Production of Sporangia by *Phytophthora cinnamomi* and *P. palmivora* in Soils at Different Matric Potentials

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ABSTRACT

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The production of sporangia by *Phytophthora cinnamomi* and *P. palmivora* was studied in a sandy loam and a clay soil at matric potentials between 0 and −15 bar. There was a strong correlation between the number of sporangia produced and matric potential, but there was no direct relationship between sporangium production and the water content of the soil. For each species, numbers of sporangia formed at the same matric potential were similar for both soil types. When mycelial inoculum was buried under 5 mm of natural soil, *P. cinnamomi* produced the most sporangia at −160 millibar (mb), with upper and lower matric potential limits of about −10 mb and −2,500 mb, respectively. The number of *P. palmivora* sporangia formed on inoculum buried in flooded soil was about 20%, and at −15 bar about 5% of the maximum observed, with buried inoculum yielding the maximum numbers of sporangia in both soils at −10 mb and inoculum on the soil surface yielding the maximum at −5 mb. Maximum numbers of sporangia were produced by *P. cinnamomi* on the soil surface only under flooded (+ 1 mb) and saturated (0 mb) conditions. Daily drying and rewetting of the sandy loam soil did not induce significantly more sporangia than were produced by *P. cinnamomi* at constant matric potentials. The ratio between the numbers of sporangia produced by *P. cinnamomi* and *P. palmivora* was about 1:200.

Soil moisture is one of the most important environmental factors affecting *Phytophthora cinnamomi,* in relation to growth and sporulation as well as to the resultant plant root infection process (35). A recent review of the water relations of water molds by Duniway (8) includes a general discussion of the significance of osmotic and matric water potentials during the different developmental phases of selected Oomycetes, including *Saprolegnia, Aphanomyces, Pythium,* and *Phytophthora* spp. Although early workers recognized the central importance of water status as a factor influencing fungal growth in soil (16) and the development of root diseases caused by soilborne fungi, only recently have precise methods been used to obtain quantitative data under comparable soil conditions. Studies by Adebayo and Harris (1) and Sterne et al (28) dealt primarily with the mycelial growth interactions. The detailed studies by Duniway (5–7), MacDonald and Duniway (19,20), and Pfender et al (23) yielded information on the formation of sporangia and release of zoospores by *Phytophthora cryptogea* and *P. megasperma.* Phytophthora root rot of *Persea indica,* in relation to soil osmotic and matric water potentials, was studied by Sterne et al (29,30).

The production of sporangia by *Phytophthora cinnamomi* in axenic cultures generally requires nutrient depletion and the presence of certain ions (4); in soil extracts or natural soils, sporulation may be stimulated by bacteria or their exudates (2,3,34,35). This behavior is in contrast to that of *P. palmivora,* which sporulates readily under many environmental conditions (26,33). Except for studies at a few moisture contents by Reeves (24) and Nesbitt et al (22), no data are available on the influence of matric water potential on the formation of sporangia by *P. cinnamomi* over the whole soil moisture range from saturation to the permanent wilting point. Furthermore, there have been few quantitative comparisons of the number of sporangia formed by *Phytophthora* spp. at different matric potentials, and also of the optimal as well as upper and lower water potential limits. One goal of this study is to provide data on the formation of sporangia by *P. cinnamomi* and *P. palmivora* in two different soil types at different matric potentials. An attempt also will be made to compare these results with data from the literature.

MATERIALS AND METHODS

Isolates. Isolates used were: Pc79, *P. cinnamomi* Rands mating type A^2, isolated from avocado in California; P255, *P. palmivora* Butler (MF 1), mating type A^2, isolated from cacao in Costa Rica; and P1034, *P. cactorum* (Leb. et Cohn) Schroet., from apple trunk, Germany (isolated by F. J. Schwinn). Cultures were from the Phytophthora collection, Department of Plant Pathology, University of California, Riverside.

Inoculum. The synthetic medium (SM) of Ribeiro et al (25) was used for growing inoculum. Disks (5 mm in diameter) cut from the margins of colonies on SM agar were transferred to SM liquid (one disk and 7 ml of medium per 60-mm diameter dish) and were incubated in the dark for three days at 25 C. After incubation,

