CMA and blended FLBA (control). Media were prepared as in the previous test, except the principal vegetable ingredients, i.e., beans or peas, used in the autoclaved treatments were autoclaved for only 30 min rather than for 2 hr. Plates were inoculated with races A-1, A-2, A-3, A-4, or A-6 and incubated as described for the previous test. A randomized complete block design with four replicates was employed.

Overall analysis of the data as well as analysis by race and by medium with ANOVA procedures were done to calculate the protected LSDs used in mean separations for both studies.

**Antibiotic study.** The term antibiotic is defined in this article as any chemical substance capable of inhibiting or preventing the growth of a microorganism. Races A-2 and A-6 were grown on clarified FPA (peas autoclaved for 30 min, 2% agar, pH adjusted to 6.5 ±0.1) containing antibiotics at the following concentrations (in micrograms of active ingredients per milligram): benomyl at 10, 100, 500, and 1,000; hymexazol at 25, 100, 500, and 1,000; iprodione at 10, 100, 250, and 500; nystatin (mycostatin, 100,000 units per gram) at 25, 50, 100, and 250; PCNB at 25, 100, 200, and 500; pimaricin at 10, 50, 100, and 250; propamocarb (hydrochloride) at 10, 100, 500, and 1,000; chloramphenicol at 10, 100, 250, and 500; gallic acid at 200, 400, 600, and 1,000; penicillin (benzylpenicillin-potassium salt, 1,590 units per milligram) at 50, 200, 500, and 1,000; polymyxin B sulfate (7,600 USP units per milligram) at 10, 50, 250, and 500; rifamycin (828 mg rifamycin SV acid per gram) at 10, 100, 500, and 1,000; rose bengal at 30, 150, 300, and 500; streptomycin sulfate (750 units per milligram) at 25, 50, 100, and 250; and vancomycin (hydrochloride, 1,030 μg/ml) at 100, 200, 500, and 1,000. The concentrations for each antibiotic were chosen to allow growth at lower concentrations and to establish an upper tolerance limit for the antibiotic under investigation.

The individual antibiotics were dissolved in 95% ethanol, dilutions were made, and 1 ml of each antibiotic treatment was incorporated into 100 ml of FPA at 45 C. Each antibiotic-medium combination was dispensed into 100-mm-diameter plastic petri dishes (15 ml in each), which were stored overnight in a microvoid hood with the fan running to speed evaporation of the ethanol. Growth was compared with that on FPA amended with an equivalent amount of ethanol.

A 7-mm-diameter plug from a 16-day-old FLBA culture of race A-2 or A-6 was inverted and placed in the center of each plate. Six plates per race per antibiotic combination were arranged in a randomized complete block design. After 14 days of incubation at 20 C in darkness, colony diameter, mycelial density (sparse to dense), and clarity of the medium were assessed for each plate. The average reading in the first two categories for six antibiotic-containing plates of each race was divided by the average of the six control plates for that race.

Eight antibiotics were also tested for their effects on germination and germ tube elongation of zoospores of race A-2. These included (concentrations in micrograms of active ingredient per milliliter) hymexazol at 25, 50, 100, and 200; nystatin at 25, 50, 100, and 250; PCNB at 25, 100, 150, and 200; gallic acid at 200, 300, 400, and 500; penicillin at 100, 250, 500, and 1,000; polymyxin B sulfate at 25, 50, 75, and 100; rifamycin at 10, 25, 50, and 75; and vancomycin at 100, 250, 500, and 1,000.

The individual antibiotics were dissolved in 95% ETOH, and dilutions were made before 0.5 ml of each treatment was incorporated into 50 ml of FPA (prepared as described in the antibiotic-mycelium study) at 45 C. About 0.1 ml of a motile zoospore suspension ($4.3 \times 10^4$ zoospores per milliliter) of race A-2 was placed in each plate. After 24 hr of incubation at 11 C under continuous incandescent light (about 2,700 lux), 100 zoospores were examined for germination and the lengths of 25 germ tubes were determined for each plate. Three plates per treatment per experiment were arranged in a randomized complete block design, and the entire experiment was repeated.

