Characteristics of Asexual Sporulation in Phytophthora palmivora and Phytophthora parasitica nicotianae

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ABSTRACT

Sporangial and zoospore production in vitro was measured in two isolates of *Phytophthora palmivora* and two isolates of *P. parasitica nicotianae*. The greatest number of sporangia were produced on agar supplemented with nutrients under continuous light (50 ft-c) at 25 C for all isolates except IS7. On water agar, *P. palmivora* isolates did not produce sporangia under continuous darkness, but *P. parasitica nicotianae* isolates produced sporangia under continuous light or darkness. Many zoospores of *P. palmivora* were observed in flooded lima bean agar plates at 10 C, but few at 25 and none at 30 C. *Phytophthora parasitica nicotianae* were active at 10, 25, and 30 C. There was a wide range of sporangial sizes where indirect germination took place, even though most of the germinated sporangia were of average size of all sporangia produced in the medium. Apparently some of the zoospores observed in sporangia did not become active (swimming) in the solutions tested.

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The importance of sporangia and zoospores of *Phytophthora* species in the infection of susceptible hosts is widely recognized. Methods to determine resistance in plant species to *P. palmivora* and *P. parasitica nicotianae* include the use of sporangia and zoospores (2, 5, 8), and similar methods are widely used for other species of *Phytophthora*. Reports of asexual sporulation document the variability of *Phytophthora* species and their requirements in vitro (4).

Our studies compare two isolates each of *P. palmivora* (Butl.) Butl. and *P. parasitica* Dastur var. *nicotianae* Tucker in their ability to produce sporangia and swimming zoospores under several conditions in vitro.

MATERIALS AND METHODS.—The ability of *P. palmivora* isolates P7 and PBr, pathogenic to *Piper nigrum*, and *P. parasitica nicotianae* isolates STW1 and IS7, pathogenic to *Tephrosia vogelii* to produce sporangia was tested. Sterile petri plates with 15 ml of a substrate of water agar with thiamine (0.002 g/liter) or a nutrient agar containing $NaNO_3$, 1.5 g; KH_2PO_4, 1.0 g; $MgSO_4$, 0.5 g; glucose, 5.4 g; agar, 30 g; and thiamine 0.002 g/liter of distilled water with or without cholesterol, exposed to continuous light (50 ft-c) or continuous darkness at 25 C were used. Cholesterol was added to the medium before autoclaving as a solution in acetone.

The fungal cultures used as inoculum had been grown in plates with nutrient agar without cholesterol. A piece of a 2-week-old culture grown at 25 C under continuous light (50 ft-c) was taken with a sterilized No. 1 cork borer and placed in the center of the petri plates with the test media for the trials.

Sporangial production of isolates at 10, 25, and 30 C was compared on commercial lima bean agar (LBA) versus nutrient agar containing 10 mg/liter of cholesterol. Plates were incubated for 2 weeks under continuous light (50 ft-c). We measured zoospore production for four isolates under these conditions by counting 20 fields of low power (X 100). The frequency of germinated sporangia by size was noted from plates incubated at 10 C. Plates were flooded with approximately 10 ml of distilled water to induce zoospore release after the 2-week incubation period. The water temperature when applied was at the same level as the incubation temperature of the plates. Continuous darkness was achieved by wrapping the individual plates with aluminum foil. Each test had 10 replicates and was done twice.

RESULTS.—Table 1 summarizes sporangial production by the four isolates under continuous light and darkness on water agar and on the nutrient medium with and without cholesterol at 25 C. The greatest number of sporangia were produced under continuous light on nutrient agar for all isolates except IS7. The least number of sporangia were produced on water agar in the dark. Under these latter conditions, no sporangia were observed on either isolate of *P. palmivora*. Sporangial production in both isolates of *P. parasitica nicotianae* was low in water agar under continuous light or darkness. Under light, nutrient agar stimulated sporangial formation when compared to water agar. This effect was also observed with isolates of *P. parasitica nicotianae* grown in the dark, but not with isolates of *P. palmivora*.

The effect of cholesterol was variable. It stimulated sporangial production in both isolates of *P. palmivora*, and at higher concentrations (10 to 20 mg/liter) in isolate STWI of *P. parasitica nicotianae*, but not of isolate IS7. When stimulation occurred, optimal concentration of cholesterol in the media was generally between 5 and 10 mg/liter.

