Comparison of Two Media Selective for *Phytophthora* and *Pythium* Species

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ABSTRACT

Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Disease 70:1038-1043.

The standard selective medium for isolating pythiaceous fungi, $P_{10}VP$ (cornmeal agar amended with 10 mg/L pimaricin, 200 mg/L vancomycin, and 100 mg/L PCNB), was compared with a modified version of this medium, P_5ARP (pimaricin reduced to 5 mg/L and vancomycin replaced by 250 mg/L ampicillin + 10 mg/L rifampicin). Hymexazol (50 mg/L) was added (i.e., $P_{10}VPH$ and P_5ARPH) to suppress unwanted *Pythium* colonies for direct enumeration of *Phytophthora* from soil. $P_5ARP(H)$, when compared with $P_{10}VP(H)$, 1) detected more germinable propagules of *P. cinnamomi, P. parasitica* var. *nicotianae*, and *Pythium ultimum* from naturally infested soils, 2) suppressed contaminating bacteria much better in the test soil, 3) was equally effective in isolating *P. megasperma* and *P. cactorum* from apple twigs, 4) was much less expensive to prepare, and 5) supported mycelium growth of 42 isolates of *Phytophthora* (13 species) and 10 isolates of *Pythium* (six species) equally well. PCNB was beneficial for reducing or eliminating background mycoflora on soil dilution plates. Hymexazol added to P_5ARP greatly inhibited *P. cactorum* and drastically limited the radial growth of one isolate each of *P. megasperma* f. sp. *medicaginis, P. megasperma* f. sp. *glycinea*, and *P. capsici*. $P_5ARP(H)$ is an improvement over $P_{10}VP(H)$ as a multipurpose selective medium for isolating *Phytophthora* and *Pythium* species from plant tissue and soil.

Phytophthora species were notoriously difficult to isolate from plant tissue until the incorporation into selective media of pimaricin, a polyene antibiotic that suppresses nearly all nonpythiaceous fungi (3). Direct isolation of *Phytophthora* species from soil was not achieved until the pimaricin concentration was reduced enough to allow germination of survival propagules, chlamydospores and oospores, on the selective medium (4,31). These selective media have also been useful for isolating *Pythium* species from plant tissue or soil (3,31). The incorporation of hymexazol (3-hydroxy-5-methylisoxazole) into selective media selectively inhibited most *Pythium* and *Mortierella* species, which can overrun or mask developing *Phytophthora* colonies on isolation plates, while allowing most species of *Phytophthora* to develop uncontested (11,30). It is especially useful for direct isolation and quantification of propagules from soil. *Phytophthora cactorum, P. palmivora,* and *P. lateralis* are sensitive to hymexazol in selective media (8,30), and *P. infestans* is completely intolerant of it (20). Shew (21) found that zoospore germination and colony formation of *P. parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker (= *P. nicotianae* Breda de Haan var. *nicotianae*) (*P. p.* var. *nicotianae*) were adversely affected by hymexazol, especially in combination with the fungicide PCNB, another common ingredient in these selective media (28,29). In 1970, Tsao (28) reviewed the development and use of selective media for isolating *Phytophthora* and *Pythium* species, and his review in 1983 (29) contains an excellent summation of the use of selective media for recovering and quantifying *Phytophthora* propagules from soil.

The $P_{10}VP$ selective medium (cornmeal agar amended with 10 mg/L pimaricin, 200 mg/L vancomycin-HCl, and 100 mg/L PCNB), either with (30) or without (31) hymexazol, and modifications of this medium have been invaluable and used widely for studying the etiology, ecology, and epidemiology of diseases associated with *Phytophthora* (6,10,13, 15,19) and *Pythium* species (12,14,16–18). Twenty-two of 36 research papers involving isolation of either *Phytophthora* or *Pythium* in volumes 67 and 68 (1983 and 1984) of PLANT DISEASE mentioned use of some variation of $P_{10}VP$ medium.

Kannwischer and Mitchell (9) modified the $P_{10}VP$ medium by replacing vancomycin with ampicillin + rifampicin (PARP), the same antibacterial antibiotics used by Masago et al (11), to enumerate *P. p.* var. *nicotianae* propagules in tobacco soils. Since then, PARP and its modifications have received only limited use (2,10,22,25). Jeffers and Munnecke (*unpublished*), however, used PARP + hymexazol (PARPH) successfully to investigate the population dynamics of *P. cinnamomi* in avocado soils in southern California and noted less interference from contaminating bacteria on soil dilution plates than with $P_{10}VPH$. In New York State, PARP(H) (PARP with or without hymexazol) has been used extensively to investigate Phytophthora root and crown rots of apple, cherry, and other deciduous fruit crops since 1981, and PARP is currently being used to study the ecology of *Pythium* species attacking beans and beets. However, the amount of pimaricin has been reduced to 5 mg/L (i.e., P_5ARP) because of reported deleterious effects on certain *Phytophthora* species at 10 mg/L (1,15,24) and more successful isolation of both *Phytophthora* and *Pythium* at the lower pimaricin concentration (12,13,23). $P_5ARP(H)$ has proven to be an extremely useful selective medium for all *Phytophthora* and *Pythium* species studied so far.