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colonies were placed with the growth medium in a sterile 360-ml capacity blender cup and were comminuted for 5 sec at low speed. Blended mycelium (5 ml suspension + 10 ml fresh SM liquid per dish) was then transferred to 9-cm diameter petri dishes, each containing an 8-cm-diameter circle of sterile nylon or teflon mesh (100- or 120-μm mesh). Cultures with mesh circles were incubated at 25 C in the dark for 1 day before 150 ppm of the optical brightener, Diethanol (9), was added. Incubation was continued for another day before the mats were subjected to nutrient depletion, according to the principles elucidated by Chen and Zentmyer (4). In early experiments, the mineral salt solution of Chen and Zentmyer was used; however, a more favorable pH (6.0) and better zoospore release was obtained with a chloride salt solution (CSS) containing 0.01 M $CaCl_2$, 0.005 M KCl, and 0.004 M $MgSO_4$; plus 1 ml of a chelated iron solution, as prepared by Chen and Zentmyer (4). Mesh disks bearing mycelium were removed from the SM and were rinsed by soaking for 1 hr each in two dishes containing about 35 ml of CSS. The mesh was then cut into 1 cm × 1-cm squares that were soaked for an additional 1–2 hr in fresh CSS.

Control of matric potential. For regulation of soil matric potentials between 0 and −320 mbar, tension cups were used, each consisting of a ceramic plate with a plexiglass cylinder 7.7 cm in diameter × 7.7 cm tall attached to form a cup. Further details were described by Sterne et al (29,30). The matric potential in the ceramic plate and the 1.5-cm layer of soil in the cup was maintained by the height of a water column between the soil surface and a water reservoir (7).

The ceramic plates were saturated overnight, then a 1-cm layer of air-dried, sieved (2.0-mm screen) sandy loam soil (water content 8.2%,w/w) or clay soil (water content 4.2%,w/w) was placed on the ceramic plate. The soil was immediately tamped gently with the bottom of a beaker with the same diameter as the cup (pressure about 6 g/cm^2), and then was allowed to stand for several hours. During this time the soil was saturated by lowering the tension cups and allowing capillary forces to bring water from the bottom to the soil surface. This method does not disturb the soil structure, and results in a minimum of air being trapped in narrow soil pores. When excess water was visible on the soil surface, the mesh squares with mycelium (4 to 10 per cup) were placed on the soil surface and gently pressed with forceps against the surface. For the studies with buried inoculum, after removing the excess surface water, a 5-mm layer of air-dried soil was added to cover the fungus and was tamped as described previously. The cups again were lowered for 2–3 hr to saturate the second layer of soil. At this point, for some treatments, inoculum was placed on the soil surface in the same cups as the buried inoculum. In other cases, the surface inoculum was placed on a single 1.5-cm layer of saturated soil. After saturation and inoculations were completed, the cups were covered with a petri dish lid and aluminum foil to reduce evaporation and exclude light. The height of the water column was then adjusted. Water columns corresponding to matric potentials of +1 mb (soil flooded with a 1-cm layer of water = waterlogged soil), 0 mb (saturated soil), −5, −10, −20, −40, −80, −160, and −320 mb were chosen and were maintained during the 2-day incubation period (1 bar = 1,000 mb = 1,020 cm water column = 0.987 atm; site of mesh square = reference of zero point for millibar values). In one experiment (Table 4), the matric potential was varied cyclically every 24 hr, with 15 hr each day at one of the eight chosen potentials (0, −5, −10, −20, −40, −80−160, or −320), followed by 6 hr at −320 mb and 3 hr at saturation (hereafter called the 15-6-3-hour cycle). Replicates were removed and observed after 1, 2, and 4 days.

Matric potentials between −1 and −15 bar were adjusted with a pressure plate apparatus (14). Up to 12 soil samples in rubber rings (5.1 cm in diameter × 1.0 cm tall, covered on the bottom with a nylon mesh) were placed on a 15-bar ceramic pressure plate 28 cm in diameter (Soil Moisture Equipment Corp., Santa Barbara, CA 93105). The soil samples were saturated and inoculated as described above for the tension cups. The pressure was maintained throughout the incubation period by using a valve system and a tank of compressed air. Incubation, both in tension cups and on the pressure plate, was at room temperature (23–27 C).

Inoculum recovery and observations. Mesh squares bearing mycelium and any spores formed were recovered and rinsed gently in water to eliminate the largest soil particles and were mounted on microscope slides in water. Sporangia of *P. palmivora* and *P. cactorum* were counted in at least five randomly chosen microscope fields (magnification × 65, diameter 0.75 mm) on each of two squares. Since *P. cinnamomi* produced fewer sporangia, 48 microscope fields (in four lines) were counted on each of two squares. Sporangia were counted under UV illumination on a Zeiss epifluorescence microscope.