Table 2 shows percent indirect germination of sporangia in the four isolates as observed under the microscope by low (X 100) magnification. Germination was observed 1 and 3 cm away from the center of the plates at 10 C on the LBA and cholesterol media in all four isolates. This was also observed in both isolates of *P. parasitica nicotianae*, except in

TABLE 1. Sporangial production by *Phytophthora palmivora* (P7, PBr) and *P. parasitica nicotianae* (STWI, IS7) isolates at 25 C on water agar and nutrient agar under continuous light or darkness with different cholesterol concentrations in the culture media

	Light								Dark							
	Amended nutrient agar				Amended H_2O agar				Amended nutrient agar				Amended H_2O agar			
	Cholesterol (mg/liter)				Cholesterol (mg/liter)				Cholesterol (mg/liter)				Cholesterol (mg/liter)			
	0	5	10	20	0	5	10	20	0	5	10	20	0	5	10	20
P7	45[a]	125	120	55	1	4	4	2	1	7	7	2	0	0	0	0
PBr	450	650	540	400	8	8	15	12	0	1	1	1	0	0	0	0
STWI	25	25	75	75	2	2	7	7	13	12	12	7	1	2	2	2
IS 7	30	30	28	30	8	6	8	5	30	30	70	18	8	7	6	8

[a] Number of sporangia per low power field, average of two trials of 20 random readings each.

TABLE 2. Indirect sporangial germination of *Phytophthora palmivora* (P7, PBr) and *P. parasitica nicotianae* (IS7, STWI) isolates at 10, 25, and 30 C in lima bean agar (LBA) and nutrient agar amended with cholesterol at the rate of 10 mg/liter

	Temperature											
	10 C				25 C				30 C			
	LBA		Cholesterol		LBA		Cholesterol		LBA		Cholesterol	
	1 cm	3 cm	1 cm[b]	3 cm[b]	1 cm	3 cm	1 cm	3 cm	1 cm	3 cm	1 cm	3 cm
P7	59	5[a]	56	84	4	3	0	0	0	0	0	0
PBr	77	60	31	22	0	0	10	0	0	0	0	0
IS7	36	29	26	57	43	52	3	7	35	25	18	21
STWI	38	38	21	35	5	17	15	34	35	23	0	0

[a] Per cent sporangial germination per 100 times power field, average of two trials of 20 random readings each.
[b] One and 3 cm from the center of the plate.

TABLE 3. Swimming zoospores in *Phytophthora palmivora* (P7, PBr) and *P. parasitica nicotianae* (IS7, STWI) isolates at 10, 25, and 30 C in lima bean agar (LBA) and nutrient agar amended with cholesterol at the rate of 10 mg/liter

	Temperature					
	10 C		25 C		30 C	
	LBA	Cholesterol	LBA	Cholesterol	LBA	Cholesterol
P7	1.38×10^6[a]	6.9×10^3	1.6×10^3	0	0	0
PBr	1.21×10^6	6.9×10^3	0	0	0	0
IS7	1.90×10^6	5.2×10^3	2.8×10^6	6.9×10^3	1.7×10^3	1.7×10^3
STWI	3.90×10^6	1.6×10^3	3.9×10^6	1.7×10^3	3.4×10^3	0

[a] Number of spores per ml, average of 20 squares (1/16 cm^2) on a hemocytometer per isolate.

the cholesterol medium at 30 C in STWI. No germination was observed in either *P. palmivora* isolate at 30 C, and only relatively few at 25 C in the P7 isolate on the LBA medium. Only 10% of the sporangia observed germinated 1 cm away from the center of the plate at 25 C on the cholesterol medium in isolate PBr. There was no other germination observed in this isolate at 25 and 30 C.