The purpose of this investigation was to compare and evaluate $P_{10}VP(H)$, the standard selective medium for *Phytophthora* and *Pythium*, and $P_5ARP(H)$, our modified version of this medium. The following criteria were evaluated: 1) quantification of naturally occurring *Phytophthora* and *Pythium* populations in soil, 2) ability to inhibit growth of soil bacteria, 3) isolation and sporulation of *Phytophthora* species from infected plant material, 4) costs of antimicrobial amendments, and 5) mycelium growth by selected *Phytophthora* and *Pythium* species. We were also interested to learn if PCNB was a necessary ingredient or if it could be omitted as implied by the selective media of others (6,18,23).

MATERIALS AND METHODS

Media recipes. Several media were used so that $P_{10}VP$ and P_5ARP could be compared meaningfully. In all media, Difco cornmeal agar (CMA) was the basal medium (17 g/L); all amendments were either suspended or dissolved in 10-ml sterile distilled water blanks and were added to CMA after it was autoclaved and cooled to 50 C in a water bath. Each medium was mixed thoroughly with a magnetic stirrer before plates were poured. Active ingredients per liter of CMA for each selective medium were as follows: $P_{10}VP$ = 10 mg of pimaricin (Delvocid Instant, 50% a.i., Gist-Brocades Fermentation Industries, Inc., Charlotte, NC), 200 mg of vancomycin-

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Accepted for publication 5 July 1986 (submitted for electronic processing).

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hydrochloride (Eli Lilly and Co., Indianapolis, IN), and 100 mg of PCNB (Terraclor, 75% a.i.); $P_5VP = P_{10}VP$ with only 5 mg of pimaricin; $P_{10}ARP = 10$ mg of pimaricin, 250 mg of sodium ampicillin (Bristol Laboratories, Syracuse, NY), 10 mg of rifampicin (Sigma Chemical Co., St. Louis, MO) dissolved in 1 ml of DMSO, and 100 mg of PCNB; $P_5ARP = P_{10}ARP$ with only 5 mg of pimaricin; and $P_5AR = P_5ARP$ without PCNB. Hymexazol (Tachigaren, 70% a.i., Sankyo Co., Tokyo, Japan) at 50 mg/L could be added to any of the media (e.g., $P_{10}VPH$, $P_{10}ARPH$, etc.). Media were dispensed into 9-cm-diameter plastic petri dishes and stored at 4 C in the dark until used 1–3 days later.

Quantification of soil populations. $P_{10}VPH$, $P_{10}ARPH$, P_5ARPH, and P_5ARH were used to estimate germinable propagule density of *Phytophthora* in each of two naturally infested North Carolina soils (received from H. D. Shew, Department of Plant Pathology, North Carolina State University, Raleigh), a Fraser fir nursery soil containing *P. cinnamomi* Rands and a tobacco soil containing *P. p.* var. *nicotianae*, using a surface soil-dilution-plating technique (12). Soils were sieved through a 5-mm mesh screen and then through a 2-mm mesh screen before being stored in polyethylene bags at 2 C in the dark for 2 mo.

To quantify *P. cinnamomi*, 60 ml of Fraser fir soil was mixed into 200 ml of 0.3% water agar (WA) with a magnetic stirrer. A 1-ml aliquot of this slurry was spread evenly onto each of 10 plates for each medium, and five additional 1-ml aliquots were air-dried and weighed to estimate the amount of soil being assayed by a given plate. Soil dilution plates were incubated in the dark at 22 C for 3 days, then the diluted soil was washed from the plates and macroscopically visible colonies of *P. cinnamomi* were counted. Washed plates were incubated for an additional 48 hr on the laboratory bench (25 C), then colonies were recounted. Population estimates were reported as number of germinable propagules per gram of air-dried soil (GPPG). A similar protocol was used for estimating *P. p.* var. *nicotianae* population density with the following exceptions: 150 ml of tobacco soil was mixed into 150 ml of 0.3% WA and soil dilution plates were initially incubated in the dark at 25 C. The experiment was repeated using five plates for each medium, and the data were combined for analysis. Treatment means were compared using single degree of freedom, nonorthogonal linear contrasts in an analysis of variance.

$P_{10}VP$, P_5VP, $P_{10}ARP$, P_5ARP, and P_5AR were used to estimate population density of low-temperature *Pythium* species, mostly *P. ultimum* Trow (16), in two naturally infested soils that had been previously cropped to table beets and snap beans. Both soils were sieved and stored as described previously. Dilutions for beet and bean soils consisted of 10 g of soil mixed in 500 or 200 ml of 0.3% WA, respectively, based on preliminary estimates of GPPG. The amount of soil assayed per plate was estimated as described previously, correcting for the agar weight. Aliquots of 1 ml were spread evenly onto each of 10 plates for each soil-medium combination, and these plates were incubated in the dark at 19 C. After 22 hr, soil was washed from each plate, rapidly growing *P. ultimum*-like colonies were counted, and the population density was recorded as number of GPPG. The experiment was repeated once, and the data were combined for analysis. Treatment means were computed using single degree of freedom, orthogonal linear contrasts in an analysis of variance.