Soil characteristics. The two soil types were compared physically by constructing their soil moisture characteristic curves (Fig. 1). In order to be able to associate a certain matric potential with the amount of water-free and water-filled pore volume (Fig. 1), the gravimetric water content (grams of H_2O per 100 g of oven-dried soil) was multiplied by the bulk density (ρ_a, grams per cubic centimeter). Bulk density was determined by filling a graduated cylinder to 500 cm^3 with air-dried soil, using the same method as described for filling tension cups; this volume of soil was then weighed; calculation of bulk density was made considering the water content of the air-dried soil. The total pore volume can be compared with the value of the maximal moisture holding capacity (Table 1).

The two soil types used in these studies were a sandy loam from an avocado grove on the University of California's Riverside campus, and a clay (omni soil series) from the Rio Vista area of Solano County, California (29,30). The textural composition of the sandy loam was 52% sand, 28% silt, 20% clay and that of the clay soil was 14% sand, 32% silt, 54% clay. The bulk densities were 1.32 for the sandy loam and 1.15 g/cm^3 for the clay. Soil chemical characteristics of the sandy loam and the clay soils, respectively, were as follows: pH 7.7 and 8.0 for saturation pastes, and 8.1 and 8.5 for saturation extracts; organic carbon 1.69% and 1.58%; C/N ratio 16.3 and 11.9. Osmotic potentials of saturation extracts were measured with a vapor pressure osmometer (model 302B, Hewlett-Packard Co., Palo Alto, CA 94304) which indicated values of −0.10 bar for the sandy loam and −0.53 bar for the clay soil.

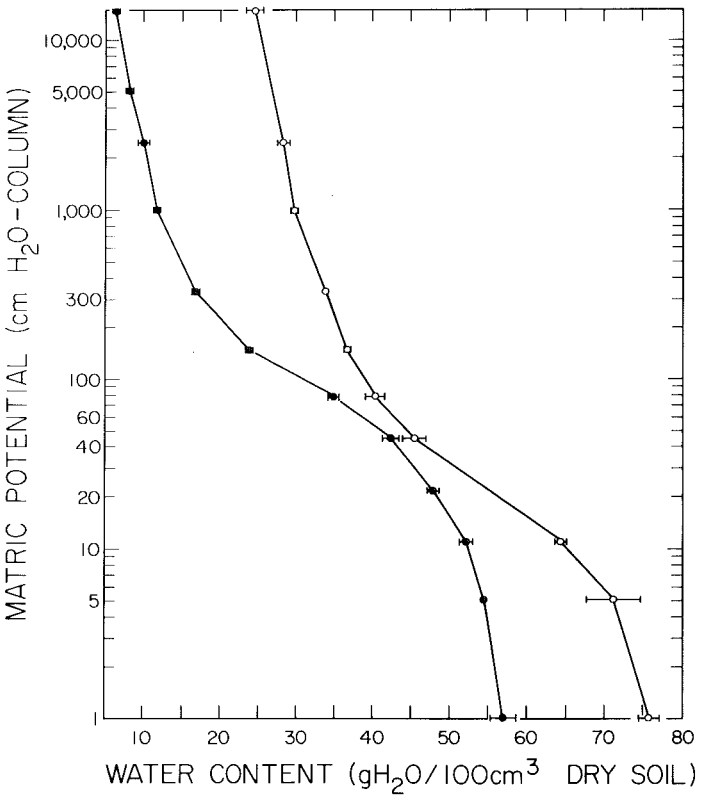

Fig. 1. Relationship between matric potential and water content for a sandy loam (●) and a clay soil (○). Confidence levels of $P = 0.1$. (conversion of units: 1 mb = 1.02 cm H_2O column).

RESULTS

As shown in Fig. 1 and Table 1, the physical characteristics of the sandy loam and the clay soil differ sufficiently to result in significantly different water contents at the same matric potential, especially between 0 and −20 mb and below −160 mb. Thus, these two soils provide a good system for investigating the relative effects of matric potential and water content of the soil on the production of sporangia.

Large differences were found in the numbers of sporangia produced by the species of *Phytophthora* examined. The ratios between the maximum numbers of sporangia produced by *P. cinnamomi* and *P. palmivora* were about 1:200 for buried mycelium and about 1:400 for mycelium on the soil surface (Tables 2 and 3). Fewer sporangia were produced by *P. cinnamomi* in soil than in the mineral salt solution (CSS), whereas *P. palmivora* exhibited reverse behavior (Table 2).

