Biology of Chlamydospores, Sporangia, and Zoospores of Phytophthora cinnamomi in Soil

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ABSTRACT

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Among three spore types of *Phytophthora cinnamomi* tested, chlamydospores were the most persistent in soil, sporangia were intermediate, and zoospores were least persistent. Zoospores were capable of extending the survival time by colonization of dead plant tissues or parasitization of live plant roots. Survival of *P. cinnamomi* in either artificially or naturally infested soils was better under moist than submerged conditions. Colonies of *P. cinnamomi* recovered from natural soil originated mainly from free chlamydospores and chlamydospores imbedded in organic matter. Sporangia of *P. cinnamomi* also were detected in natural soil. At high inoculum levels, encysted zoospores were less infective to ohia seedlings than chlamydospores and motile zoospores. There was no difference in infection potential between chlamydospores and motile zoospores at any level of inoculum. Among the three spore types tested, chlamydospores were the most effective in colonizing dead tissues in soil, whereas colonization potential of motile and encysted zoospores was about the same.

Additional key words: nonspecialized parasities, root-infecting fungi.

In Hawaii, *Phytophthora cinnamomi* Rands has been isolated from soils and roots of a wide range of plant species (5, 10, 11). The fungus produces sporangia on the root surfaces and chlamydospores in root tissues (5, 9). Zoospores of *P. cinnamomi* are released in soil and dispersed by rain splash or runoff water during the rainy periods (11). Since very little is known about the behavior of these propagules in nature, we compared the ability of zoospores, sporangia, and chlamydospores of *P. cinnamomi* to survive, induce disease in host plants, and colonize substrates in soil. Oospores were not included in these studies because they were not found in roots and soils collected from the field (5, 10).

MATERIALS AND METHODS

Preparation of fungal propagules.—*Phytophthora cinnamomi* (isolate 58F) obtained from an ohia (*Metrosideros collina* subsp. *polymorpha*) rootlet was maintained on vegetable juice agar (per liter: V-8 juice, 200 ml; $CaCO_3$, 2 g; agar, 20 g). Sporangia, zoospores, and chlamydospores were obtained as previously reported (6, 8). Zoospores were induced to encyst by agitating zoospore suspensions in a test tube for 1.0 - 1.5 min with a Vortex mixer. Concentration of spore suspensions was determined by the microsyringe method (12).

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Survival in soil.—Fifty ml of zoospore and sporangium suspensions at concentrations of 2.6×10^6/ml and 1.2×10^3/ml, respectively, were separately mixed with 400 g of air-dry natural soil (Inceptisols, clay loam). After thorough mixing, half of each soil was placed in a 400-ml beaker and adjusted to 60% water by weight (moist, but not sticky) and the other half was submerged in water. Studies of survival of chlamydospores in moist and submerged soils were conducted separately. Twenty-five ml of a chlamydospore suspension at 1.2×10^3 or 3.4×10^3/ml was added to 200 g of air-dry soil adjusted to either moist or submerged conditions as described above. Soils were incubated in moist chambers at 24 C following moisture adjustment. At various time intervals, 5 g of soil was removed and the population of each propagule type in the soil sample was determined by plating the diluted soil suspension on a selective medium (14). Two replicates with five plates per replicate were used.

To study survival of *P. cinnamomi* in root tissues in soil, ohia root segments 10 mm long and 3-10 mm in diameter were washed in running tap water for 1 hr, surface sterilized with 75% alcohol for 10 min, and inoculated with *P. cinnamomi*. After 2-wk of incubation in petri dishes at 24 C, root segments were removed and buried in soil adjusted to 60% moisture. At various time intervals during 1 yr, 50 segments were removed, washed, and plated on a selective medium. Naturally infected avocado roots collected from a severely declining avocado tree also were tested.

Survival of *P. cinnamomi* in naturally infested soil also was studied using soil collected from the root zone of a declining avocado tree. After larger rocks and nondecomposed organic matter were removed, 500 g of soil was placed in a beaker and submerged with water; another 500 g was adjusted to 60% moisture. Soils were incubated in a moist chamber at 24 C for 1 yr. At various time intervals, 30 g of soil was removed and the population of *P. cinnamomi* was determined by the wet-sieving method and plating the sievings on a selective medium.