Inhibition of soil bacteria. Three media ($P_{10}VP$, $P_{10}ARP$, and P_5ARP) were used to compare the effectiveness of vancomycin with that of ampicillin + rifampicin in inhibiting growth of unwanted soil bacteria from an apple orchard soil. Dilutions of 1:10, 1:100, and 1:1,000 were prepared using 1 g of sieved soil and 9-ml sterile distilled water blanks. For each medium, a 0.1-ml aliquot was spread onto each of five replicate plates from each dilution. Soil dilution plates were incubated in the dark at 25 C for 3 days. Total colonies of bacteria were then counted, and the mean number of colonies per plate was calculated for each dilution on each medium. The experiment was repeated.

Isolation from plant material and sporulation. Isolations were attempted on $P_{10}VP$, $P_{10}ARP$, P_5ARP, and P_5AR from woody apple twigs individually inoculated with each of five isolates, two of *P. cactorum* and three of *P. megasperma*. Plates were incubated in the dark at 22 C for 5 days and were examined for mycelium growth, presence of oospores and sporangia (*P. cactorum* only), and contaminating bacteria.

Costs of antimicrobial amendments. To make this comparison, four catalogs from well-known chemical suppliers were surveyed. Two or three 1984 prices were used to obtain a mean price and to calculate the cost per liter for pimaricin (Pimafucin), vancomycin-HCl (Vancocin), sodium ampicillin, and rifampicin (Rifampin) needed to prepare $P_{10}VP$ and P_5ARP. The most economically priced quantities available from the individual suppliers were used for comparison. Because the amount of PCNB used in both media was the same, it was not included in the cost comparison.

Mycelium growth. All isolates tested were from the collection maintained by the first author. Isolate designations beginning with "P" were originally from the *Phytophthora* collection of the Department of Plant Pathology, University of California, Riverside. Various other isolates were donated by colleagues in the United States and Australia. The isolates tested represented a diverse group originating from many hosts (e.g., apple, avocado, cacao, cherry, strawberry, tobacco) in seven countries (Australia, Brazil, Canada, Costa Rica, Ireland, Jamaica, and United States). Mycelium growth of 42 isolates of *Phytophthora*, representing 13 species (Table 1), was compared on CMA, $P_{10}VP$, P_5ARP, and P_5ARPH. Three replicate plates, each containing 15 ml of medium, were used for each isolate on each medium. A 5-mm plug from the advancing edge of a colony on CMA was transferred to the center of each plate, and plates were incubated in the dark at 22 C (19 C for *P. syringae*) for 5 days. The average colony radius was measured for each plate, and colony morphology on $P_{10}VP$, P_5ARP, and P_5ARPH was compared with that on CMA.

Mycelium growth of 10 isolates of *Pythium*, including six species and two unidentified isolates (Table 1), was compared in a similar manner with two exceptions: P_5ARPH was not tested because hymexazol is inhibitory to most *Pythium* species and incubation was for only 2 days because of the rapid growth of several isolates. The entire experiment was repeated, and data were combined for analysis. Treatment means for each isolate were compared using single degree of freedom, nonorthogonal (for *Phytophthora* isolates), or orthogonal (for *Pythium* isolates) linear contrasts in two analyses of variance.

RESULTS

Quantification of soil populations. The population density of *P. p.* var. *nicotianae* in the tobacco soil was 2–3 GPPG (Table 2); this low level may have resulted from storing the soil at 2 C for 2 mo (10,27). Consequently, the differences in GPPG among media were not as dramatic as they were with the Fraser fir soil. Significantly ($P = 0.01$) greater numbers of GPPG were recovered on $P_{10}ARPH$ than on $P_{10}VPH$ for both *P. cinnamomi* (37.7 vs. 14.8) and *P. p.* var. *nicotianae* (2.1 vs. 0.0) (Table 2, contrast 1). No colony of *P. p.* var. *nicotianae* was observed on any of the 15 replicate plates of $P_{10}VPH$ used in two experiments with the test soil. More than three times as many GPPG of *P. cinnamomi* were observed on P_5ARPH as on $P_{10}ARPH$, but for *P. p.* var. *nicotianae*, there was no real difference between these two media (Table 2, contrast 2). The effect of PCNB in the selective medium on quantification of either *P. cinnamomi* or *P. p.* var. *nicotianae* was insignificant (Table 2, contrast 3), although it did limit the size of slow-growing, nonpythiaceous fungi that developed on $P_{10}ARPH$, P_5ARPH, and P_5ARH plates containing tobacco soil dilutions. These fungi did not

develop on P_{10}VPH. Of 10 slow-growing colonies subcultured, eight were identified as *Gongronella butleri* (Lendner) Peyronel & Dal Vesco, one was a *Fusarium* species, and one was not identified.

