Survival of *Phytophthora colocasiae* in Field Soil at Various Temperatures and Water Matric Potentials

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ABSTRACT

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A large number of the zoospores of *Phytophthora colocasiae* incorporated into moist soils germinated by zoospore discharge, and/or lysed in the soil during the first 5 days of incubation, decreasing the initial number of colony-forming units from 1×10^4 to 1×10^2 per g of soil in all treatments. Eighteen days after incorporation, the viable zoospores present in moist soils had thickened their cell walls and germinated only directly, often producing smaller zoospores. A few thick-walled chlamydospores were observed, and they germinated only directly. Zoospores in soils at $-1,500$ J/kg matric potential survived longer than 107 days, and the amount of viable zoospores present at that time was approximately 0.1×10^2 CFU/g of soil. Apparently the great majority of the thin-walled zoospores produced on V8 agar, when incorporated into moist soil, germinated indirectly in the first 5 days of incubation. Zoospores that did not germinate became resting zoospores by increasing their wall thickness or by producing chlamydospores. These enabled the pathogen to survive in soil at $-1,500$ J/kg matric potential for more than 3 months. However, in the absence of the host, the pathogen is predicted to survive less than 1 year due to its lack of saprophytic ability to colonize nonhost tissues.

Additional keywords: bait, resting mycelia, sieving-selective medium technique, survival propagule

Phytophthora spp. survive in soil in the absence of the hosts as resting mycelia, encysted zoospores, overwintering zoospores, chlamydospores, or oospores (3–9,12–16,19,21,24). Survival as mycelium is relatively short because, in the absence of the host, most *Phytophthora* spp. lack saprophytic ability in field soil (23,24). Therefore, mycelial growth in soil is limited and usually is lysed by other soil microorganisms very rapidly (17). The exceptions are *P. cryptogea*, which persists in soil by colonizing dead organic matter in competition with other soil microorganisms (3), and *P. cinnamomi*, which apparently has a saprophytic phase in soil (27). The encysted zoospore's ability to survive in soils is short-lived in some species (3,26) and long-lived in others (9,14). Resting zoospores are the persistent propagule of *P. infestans* (1,26) and of *P. palmivora* in moist soil (24). However, chlamydospores are considered the primary survival structure of most *Phytophthora* spp. in soil (1, 5,12,13,22). The thick walls of the chlamydospores enhance their survival capabilities under unfavorable envi-

ronmental conditions. Chlamydospores of *P. cinnamomi* persist in water-submerged soil for only 3 months but may persist in moist soil for more than 1 year (9). The extended viability of this pathogen has been attributed to its saprophytic ability in the presence of dead avocado feeder roots in the soil, which become reservoirs for chlamydospores (27). The role of oospores in the survival of most homothallic *Phytophthora* spp. in soil is well established (18,19). However, oospores of heterothallic species in naturally infested soils are rare (25). *Phytophthora* spp. that require sexually compatible mating types to form oospores normally produce these in culture dishes but rarely in naturally infected host tissues (4). Oospores produced in vitro germinate at different times, suggesting different maturation periods for individual oospores. *P. megasperma* oospores in soybean residue in soil have long survival capabilities, up to 4 years (18).

Survival in nature of *P. colocasiae*, the cause of taro leaf blight of *Colocasia esculenta* (L.) Schott, is poorly understood. Oospores and chlamydospores, which are readily formed in culture, have not been found in naturally infested soil or in association with naturally infected host tissues (3,11). This species is heterothallic, requiring two mating types to form oospores (11). Zoospores, which are produced abundantly on the surfaces of infected leaves (21), are considered the most important survival structures of this pathogen.

Soil survival of *P. colocasiae* zoospores is ephemeral, as the pathogen has been reported to persist for less than 21 days in both natural and artificially infested soil (6). However, research done in the Philippines suggested that zoospores survive for 3 months on taro leaf tissue (7).

Preliminary work done in our laboratory indicated that diseased leaves bearing zoospores, when stored in moist conditions, are rapidly colonized by the saprophytic flora present on the leaf surface, and the zoospores undergo lysis shortly thereafter. When diseased leaves are allowed to dry in the shade, the viability of the spores is lost in less than 2 days (21). To corroborate information on survival capabilities of *P. colocasiae* zoospores in nature, zoospores of *P. colocasiae* produced on V8 agar were mixed with soil and stored at three soil water matric potentials and four temperatures. The viability was determined at weekly intervals for 10 weeks.