Nature of propagules recovered from soil.—Two avocado field soils and two ohia forest soils known to have high population of *P. cinnamomi* were processed through the same procedure as that used in determining *P. cinnamomi* population in soil (5, 7). Both filtrate and the materials retained on the sieve were plated on the selective medium, and 10 plates each were used for each soil sample. After 36 hr at 24 C, plates were washed gently to remove soil particles with running tap water. Each colony of *P. cinnamomi* on the plate was marked on the bottom of the petri dish under a dissecting microscope. The origin of each colony was determined under a microscope at ×100. Whenever pieces of organic matter were recognized as the origin of colonies, the material was removed with a needle to a glass slide, pressed under the cover glass, and examined for the presence of fungal propagules.

Infection potential of motile zoospores, encysted zoospores, and chlamydospores.—Ohia seeds were sowed on a layer of mica peat (about 3 mm thick) laid on the surface of 200 g of soil in a plastic tray (12 × 12 × 2.5 cm). Seedlings were thinned to 70 per pot 3 wk after sowing. Inoculations with motile zoospores, encysted

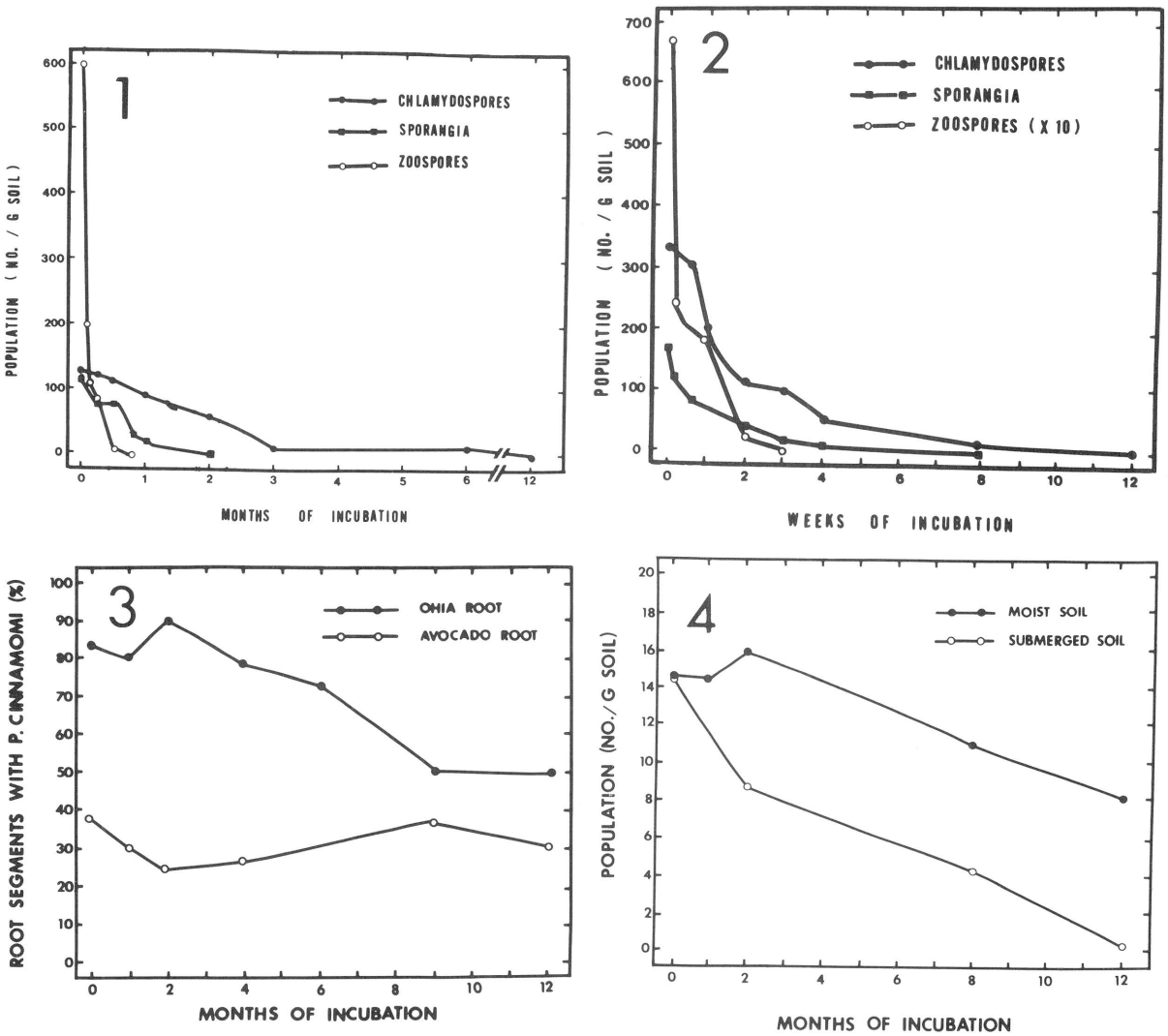

Fig 1-4. Survival of *Phytophthora cinnamomi* in soil (Inceptisols, clay loam). **1)** Persistence of chlamydospores, sporangia, and zoospores of *P. cinnamomi* in moist (60% water by weight) soil. **2)** Persistence of chlamydospores, sporangia, and zoospores of *P. cinnamomi* in submerged soil. **3)** Persistence of *P. cinnamomi* in diseased avocado roots and in artificially inoculated ohia roots buried in moist soil. **4)** Survival of *P. cinnamomi* in naturally infested avocado field soil adjusted to moist and submerged conditions.