The only significant ($P = 0.05$) difference in number of GPPG of low-temperature *Pythium* species occurred in the bean soil between P_{10} and P_5 (Table 3, contrast 1). Media with 10 mg/L pimaricin (P_{10}VP and P_{10}ARP) yielded a lower GPPG number than did media with 5 mg/L pimaricin (P_5VP, P_5ARP, and P_5AR). Ampicillin + rifampicin in selective media yielded numbers of GPPG similar to those obtained with vancomycin (Table 3, contrasts 2 and 3). Omitting PCNB from P_5ARP (i.e., P_5AR) did not affect population estimates (Table 3, contrast 4). As with tobacco soil, many more small, slow-growing fungus colonies developed on medium without PCNB (P_5 AR) than on medium with PCNB (P_5ARP). Most of these colonies were tentatively identified as members of the genus *Pythium*.

Table 1. Mean radial growth[u] of mycelia of selected *Phytophthora* and *Pythium* species on amended and unamended cornmeal agar (CMA)

Species	Isolate	Medium[v] CMA	P_{10}VP	P_5ARP	P_5ARPH	Linear contrast[w] 1	2	3
Phytophthora cactorum	NY.097	20.7	18.2	18.0	8.8 d[x]	**	NS	**
	NY.147	18.2	14.8	14.3	5.8 d	**	NS	**
	NY.188	22.2	19.5	20.7	8.0 d	**	NS	**
	NY.194	22.5	20.0	20.5	9.2 d	**	NS	**
	A.P58	19.5	16.8	16.8	7.8 d	**	NS	**
	A.FG3	21.5	18.0	18.7	7.8 d	**	NS	**
P. megasperma	NY.055	22.7	19.0	18.5	18.5	**	NS	NS
	NY.128	22.5	11.3	14.3	6.0	**	**	**
	NY.143	25.2	22.5	22.2	18.0 d	**	NS	**
	NY.150	12.3	14.5	14.2	14.2	*	NS	NS
	NY.153	27.8	26.2	26.8	25.0	**	NS	**
	NY.169	14.7	10.2	12.8	3.3	**	**	**
P. cryptogea	NY.001	19.8	18.0	17.2	15.2 d	**	NS	**
	NY.154	19.0	16.8	16.8	13.2	**	NS	**
	A.P8	37.7	32.5	30.0	26.8 d	**	**	**
	P1088	30.2	27.3	24.5	24.5	**	*	NS
P. cambivora	NY.113	13.8	9.8	10.2	11.8	**	NS	**
	NY.141	21.7	18.3	17.3	16.8	**	NS	NS
	NY.151	18.0	14.3	15.3	14.7	**	**	*
	NY.187	15.5	13.7	12.8	14.5	**	NS	*
	A.P5	23.7	19.7	22.3	21.3 d	**	**	NS
	A.P6	8.8	7.8	6.0	7.0	**	**	*
P. drechsleri	P1087	26.0	26.2	25.3	22.5	NS	*	**
	NY.152	26.8	23.7	24.2	21.3	**	NS	**
P. cinnamomi	Pc40	34.0	29.5	29.2	28.3	**	NS	NS
	Pc138	23.8	19.7	17.5	19.8	**	**	**
	NY.200	22.0	17.8	16.0	18.5 d	**	**	**
P. parasitica	NY.162	14.7	14.0	17.2	13.8	NS	NS	NS
	NY.163	19.5	16.5	17.2	15.2	**	NS	*
P. parasitica var. *nicotianae*	P1063	21.2	22.8	25.8	15.5 d	**	**	**
	NY.202	13.7	11.2	12.2	10.7	**	NS	*
P. citricola	P1100	35.5	25.2	27.0	19.0 d	**	*	**
	NY.213	32.0	24.7	25.8	20.5 d	**	NS	**
P. palmivora	P550	23.2	19.8	23.0	17.7 d	*	**	**
	P255	23.5	18.5	18.3	16.7	**	NS	**
P. capsici	P780	32.0	29.0	30.3	10.0 d	**	*	*
	P1043	35.8	28.2	27.7	22.3	**	NS	**
P. erythroseptica	NY.189	30.0	29.5	29.2	26.7	**	NS	**
P. citrophthora	P479	28.7	22.7	22.8	19.7	**	NS	**
	P1212	32.2	24.7	25.8	23.7	**	*	**
P. syringae	NY.219	8.8	5.0	5.0	5.0	**	NS	NS
	A.P81	8.2	4.0	3.8	3.0	**	NS	NS
Pythium ultimum	NY.224	40+[y]	26.8	30.5	–[z]	**	**	–
	NY.225	30.3	23.2	25.2	–	**	*	–
P. irregulare	NY.022	40+[y]	39.5	39.3	–	NS	NS	–
	NY.095	40+[y]	39.5	39.5	–	NS	NS	–
P. splendens	NY.024	32.7	26.7	25.8	–	**	NS	–
P. acanthicum	NY.226	20.5	11.5	10.5	–	**	NS	–
P. vexans	NY.205	22.2	15.2	14.2	–	**	NS	–
P. aphanidermatum	NY.215	40+[y]	38.5	38.8	–	*	NS	–
Pythium species	NY.005	20.0	16.7	16.5	–	**	NS	–
	NY.041	19.5	12.7	13.5	–	**	NS	–

[u] Each mean is from six replicate plates (three replicates per isolate in each of two trials). *Phytophthora* isolates were grown for 5 days at 22 C in darkness (19 C for *P. syringae*). *Pythium* isolates were grown for 2 days at 22 C in darkness.

