Variability in Growth of Phytophthora cinnamomi in Relation to Temperature

G. A. Zentmyer, J. V. Leary, L. J. Klure, and G. L. Grantham

Professor, Assistant Professor, and Staff Research Associates, Department of Plant Pathology, University of California, Riverside 92502.

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ABSTRACT

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Growth of 187 isolates of *Phytophthora cinnamomi* (20 A^1, 167 A^2 mating type) from 24 countries and 59 hosts was compared at 25 C on potato-dextrose agar. The frequency distribution obtained for these isolates closely approximated a normal curve, with 90 isolates below the mean and 97 above. Colony diameters after 4 days of growth ranged from 41 to 85 mm. Nutrition affected the growth-temperature response; some isolates grew rapidly on one medium and slowly on another medium at 25 C. On minimal medium at 25 C with and without β-sitosterol the mean growth rate for A^1 mating type isolates of *P. cinnamomi* was significantly slower than for A^2 isolates; on clear V-8 agar there was no significant difference between the two mating types. At 30 and 33 C on minimal medium, three A^1 isolates grew more rapidly than 16 other isolates that included 12 A^2 and 4 A^1 types. Our studies, plus other reports, indicate that the cardinal temperatures for *P. cinnamomi* range from: minimum, 5-16 C; optimum, 20-32.5 C; and maximum, 30-36 C.

Additional key words: nutrition, mating type.

Temperature is one of the most significant environmental factors in growth, reproduction, and pathogenesis of plant pathogenic fungi. This paper reports studies of vegetative growth in relation to temperature, involving isolates of *Phytophthora cinnamomi* Rands from various parts of the world and from many different hosts. This fungus is being increasingly recognized in recent years as a highly destructive pathogen on many plants. Pathologists in Australia have become particularly aware of its potentialities in relation to "jarrah dieback" in the past 10 years (10, 11) and much research is being reported on Australian isolates.

There have been a number of reports of the effect of temperature on *P. cinnamomi* (2, 3, 5, 6, 7, 9, 14, 15, 17); most of these have included only a few isolates and have indicated little variability in response. In general, these studies showed that *P. cinnamomi* is a moderate-temperature fungus, with little growth below 10 C or above 33 C, and optimum temperatures ranging from 20 to 27 C. The extensive study by Shepherd and Pratt (12) involved many isolates of *P. cinnamomi* from Australia; they reported variation in rates of growth at 25 C and in cardinal temperatures. Shepherd et al. (13) found that the mean growth rates of A^2 isolates were slightly higher than those of A^1 isolates between 15 and 30 C, but at 32.5 the mean growth rate of A^1 isolates was significantly higher than that of A^2 isolates. Four of their A^1 isolates grew at 34 C whereas no A^2 isolates grew at that temperature.

MATERIALS AND METHODS

Isolates of *P. cinnamomi* were obtained from the following countries (the number of isolates from each area follows the name): Australia (New South Wales, Queensland, S. Australia, Victoria, W. Australia), 50; Brazil, 3; Cameroon, 3; Canada, 1; Colombia, 3; Congo, 2; Costa Rica, 5; El Salvador, 1; England, 5; France, 1; Germany, 1; Honduras, 1; Indonesia (Sumatra), 1; Ivory Coast, 4; Jamaica, 2; Malagasy Republic, 2; Mexico, 9; New Zealand, 7; Papua New Guinea, 1; Peru, 2; South Africa, 5; Trust Territory of the Pacific (Ponape), 1; United States (California, 44; Georgia, 2; Hawaii, 8; Maryland, 3; North Carolina, 4; Ohio, 3; Oregon, 3); Virgin Islands (U.S., St. Croix), 1. These isolates were from 59 different hosts. The mating types of the *P. cinnamomi* cultures were related to the original designations of A^1 and A^2 in *P. infestans,* as established by Gallegly and Galindo (4).

The synthetic minimal medium (MM) was slightly modified from that of Bartnicki-Garcia (1) and contained per liter: KH_2PO_4, 2.0 g; $CaCl_2 \cdot 2H_2O$, 3.4 mg; chelated iron, 1 ml ($FeCl_3 \cdot 6H_2O$, 1.0 mg, ethylenedinitrilotetraacetic acid disodium salt, 10.0 mg); $ZnSO_4 \cdot 7H_2O$, 1.8 mg; $MnSO_4 \cdot H_2O$, 0.3 mg; $CuSO_4 \cdot 5H_2O$, 0.4 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.3 mg; $MgSO_4 \cdot 7H_2O$, 0.5 g; thiamine HCl, 1.0 mg; $NaNO_3$, 0.5 g; glucose, 20 g; and Noble agar, 15 g. The pH was adjusted to 6.0 before the solution was autoclaved.