Very young fungal structures formed in soil did not fluoresce (Fig. 2A–B), whereas mature sporangia exhibited a typical fluorescence of the secondary wall (12), with brighter spots just below the apex and in regions where a germ tube had emerged (Figs. 2 and 3). Sporangia produced at matric potentials lower than −1 bar did not fluoresce brightly, possibly due to poor diffusion of the brightener in drier soils. Sporangia of *P. cinnamomi* were not produced uniformly throughout the mycelial mat; especially under drier conditions, sporangia were often found at the edges of the mat, in places where growth was sparse, and also close to optically dense soil particles (Fig. 3).

In experiments with buried mycelial mats, *P. cinnamomi* produced the most sporangia at a matric potential of −160 mbar in both the sandy loam and the clay soils (Table 2); no sporangia were produced at matric potentials higher than −10 mb, or lower than −2.5 bar. The matric potential range for sporangium production by *P. cinnamomi* in the clay soil shifted to slightly higher values than in the sandy loam (Table 2). In both soils the optimal matric potential for sporangium production by *P. palmivora* was −10 mb. In most cases, no significant differences were observed between numbers of *P. palmivora* sporangia formed at the same potential in the two soils (Table 2). In contrast to *P. cinnamomi,* upper and lower limits were not found for *P. palmivora;* 1,900 to 2,800 sporangia per square centimeter were produced under flooded conditions, and in the remarkably dry condition of −15 bars (permanent wilting point for most higher plants) about 500 sporangia/per square centimeter were formed. Numbers of sporangia decreased steadily between −20 mb and −15 bars,

In another group of experiments, the effect of aeration on the production of sporangia was investigated by placing mycelial mats on the soil surface. At matric potentials higher than −40 mb, the exposure of *P. palmivora* on the surface of both soils resulted in the production of two to nine times more sporangia than those produced by buried mycelia. At matric potentials lower than −40 mb, no significant differences in sporangium production were observed between *P. palmivora* mycelia buried in or placed on the surface of the sandy loam, but more sporangia were produced on the surface of the clay soil down to at least −160 mb (Tables 2 and 3). The behavior of *P. cinnamomi* on the soil surface was quite different from that of *P. palmivora.* Except in flooded and saturated soils, *P. cinnamomi* formed few sporangia on the soil surface (Table 3). No significant differences in the numbers of sporangia produced on the surface of the sandy loam and the clay soil could be detected except between the flooded soils.

In another experiment, matric potentials were varied cyclically to approximate the fluctuations that occur in the field, and to investigate whether re-aeration during brief dry cycles would result in the formation of more sporangia in the wet sandy loam. The influence of the incubation time on the production of sporangia also was studied. One difference observed between *P. palmivora* and *P. cinnamomi* was that the number of *P. palmivora* sporangia increased from the first to the fourth day in the soil, in contrast to a more or less constant or even decreasing number produced by *P. cinnamomi* (Table 4). By the fourth day, lysis of the mycelial mat was quite evident; the hyphal walls were still clearly visible under

TABLE 2. Effect of soil matric potential on production of sporangia by *Phytophthora cinnamomi* and *P. palmivora* on mycelium buried for 2 days under a 5-mm layer of sandy loam or clay soil

Matric potential (mb)	Number of sporangia per cm²[a]			
	P. cinnamomi[b]		*P. palmivora* × 100[c]	
	sandy loam	clay	sandy loam	clay
+1	...[d]	0	19 ± 3	28 ± 5
0	...	0	23 ± 3	32 ± 11
−5	...	0	33 ± 9	49 ± 16
−10	0	8 ± 7	141 ± 50	107 ± 17
−20	4	10 ± 8	82 ± 10	87 ± 16
−40	15 ± 9	28 ± 8	74 ± 12	78 ± 11
−80	46 ± 26	49 ± 7	69 ± 11[e]	70 ± 6
−160	52 ± 29	63 ± 14	57 ± 10	54 ± 7
−320	26 ± 20	24 ± 20	54 ± 9	51 ± 8
−1,000	18	7	44 ± 7	47 ± 10
−2,500	7	0	24 ± 4	34 ± 4
−5,000	0	0	...	26
−15,000	0	0	5 ± 2	5 ± 4

[a] Means with confidence levels $P = 0.1$. Mycelial mats incubated at ~25 C in darkness.
[b] Control in the mineral solution (CSS): 97 ± 14 sporangia per square centimeter.
[c] Control in the mineral solution (CSS): 1,300 ± 400 sporangia per square centimeter.
[d] Not tested.
[e] Under the same conditions, *P. cactorum* produced 400 sporangia per square centimeter.

