Variability in Growth of *Phytophthora cinnamomi* Isolates in Response to Antibiotics

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ABSTRACT

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Growth rates of 35 isolates of *Phytophthora cinnamomi* (16 A^1, 19 A^2 mating type) from eight countries and 18 different hosts were compared at 25 C on media containing one of the following antibiotics: streptomycin, chlortetracycline, cycloheximide, nystatin, ethidium bromide, or chloramphenicol. Significant differences among isolates were observed in the presence of every single antibiotic, and several patterns of differential responses were apparent when the data were combined. The effect of streptomycin on *P. cinnamomi* was influenced by the nutrient medium used. On the average, the A^1 isolates were significantly more resistant to cycloheximide than the A^2 isolates. Most *P. cinnamomi* isolates were relatively sensitive to chlortetracycline and resistant to nystatin. Sensitivity of isolates to chloramphenicol correlated significantly with sensitivity to ethidium bromide and the response to chloramphenicol also correlated with the response to chlortetracycline. However, there was not a significant correlation between the effects of ethidium bromide and of chlortetracycline. Isolates with similar growth/temperature responses or isolates from the same host or geographic origin did not necessarily show the same pharmacological responses.

Additional key words: adaptation, selective media, β-sitosterol, physiological races, polyene.

Phytophthora cinnamomi Rands is recognized as an important pathogen of a wide range of economically important plants in many parts of the world (28). To understand and combat the fungus in such diverse environmental situations, several researchers have realized the need to define the range of intraspecific variability with respect to such characters as growth rate, response to temperature, morphology, etc. (19,28,29).

The effects of some antibiotics on *Phytophthora* spp. were studied in relation to their potential for disease control (24,27), in the development of selective media (2,4,6,12,20,22,23) and as a tool for genetical studies (7,11). However, most of these studies involved only a few isolates. To use antibiotics more effectively in these and other types of research on *Phytophthora* spp., it is important to know the variation in responses to antibiotics within one species. Differences in responses to some inhibitors have been found among Australian isolates of *P. cinnamomi* (19), indicating that similar studies of isolates from other parts of the world could be fruitful.

Compared to other soil fungi, species of *Phytophthora* and *Pythium* generally are considered to be unusually sensitive to many "antibacterial" antibiotics (22). Understanding the pharmacological responses of *Phytophthora* spp. may therefore be pertinent to our basic knowledge of the physiology, evolution, and taxonomy of these pathogens.

We studied the effect of six antibiotics on vegetative growth of 35 isolates of *P. cinnamomi* from various hosts and countries. We also considered whether these pharmacological responses could be correlated with geographic or host origin, mating type, or the previously demonstrated variation in response to temperature (29).

MATERIALS AND METHODS

Isolates of *P. cinnamomi* are listed in Table 1. Numbers refer to the *P. cinnamomi* collection, Department of Plant Pathology, University of California, Riverside. The majority of isolates were collected by G. A. Zentmyer; additional cultures were provided by H. T. Brodrick, Atomic Energy Board, South Africa; M. Bumbieris, Waite Agric. Res. Inst., Australia; B. B. Huguenin, Centre ORSTOM, Ivory Coast; K. G. Pegg, Dept. of Primary Industries, Queensland, Australia; B. H. Pratt, Conservation and Agric. Dept., A. C. T., Australia; M. Tarjot, IFCC, Ivory Coast; B. Zak, U.S. Forest Service, Georgia; and also by the California Department of Food and Agriculture, and the Commonwealth Mycological Institute.

Cultures were transferred originally from V-8 agar stocks and, during the course of these experiments, cultures were maintained as mass transfers on synthetic minimal medium without sterol (MM) (29).

Growth response was tested on MM or CV-8A containing antibiotics at the following concentrations: streptomycin sulfate (B-grade, Calbiochem), 200 μg/ml of medium; chlortetracycline (hydrochloride, Calbiochem), 1 μg/ml; cycloheximide (Sigma), 1 μM (0.28 μg/ml of medium); nystatin (mycostatin, B-grade, Calbiochem), 100 μg/ml; ethidium bromide (Calbiochem), 5 μg/ml; and chloramphenicol (B-grade, Calbiochem), 100 μg/ml. Concentrations used for each antibiotic were chosen by reference to previous reports, or by conducting a pilot experiment using a range of concentrations. Studies were conducted deliberately using concentrations that would allow at least some growth by all isolates in order to see the full range of variability in response to sublethal dosages.