Table 3 shows numbers of swimming zoospores of all four isolates observed in water solutions over LBA and cholesterol media incubated at 10, 25, and 30 C. Water solutions over LBA agar at 10 C consistently had a high number of swimming zoospores in all isolates. Water solutions over the cholesterol medium had fewer active zoospores at 10 C than over the LBA medium, and none at 25 and 30 C in both isolates of *P. palmivora*. At these two higher temperatures, active zoospores were observed in this species only in isolate P7 in LBA at 25 C, and much fewer than were observed in this isolate at 10 C. Sporangia of *P. palmivora* isolates produced in plates incubated at 30 C were able to germinate and produce numbers of

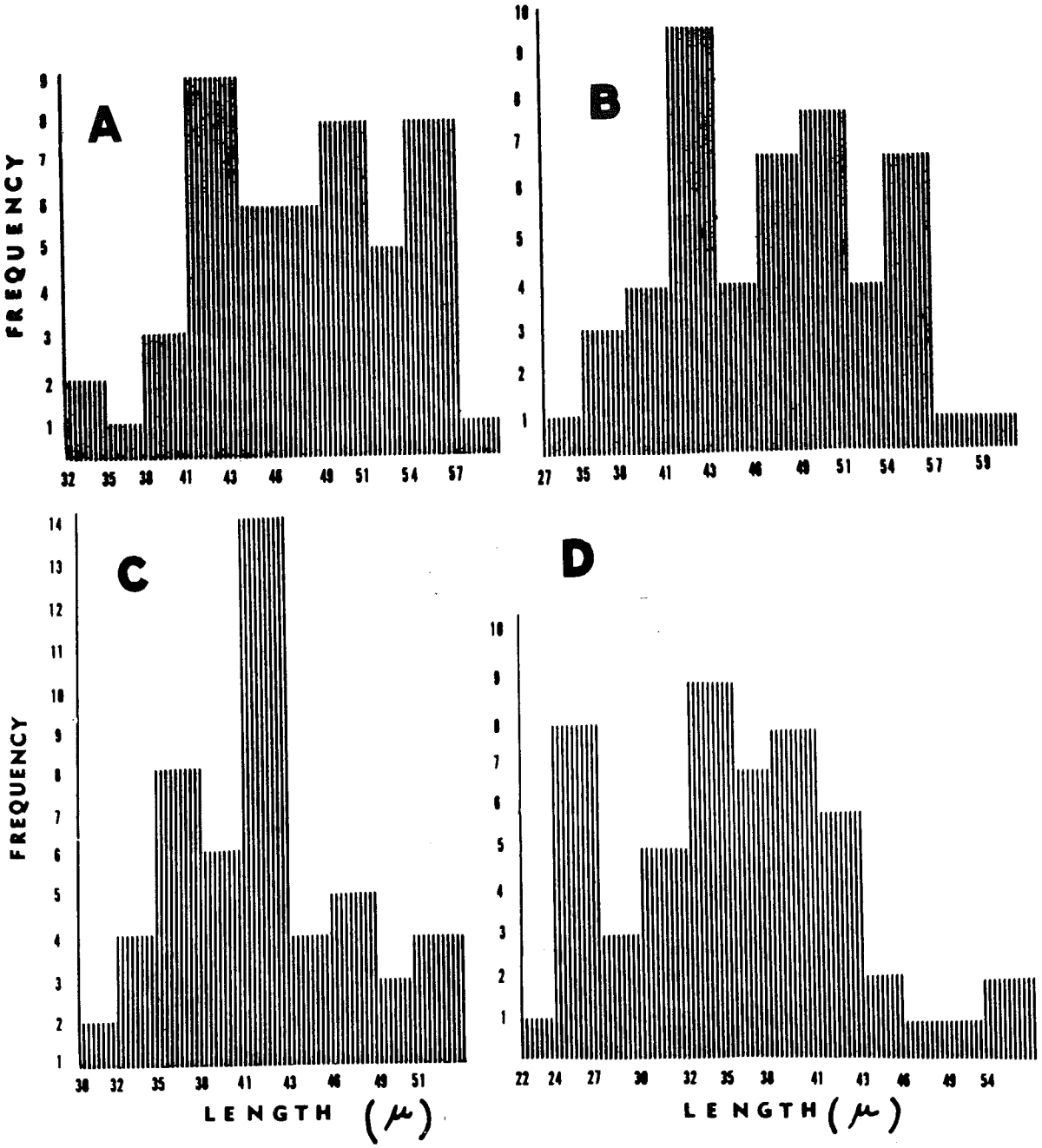

Fig. 1. Frequencies by sporangial length of isolates PBr and P7 of *Phytophthora palmivora* which germinated by zoospores. Counts made at random of 50 sporangia, average of two trials. A) Length of sporangia produced by isolate PBr on nutrient agar amended with 10 mg/liter of cholesterol. B) Length of sporangia produced by isolate PBr on lima bean agar. C) Length of sporangia produced by isolate P7 on nutrient agar amended with 10 mg/liter of cholesterol. D) Length of sporangia produced by isolate P7 on lima bean agar.

active zoospores similar to those incubated at 10 C when flooded with distilled water at 10 C. *Phytophthora parasitica nicotianae* zoospores were active in all three temperatures tested, except in the cholesterol medium at 30 C in STWI.