Cleared V-8 juice agar (CV-8A) was prepared by mixing 2 g of $CaCO_3$ with 200 ml of Campbell's V-8 juice, centrifuging at 3,500 *g* for 20 minutes, diluting 200 ml of the supernatant with 800 ml of deionized water, and adding 15 g agar. Fresh potato dextrose agar (PDA) was prepared by autoclaving for 10 minutes 250 g of potatoes covered with demineralized water, straining the juice through cotton, and bringing the volume up to 1 liter, and adding 20 g glucose and 20 g agar.

Isolates were maintained as mass transfers on V-8 slants at 15 C. At least two transfers at room temperature were made before conducting tests, especially in the case of studies on MM, where cultures were serially transferred to eliminate carry-over of nutrients.

Vegetative growth at temperatures ranging from 6-36 C was studied using controlled-temperature incubators maintained at intervals of 3 C. Inoculum disks (5 mm) from the margins of cultures growing on MM were transferred to 15 ml MM plates and were incubated in darkness. Colony diameters were measured at 3 and 5 days. There were three replicates per culture per temperature in each experiment, and experiments were replicated at least three times.

RESULTS

Growth of 187 isolates (20 A^1 and 167 A^2 mating type) on PDA at 25 C for 4 days ranged from 41 to 85+ mm. Distribution of colony size is shown in Fig. 1. Of six cultures in the slowest growth class, two each were from South Africa and Mexico, and one each from Costa Rica and Hawaii. Five were A^2 compatibility type, and one was A^1. Eight of sixteen cultures in the most rapid growth class were from Australia, two each from Hawaii, Jamaica, and California, and one from England and Ohio. All were A^2 isolates. Rands' type culture (Pc 110) was intermediate in growth rate, in the 67.5 - 72.5 mm class. The cultures showed a remarkably normal

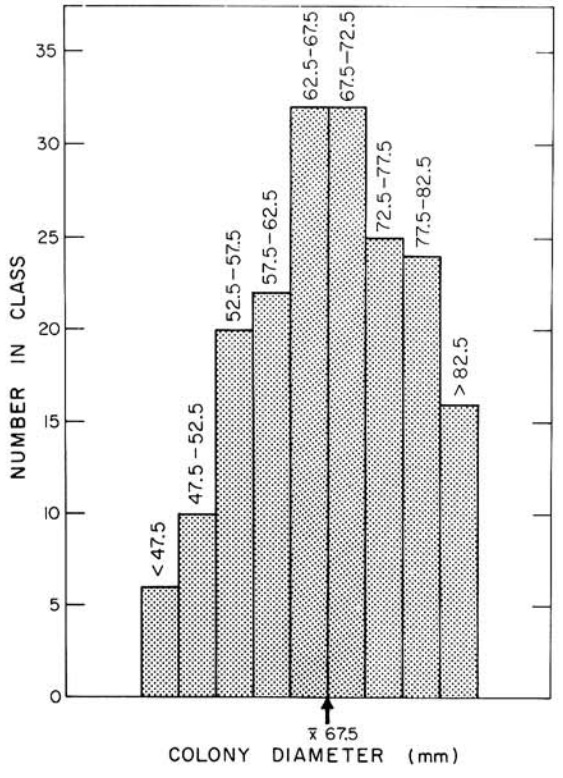

Fig. 1. Frequency distribution of colony diameters of 187 isolates of *Phytophthora cinnamomi* grown on potato dextrose agar for 4 days at 25 C.

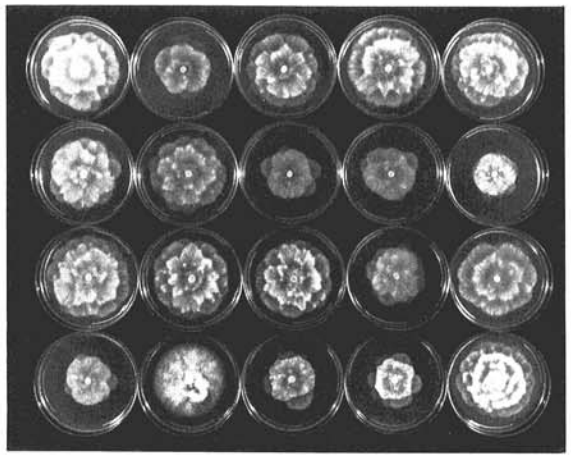

Fig. 2. Cultures of *Phytophthora cinnamomi* grown for 4 days on potato dextrose agar at 25 C. Morphology of the culture in lower right corner was typical of the majority of isolates.