Media were prepared in lots of 2 L each; every isolate within an experiment was tested on each medium from the same flask. Antibiotics were incorporated after the agar had been autoclaved and allowed to cool to about 60 C. Each medium was dispensed uniformly into 100-mm-diameter plastic petri dishes at 15 ml per plate. In the case of nystatin only, the antibiotic was dissolved in 10 ml of 95% ethanol per 2 L of medium before addition to the medium, and growth was compared to control plates containing the same concentration of ethanol. In the nystatin experiments only, 30 μg of β-sitosterol per milliliter of medium was added to both treated and control media.

Each plate was inoculated with a 5-mm-diameter disk cut with a cork borer from the margin of an actively growing colony on

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control media. There were three plates per isolate per antibiotic concentration per experiment. Experiments were repeated at least three times. Plates were incubated at 25 C in darkness. Colony diameters were measured 5 and 7 days after inoculation on MM, and 3, 4, and 5 days after inoculation on CV-8A (29). Two perpendicular diameters (less the 5 mm of the inoculum disk) were averaged for each plate. The average diameter for three drug-containing plates of one isolate was divided by the average of the three control plates for that isolate. Experimental means for each isolate were used as replicates in statistical analyses.

With chloramphenicol, chlortetracycline, and ethidium bromide, 5 g of L-sorbose per liter of medium was added to both the treated and control media in an attempt to restrict hyphal extension and to keep colony density similar in antibiotic and control plates (7).

Due to data storage limitations, some isolates were excluded from statistical analyses; isolates excluded were usually chosen because of unequal numbers of replicates or because of inconsistent behavior in replicated experiments. Using Duncan's multiple range test, significant differences between isolates were obtained at $P = 0.01$ with all antibiotics. However, most of the data in tables are presented at $P = 0.05$, since at this level there is less overlap between ranges and trends can be seen more clearly.

RESULTS

Among the 35 isolates studied, considerable variability was observed in the amount of growth in the presence of individual antibiotics and in the patterns of responses to the group of antibiotics tested. The demonstrated variability could not be correlated with countries of origin of isolates or hosts from which isolates were originally obtained.

TABLE 1. Origins of isolates of *Phytophthora cinnamomi* isolates

Isolate	Isolate from: Host plant	Source
A^1		
21[a]	*Camellia japonica*	California, USA
62	*Macadamia integrifolia*	Hawaii, USA
67	*Camellia japonica*	California, USA
68	*Camellia japonica*	California, USA
96	*Camellia japonica*	California, USA
97, ATCC 32993	*Camellia japonica*	California, USA
100	*Camellia japonica*	California, USA
101	*Camellia japonica*	California, USA
104	*Camellia japonica*	California, USA
121	*Persea americana*	Malagasy Rep.
122	*Persea americana*	Malagasy Rep.
138, ATCC 38581	*Persea americana*	California, USA
152, IMI 158786	*Tristania conferta*	Queensland, Aust.
159, IMI 157799	*Vitis* sp.	South Africa
160, IMI 157800	*Vitis* sp.	South Africa
184	*Eucalyptus globoidea*	New South Wales, Aust.
A^2		
3	*Leucopogon verticillata*	W. Australia
6	*Xylomelum occidentalis*	W. Australia
7	*Hovea elliptica*	W. Australia
8	*Lomandra* sp.	W. Australia
13	*Lasiopetalum floribundum*	W. Australia
16	*Hibbertia cunninghamii*	W. Australia
17	*Eucalyptus marginata*	W. Australia
18	*Acacia huegelii*	W. Australia
40, ATCC 32992	*Persea americana*	California, USA
45	*Pinus echinata*	Georgia, USA
53	*Persea americana*	California, USA
55	*Macadamia* sp.	California, USA
65	*Persea americana*	E. Caroline Islands
73	*Eucalyptus marginata*	W. Australia
74	*Persea americana*	California, USA
93	*Persea americana*	California, USA
110, IMI 22938	*Cinnamomum burmanni*	Sumatra, Indonesia
135	*Pinus radiata*	S. Australia
382	*Erica gracilis*	W. Germany

[a] Numbers are those used by the *P. cinnamomi* Collection, U.C. Riverside, except ATCC = American Type Culture Collection, and IMI = Commonwealth Mycological Institute.