Figures 1 and 2 illustrate the wide range of sporangial size where zoospore germination was observed. The histograms show the frequency by size (length) of germinated sporangia as observed through the microscope at X 100 magnification. They represent an average of two trials of measurements taken at random from colonies grown on lima bean agar and

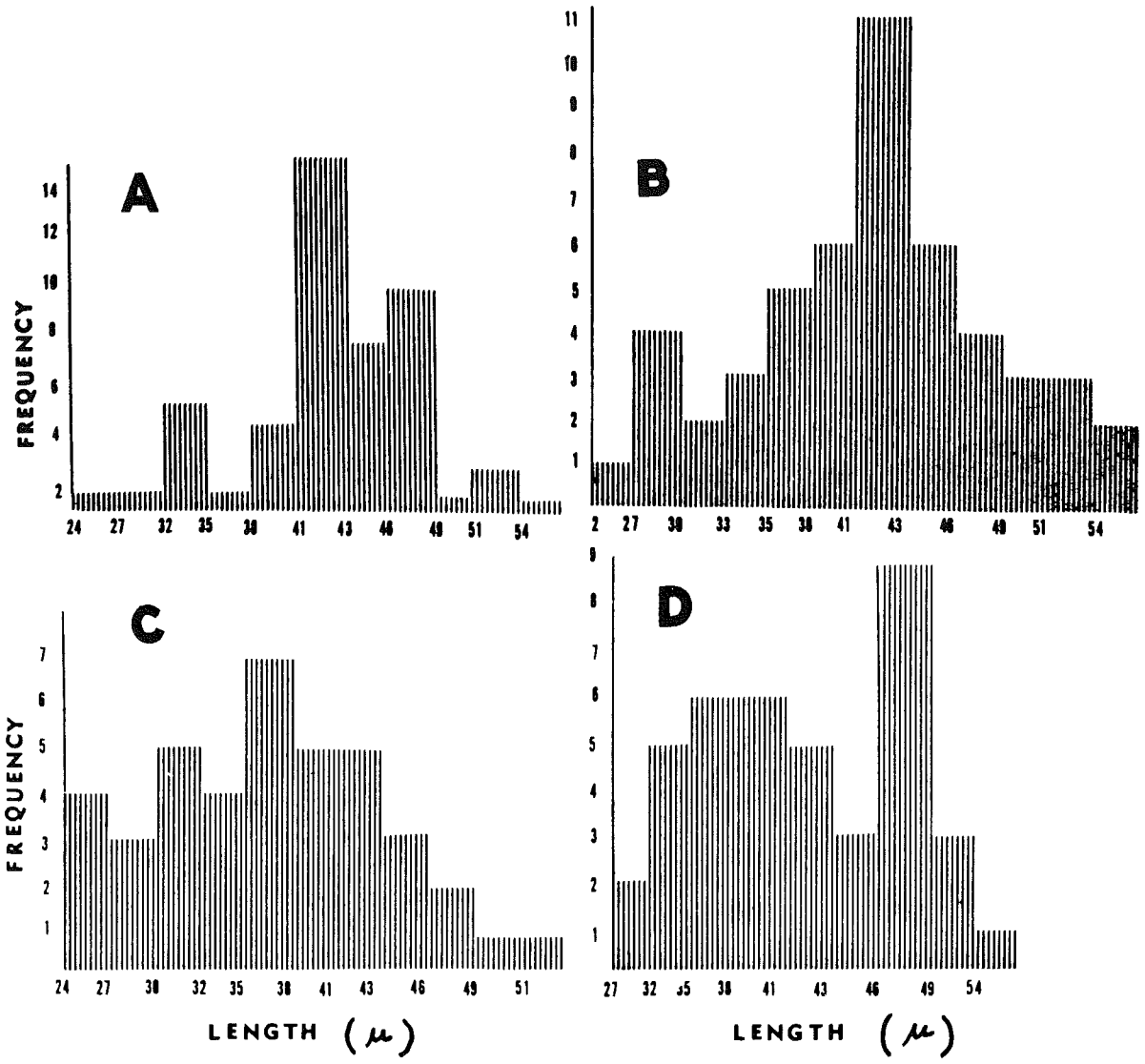

Fig. 2. Frequencies by sporangial length of isolates STWI and IS7 of *Phytophthora parasitica nicotianae* which germinated by zoospores. Counts made at random of 50 sporangia, average of two trials. **A)** Length of sporangia produced by isolate STWI on nutrient agar amended with 10 mg/liter of cholesterol. **B)** Length of sporangia produced by isolate STWI on lima bean agar. **C)** Length of sporangia produced by isolate IS7 on nutrient agar amended with 10 mg/liter of cholesterol. **D)** Length of sporangia produced by isolate IS7 on lima bean agar.

nutrient agar with cholesterol (10 mg/liter), and flooded with distilled water at 10 C. The average size of all sporangia formed on lima bean agar was 41 X 33 μ for IS7, 41 X 33 μ for STWI, 34 X 27 μ for P7, and 43 X 27 μ for PBr. Although the highest frequency of germinated sporangia was at or near the average size of all sporangia produced in that medium, there is apparently a fairly wide range of sizes that contributes to the total population of zoospores.

DISCUSSION.—Several culture media are adequate to produce large numbers of sporangia of *Phytophthora* species. Generally, the cultures are then chilled with cold water to induce zoospore formation and release. Blackwell & Waterhouse (4) discussed conditions necessary for sporangial germination, and indicate that investigators had found differences in optimal conditions for different species. There have been many studies published about these and related factors and many of the questions posed by Blackwell & Waterhouse (4) are still subjects for further study.

We found the response between isolates of the same species to be generally more similar than between isolates of different species. This was the case in the production of sporangia in continuous darkness on water agar by *P. parasitica nicotianae*, whereas none was observed in *P. palmivora*. It was also true in the per cent germination of sporangia at 25 and 30 C