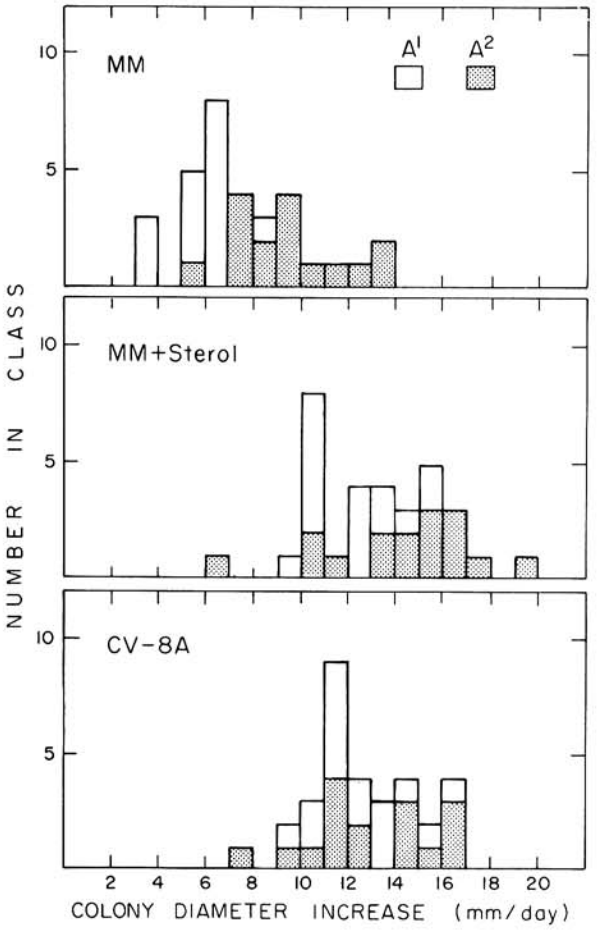

Fig. 3. Frequency distributions of growth rates of 16 A^1 and 16 A^2 isolates of *Phytophthora cinnamomi* grown on minimal medium (MM), MM + β-sitosterol, and clear V-8 agar (CV-8A) at 25 C.

distribution curve, with a chi-square value of 4.76. The hypothesis that this is a normal distribution would not be rejected.

Of 90 cultures below the mean, 18 were A^1 and 72 were A^2 isolates. Ninety-five of ninety-seven cultures above the mean were A^2 and two were A^1. Fifty cultures from Australia were tested; 21 were below the mean and 29 above. Of 44 cultures from California, 21 were below the mean and 23 above. There was no obvious correlation between the culture origin and growth rate. Cultures from warm tropical areas (Costa Rica, Hawaii, Mexico) were scattered throughout various growth classes, as were cultures from the more temperate regions (e.g. England, New Zealand, South Africa, North Carolina).

There was no observed correlation between host and growth rate. Eighty isolates from avocado occurred in all but the lowest growth class, and when plotted separately, resembled a normal frequency distribution. Of eight isolates from camellia (all A^1), six were below the mean and two above the mean. Figure 2 shows the variable growth and phenotype of some of the cultures grown on PDA at 25 C.

Growth of 26 cultures was studied on MM at 25 C (Table 1). Growth ranged from 3.0 to 7.7 mm per day on MM, as compared with 11.2 to >21 mm per day on PDA. Compared to the mean for each medium, the growth rates of some isolates were strikingly different on MM and PDA. An A^1 isolate from macadamia trees in Hawaii made the most rapid growth on MM but was in the slowest-growing class on PDA. The slowest-growing culture on MM (an A^1 isolate from camellia) was in the class just above the mean on PDA. The type culture (Pc 110) was intermediate in growth rate on both media. Of the 15 A^1 cultures tested, 10 were below the mean, compared to only two of the 11 A^2 cultures below the mean.