[v] P_{10}VP = cornmeal agar (CMA) + 10 mg/L pimaricin, 200 mg/L vancomycin-HCl, and 100 mg/L PCNB; P_5ARP = CMA + 5 mg/L pimaricin, 250 mg/L sodium ampicillin, 10 mg/L rifampicin, and 100 mg/L PCNB; and P_5ARPH = P_5ARP + 50 mg/L hymexazol.

[w] Single degree of freedom contrasts of treatment means are as follows: 1 = CMA vs. (P_{10}VP + P_5ARP), 2 = P_{10}VP vs. P_5ARP, and 3 = P_5ARP vs. P_5ARPH. A contrast is significant at $P = 0.01$ (**) or $P = 0.05$ (*) or not significant (NS).

[x] d = Colony morphology distinctively different from that on CMA.

[y] Colony radius had reached the edge of the petri plate.

[z] Isolate was not grown on this medium, and the corresponding contrast was not made.

Inhibition of soil bacteria. The antibacterial antibiotics ampicillin + rifampicin were much more effective than vancomycin alone in inhibiting the development of bacteria at all three dilutions of the orchard soil tested. After 3 days of incubation at 25 C, no colonies of bacteria developed on any plate of either P_{10}ARP or P_5ARP at any dilution, whereas the mean numbers of colonies that developed on P_{10}VP were 143.2, 15.6, and 1.6 at dilutions that delivered 10^{-2}, 10^{-3}, and 10^{-4} g of soil per plate, respectively. All plates of P_{10}ARP and P_5ARP remained free of bacteria after two additional days of incubation at 25 C while the colonies of bacteria on P_{10}VP continued to increase in both size and

Table 2. Number of germinable propagules of *Phytophthora cinnamomi* and *P. parasitica* var. *nicotianae* recovered from two naturally infested soils by soil dilution plating onto selective media

Medium[a]	Soil infested with: *P. cinnamomi*	*P. parasitica* var. *nicotianae*
P_{10}VPH	14.8[b]	0.0
P_{10}ARPH	37.7	2.1
P_5ARPH	116.3	2.9
P_5ARH	110.2	2.6
Linear contrasts of treatment means[c]		
1. P_{10}VPH vs. P_{10}ARPH	**	**
2. P_{10}ARPH vs. P_5ARPH	**	NS
3. P_5ARPH vs. P_5ARH	NS	NS

[a] P_{10}VPH = cornmeal agar (CMA) + 10 mg/L pimaricin, 200 mg/L vancomycin-HCl, 100 mg/L PCNB, and 50 mg/L hymexazol; P_{10}ARPH = CMA + 10 mg/L pimaricin, 250 mg/L sodium ampicillin, 10 mg/L rifampicin, 100 mg/L PCNB, and 50 mg/L hymexazol; P_5ARPH = P_{10}ARPH with only 5 mg/L pimaricin; and P_5ARH = P_5ARPH without PCNB.

[b] Mean number of germinable propagules per gram of air-dried soil from 15 replicate plates per medium.

[c] Single degree of freedom, nonorthogonal contrasts were significant at $P = 0.01$ (**) or not significant (NS).

Table 3. Number of germinable propagules of low-temperature *Pythium* species recovered from two naturally infested soils by soil dilution plating onto selective media

Medium[a]	Beet soil	Bean soil
P_{10}VP	553[b]	236
P_{10}ARP	601	346
P_5VP	663	362
P_5ARP	646	425
P_5AR	665	425
Linear contrasts of treatment means[c]		
1. P_{10} vs. P_5	NS	*
2. P_{10}ARP vs. P_{10}VP	NS	NS
3. P_5VP vs. (P_5ARP + P_5AR)	NS	NS
4. P_5ARP vs. P_5AR	NS	NS

[a] P_{10}VP = cornmeal agar (CMA) + 10 mg/L pimaricin, 200 mg/L vancomycin-HCl, and 100 mg/L PCNB; P_{10}ARP = CMA + 10 mg/L pimaricin, 250 mg/L sodium ampicillin, 10 mg/L rifampicin, and 100 mg/L PCNB; P_5VP = P_{10}VP with only 5 mg/L pimaricin; P_5ARP = P_{10}ARP with only 5 mg/L pimaricin; and P_5AR = P_5ARP without PCNB.

[b] Mean number of germinable propagules per gram of oven-dried soil from 20 replicate plates per medium.

[c] Single degree of freedom, orthogonal contrasts were significant at $P = 0.05$ (*) or not significant (NS).

number. Similar data resulted when the experiment was repeated.

Isolation from plant material and sporulation. On all four media, isolation of *P. cactorum* and *P. megasperma* from apple twigs was equally successful; both isolates of *P. cactorum* formed oospores and sporangia, and all three isolates of *P. megasperma* formed oospores. Although the relative abundance of oospores or sporangia varied among isolates, no noticeable difference was observed among the four media for a given isolate. There was a noticeable difference, however, in presence or absence of contaminating bacteria produced on the various media. After 5 days, a ring of bacterial ooze surrounded most of the apple bark pieces on P_{10}VP, but none appeared around the pieces on P_{10}ARP, P_5ARP, and P_5AR.