The mean percent germination and germ tube length for each treatment in the two experiments was expressed as a percentage of the results obtained from the six control plates. The same treatments listed in the antibiotic-zoospore germination study were also evaluated for their abilities to retard bacterial and unwanted fungal growth. About 0.1 ml of a nonsterile soil leachate (10 g of loam topsoil per liter of deionized water) was placed in each plate, then the plate was rotated to better distribute the liquid. After 72 hr of incubation at 20 C under continuous fluorescent light (about 2,500 lux), visual ratings (expressed as percentage of control) were made of the extent of bacterial and fungal growth. Replicates and experimental design were the same as in the antibiotic-zoospore germination study.

**Isolation.** Oospores of *P. fragariae* were observed microscopically in roots of Tennessee Beauty plants 17 days after inoculation with race A-6. Nonsterile soil leachate was dispensed into 60-mm-diameter plastic petri dishes (6 ml per plate). About 200 segments (1 cm long) of the infected root tissue were placed in each plate. After 48 hr of incubation at 11 C, an average of $8 \times 10^3$ zoospores per milliliter were present in the flooding liquid. Disks (5 mm in diameter) of leaf tissue were removed from 4-wk-old Tennessee Beauty plants and immersed (20 disks per plate) in the soil leachate containing the root segments. After 48 hr of incubation at 15 ± 2 C, the leaf disks were surface-disinfested and plated on the selective medium described later. Attempts to isolate the fungus from root segments (1 cm long) of Tennessee Beauty plants were made 17 days after inoculation with races A-1 and A-2. Oospores characteristic of *P. fragariae* were also observed in the roots of these plants.

The leaf disks and root segments to be plated directly were surface-disinfected in either 50% teepol (Teepol L, 27% a.i., Shell) for 60 sec (8) or 70% ethanol (ETOH) for 10 sec, both followed by two rinses in sterile deionized water. The tissue was air-dried before plating on a basal medium that contained benomyl, pimaricin, hymexazol, and rifamycin at 10, 10, 50, and 10 μg/ml, respectively (8). These antibiotics were dissolved in 95% ETOH and incorporated into FPA as described for the antibiotic study. The plates were incubated under continuous incandescent light (about 2,700 lux) at either 11 or 20 C for 4–7 days. Five plates per treatment (five pieces of plant tissue per plate) were arranged in a randomized complete block design. Identification of the coenocytic hyphae emerging from infected root segments and leaf disks was made by inoculating roots of Tennessee Beauty plants with 7-day-old mycelial mats produced from these hyphae and examining roots for oospores characteristic of *P. fragariae* after 14 days of incubation at 15 ± 2 C.

## RESULTS

**Basal medium.** Data for races A-1 and A-4 indicated that FLBA autoclaved, FGBA blended, and FPA both blended and autoclaved gave the best combination of mycelial growth and medium clarity. Colony diameters (in millimeters) for races A-1 and A-4 on FLBA, FGBA, and FPA (blended or autoclaved) were 71 and 48, 69 and 42, 74 and 36, and 70 and 31, respectively. Colony diameters for races A-1 and A-4 were greater in these media than in CMA (61 and 29 mm), V-8A (64 and 31 mm), and WA (39 and 9 mm). Mycelial growth on the nonclarified control medium was 74 and 36 mm for races A-1 and A-4, respectively. Clarity of the control medium was very poor.