zoospores, or chlamydospores were made 3 mo after sowing when ohia seedlings had four to six leaves and were about 10 mm in height. Thirty ml of propagule suspension was evenly distributed over the soil surface in each pot with a disposable pipette. All seedlings were flooded with water for 24 hr following treatment and then watered once daily. Each treatment was replicated twice and the experiment was repeated once. After 1 mo, the number of ohia seedlings killed in each pot was recorded.

Colonization potential of motile zoospores, encysted zoospores, and chlamydospores.—Thirty ml of propagule suspension was mixed with 200 g of soil in a 500-ml beaker. The soil moisture was adjusted to near saturation (70% on an oven dry weight basis). One-hundred stem segments (15 mm long and about 2 mm in diameter) obtained from young shoots of ohia trees were dried overnight in an oven at 65 C and buried in the infested soil. After 1 wk at 24 C, 50 segments were removed from soil, washed in running tap water for 2 hr, surface sterilized in 0.5% NaOCl for 30 sec, rinsed once in sterile distilled water, and placed on selective medium. The number of segments containing *P. cinnamomi* was recorded. The experiment was repeated once.

RESULTS

Survival in soil.—Chlamydospores were the most persistent in soil, sporangia were intermediate, and zoospores were the least persistent (Fig. 1 and 2). The population of chlamydospores decreased to a nondetectable level after 1 yr under moist conditions, and after 3 mo under submerged conditions. Sporangia and zoospores were not recovered from moist and submerged soils after 2 mo and 3 wk, respectively.

To test if sporangia released zoospores in soil, 10 g of soil used in the survival study was removed, and the population of zoospores in soil was determined by the following method. The soil suspension was passed through a 38-μm sieve which retained sporangia and allowed zoospores to pass through. The filtrate was incubated on the selective medium, and the number of colonies of *P. cinnamomi* that originated from zoospores on the isolation plates was counted after 36 hr of incubation.

Zoospores were recovered from both moist and submerged soils over a 2-wk period after sporangia were added to the soil. After incubation for 1 day, the ratio of zoospores to sporangia was about 4:1 and 1:1 in submerged and moist soils, respectively. The population of zoospores was maximum on the 4th day in both soils, and then decreased rapidly to a nondetectable level after 2 wk. Sporangia were still recoverable from soils of both moisture levels after 2 wk, but were not detectable at both moisture levels after 8 wk.

When artificially inoculated ohia roots were buried in moist soil and incubated at 24 C, the percentage of root segments infested with *P. cinnamomi* decreased only from 83% to 50% over a 12-mo period (Fig. 3). Similar results were obtained when naturally infected avocado roots were used. Chlamydospores of *P. cinnamomi* were observed in both avocado and ohia root tissues.

Phytophthora cinnamomi also survived better under moist than under submerged conditions in naturally infested soil. During the 12-mo period the population of *P. cinnamomi* decreased only from 14.3 to 8.0 propagules/g of soil under moist conditions, but from 14.3 to 0.6 propagules/g of soil under submerged conditions (Fig. 4).

Factors affecting the survival of zoospores in soil.—Zoospores of *P. cinnamomi* were very short-lived in soil, surviving only 3 wk as indicated in the above study. However, when they were added to moist soil mixed with ohia stems (2.5 × 10 mm) or leaves, they colonized these tissues. *Phytophthora cinnamomi* was isolated consistently from stem segments and leaves for 1 yr and 9 mo, respectively. Leaf tissues were decomposed completely after 9 mo in soil, but the fungus, in the form of free chlamydospores, was still recovered from soil when the last isolation was made after 1 yr of incubation.

When zoospores were added to soils planted with ohia, papaya, tomato, or pepper seedlings, they infected roots of these plants and thus extended the survival time of the fungus in soil. One month after inoculation, all the ohia seedlings were killed, and roots of papaya, tomato, and pepper seedlings were infected with *P. cinnamomi* without showing visible symptoms. The fungus was recovered from roots of all the ohia seedlings, 70% of tomato, 11% of papaya, and 14% of pepper seedlings after 1 mo. *Phytophthora cinnamomi* produced sporangia on the root surface and chlamydospores in root tissue of ohia seedlings. No spores were observed in root tissues of other plant species.