TABLE 1. Pore space composition in a sandy loam and a clay soil, expressed as percent of the total soil volume (% SV) and as percent of the total pore volume (% PV)

Matric potential range[b] (−mb)	Theoretical diameters of pore necks[b] (μm)	Percent of total volume[a]			
		% SV		% PV	
		sandy loam	clay	sandy loam	clay
0	...	57	76	100	100
0–80	>37	22	36	39	47
80–320	37–9	18	6	31	8
320–15,000	9–0.2	10	9	18	12
>15,000	<0.2	7	25	12	33

[a] Values given are based on grams per hundred cubic centimeters dry soil and refer to the water-filled pore space at a certain matric potential. The maximal moisture holding capacities, expressed as grams of water per hundred grams of dry soil, are 43% for the sandy loam and 66% for the clay soil.
[b] For theoretical connection between matric potential and diameter of pores see Vomocil (32).

TABLE 3. Effect of soil matric potential on production of sporangia by *Phytophthora cinnamomi* and *P. palmivora* on mycelium incubated for 2 days on the surface of sandy loam or clay soil

Matric potential (mb)	Number of sporangia per cm²[a]			
	P. cinnamomi		*P. palmivora* × 100	
	sandy loam	clay	sandy loam	clay
+1	104 ± 18	63 ± 17	100 ± 17	244 ± 25
0	58	...[b]	216 ± 50	302 ± 30
−5	24 ± 11	20 ± 5	320 ± 35	335 ± 47
−10	22 ± 11	18 ± 7	220	248 ± 53
−20	15 ± 10	...	139 ± 20	...
−40	12 ± 7	15 ± 8	99 ± 13	188 ± 18
−80	11 ± 11	13 ± 11	88 ± 22	104 ± 15
−160	9 ± 7	7 ±10	57 ± 12	74 ± 5
−320	0	0	55 ± 12	...
−1,000	...	...	44 ± 7	...

[a] Means with confidence level, $P = 0.1$. Mycelial mats incubated at ~25 C in darkness.
[b] Not tested.

UV illumination, but in normal light the fungal structures were barely recognizable between soil particles. Recovery of the mycelial mats at 4 days often was complicated by the adhesion of the mycelium to soil particles and by fragmentation of the mycelial mat. Lysis was most pronounced at matric potentials between −40 and −320 mb.

Varying the matric potential (15-6-3 hr cycles daily), did not result in the production of significantly more sporangia by *P. cinnamomi* than were produced at corresponding fixed potentials (compare Tables 2 and 4, since *P. cinnamomi* formed few or no sporangia at potentials between 0 and −20 mb these data are not included in Table 4). After 4 days of incubation, the appearance of sporangia in soil with varied potentials differed (smaller, more irregular in shape) from those formed under constant conditions. The fluorescence of sporangia recovered from soils with varied potentials was often not bright, whereas under constant conditions, the fluorescence remained bright and clear (Fig. 3) even after 4 days in the soil.