Growth of 16 A^1 and 16 A^2 isolates were compared on MM, MM + β-sitosterol (30 mg/liter), and CV-8A at 25 C (Fig. 3). The mean growth rate of the A^1 isolates was significantly ($P = 0.01$) slower on MM than that of the A^2 isolates. On MM + β-sitosterol the A^1 mean was significantly ($P = 0.05$) slower than the A^2 mean, and the mean growth rates of both groups were significantly faster than their respective means on MM without sterol. On CV-8A the mean growth rates of the two groups were not significantly different. Individual isolates varied considerably in their growth rates on different media. For example, Pc 97 and Pc 65, which were in the slowest groups on MM, were both in the fastest group on CV-8A; conversely, Pc 18, in the slowest group on CV-8A was in the fastest group on MM.

Growth of 19 isolates was studied on MM at temperatures ranging between 6-36 C (Table 2). Differences in growth rates between isolates were greatest

TABLE 1. Mean colony diameters of 26 *Phytophthora cinnamomi* isolates grown at 25 C on minimal medium agar and potato dextrose agar

Isolate no.	Source of isolate	Mating type	Minimal Medium: Colony diameter[a] mm (5 days)	Minimal Medium: Homogeneous subgroups[b]	PDA: Colony diameter[c] mm (4 days)
Pc 62	*Macadamia integrifolia*, Hawaii	A^1	38.5	A	45
Pc 16	*Hibbertia cunninghamii*, Australia	A^2	34.4	B	79
Pc 13	*Lasiopetalum floribundum*, Australia	A^2	33.5	BC	82
Pc 40	*Persea americana*, California	A^2	32.9	BCD	75
Pc 184	*Eucalyptus globoidea*, Australia	A^1	31.0	CDE	53
Pc 8	*Lomandra* sp., Australia	A^2	30.8	CDEF	85+
Pc 7	*Hovea elliptica*, Australia	A^2	30.5	CDEFG	83
Pc 152	*Tristania conferta*, Australia	A^1	29.8	DEFGH	58
Pc 3	*Leucopogon verticillata*, Australia	A^2	28.5	EFGHI	85+
Pc 159	*Vitis* sp., South Africa	A^1	27.8	FGHI	68
Pc 100	*Camellia japonica*, California	A^1	27.6	GHI	66
P 382	*Erica gracilis*, Germany	A^2	27.5	GHI	...
Pc 110	*Cinnamomum burmanni*, Sumatra	A^2	27.3	HI	68
Pc 6	*Xylomelum occidentalis*, Australia	A^2	26.8	HIJ	80
Pc 93	*Persea americana*, California	A^2	25.5	IJK	56
Pc 160	*Vitis* sp., South Africa	A^1	24.1	JKL	59
Pc 21	*Camellia japonica*, California	A^1	23.4	KL	65
Pc 65	*Persea americana*, E. Caroline Islands	A^2	23.2	KL	59
Pc 96	*Camellia japonica*, California	A^1	23.0	KL	60
Pc 121	*Persea americana*, Madagascar	A^1	22.5	KL	61
Pc 122	*Persea americana*, Madagascar	A^1	22.1	L	58
Pc 101	*Camellia japonica*, California	A^1	19.1	M	66
Pc 104	*Camellia japonica*, California	A^1	18.7	M	62
Pc 138	*Persea americana*, California	A^1	15.8	N	65
Pc 97	*Camellia japonica*, California	A^1	15.4	N	64
Pc 67	*Camellia japonica*, California	A^1	15.0	N	71

[a]Colony diameters (5-mm diameter inoculum plug subtracted) were derived from the means of three replicate cultures from 11 separate experiments.

[b]Duncan's multiple range, significance at $P = 0.01$.

[c]Data from one experiment.

at 30 C. The optimum temperature for growth ranged from 21-30 C, but most cultures grew best between 24-27 C. Generally, daily growth was linear for each isolate within the range of 21-30 C, but outside this range, some isolates exhibited a slight lag during the first 3 days, or a slightly slower growth rate during the 3- to 5-day period. These growth rate differences generally were not more than 1 mm/day, and could be related to minor fluctuations in temperature or to variable growth during the period before equilibrium was reached.

DISCUSSION

Isolates identified by various workers as *P. cinnamomi* have exhibited in vitro cardinal temperatures for growth within the following ranges: minimum, 5-16 C; optimum, 20-32 C; maximum, 30-36 C. Waterhouse (16) indicated the following cardinal temperatures for *P. cinnamomi*: minimum, approximately 5 C; optimum, 24-28 C; maximum, 32-34 C. Our work with this species, along with other reports (5, 6, 12, 14, 15) supports the conclusion that the cardinal temperatures for the species should be revised.