Statistical analysis of data from all five races indicated a significant ($P = 0.01$) race × medium interaction for the variable colony diameter, so response to the various media was analyzed by individual race. FPA (autoclaved 30 min) resulted in significantly ($P = 0.05$) greater colony diameters for races A-2 and A-6 (Table 1). It also provided the best combination of medium clarity and mycelial growth for the five races of *P. fragariae* tested. This medium also resulted in significantly ($P = 0.05$) greater

colony diameters for all five races tested than did CMA, with increases of 200% recorded for races A-2 and A-6 and 300% for A-4. Mean density of mycelium was not significantly different at $P = 0.05$ and a significant ($P = 0.01$) medium × autoclaved or blended interaction was evident for all three variables.

**Effects of selected antibiotics on mycelial growth.** Considerable variation was observed in the amount of mycelial growth in the presence of individual antibiotics (Table 2). Good to excellent growth was recorded for *P. fragariae* races A-2 and A-6 on hymexazol through 100 μg/ml of medium, but these races were very sensitive to benomyl at 100 μg/ml of medium and to streptomycin sulfate and rose bengal even at the lowest concentrations tested. Colony diameters of A-2 and A-6 for these three antibiotic concentrations were 1 and 2, 9 and 11, and 24 and 24 mm, respectively. Races A-2 and A-6 showed similar responses to most of the antibiotics tested.

**Effects of selected antibiotics on zoospore germination and growth.** Nystatin and rifamycin reduced germination of zoospores of *P. fragariae* even at the lowest concentrations tested (Table 3). Increasing the concentration of these two antibiotics to 250 and 75 μg/ml, respectively, had little or no effect on percent germination; however, these antibiotics did not appear to suppress germ tube growth as drastically as zoospore germination. Percent germination of race A-2 was strongly inhibited (18%) by gallic acid at 500 μg/ml. Zoospore germination and growth was very good on hymexazol, vancomycin, and penicillin through concentrations of 100, 1,000, and 1,000 μg/ml of medium, respectively.

**Effects of selected antibiotics on suppression of fungal and bacterial contaminants.** Bacterial contamination was extremely severe in the control and various antibiotic treatments (Table 3) after 72 hr; however, only 2% of the plates showed any evidence of fungal contamination (*Rhizoctonia* sp.). Poor bacterial suppression was observed for nystatin, PCNB, gallic acid, penicillin, and vancomycin at concentrations through 250, 200, 300, 1,000, and 1,000 μg/ml of medium, respectively, whereas moderate suppression was recorded for hymexazol, polymyxin B sulfate, and gallic acid at concentrations of 200, 75, and 400 μg/ml of medium, respectively. Excellent suppression of bacterial contaminants was observed for gallic acid at 500 μg/ml of medium and for rifamycin at 10 μg/ml, the lowest concentration tested.

**Effects of isolation techniques.** Considerable variation was observed in percent recovery of *P. fragariae* in response to various isolation techniques (Table 4). Surface disinfection with 70% ETOH increased recovery of the target organism while reducing the percentage of colonies contaminated with bacteria. Teepol was detrimental to the growth of *P. fragariae*, particularly from leaf disks. There was little difference between the teepol or ETOH treatments in the bacterial contamination of plant tissue pieces that did not support growth of the target organism.

Incubation at 20 C resulted in somewhat greater recovery of *P. fragariae*, with a lower percentage of colonies showing bacterial contamination, than did incubation at 11 C. Incubation temperature had little effect on bacterial contamination in the absence of the target organism.

Mean recovery from root segments was substantially greater than from leaf disks at both 11 and 20 C (Fig. 1) primarily because of the deleterious

**Table 1.** Influence of clarified and nonclarified basal media on mycelial growth of races A-1, A-2, A-3, A-4, and A-6 of *Phytophthora fragariae*[v]