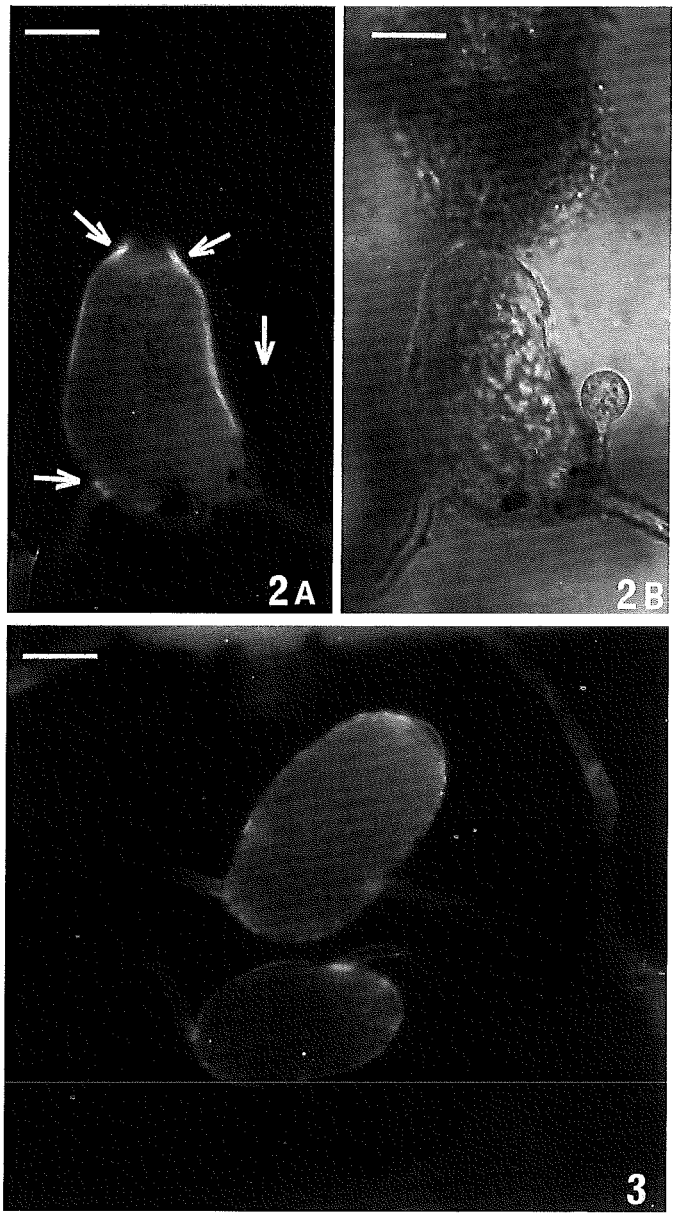

Fig. 2,3. Sporangia produced by *Phytophthora cinnamomi* buried in sandy loam soil at −80 mb matric potential. **2B,** normal light; **2A** and **3,** UV illumination. Notice the intense fluorescence of the sporangial wall. Further details are described in the test. (Scale bar=20 μm).

DISCUSSION

The sensitivity of *Phytophthora* to water stress is characterized by different levels of water potential affecting mycelial growth, sporangium formation, and zoospore release. Mycelial growth is completely suppressed in soil at matric potentials of about −20 to −35 bars (1). The production of sporangia is limited (<10% of maximum) at matric potentials between about −2 and −5 bars, the exact values depending on the inoculation method as well as on the species. Zoospore release is restricted at matric potentials of about −50 mb (7,19,23). In general, *Phytophthora* spp. appear to be much more sensitive to matric than to osmotic water potentials; the limiting levels for growth are about 2× lower for osmotic than for matric potentials (1,28), and osmotic potentials which prevent zoospore release are about 10–100× lower than inhibitory matric potentials (11,19).

When washed mycelium is used as inoculum, the lower limiting matric potential for production of sporangia in soil is about −2.5 bars for *P. cinnamomi* (Table 2), −4 to −5 bars for *P. cryptogea* (6) and *P. cactorum* (21), and −10 bars for *P. palmivora* (Table 2). The relatively low value for *P. palmivora* reflects the ability of this species to tolerate drier conditions and to colonize microhabitats in which moisture conditions can fluctuate drastically, such as cacao pods or other aerial plant parts (26). Reports by Pfender et al (23) and Sugar (31) indicate much higher limiting matric potentials, at about −250 mb (*P. megasperma*) or even −10 mb (*P. cambivora*) (compare Fig. 4F–G). These findings are probably due to differences in inoculation methods (infected tissues versus washed mycelial mat) rather than to differential sensitivity of the species to matric potentials. A colonized radicle (23) or leaf disk (31) represents a different microhabitat for the fungus in that nutrient sources, water potential, and microbial interactions are different from those of a washed mycelial mat. The use of colonized plant parts instead of mycelial mats as inocula may yield data more closely approximating the behavior of the fungus under field