Growth with streptomycin. *P. cinnamomi* isolates were tolerant of streptomycin; with most isolates vegetative growth was inhibited less than 50% at 100 and 200 μg/ml. When *P. cinnamomi* isolates were grown on MM with streptomycin at 200 μg/ml, the overall inhibition of A^1 and A^2 isolates combined was significantly greater ($P = 0.01$) than when 200 μg streptomycin per milliliter of medium was incorporated in CV-8A (Table 2). However, A^1 isolates grew significantly better ($P = 0.01$) on MM + 200 μg streptomycin per milliliter of medium (mean percentage of control = 73) than on CV-8A with 200 μg streptomycin per milliliter of medium (mean percentage of control = 57). The A^1 mean was significantly higher ($P = 0.01$) than the A^2 mean on MM + 200 μg streptomycin per milliliter of medium, whereas at the same concentration on CV-8A the mean percentage of the control for A^2 isolates was significantly higher ($P = 0.01$). With MM and CV-8A data combined, the A^1 and A^2 means for 200 g streptomycin per milliliter of medium were not significantly different. In all cases there were significant differences among individual A^1 and A^2 isolates, with the growth of some isolates reduced by 67–70%, whereas others appeared completely resistant to the drug.

Growth with chlortetracycline. *P. cinnamomi* isolates were sensitive to chlortetracycline; in pilot experiments with four isolates, inhibition was complete at 25 μg/ml and 70–80% at 5 μg/ml. At 1 μg chlortetracycline per milliliter of medium, several isolates grew sparsely or behaved inconsistently, but the colony diameters of 28 isolates averaged 47% of the controls (Table 3). The A^1 and A^2 means were not significantly different.

Growth with cycloheximide. Only a few *P. cinnamomi* isolates

TABLE 2. Effect of streptomycin[a] on growth of *Phytophthora cinnamomi* on minimum medium and on clarified V-8 agar

Minimal medium		Clarified V-8 agar	
Isolate	Mean % of control[b]	Isolate	Mean % of control[b]
62 A^1	101 A[c]	55	98 N[c]
73[d]	95 AB	100 A^1	97 N
96 A^1	90 ABC	97 A^1	92 NO
100 A^1	88 ABCD	62 A^1	89 NO
104 A^1	88 ABCD	3	84 OP
68 A^1	85 ABCD	16	82 OP
55	84 ABCD	7	81 OP
101 A^1	84 ABCD	18	76 PQ
21 A^1	75 BCDE	13	75 PQ
93	73 BCDE	17	74 PQR
8	72 BCDE	65	65 QRS
122 A^1	72 CDEF	93	63 RST
121 A^1	70 CDEFG	40	62 STU
74	68 CDEFGH	160 A^1	58 STUV
40	68 CDEFGH	73	58 STUV
152 A^1	67 CDEFGH	101 A^1	57 STUVW
159 A^1	66 DEFGH	104 A^1	57 STUVW
13	66 DEFGH	8	53 STUVWX
184 A^1	65 DEFGH	96 A^1	52 TUVWX
160 A^1	60 EFGH	53	52 TUVWX
7	59 EFGH	122 A^1	51 TUVWX
16	55 EFGHI	121 A^1	51 TUVWX
97 A^1	54 EFGHI	6	51 TUVWX
67 A^1	53 EFGHI	68 A^1	51 TUVWX
110	53 EFGHI	138 A^1	49 TUVWX
3	52 EFGHI	159 A^1	48 VWXYZ
138 A^1	52 EFGHI	74	46 VWXYZ
65	48 FGHI	110	46 VWXYZ
17	47 GHI	67 A^1	45 WXYZ
6	46 HI	152 A^1	43 XYZ
53	35 I	184 A^1	38 YZ
18	33 I	21 A^1	36 Z

[a] Concentration, 200 μg/ml.
[b] Based on colony diameter, average of three experiments.
[c] Duncan's multiple range, $P = 0.05$; data also significant at $P = 0.01$.
[d] Isolates without mating-type designations are A^2.