| Treatment | Colony diameter (mm)[w,x] | | | | | Medium clarity[y] |
|---|---|---|---|---|---|---|
| | A-1 | A-2 | A-3 | A-4 | A-6 | |
| Clarified | | | | | | |
| Autoclaved | | | | | | |
| FLBA | 71 bc | 29 b | 11 b | 32 c | 26 c | Good |
| FGBA | 58 d | 14 cd | 8 d | 11 e | 13 e | Excellent |
| FPA | 74 a | 36 a | 11 b | 36 b | 34 a | Excellent |
| Blended | | | | | | |
| FLBA | 71 bc | 31 b | 11 b | 43 a | 29 b | Fair |
| FGBA | 60 d | 29 b | 10 bc | 26 d | 16 d | Excellent |
| FPA | 73 ab | 16 c | 7 d | 24 d | 14 de | Fair |
| Nonclarified | | | | | | |
| CMA | 58 d | 13 d | 9 cd | 12 e | 14 de | Excellent |
| Control[z] | 72 bc | 31 b | 32 a | 34 bc | 35 a | Very poor |

[v] Data are means of four plates per treatment.
[w] Colony diameters recorded after 8 days of incubation at 20 C.
[x] Means in the same column followed by the same letter are not statistically different ($P = 0.05$). Protected LSDs for mean separation in races A-1, A-2, A-3, A-4, and A-6 are 2.2, 2.6, 2.1, 3.4, and 2.6, respectively.
[y] Races A-1, A-2, A-3, A-4, and A-6 combined.
[z] Control = FLBA (blended, not clarified).

**Table 2.** Influence of individual antibiotics on mycelial growth of races A-2 and A-6 of *Phytophthora fragariae*[a]

| Antibiotic | Rate (μg/ml)[b] | Colony diameter[c] (% of control) | | |
|---|---|---|---|---|
| | | A-2 | A-6 | Mean |
| Benomyl | 10 | 80 | 74 | 77 |
| | 100 | 2 | 2 | 2 |
| Hymexazol | 25 | 90 | 81 | 86 |
| | 100 | 66 | 76 | 71 |
| | 500 | 2 | 2 | 2 |
| Pimaricin | 10 | 70 | 57 | 64 |
| | 50 | 6 | 8 | 7 |
| Rifamycin | 10 | 58 | 72 | 65 |
| | 100 | 14 | 17 | 16 |
| Chloramphenicol | 10 | 92 | 82 | 87 |
| | 100 | 10 | 15 | 13 |
| Nystatin | 25 | 107 | 95 | 101 |
| PCNB | 25 | 102 | 96 | 99 |
| Penicillin | 50 | 98 | 102 | 100 |
| Polymixin B sulfate | 50 | 80 | 98 | 89 |
| | 250 | 40 | 21 | 31 |
| | 500 | 15 | 17 | 16 |
| Streptomycin sulfate | 25 | 9 | 11 | 10 |
| Gallic Acid | 400 | 70 | 79 | 75 |
| | 600 | 13 | 45 | 29 |
| | 1,000 | 1 | 14 | 8 |
| Rose bengal | 30 | 24 | 24 | 24 |
| | 300 | 3 | 12 | 8 |
| Propamocarb | 10 | 48 | 49 | 49 |
| Vancomycin | 1,000 | 63 | 80 | 72 |
| Iprodione | 10 | 101 | 96 | 99 |
| | 250 | 48 | 47 | 48 |
| | 500 | 13 | 15 | 14 |

[a] Data are means of six plates per treatment.
[b] Antibiotic concentrations at which little or no change occurred in fungal response, compared with either lesser or greater concentrations of the same antibiotic, are presented.
[c] Colony diameters recorded 14 days after incubation at 20 C.

**Table 3.** Influence of individual antibiotics on zoospore germination and germ tube elongation of race A-2 of *Phytophthora fragariae* and on suppression of bacterial contaminants

| Antibiotic | Rate (μg/ml) | Percent germination (% of control) | Germ tube length (% of control) | Bacterial contamination (% of control) |
|---|---|---|---|---|
| Nystatin | 25[a] | 40[b] | 63[c] | 100[d] |
| PCNB | 200 | 78 | 47 | 100 |
| Hymexazol | 100 | 88 | 41 | 63 |
| Polymyxin B sulfate | 100 | 66 | 51 | 79 |
| Gallic acid | 300 | 89 | 65 | 96 |
| Rifamycin | 10 | 47 | 61 | 0 |
| Penicillin | 1,000 | 105 | 96 | 100 |
| Vancomycin | 1,000 | 98 | 45 | 79 |