in lima bean agar and nutrient agar with cholesterol. When swimming zoospores were counted in flooded culture plates at 10, 25 and 30 C, species differences were clear at the two higher temperatures. While it is necessary to have water at 10 C to induce production of active (swimming) zoospores in our *P. palmivora* isolates, except in isolate P7 at 25 C in LBA medium, our *P. parasitica nicotianae* isolates produced them in similar quantities at 25 C, and even a fair number at 30 C. Since we used distilled water incubated at the same temperatures as the plates to flood them, there probably was no appreciable change in temperature between water and fungus colony. Cultures of *P. palmivora* incubated at 30 C had apparently normal sporangia, since these can be induced to produce active zoospores if flooded with distilled water at 10 C.

Some of the zoospores observed in the sporangia were either not released or did not become active at 30 C in *P. parasitica nicotianae*. Although the highest size frequency of germinated sporangia was near the average size of all sporangia for each isolate, it was apparent that there was a great range of sizes where sporangia formed zoospores.

Bimpong & Clerk (3) reported abundant production of actively moving zoospores in sporangia of *P. palmivora* at 22 C, three degrees lower than the incubation temperature of their fungus colonies. The motility of the zoospores of their isolates was uniform for 72 to 84 hr at concentrations of 10^3 to 700 $\times$ 10^3 spores/ml. This is in contrast to what Gooding & Lucas (6) found with *P. parasitica nicotianae* where a dilution from 10^6 spores/ml to 10^3 reduced the motility markedly. Our observations did not include the study of factors affecting motility of zoospores. It is apparent from our observations, however, that these two species of *Phytophthora* also differ in their requirements for sporangial and zoospore production. These differences may be useful in further studies on the role of maturation, temperature, and other factors in the induction of zoospore production and release in this genus.

Irradiation was clearly stimulating for sporangial production of both isolates of *P. palmivora* in amended nutrient agar with cholesterol and water agar with cholesterol. These results are similar to those observed by Hendrix with this species (7). The two isolates of *P. parasitica nicotianae* differed in this respect. There was a stimulation by light in isolate STWI, but none was observed in isolate IS7. The use of water agar amended with cholesterol resulted in poor sporangial production with both isolates. Aragaki & Hine (1) obtained a great stimulation of sporangial production of *P. parasitica* Dast. These workers used natural media in their studies. Their use of natural media and *P. parasitica* instead of *P. parasitica nicotianae* could explain the differences observed.

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