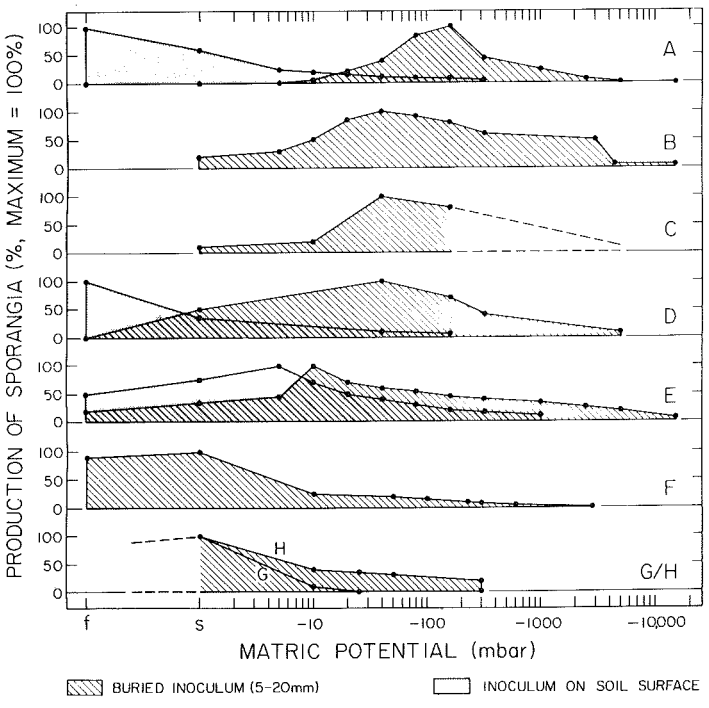

Fig. 4. Effect of soil matric potential on production of sporangia by *Phytophthora* spp, expressed as percent of the maximal number (= 100%) observed after 2–4 days. (S = saturated soil; f = flooded soil). **A,** *P. cinnamomi* (this publication). **B,** *P. cryptogea* (5,6); analogous behavior for *P. parasitica* (15). **C,** *P. megasperma* (17,18). **D,** *P. cactorum:* surface (10), buried (21,27). **E,** *P. palmivora* (this publication). **F,** *P. megasperma* (17,23, 31). **G,** *P. cambivora* (31). **H,** *P. drechsleri* (31).

conditions. Thus, the different results obtained by various workers are not necessarily contradictory because they are not directly comparable. MacDonald (18), using washed mycelium of *P. megasperma* as inoculum, obtained results more similar to those observed with other species when similar methods were used (5,6,21, and this publication) than to those Sugar (31) obtained with leaf disks colonized by *P. megasperma* (Fig. 4). Data from the literature representing the two main inoculation methods are compared (Fig. 4) for those studies in which sufficient information was given on the numbers of sporangia and the soil moisture conditions (water potentials, or percent moisture holding capacity).

In studies of the influence of very high matric potentials on the production of sporangia in soil, the amount of reported diversity that is inherent among *Phytophthora* species is even more pronounced (8). From the results presented in Tables 2 and 3, it is apparent that *P. cinnamomi* is more sensitive to poor aeration than is *P. palmivora;* the rapid decline in number of sporangia produced in wet soils begins at matric potentials ≥ -40 and ≥ -5 mb, respectively. Although *Phytopthora* species differ somewhat in sensitivity to poor aeration, *P. cinnamomi, P. palmivora, P. cactorum* (21), *P. cryptogea* (5,6), *P. parasitica* (15), and *P. megasperma* (18) all produced comparatively fewer sporangia when mycelium was buried in very wet soils (Fig. 4). In contrast, other results with *P. megasperma* (23,31), *P. drechsleri,* and *P. cambivora* (31) do not indicate substantial reductions in numbers of sporangia in flooded soil, even though the inoculum was buried more deeply (Fig. 4). These apparent discrepancies could reflect inter- and intraspecific differences in aeration and/or moisture requirements, but it seems much more likely that the inoculation methods (colonized plant tissue versus washed mycelium) influenced the results. This suggestion is supported by a recent study (17) of the production of sporangia in soil by *P. megasperma* f. sp. *medicaginis;* the results indicated that oospores or infected alfalfa roots as inoculum produced sporangia in the manner shown in Fig. 4F, whereas the behavior of mycelial inoculum was similar to the characteristics described by MacDonald (18, Fig. 4C).

At matric potentials higher than -20 mb, a two- to tenfold increase in the number of *P. palmivora* sporangia occurred when the mycelium was placed on the soil surface rather than buried (Tables 2 and 3); a similar observation was also reported by Duniway (5) for *P. cryptogea* in saturated soil conditions. The sporulation curve for *P. palmivora* is only slightly shifted to wetter conditions if the mycelium is incubated on the soil surface (Fig. 4). In contrast, with *P. cinnamomi,* the maximal number of sporangia shifted from -160 mb matric potential for buried inoculum, to flooded conditions for inoculum on the soil surface (Fig. 4). Between -5 and -40 mb, little or no stimulation resulted from the improved aeration on the surface (Table 3), and cyclically varying the matric potential also failed to increase sporulation. This suggests that the aeration alone is not responsible for stimulating sporangial production at the surface by this species, but some other factor such as close contact of the inoculum to soil particles or soil solution may be involved. This contact allows much more interaction with soil microorganisms and their exudates, which are known to be important for sporangium formation in *P. cinnamomi* (2,34). Several details observed in these studies contribute to this theory: (i) in experiments in which the soil was prewetted and incubated in the ceramic cups for 2 days before the soil was inoculated, more sporangia were formed at matric potentials between -80 and -320 mb (Table 4) than in soil wetted as described in Materials and Methods. This could be a response to increased activity by the microflora in the prewetted soils. Increased sporangium formation has also been found (G. D. King, *personal communication*) in extracts from prewetted soils. (ii) Frequently the sporangia were observed in close contact with soil particles (Fig. 3). (iii) When the inoculum was on the soil surface, the largest numbers of sporangia were produced by *P. cinnamomi* under flooded conditions, and more in the sandy loam than in the clay soil (Table 3); under these conditions, some diffusates from organic matter or soil bacteria may have differentially affected sporangium formation. Some experiments with saturation extracts from the two soil types showed the same trend of higher numbers of sporangia in the sandy loam extract.