made very slight growth on MM containing cycloheximide at 10 μM. At 1 μM (0.28 μg/ml) all isolates grew, but there were more inconsistencies in the response of individual isolates, especially of A^2 isolates, in different replicate experiments than there were with other antibiotics. With 1 μM cycloheximide in MM (Table 4), the average for 17 A^1 isolates was significantly greater ($P = 0.01$) than the average for 15 A^2 isolates; the A^1 mean was 62, A^2 mean 45, and the overall mean 54% of the controls. There were significant differences between individual A^2 isolates, but not between A^1 isolates. When nine isolates (Pc 18, 21, 68, 100, 101, 104, 110, 138, and 152) were maintained on 1 μM cycloheximide for one to three transfers, they subsequently were able to grow on 10 μM cycloheximide. Throughout five to six serial transfers on 10 μM cycloheximide, growth continued and, in most cases, progressively improved.

Growth with nystatin. When nystatin was incorporated into MM without sterol, linear growth of all *P. cinnamomi* isolates was faster on media with the antibiotic than on control media. Greater differences between isolates were obtained when nystatin was tested in MM containing β-sitosterol than in MM without the sterol; at $P = 0.05$ ranges for means of 112% and above did not overlap with means below 100% of the controls (Table 4). With 100 μg nystatin per milliliter of medium (dissolved in ethanol) in MM + β-sitosterol, the average for 16 A^2 isolates (105% of control) was higher, but not significantly different from the A^1 average (100%). Similar results were obtained when nystatin was dissolved in dimethylsulfoxide.

Growth with ethidium bromide and chloramphenicol. In pilot experiments with ethidium bromide, the effect on linear growth of eight *P. cinnamomi* isolates was the same at antibiotic concentrations of 5, 25, 50, and 100 μg/ml. With ethidium bromide at 5 μg/ml the response of individual isolates varied from 9–76% of the controls (Table 5). Although all but one of the most resistant isolates were A^1, there were also A^1 isolates in the lowest range group. The mean percentage of control for 15 A^1 isolates was higher, but not significantly different from the mean for 20 A^2 isolates.

P. cinnamomi was more sensitive to ethidium bromide (overall mean = 40% of the control at 5 μg/ml) than to chloramphenicol (overall mean = 35% at 100 μg/ml). There was, however, a positive correlation between the responses of *P. cinnamomi* isolates to chloramphenicol and to ethidium bromide ($R = 0.7037$, significant at $P = 0.01$).

On MM + 100 μg chloramphenicol per milliliter of medium, the overall average was 35% of the control (Table 5). Isolates of both mating types were spread fairly evenly among the range groups, and the A^1 and A^2 means were not significantly different. Some isolates were less than 50% inhibited on the first exposure to 100 μg chloramphenicol per milliliter of medium. However, when they were transferred from MM + chloramphenicol to MM + chloramphenicol, at the same concentration, they grew poorly or not at all on the second transfer.

In Table 6, *P. cinnamomi* isolates are arranged in four general patterns of responses to streptomycin, chloramphenicol, ethidium bromide, and chlortetracycline: generally sensitive to all four drugs; resistant to streptomycin but sensitive to the other three; resistant to streptomycin and also showing resistance to one or more of the other drugs; and intermediate reaction to streptomycin but resistant to chloramphenicol, ethidium bromide, and chlortetracycline.

TABLE 3. Effect of chlortetracycline[a] on growth of *Phytophthora cinnamomi* in minimal medium

Isolate	Chlortetracycline (Mean % of control)[b]
184 A^1	75 A[c]
17[d]	72 AB
104 A^1	72 AB
40	71 ABC
62 A^1	67 ABCD
160 A^1	66 ABCD
110	66 ABCD
121 A^1	65 ABCDE
122 A^1	65 ABCDE
159 A^1	63 ABCDEF
8	59 ABCDEF
152 A^1	59 ABCDEF
55	57 BCDEF
96 A^1	54 CDEFG
7	52 DEFG
16	49 EFGH
6	47 FGH
3	39 GHI
18	34 HIJ
93	30 IJK
74	29 IJK
135	26 IJKL
101 A^1	25 IJKL
68 A^1	24 IJKL
53	22 JKL
67 A^1	18 JKL
97 A^1	17 KL
138 A^1	11 L

[a] Concentration, 1 μg/ml.
[b] Based on colony diameter, average of three experiments.
[c] Duncan's multiple range, $P = 0.05$; data also significant at $P = 0.01$.
[d] Isolates without mating type designations are A^2.