Costs of antimicrobial amendments. Of the four catalogs surveyed, two carried pimaricin, three carried vancomycin, two carried ampicillin formulated as sodium salt, and three carried rifampicin. The average price for 1 g of vancomycin-HCl was $35.50 (based on 5-g, 1-g, and 250-mg quantities), giving a cost of $7.10 for the 200 mg needed to prepare 1 L. Sodium ampicillin averaged $1.88/g (based on 25-g quantities), or $0.47 for 250 mg/L. Rifampicin averaged $15.83/g (based on 1-g quantities), or $0.16 for 10 mg/L. Pimaricin (Pimafucin) is marketed in 20-ml quantities as a 2.5% sterile suspension at $74.05 on the average ($148.10/g). In 1 L of medium, 10 and 5 mg/L would cost $1.48 and $0.74, respectively. The costs of amendments for preparing 1 L each of P_{10}VP or P_5ARP (less PCNB) were $8.58 and $1.37, respectively. For the antibacterial ingredients alone, vancomycin cost more than 11 times as much as ampicillin + rifampicin.

Mycelium growth. Radial growth on the unamended CMA often varied among isolates of the same species, and these isolates did not always respond similarly to amended media (Table 1). This was expected, hence the reason for analyzing the data at the isolate level and not at the species level. Forty-six of the 52 isolates grew significantly less on P_{10}VP and P_5ARP than on unamended CMA (Table 1, contrast 1). In only two instances, *P. p.* var. *nicotianae* P1063 and *P. megasperma* NY.150, was growth significantly greater on the amended media than on CMA. Growth of 35 of the 52 isolates on P_{10}VP did not differ significantly from that on P_5ARP. Of the 17 isolates that responded differentially, five grew better on P_{10}VP and 12 grew better on P_5ARP. Adding hymexazol to P_5ARP significantly restricted the growth of 28 of 42 *Phytophthora* isolates (Table 1, contrast 3). Those affected most severely were all *P. cactorum* isolates (57% mean growth reduction), *P. megasperma* NY.128 (58% reduction), *P. megasperma* NY.169 (74% reduction), and *P. capsici* P780 (67% reduction). Isolates NY.128 and NY.169 are *P. m.* f. sp. *medicaginis* (host = alfalfa) and *P. m.* f. sp. *glycinea* (host = soybean), respectively. The other *P. megasperma* isolates, those much less affected by hymexazol, were isolated from apple or cherry. Growth of five *Phytophthora* isolates—*P. cambivora* NY.113, NY.187, and A.P6 and *P. cinnamomi* NY.200 and Pc138—was significantly greater on P_5ARPH than on P_5ARP. Nine isolates were unaffected by hymexazol.

Hymexazol also distinctively altered the colony morphology of 15 *Phytophthora* isolates (Table 1). Isolates of *P. cactorum* all responded similarly; in addition to being restricted, mycelium growth was extremely sparse and lacked uniformity (e.g., *P. cactorum* NY.188 in Fig. 1A). Observation at 100× revealed individual hyphae growing abnormally and without direction. Isolates of other species were affected less dramatically; mycelia were either less dense (e.g., *P. cinnamomi* NY.200 in Fig. 1B) or lacked the characteristic pattern exhibited on the other three media (e.g., *P. citricola* NY.213 in Fig. 1C).

DISCUSSION

P_5ARP(H) was much more effective than P_{10}VP(H) in detecting *P. cinnamomi*, *P. p.* var. *nicotianae*, or low-temperature *Pythium* species (bean soil) in naturally infested soils. Almost eight times as many GPPG of *P. cinnamomi* were recovered on P_5ARPH (116.3) as on P_{10}VPH (14.8). At the low population density in our test soil, the difference between media in recovering *P. p.* var. *nicotianae* was both qualitative and quantitative; 2.9 GPPG were recovered on P_5ARPH but none was recovered on P_{10}VPH. Twice as many colonies of low-temperature *Pythium* species from bean soil were recovered on P_5ARP as on P_{10}VP (425 vs. 236), but recovery from beet soil on the two media was similar.

One contributing factor to these differences in recovery was the reduction in pimaricin from 10 to 5 mg/L; this significantly increased recovery of *P. cinnamomi* and was the only significant factor affecting recovery of low-temperature *Pythium* species. Similar responses to pimaricin concentration have been reported: 2 mg/L pimaricin was "critical" for direct isolation of *P. m.*