Our data indicate that it is primarily the matric water potential, rather than the water content, that is responsible for the control of sporangium production in soil. This relationship was suggested by Reeves (24) for *P. cinnamomi,* but is clearly demonstrated here for the first time for both *P. cinnamomi* and *P. palmivora.* For the entire range of potential levels, we found no significant differences in the numbers of sporangia produced on inoculum buried at a certain matric potential in the two soil types, for both *P. cinnamomi* and *P. palmivora* (Table 2), but there were large differences in the water contents of the two soils (Fig. 1). For *P. palmivora,* the slightly higher numbers of sporangia in the clay soil for both exposure methods (Tables 2 and 3) may be related to the somewhat larger amount of the very coarse pores (>37 μm, Table 1) in the clay soil. Furthermore, the percent of the total soil water that is not available for production of sporangia (water bound in soil pores <0.2 μm) is 12% in the sandy loam, and 33% in the clay soil (Table 1).

The observation that sporangium production by *P. cinnamomi* in soil never reached a value as high as the number of sporangia produced in the mineral solution (Table 2) indicates that the chosen soil conditions were not optimal. Nevertheless, the maximal numbers per unit area reached by *P. cinnamomi* in these studies are comparable to values reported by Sugar (31) for *P. cambivora, P. drechsleri,* and *P. megasperma* (100–300 sporangia per square centimeter). The data for *P. palmivora* are similar to the data given by Gooding and Lucas (13) for *P. parasitica* var. *nicotianae* in different solutions (maximum 60,000 sporangia per square centimeter). The smaller number of sporangia of *P. palmivora* in the mineral solution (CSS), compared with the soil experiments (Table 2), indicates that this solution is not favorable for *P. palmivora.* To give an approximate order of magnitude of the ability to produce sporangia, the maximal numbers of sporangia formed at the surface were compared between three species. The observed ratio of 1:20:400 for numbers of *P. cinnamomi, P. cactorum,* and *P. palmivora* sporangia does not necessarily result in a differential infection potential, because the sporangia are also different in size, with a much higher number of zoospores per sporangium in *P. cinnamomi* than in the other two species (U. Gisi and G. A. Zentmyer, *unpublished*). Also, the numbers of sporangia formed by these species appeared to be inversely proportional to the amount of growth in soil; on recovered inocula of *P. cinnamomi* numerous nonfluorescent hyphae were observed, indicating that new growth occurred in soil.

The gradually increasing number of sporangia with increasing time for *P. palmivora* (Table 4) corresponds to the behavior of *P. cactorum* (10,27), *P. cryptogea* (5), and other species, whereas the

TABLE 4. Effect of constant or cyclically varied[a] soil matric potential and of incubation time on production of sporangia by *Phytophthora cinnamomi* and *P. palmivora* on mycelium incubated in sandy loam soil

Matric potential	Number of sporangia per cm^2[b]					
	P. cinnamomi[c]			*P. palmivora* × 100		
(mb)	day 1	day 2	day 4	day 1	day 2	day 4
−40	...[d]	15	...	74	99	105
−40[a]	15	18	17	...	...	...
−80	96	88	60	63	88	91
−80[a]	120	78	34	...	...	...
−160	...	...	...	41	57	72
−160[a]	184	176	96	...	...	...
−320	...	26	...	39	55	69
−320[a]	100	32	10	...	...	...

[a] 15-6-3-hour cycles daily, see Methods.
[b] Mycelial mats incubated at 25 C in darkness. *P. cinnamomi* was buried under a 5-mm soil layer, and *P. palmivora* was on the soil surface.
[c] Absolute numbers of sporangia shown for *P. cinnamomi* in this table sometimes differ from those given in Table 2, because of differences in experimental design (pre-wetting of soil, see Discussion).
[d] Not tested.

declining tendency with increasing time for *P. cinnamomi* (Table 4) agrees with data reported by Pfender et al (23) for *P. megasperma.* This decrease in number of *P. cinnamomi* sporangia is probably due to the disappearance of a large percentage of the sporangia by indirect germination and subsequent rapid lysis of the empty sporangia.

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