TABLE 4. Growth of A^1 and A^2 mating-type isolates of *Phytophthora cinnamomi* in the presence of cycloheximide and nystatin

Isolate	Mean percentage of control colony diameter[a]	
	Cycloheximide	Nystatin
A^1		
21	59 ABC[b]	109
62	70 A	97 WX[c]
67	67 A	103
68	56	104
96	57 ABC	99 VWX
97	67	98 VWX
100	64 AB	106
101	61 AB	100 VWX
104	60 ABC	101
121	64 AB	99 VWX
122	63 AB	99 VWX
138	64	99 VWX
152	65 A	97 VWX
159	69 A	102
160	57 ABC	102
184	69 A	99
A^2		
3	57 ABC	101
6	61 AB	99
7	39	111
8	65 A	115 PQR
13	57 ABC	103
16	46	102
17	55	124 P
18	23	113 PQRST
40	54 ABC	105
45	19	108
53	23	114 PQRS
55	42 C	89 X
65	37	115 PQ
73	72	100 VWX
74	...	97 WX
93	46 BC	100 UVWX
110	65 A	104
135	75	106
382	41	112 PQRSTU

[a] Growth on synthetic minimal media; β-sitosterol added in the nystatin experiment. Cycloheximide, 1 μM; nystatin, 100 μg/ml.
[b] Duncan's multiple range, significant at $P = 0.01$, isolates without letters were not included in analysis.
[c] Duncan's multiple range, significant at $P = 0.05$, isolates without letters fell into intermediate, overlapping ranges.

DISCUSSION

One of our intentions in these studies was to determine whether the response of numerous isolates to several common antibiotics would provide an additional criterion for the definition of physiological races of *P. cinnamomi*.

When the isolates studied were grouped according to their growth response in the presence of the antibiotics tested (Table 6), this grouping did not correlate consistently with the growth temperature responses (29) or the pathogenicity to various plants (*unpublished*), or the host and geographic origins of the isolates studied. There were, however, a number of isolates that responded similarly in the temperature response tests (29) and in these studies. For example, the A^1 isolates from camellia, Pc 67 and 97, and the A^1 isolate from avocado, Pc 138, all grew slowly, had similar cardinal temperatures (29), and showed uniform sensitivity to the antibiotics tested. Pc 138 is the only known A^1 culture isolated from avocado in California. Other California A^1 isolates from camellia (Pc 68, 96, 100, 101, and 104) were markedly more resistant to streptomycin and also grew more rapidly (L. J. Klure, *unpublished*).

A number of workers noted striking differences in the response of various *Phytophthora* spp. or isolates to streptomycin, ranging from nearly complete inhibition to no inhibition of growth at 100 μg/ml (2,9,24,26). Some of these differences could be related to our observation that the effect of streptomycin on growth of various *P. cinnamomi* isolates was modified in different ways by nutrition. On MM the A^1 mean was higher (indicating more resistance) than the A^2 mean, whereas on CV-8A the A^2 mean was higher. Nutrition strongly affected the response of individual isolates; for example, on MM + streptomycin at 200 μg/ml, Pc 18 grew only 33% of the control, whereas on CV-8A + streptomycin at 200 μg/ml, Pc 18 grew 76% of the CV-8A control. Further study of the response to streptomycin by sexual and asexual progeny of *P. cinnamomi* would be interesting in comparison to the data obtained with *P. cactorum* (16).

Two of the isolates used in the present study, Pc 62 and Pc 152, grew unusually well at high temperatures. However, they differed markedly from each other in their growth response in the presence of the various antibiotics.

Differences in growth rate and cardinal temperatures among isolates from Australia have been reported previously (19,29). We found at least three different patterns of response to the antibiotics among the Australian isolates. Similar groups were also found among isolates from avocado. The significant differences between Pc 40 and Pc 53 in sensitivity to four antibiotics are particularly striking, since these cultures were obtained as single zoospores from the same field isolate. Why such marked differences should occur with two isolates from the same source cannot be explained at this time. The data would suggest that the field isolate was not genetically "pure" and may have been a heterokaryon or a heteroplasmon. Also supporting this idea is the fact that several isolates (Pc 13, 16, 17, 21, 45, 65, and 74) behaved inconsistently during the course of these studies. These inconsistencies were manifested as changes in morphology, sectoring of the colony, and variable growth response to one or more of the antibiotics. It is possible that this behavior was due to genetically different nuclei or cytoplasms.