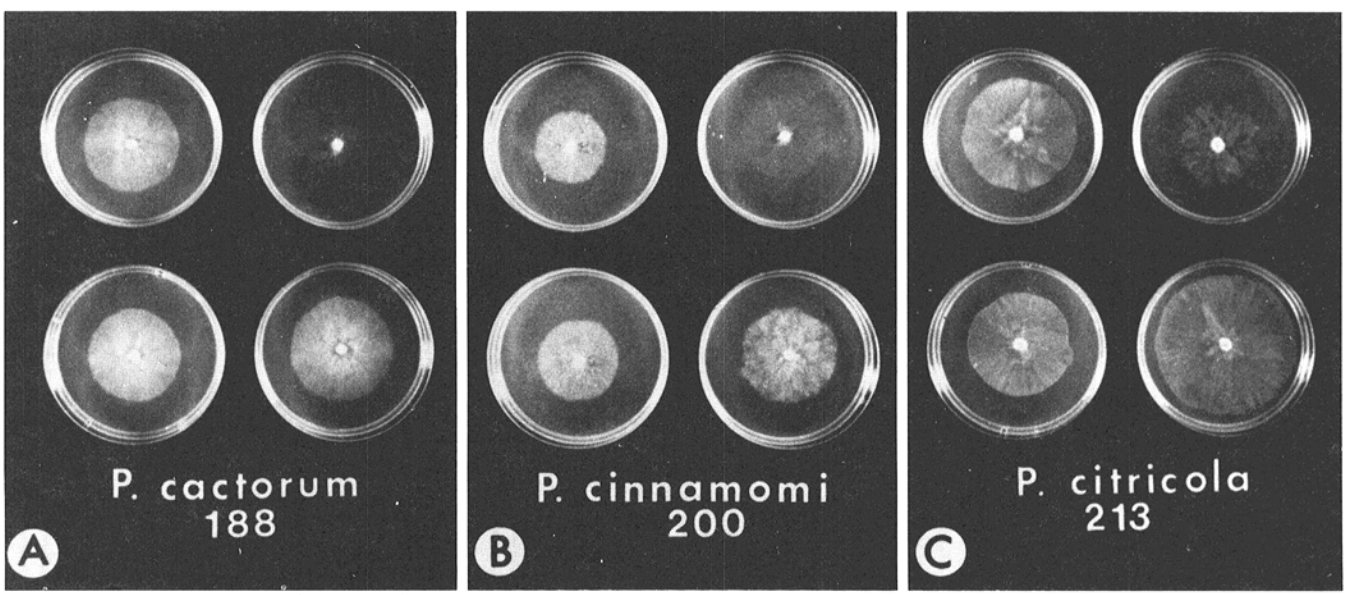

Fig. 1. Comparative mycelium growth and colony morphology of three *Phytophthora* species on cornmeal agar (CMA) and three selective media (clockwise from bottom right: CMA, P_{10}VP, P_5ARP, and P_5ARPH). Addition of hymexazol (i.e., P_5ARPH) **(A)** severely restricted growth and development of *P. cactorum* NY.188, **(B)** created a less dense colony of *P. cinnamomi* NY.200, and **(C)** changed the colony pattern of *P. citricola* NY.213. Effects from other amendments (i.e., P_{10}VP and P_5ARP) were less striking.

var. *sojae* (= *P. m.* f. sp. *glycinea*) from soil (4), germination of oospores and zoospores of *P. cactorum* was reduced by 10 mg/L pimaricin (1,24), isolation of *Phytophthora* and *Pythium* species was more successful using 5 mg/L pimaricin in modified $P_{10}VP$ (12,13), and 10 mg/L pimaricin in selective medium was toxic to one isolate of *P. capsici* tested (15). Other selective media developed for *Phytophthora* and *Pythium* species have used 5 mg/L as opposed to 10 mg/L pimaricin (14,16,17,23).

Replacing vancomycin with ampicillin + rifampicin was the other factor contributing to the more effective recovery of *Phytophthora* propagules from soil. It significantly increased *P. cinnamomi* recovery by 2.5 times and made possible the recovery of *P. p.* var. *nicotianae*, which was not accomplished on $P_{10}VPH$. Although we have found no evidence in the literature for the inhibitory effect of vancomycin in selective media on fungus propagules, the germination or subsequent development of both *Phytophthora* species assayed in our tests were inhibited. It also apparently inhibited all the slow-growing, contaminating fungi that were observed developing on either $P_{10}ARPH$, P_5ARPH, or P_5ARH with tobacco soil dilutions. This effect was not evident on the *Pythium* dilution plates, but different species of fungi were involved.

Ampicillin + rifampicin were more effective than vancomycin in limiting the development of contaminating bacteria on dilution plates of an apple orchard soil and on apple twig isolation plates. In both instances, bacteria grew on $P_{10}VP$ but not on $P_{10}ARP$. The growth of bacteria on Fraser fir and tobacco soil dilution plates may well have contributed to the reduced number of GPPG of *P. cinnamomi* and *P. p.* var. *nicotianae*, respectively. Consequently, the inhibitory effect of vancomycin may be indirect, caused by failure to suppress bacteria on soil dilution plates. In his recent review (29), Tsao stated, "The combination of ampicillin and rifampicin may suppress bacterial growth more efficiently than vancomycin alone in some, but not all, soils tested." The apparent dissatisfaction with vancomycin at 200 mg/L is reflected in the numerous recipes that have either increased the concentration to 300 mg/L or have supplemented media with other antibacterial amendments (6,12–16,24).