MATERIALS AND METHODS

Ten isolates of *P. colocasiae* were obtained from diseased taro leaves randomly collected in Hakalau, Hawaii. Cultures were maintained at 24°C on V8 agar medium consisting of 20% V8 juice, 2% CaCO_3 , and agar. The Makiki clay loam (MCL) used in this study is classified as Andic Ustic Humitropept, fine, mixed, and isohyperthermic (20). It was adjusted to 75, 90, and 100% moisture-holding capacity by the addition of 26, 32, and 38 cm^3 of water per 100 cm^3 of oven-dry soil, which corresponds to soil matric potential (ψ_m) values of $-1,500$, -20 , and -3 J/kg, respectively (2). The soil used was free of zoospores of *P. colocasiae* as determined by sieving a soil-water solution through a 21- μm screen (15) and plating the residue on the *Phytophthora* selective medium P₅ARP (10). Zoospores from 10-day-old cultures were harvested by adding to each plate 10 g of soil at -20 J/kg ψ_m , previously screened through a 2-mm sieve. The soil was gently mixed with the spores present on the surface of the culture by using a cell scraper, COSTAR 3011 (Costar Corporation, Cambridge, MA). After harvesting of the zoospores, the aliquots of soil containing the pathogen were bulked in a large plastic container and mixed manually to establish an even propagule distribution.

The zoospores from 40 cultures harvested in soil at -20 J/kg were mixed thor-

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oughly with soil at $-4,000 \text{ J/kg } \psi_m$ in a 1:12 wt/wt ratio. The infested soil was divided into three 1,600-kg portions, and each was adjusted to moistures of $-1,500$, -20 , or $-3 \text{ J/kg } \psi_m$ by addition of the necessary amount of water. When the desired water content was established in the soils, each portion was divided into 400-g aliquot samples, placed in vented containers, and stored in controlled temperature incubators at 20, 24, 28, or 30°C. Soils were sampled before incubation to determine the approximate number of zoosporangia present in each soil at 0 storage time.

To avoid mite contamination of incubators, all soil treatments at each temperature were placed in a larger plastic container with 1 cm of water. The water also served to maintain high humidity around the samples, thus reducing loss of water. Soil moisture was adjusted every 5 days by addition of the required amount of sterile distilled water (SDW) to compensate for the loss of weight due to soil-water evaporation. Treatments were sampled weekly by removing four 5-g aliquots per treatment, and each sample was placed in a 60-ml Qorpak tablet bottle no. 7521 (Fisher Scientific, Pittsburgh, PA) containing 10

ml of SDW. Samples were shaken vigorously for 30 s, then gently washed through a series of Tyler screens, from 70 to 38 to 25 μm , using a backwashing method (15). All particles retained in the 25- μm sieve were collected in a small beaker and brought up to 10 ml prior to placing five 20- μl droplets on four plates of the selective medium P₅ARP. Colony-forming units (CFU) of *P. colocasiae* recorded represent germinated zoosporangia observed on the surface of P₅ARP medium with a compound microscope at 100 $\times$ 12 to 24 h after plating. Empty zoosporangia, which had germinated by zoospore release, were re-

Fig. 1. Behavior of *Phytophthora colocasiae* zoosporangia produced on V8 incorporated in moistened Makiki clay loam at $-1,500$ to $-3 \text{ J/kg } \psi_m$ and stored at four temperatures. (A) Zoosporangia that germinated in soil at -20 and $-3 \text{ J/kg } \psi_m$ after 3 days. Scale bar = 72 μm . (B) Germination of thick-walled zoosporangia after 40 days. Scale bar = 43 μm . (C) Zoosporangia after 15 days in soil at $-1,500 \text{ J/kg } \psi_m$ and 20°C with thick walls found germinating to produce thin-walled smaller zoosporangia. Scale bar = 43 μm . (D) Thick-walled chlamydospores found at $-1,500 \text{ J/kg } \psi_m$ after 40 days. Scale bar = 72 μm .

corded as indirect germination. Zoosporangia that germinated by germ tube and hyphal production were recorded as direct germination. Experiments as described were repeated with similar results. When *P. colocasiae* CFU were no longer detectable by the procedure previously described, detection was attempted with a bioassay using taro leaf disks as pathogen bait. The bioassay consisted of a taro leaf disk (9 cm diameter) floating on 5 ml of SDW in a 90-mm-diameter plate. Ten rubber rings, 0.5 cm diameter, were placed on the taro surface containing 100- μ l aliquots of soil solution made from soil treatments. One gram of soil from each treatment was diluted into 10 ml of sterile water, and the whole gram was baited as 100- μ l aliquots on 10 taro disks. The taro disks were incubated at 25°C for 4 days to allow for lesion development. Positive bioassay was identified when *P. colocasiae* lesions developed on the taro disk surface under the rubber ring and sporulation was visible after 4 to 5 days of incubation. Each lesion was arbitrarily considered to be 1 CFU. The soil at -1,500 J/kg ψ_m was sampled by bioassay up to 107 days after inoculation. In order to compare differences in viability, the data were transformed by calculating the log of the percent germination. The data could then be plotted as linear functions for comparison. The compiled data were analyzed

using Statistix (Analytical Software, Tallahassee, FL). A one-way ANOVA and LSD comparison of means was used to compare the slopes.