Drug resistance, including resistance to some of the same antibiotics we have used, has provided useful markers in several studies of *Phytophthora* spp. genetics (7,11,15–17). Naturally occurring differences between wild-type isolates, such as we have

TABLE 5. Effect of ethidium bromide[a] and chloramphenicol[b] on growth of *Phytophthora cinnamomi* in minimal medium

Ethidium bromide[a]		Chloramphenicol[b]	
Isolate	Mean % of control[c]	Isolate	Mean % of control[c]
100 A^1	76 A[d]	40	61 P[d]
184 A^1	69 AB	184 A^1	57 PQ
160 A^1	66 AB	110	53 PQR
122 A^1	65 AB	7	51 PGRS
135 [e]	65 AB	121 A^1	50 PQRST
152 A^1	63 AB	122 A^1	50 PQRST
121 A^1	60 ABC	152 A^1	49 PQRST
104 A^1	54 BCD	159 A^1	47 PQRSTU
110	52 BCDE	8	44 PQRSTUV
13	50 BCDEF	104 A^1	43 PQRSTUVW
40	44 CDEFG	17	43 PQRSTUVW
74	40 DEFG	160 A^1	42 PQRSTUVW
6	38 DEFG	135	39 QRSTUVWX
68 A^1	38 DEFG	16	38 QRSTUVWXY
21 A^1	37 DEFG	73	37 QRSTUVWXY
3	35 DEFGH	21 A^1	35 RSTUVWXY
67 A^1	35 DEFGH	3	31 STUVWXYZ
18	34 EFGH	6	30 STUVWXYZ
7	34 EFGHI	62 A^1	27 UVWXYZ
16	33 EFGHI	96 A^1	25 VWXYZ
73	31 FGHI	18	23 WXYZ
97 A^1	30 FGHI	67 A^1	22 XYZ
101 A^1	28 GHIJ	55	20 XYZ
138 A^1	26 GHIJ	101 A^1	20 XYZ
96 A^1	25 GHIJ	97 A^1	20 XYZ
62 A^1	24 GHIJ	53	18 YZ
93	16 HIJ	93	14 Z
17	14 IJ	382	12 Z
53	14 IJ		
55	9 J		

[a] Concentration, 5 μg/ml.
[b] Concentration, 100 μg/ml.
[c] Based on colony diameter, average of three experiments.
[d] Duncan's multiple range, $P = 0.05$; data also significant, $P = 0.01$, data for each antibiotic analyzed separately.
[e] All isolates without mating-type designations are A^2.

TABLE 6. Comparative sensitivity (S) and resistance (R) of *Phytophthora cinnamomi* isolates to several antibiotics[a]

Isolate	Streptomycin[b]	Chloramphenicol[c]	Ethidium bromide[d]	Chlortetracycline[e]
67 A^1[f]	S	S	I–S[g]	S
97 A^1	S	S	I–S	S
138 A^1	S	S*[h]	S	S
3	S	S	I–S	I–S
6	S	S	I–S	I
18	S	S	I–S	I–S
53	S	S	S	S
68 A^1	R	S*	I–S	S
96 A^1	R	S	S	Var[i]
101 A^1	R	S	S	S
55	R	S	S	Var
73	R	I	I–S	I–S
93	I–R	S	S	I–S
62 A^1	R	S	S	R
100 A^1	R	Var	R	I–R
104 A^1	R	I–R	I–R	R
135	R*	I	R	S
121 A^1	I	R	R	R
122 A^1	I	R	R	R
152 A^1	I	R	R	R
159 A^1	I	R	R	R
160 A^1	I	R	R	R
184 A^1	I	R	R	R
7	I	R	Var	I–R
8	I	R	Var	R
40	I	R	I	R
110	I	R	I–R	R