Nature of propagules recovered from soil.—To determine the presence of zoospores of *P. cinnamomi* in natural soil, 50 g of soil suspended in 300 ml of water was passed through a 38-μm sieve which permitted the passage of zoospores but not chlamydospores and sporangia. Materials retained on the sieve and filtrate were plated separately on the selective medium. *Phytophthora cinnamomi* was recovered only from materials retained on the sieve, indicating that zoospores of *P. cinnamomi* were not present in natural soil. Plates containing materials retained on the sieve were examined microscopically to determine the origin of colonies of *P. cinnamomi*. Results showed that *P. cinnamomi* existed mainly as free chlamydospores in avocado soils and as chlamydospores imbedded in organic matter in ohia forest soils (Table 1). Chlamydospores were thin-walled and appeared either singly or in clusters of two or three spores. Zoospores also were observed on the plates. Very often several zoospores were found close together, and occasionally empty sporangia were observed in the vicinity of these zoospores. This suggested that sporangia of *P. cinnamomi* may be present in natural soil.

In preliminary tests, when *P. cinnamomi* sporangia added to soil were suspended in water (50 g soil/100 ml), chilled at 5 C for 30 min, incubated at 24 C for 1 hr, and then passed through a 38-μm sieve, zoospores were detected in the filtrate. No zoospores were detected in the filtrate when *P. cinnamomi* chlamydospores added to soil were similarly tested. By using this technique, zoospores at the concentrations of 10 and 50/g of soil were detected in the filtrate of ohia forest soil and avocado soil, respectively. Without the chilling treatment, no zoospores were detected in the filtrate from either soil. These results further confirmed the previous observations that sporangia of *P. cinnamomi* also existed in natural

soil and that zoospores retained on the sieve originated from sporangia during incubation.

To determine whether chlamydospores germinated in soil, 3 ml of chlamydospore suspension at 5.0×10^5/ml were added to 10 g of soil in a small petri dish (50 × 15 mm). The soil was adjusted with water to either moist (60% water by weight) or submerged and held at 24 C. Percentage of chlamydospore germination was determined under the microscope by suspending 1 g of soil in 5 ml of distilled water. In submerged soil, 23% of chlamydospores germinated by producing sporangia on the tips of germ tubes after 1 mo of incubation. Most sporangia were empty due to release of zoospores under submerged conditions. In moist soil, 18% of chlamydospores germinated after 1 mo by production of term tubes and sporangia. Sporangia formed under such conditions did not discharge zoospores, but many of them discharged zoospores after incubation for 24 hr in water. In one moist soil to which chlamydospores were added at 1,900/g soil, the population of zoospores in soil was determined by using the combination of chilling treatment and wet sieving as described above. Zoospores, released from sporangia in soil, were consistently recovered for the first 5 mo of incubation. The population of zoospores was at the maximum (600 zoospores/g of soil) after 1 mo and declined gradually thereafter. It reached a nondetectable level after 4.5 mo. The population of chlamydospores decreased from 1,900 to 50/g of soil during the same incubation period.

Infection potential of motile zoospores, encysted zoospores, and chlamydospores.—Percentages of 3-mo-old ohia seedlings killed by motile zoospores, encysted zoospores, and chlamydospores of *P. cinnamomi* at various inoculum levels were determined after 1 mo. When the inoculum level was at or above 2.5×10^3 propagules/g of soil, encysted zoospores were the least infective to the ohia seedlings, but the infection potential of motile zoospores and chlamydospores was about the

TABLE 1. Nature of propagules of *Phytophthora cinnamomi* recovered from soil

Source of soil	Colonies examined (no.)	Origin of colony			
		Free chlamydospore (%)	Zoospore (%)	Organic matter: Chlamydospore (%)	Organic matter: Unknown (%)
Soil A (ohia)	69	9	7	58	26
Soil B (ohia)	30	30	3	50	17
Soil C (avocado)	100	71	0	20	9
Soil D (avocado)	53	60	28	12	0

TABLE 2. Comparison of infection potential of chlamydospores, motile zoospores, and encysted zoospores of *Phytophthora cinnamomi*

Propagules per gram of soil (no.)	Ohia seedlings killed[a]		
	Chlamydospores (%)	Motile zoospores (%)	Encysted zoospores (%)
5.0×10^3	...[b]	93 A[c]	42 B
2.5×10^3	45 A	65 A	18 B
5.0×10^2	13 A	25 A	5 A
2.5×10^2	11 A	3 A	...