The omission of PCNB from P_5ARPH, though not affecting population estimates, had a considerable effect on the development of background mycoflora. For the tobacco soil, it effectively limited colony diameters of slow-growing soil fungi, making enumeration and subculturing of *P. p.* var. *nicotianae* on P_5ARPH much less difficult than on P_5ARH. For table beet and snap bean soils, PCNB drastically reduced the development of slow-growing fungi (tentatively identified as species of *Pythium* different from low-temperature *P. ultimum*-types) that developed on P_5AR. Consequently, incorporation of PCNB into media is recommended for quantifying *Phytophthora* and *Pythium* propagules in soil unless a total *Pythium* population estimate is needed (e.g., ecological studies). Shew (21) indicated, however, that PCNB reduced germination of *P. p.* var. *nicotianae* zoospores, although he did not discuss the relative importance of these propagules in recovering this fungus from soil. In New York, P_5AR and P_5ARH (without PCNB) have been used successfully to isolate *Phytophthora* species from root and crown tissues of apple, apricot, cherry, peach, and raspberry, and no benefit has been observed by adding PCNB for these purposes. PCNB was omitted from various other selective media for isolating both *Phytophthora* and *Pythium* without adverse effects (6,10,14,18).

Isolations of *P. cactorum* or *P. megasperma* from apple twigs and subsequent oospore or sporangium formation were not noticeably affected by pimaricin concentration, antibacterial amendments, or presence of PCNB in the four media tested. However, isolations were made from active cankers containing viable mycelium; this is not always the case with field or diagnostic samples. Often, isolations must be made from badly decayed tissue that may contain only quiescent propagules of *Phytophthora* or *Pythium*. Therefore, the medium that permits spores to germinate and develop more readily would be preferable. The formation of oospores directly on isolation plates has been a tremendous aid to us in making tentative identifications of *Phytophthora* species attacking deciduous fruit crops. $P_{10}VP$ and P_5ARP functioned equally well in this experiment and in our previous experiences with both media. In addition, sporangium formation by *P. capsici* was one criterion used to select an appropriate medium for selective isolation of this species from soil (15).

The cost per liter of medium alone is probably not a viable criterion for choosing P_5ARP over $P_{10}VP$. However, if P_5ARP and $P_{10}VP$ were equally effective as selective media, the cost could be a deciding factor, especially for a diagnostic or research laboratory that uses such a medium on a routine basis. A savings of more than $7.00/L is realized by using P_5ARP instead of $P_{10}VP$.

Although both P_5ARP and $P_{10}VP$ supported good growth of the 52 *Phytophthora* and *Pythium* isolates tested, radial growth of mycelia was significantly less than on unamended CMA. In most instances, however, the inhibition $<$ 5 mm under our experimental conditions. Similar results were reported by Masago et al (11) in developing their BNPRAH medium selective for *Phytophthora* species. Of the 17 isolates of 52 that showed significant differential growth on P_5ARP compared with $P_{10}VP$, the differences were minimal ($<$4 mm) and were considered biologically insignificant; the two media were comparable.

The deleterious effect of hymexazol on *P. cactorum* in P_5ARPH was similar to the effect observed in $P_{10}VPH$ (30). The sensitivity of *P. cactorum* to hymexazol has been noted in at least one other paper (8). Tay et al (26), however, reported only one of four isolates of *P. cactorum* was inhibited in the manner we observed. They also reported that most isolates of species in Waterhouse's Group VI (including *P. cambivora, P. cinnamomi, P. cryptogea,* and *P. erythroseptica* tested herein) grew significantly better on $P_{10}VPH$ than on unamended medium. Our data do not support this general pattern. None of the isolates we assayed grew significantly better on P_5ARPH than on CMA. These inconsistencies may be due to the different basal medium, i.e., half-strength potato-dextrose agar, used by Tay et al (26). In addition, *P. palmivora* (30), *P. lateralis* (8), the newly described *P. pseudotsugae* (5,8), and *P. infestans* (20) were sensitive to hymexazol at the concentration that we have used, 50 mg/L.

The drastic reduction in mycelium growth of *P. m.* f. sp. *glycinea* (isolate NY.169) and *P. m.* f. sp. *medicaginis* (isolate NY.128) has not been reported elsewhere. However, no general conclusion about the sensitivity of these two formae speciales of *P. megasperma* to hymexazol should be made on the basis of our data, because we used only one isolate of each. Both fungi appeared to grow well but very slowly on P_5ARPH. The differential response to hymexazol by these two types of *P. megasperma* compared with the fruit tree types is further evidence of the variability within this species (7).

In conclusion, $P_5ARP(H)$ is an improvement over $P_{10}VP(H)$ as a multipurpose selective medium for recovering *Phytophthora* and *Pythium* species from plant tissue and soil. We have not attempted to compare this medium with the multitude of $P_{10}VP(H)$ variants and other selective media for isolating these two genera that have been described during the last 5–10 yr (29). Undoubtedly, the best medium to isolate a given species of *Phytophthora* or *Pythium* under the diverse biological and environmental conditions that exist will not always be the same. Each situation should be assessed, and the medium best suited for the particular purpose should be chosen.

ACKNOWLEDGMENTS

We wish to thank H. S. Aldwinckle, G. S. Abawi, and H. C. Hoch for supporting this project. S. B. Martin was supported by a grant from the USDA-SEA (59-2361-1-1-750-0).

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