[a] Based on percentage of control colony diameter; S = lowest percent control range group, Duncan's multiple range, $P = 0.05$; R = highest percent control range group; and I = intermediate range groups.
[b] Streptomycin 200 μg/ml in MM.
[c] Chloramphenicol 100 μg/ml in MM.
[d] Ethidium bromide 5 μg/ml in MM.
[e] Chlortetracycline 1 μg/ml in MM.
[f] All isolates without mating type designation are A^2.
[g] I–S = intermediate range groups, but closer to susceptible end of distribution; I–R = intermediate, but closer to resistant than to susceptible.
[h] * = reactions not included in statistical analysis.
[i] Var = variable behavior in replicate experiments.

found, could provide a basis for genetical studies, or such characters could supplement induced markers. With several of the compounds and concentrations we used, *P. cinnamomi* isolates generally ranged between 20–70% of the controls; enhancement of resistance through mutation, adaptation, or single-spore selection might produce more distinct phenotypes.

When sensitivity data for individual antibiotics were paired with data for other antibiotics in regression analyses, two strong correlations were observed: ethidium bromide versus chloramphenicol ($R = 0.7037$, $P = 0.01$), and chloramphenicol versus chlortetracycline ($R = 0.796$, $P = 0.01$). There was not a significant correlation between the results for ethidium bromide and for chlortetracycline. These comparisons possibly can be explained by the reports that ethidium bromide specifically inhibits nucleic acid synthesis (5,13), and chlortetracycline specifically inhibits protein synthesis (21), whereas chloramphenicol can interfere with both macromolecular synthetic processes (8,14).

We observed differential reactions with some isolates in response to cycloheximide and chlortetracycline. For example, Pc 67 was resistant to cycloheximide and sensitive to chlortetracycline, and Pc 40 was comparatively sensitive to cycloheximide and resistant to chlortetracycline. This is consistent with reports that these two antibiotics act at different specific sites in interfering with eukaryotic protein biosynthesis (25). Our contrasting observations that *P. cinnamomi* isolates could not be serially transferred on media with chloramphenicol, whereas "adaptation" could take place in vitro with cycloheximide, may reflect differences in sensitivity that relate either to differences in the sites of action of these compounds or to differences in the mechanisms by which resistance is inherited in the fungus.

Chloramphenicol is used at 10 μg/ml in a selective medium developed specifically for *P. cinnamomi* by Shew and Benson (20). The sensitivity we observed in some isolates at 100 μg/ml indicates that caution is necessary whenever this antibiotic is used for new situations.

The resistance of the vegetative stage of *P. cinnamomi* isolates to the polyene antibiotic nystatin was expected, since the Pythiaceous fungi are known to be resistant to a number of polyenes (22), and in the process of purifying these isolates they have been cultured on media containing the polyene pimaricin. It is interesting to note that compared to A^2 isolates, the A^1 isolates showed a slightly greater stimulation by β-sitosterol and a corresponding slight sensitivity to nystatin. This may be related to the observation that organisms normally resistant to polyenes may become sensitive when they have incorporated sterols into their membranes.

Shepherd et al (19) studied the growth response of 20 A^1 and 20 A^2 isolates to eight inhibitors. Except for one A^1 isolate, they did not find significant differences among isolates of the same mating type. Significant differences were found between the mating types in response to safranin O, rose bengal, and pyronin G. With acriflavine, basic fuchsin, brilliant green, malachite green, and copper sulfate, there were no significant differences between the A^1 and A^2 means. Their studies were conducted in a fairly rich sucrose/casein medium (18), which may have masked some differences. Since almost all of the isolates used were from Australia, the full range of variability for the species is not known. Intraspecific variability precludes such general applications as using rose bengal in a selective medium (22), or attempting to distinguish species with malachite green (10). However, our results and those of Shepherd and co-workers (18,19) indicate that in some instances pharmacological responses may help distinguish subspecific groups, such as mating types or physiological races.

Additional studies testing fungicides, antibiotics, and other inhibitors against *Phytophthora* spp. isolates from various parts of the world would be useful. In particular, more information is needed on the effect of antibiotics on the production and germination of various spore stages. The possibility of differential sensitivity of life cycle stages, such as has been observed for *P. infestans* (1,3), is especially relevant to the development of selective media (22,23). Sensitive and resistant isolates could also be used to further clarify specific modes of action, stage specificity, or timing effects (1) of these antibiotics.

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