[a]Average of 70 ohia seedlings per treatment.
[b]Not tested.
[c]Means followed by the same letter in each row are not significantly different, $P = 0.05$.

TABLE 3. Comparison of colonization potential of chlamydospores, motile zoospores, and encysted zoospores of *Phytophthora cinnamomi*

Propagules per gram of soil (no.)	Ohia stem segments colonized[a]		
	Chlamydospores (%)	Motile zoospores (%)	Encysted zoospores (%)
1×10^3	94 A[b]	18 B	21 B
1×10^2	77 A	8 B	19 B
1×10	52 A	3 B	3 B

[a]Average of 50 dry ohia stem segments per treatment.
[b]Means followed by the same letter in each row are not significantly different, $P = 0.05$.

same (Table 2). For instance, at 2.5×10^3 propagules/g of soil, percentages of ohia seedlings killed by encysted zoospores, motile zoospores, and chlamydospores were 18, 65, and 45%, respectively. At 5.0×10^2 propagules/g of soil, however, no significant difference was found among these three spore types tested.

Colonization potential of motile zoospores, encysted zoospores, and chlamydospores.—Percentages of dead ohia stem segments colonized by motile zoospores, encysted zoospores, and chlamydospores of *P. cinnamomi* at various inoculum levels were determined after 1 wk. Chlamydospores were the most effective in colonizing ohia stems, whereas colonization potential of motile and encysted zoospores was about the same (Table 3). For instance, at 1×10^2 propagules/g of soil, the percentages of ohia stem segments colonized by chlamydospores, motile zoospores, and encysted zoospores were 77, 8, and 19%, respectively.

DISCUSSION

Phytophthora cinnamomi survives 6 yr in avocado orchard soil and 19 mo in forest soil (13, 21). Results of the present study showed that in natural soil *P. cinnamomi* exists mainly as free chlamydospores and chlamydospores imbedded in organic matter, and less frequently as free sporangia. Free chlamydospores as the origin of colonies of *P. cinnamomi* on soil isolation plates have been reported (3, 11, 14, 17). Although chlamydospores of *P. cinnamomi* were the most persistent among the three spore types tested, their population decreased to nondetectable levels after 12 mo. When root tissues naturally or artificially infested with *P. cinnamomi* were incubated in soil, the fungus remained viable in more than 50% of root segments after 12 mo, and chlamydospores were observed within the tissues. This suggests that chlamydospores imbedded in organic matter are responsible for long-term survival. After decomposition in soil of leaf tissues colonized by the fungus, free chlamydospores were observed in soil isolation plates, indicating that these propagules were released from plant tissues. Results of this study also showed that free sporangia in soil could originate from those produced on the surface of infected root tissues or from germinating chlamydospores.

Both sporangia and zoospores of *P. cinnamomi* were relatively short-lived in soil. This is in agreement with previous reports on survival of other *Phytophthora* species in soil (4, 15, 16, 19), with the exception of *P. palmivora*. Turner (18) showed that zoospores and sporangia of this fungus remained viable in soil for 6 mo and 2 yr, respectively. However, he did not determine the exact nature of survival.

Phytophthora cinnamomi zoospores are the primary structures dispersed in rain splash and runoff water in ohia forests (11). In the presence of dead tissues, *P. cinnamomi* zoospores were able to establish themselves in soil through colonization of plant tissues and subsequent production of chlamydospores. They were also able to prolong their survival by infecting roots of both susceptible and nonsusceptible plants. This may account in part for the widespread occurrence of this fungus in ohia forests (10).

Our results suggest that *P. cinnamomi* is a good saprophyte. It was able to colonize 52% of stem segments at a population as low as 10 chlamydospores/g of soil. This is in accord with that of Zentmyer and Mircetich (21), but disagrees with that of Kuhlman (13) who reported that dead tissue in soil was rarely invaded by *P. cinnamomi*. Since *P. cinnamomi* grown in a mixture of alfalfa meal and sand was used as the inoculum by Kuhlman, activity of this fungus in soil could have been suppressed by the growth activity of other microorganisms promoted by the alfalfa meal carried along with the inoculum. Zentmyer (20) and Gilpatrick (2) had demonstrated the suppression of avocado root rot caused by *P. cinnamomi* by amendment of soil with alfalfa meal.

Garrett (1) broadly classified root-infecting fungi into specialized and nonspecialized parasites. According to the results of this study and those reported by others (11, 21), *P. cinnamomi* fits the description of a nonspecialized parasite.

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