[a] Maximum concentration of antibiotic that can be used without adverse effects on germination and growth.
[b] Mean from six plates (three per experiment), 100 zoospores counted per plate. Expressed as percent of control (basal medium + ETOH).
[c] Mean from six plates (three per experiment), 10 germ tubes measured per plate. Expressed as percent of control (basal medium + ETOH).
[d] Mean from six plates (three per experiment), expressed as percent of control (basal medium + ETOH); 100% indicates no bacterial suppression, whereas 0% denotes complete suppression.

**Table 4.** Influence of plant tissue, surface disinfectant, and incubation temperature on percent recovery of *Phytophthora fragariae* from greenhouse-inoculated plants[a,b]

| | Recovery of *P. fragariae* (%) | | | Colonies of *P. fragariae* contaminated with bacteria (%) | | | Plant tissue pieces contaminated with bacteria, no *P. fragariae* detected (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| Temperature Technique | Teepol[c] | ETOH[d] | Plant tissue mean | Teepol | ETOH | Plant tissue mean | Teepol | ETOH | Plant tissue mean |
| 11 C | | | | | | | | | |
| Leaf disk | 4 | 28 | 16 | 100 | 100 | 100 | 96 | 72 | 84 |
| Root piece | 20 | 32 | 26 | 0 | 0 | 0 | 32 | 40 | 36 |
| Temp. mean | ··· | ··· | 21 | ··· | ··· | 50 | ··· | ··· | 60 |
| 20 C | | | | | | | | | |
| Leaf disk | 4 | 36 | 20 | 100 | 33 | 67 | 96 | 64 | 80 |
| Root piece | 28 | 32 | 30 | 0 | 13 | 7 | 36 | 56 | 46 |
| Temp. mean | ··· | ··· | 25 | ··· | ··· | 37 | ··· | ··· | 63 |
| Disinfectant mean | 14 | 32 | ··· | 50 | 37 | ··· | 65 | 58 | ··· |

[a] Data are means of five plates per treatment (five pieces of plant tissue per plate).
[b] Isolation plates were incubated under continuous incandescent light (about 2,700 lux) at either 11 or 20 C for 4–7 days. The isolation medium consisted of FPA amended with the following antibiotics (concentrations in μg/ml): benomyl 10, pimaricin 10, hymexazol 50, and rifamycin 10.
[c] Plant tissues immersed in 50% teepol for 60 sec.
[d] Plant tissues immersed in 70% ethanol (ETOH) for 10 sec.

effect of teepol when leaf disks were employed. Bacterial contamination of colonies of *P. fragariae* was a much greater problem at both incubation temperatures with the leaf disk technique than with root segments.

The identity of coenocytic hyphae emerging from infected root segments and leaf disks was confirmed when oospores characteristic of *P. fragariae* were observed in the stelar tissue of Tennessee Beauty plants 14 days after inoculation with mycelial mats produced from these hyphae.

## DISCUSSION

Various synthetic and natural media have been used to grow *P. fragariae* in culture (4,5) or used as the basal medium amended with antibiotics for isolation purposes (7). However, none of these media were clarified, and their opacity makes rapid identification of the target organism difficult if not impossible. In our studies, frozen peas, lima beans, and green beans provided good bases for clarified natural media. Colony diameters for the five races of *P. fragariae* tested on FPA (autoclaved for 30 min) was significantly greater than on cornmeal agar, a basal medium often used in selective media designed to isolate *Phytophthora* spp. (3,6,9). It is important to note, however, that clarification seemed to remove nutrients vital to the vigorous growth of race A-3, thus further testing of clarified media or use of nonclarified media would be required if this race were predominant in a given region.