Our observations agree with those of Shepherd and Pratt (12); i.e., composition of the nutrient medium affects the growth-temperature relationships. More attention must be given to the growth medium if response to temperature is used as a taxonomic criterion, since nutrient composition influences the cardinal temperatures as well as the growth rate. When various isolates of *P. cinnamomi* are compared at the same temperature, individual isolates may grow rapidly on one medium and slowly on another medium, as compared to the mean growth rate for each medium. This suggests that different isolates within the species also vary in response to nutritional or environmental factors other than temperature.

Most of our studies were completed before Shepherd and Pratt published their work with Australian isolates (12, 13), and unfortunately the media and methods we had used were different. The mean radial growth rate for 50 isolates on V-8 agar at 25 C was 12.25 mm/day (12), whereas the 32 isolates we studied on CV-8A at 25 C gave a mean radial growth rate of 6.25 mm/day. Part of this difference may reflect differences in techniques, but we have used their methods with some of our isolates and obtained significantly slower growth rates than they report. Possibly there are actually growth rate differences between the two groups of isolates since it appears that some *P. cinnamomi* populations in Australia have growth rates that are among the most rapid observed for this fungus. This is also suggested by the skew toward the fast side of the growth rate frequency distribution that Shepherd and Pratt (12, Fig. 2) presented for 361 isolates. However, A^1 isolates apparently were not included in these data, and the growth rate distribution for the Australian *P. cinnamomi* population as a whole may be slightly different.

Shepherd et al. (13) indicated that their results did not support the suggestion by Galindo and Zentmyer (3) and A^1 isolates have slightly higher temperature optima than A^2 isolates. Yet their data show A^1 isolates growing almost as well at 32.5 as at 25 C, and growing nearly three times as fast as A^2 isolates at 32.5. An accurate picture of the temperature optima for the two mating types world wide will be gained only through further detailed analysis of growth curves of many isolates.

Our data in general show that daily growth increments

TABLE 2. Growth of *Phytophthora cinnamomi* isolates on minimal media agar for 5 days at various temperatures

Isolate[b] no.	Mean colony diameter (mm) at indicated temperatures[a]										
	6 C	9 C	12 C	15 C	18 C	21 C	24 C	27 C	30 C	33 C	36 C
Pc 3	0	1	5	20	19	34	35	40	35	16	0
Pc 13	0	2	10	23	22	34	49	49	40	17	0
Pc 16	0	1	6	19	20	29	36	37	33	16	0
Pc 18	0	0	1	5	4	20	30	35	25	7	0
Pc 33	0	0	1	8	9	24	34	33	39	35	1
Pc 40	0	4	12	25	30	46	46	42	29	3	0
Pc 45	0	1	3	16	16	39	34	18	25	1	0
Pc 53	0	1	8	23	21	41	48	40	25	4	0
Pc 62	0	0	2	17	22	42	42	46	48	36	1[c]
Pc 67	0	0	1	7	3	15	13	10	4	4	0
Pc 73	0	1	7	19	19	30	32	28	32	3	0
Pc 93	0	0	2	14	12	22	27	31	26	8	0
Pc 97	0	2	5	16	18	21	21	18	6	2	0
Pc 104	0	0	1	9	6	17	17	18	8	4	0
Pc 110	0	5	11	22	22	32	38	39	38	6	0
Pc 135	0	1	7	16	17	24	31	30	29	5	0
Pc 138	0	2	5	22	19	28	28	23	8	2	0
Pc 152	0	1	2	13	8	25	31	35	46	30	0
P 382	0	0	1	6	9	21	23	22	7	0	0

[a]Colony diameters (5-mm diameter inoculum plug subtracted) from at least three separate experiments, replicated three times/experiment.

[b]Isolates are identified in Table 1 except as follows: Pc18 (A^2), *Acacia huegelii*, Australia; Pc 33 (A^1), *Thuja* sp., Hawaii; Pc 45 (A^2), *Pinus echinata*, Georgia; Pc 53 (A^2), *Persea americana*, California; Pc 73 (A^2), *Eucalyptus marginata*, Australia; Pc 135 (A^2), *Pinus* sp., Australia.

[c]On PDA, this isolate grows 1-2.5 mm/day at 36 C.

at temperatures between 15-30 C are linear, especially if the first 24 hours and the last 24 hours before the plate is filled are excluded. Possibly factors other than temperature may cause nonlinear growth by *P. cinnamomi* under some conditions. Kliejunas and Ko (8) grew an isolate on a medium in tubes, and found that growth continued for 50 days. Fluctuations in the daily growth rate they obtained during the first 5 days, appear greater than those observed by us or by Shepherd and Pratt (12).

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