The difficulties in routinely isolating *P. fragariae* by plating root pieces on various media are well documented

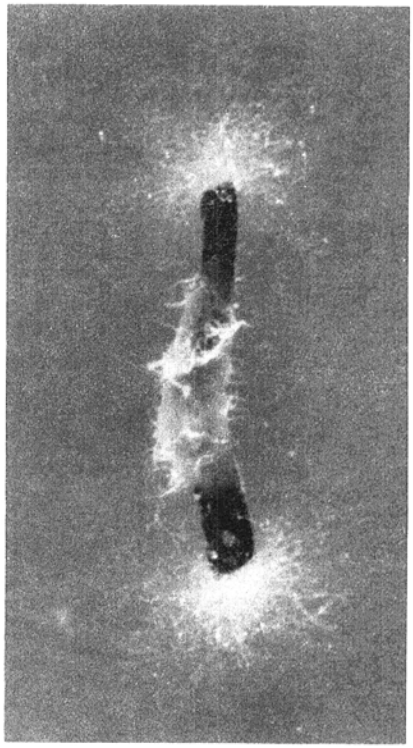

**Fig. 1.** Growth of *Phytophthora fragariae* from infected root tissue of the cultivar Tennessee Beauty 14 days after placement on a selective medium.

(1,8,11). The particularly detrimental effect of teepol on recovery of *P. fragariae* from leaf disks strongly suggests the need to tailor surface-disinfection techniques to a given isolation procedure, e.g., leaf disk, root piece, etc. Our data also indicate that incubation at 20 C, the temperature most favorable for mycelial growth of the target organism (12), provided maximum recovery of *P. fragariae* with minimum bacterial contamination. Bacterial contamination was such a serious problem with the leaf disk isolation technique that perhaps one of the fungicides in the selective medium tested should be replaced with one or more materials with antibacterial properties. Thus, it is evident that no single combination of surface disinfectant, selective medium, and incubation temperature is best for widely differing isolation techniques.

On the basis of our data, the types and concentrations of antibiotics selected can now be tailored to specific isolation techniques. For example, higher rates of many of the antibiotics tested can be employed when isolating hyphae of *P. fragariae* emerging from root pieces than when isolation is dependent on zoospore germination. Such differential response to antibiotics according to the stage of the fungal life cycle has also been reported for *P. infestans* (2). Our experience (S. W. George and R. D. Milholland, *unpublished*) has shown that increased numbers and concentrations of fungicides are required when isolating

directly from naturally infected root tissue and that antibacterial compounds are more important when direct plating of zoospores is attempted.

These results will also aid in selecting antibiotics to combat specific contaminants. For example, *P. fragariae* appears tolerant of PCNB, which could be included in a selective medium if contamination by *Rhizoctonia* spp. were a problem as it is in North Carolina (S. W. George and R. D. Milholland, *unpublished*). It also appears tolerant of hymexazol, a fungicide that gives good control of *Pythium* spp. (a most important contaminant in Japan [8]), and one that, surprisingly enough, afforded moderate bacterial control at higher concentrations.

The utility of a given antibiotic must be evaluated not only on its influence on the germination and/or growth of *P. fragariae* but on its ability to suppress the growth of contaminants as well. For example, penicillin and vancomycin at concentrations as great as 1,000 $\mu$g/ml had little adverse effect on the germination of zoospores of *P. fragariae* but they also offered little if any control of the bacterial contaminants present. Because these antibiotics are known to provide good control of many gram-positive bacteria, this is strong evidence that the bacterial contaminants present were predominantly gram-negative. Investigators may be willing to accept a 60% reduction in the growth of *P. fragariae* if a particular antibiotic provides outstanding control of certain contaminants. Such was the case with rifamycin in these studies, where even 10 $\mu$g/ml, the lowest concentration tested, reduced zoospore germination 53% but afforded excellent control of bacterial contaminants.

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