RESULTS

Microscopic observations of *P. colocasiae* zoosporangia prior to soil infestation showed viable zoosporangia having thin walls with granular protoplasm and a single large vacuole. In all treatments, the initial amount of viable zoosporangia at 0 time was approximately 1×10^4 CFU/g of soil. Zoosporangia viability in field soil decreased rapidly in the first 5 days of incubation, tapering gradually in the next 10 days to less than 1×10^2 CFU/g of soil. Zoosporangia in soil at -3 and -20 J/kg ψ_m at all incubation temperatures at 0 storage time germinated directly or indirectly in a 1:1 ratio. Less than 2% of the zoosporangia failed to germinate and were lysed by bacteria. Three days after incubation, the number of viable zoosporangia had been reduced to <50%, and these germinated directly by production of hyphae when plated on P₅ARP medium. Many of the zoosporangia that had germinated in the soil previous to plating had produced smaller, thin-walled zoosporangia (Fig. 1A). At day 18, thin-walled zoosporangia and chlamydospores attached to germinated thick-walled zoosporangia were ob-

served. However, the chlamydospores failed to germinate and were lysed by soil bacteria. All treatments at -3 J/kg ψ_m yielded <10 CFU by day 40, and all were from zoosporangia with relatively thicker walls (Fig. 1B). No viable zoosporangia could be detected thereafter with the soil sieving-selective medium technique or bioassay. The presence of *P. colocasiae* was detectable at a population of 0.1×10^2 CFU/g in soils at -20 J/kg ψ_m at 24°C after 63 days incubation, but not in the soils at 20, 28, and 30°C.

Zoosporangia of *P. colocasiae* survived best in soils at -1,500 J/kg ψ_m ; after 6 days incubation, viability was approximately 3×10^3 CFU/g at all soil temperatures. Eighteen days after incubation, the zoosporangia present in the soil had thickened their cell walls noticeably to 3 to 4 μ m wide (Fig. 1C), compared with 1.8- to 2- μ m cell wall thickness of freshly produced zoosporangia. Also, chlamydospores with thick walls were found 40 days after incubation, and these germinated readily when spotted on the P₅ARP selective medium (Fig. 1D). After 63 days of incubation, zoosporangia viability in soils at -1,500 J/kg ψ_m at the four temperatures ranged from 0.65 to 2.25×10^2 CFU/g of soil.

When zoosporangia could no longer be recovered from infested soils with the soil

Fig. 2. Taro leaf disk bioassay for the detection of low soil populations of *Phytophthora colocasiae*. (A) Taro disk with rubber rings each containing 0.01 g of *P. colocasiae*-infested soil in 100 μ l of sterile distilled water. (B) A taro leaf blight lesion from a soil microsample containing the pathogen, showing sporulation around the rubber disk 5 days after incubation. Bar = 428 μ m.

sieving-selective medium technique, taro leaf disks were used as a bioassay method to detect low populations of the fungus in soil (Fig. 2A and B). The pathogen was not

Table 1. Survival of *Phytophthora colocasiae* expressed as regression slopes in soil at -1,500, -20, and -3 J/kg matric potential stored at 20, 24, 28, and 30°C^z

Treatment (temp./J/kg ψ_m)	Slope
20°C/-1,500	-0.0334 b
24°C/-1,500	-0.0272 ab
28°C/-1,500	-0.0298 ab
30°C/-1,500	-0.0229 a
20°C/-20	-0.0509 c
24°C/-20	-0.0489 c
28°C/-20	-0.0465 c
30°C/-20	-0.0498 c
20°C/-3	-0.0641 d
24°C/-3	-0.0687 d
28°C/-3	-0.0644 d
30°C/-3	-0.0688 d

^z LSD pairwise comparisons were made of the slope between treatments. The steeper the slope, the shorter the survival period. Slope values with the same letter are not significantly different ($P \leq 0.05$, LSD = 0.0082).

recovered from soils at -3 and -20 J/kg ψ_m after 40 and 63 days of incubation, respectively. Less than 10 CFU/g was recovered from infested soils at -1,500 J/kg ψ_m after 101 days of incubation. Further bioassays indicated that zoospores of *P. colocasiae* survived more than 107 days in soils at -1,500 J/kg ψ_m .

The linear regression lines of the data show the decreasing trend in sporangia viability over time (Fig. 3). When compared for rate of change between treatments, an LSD comparison of mean slopes ($P \leq 0.05$) indicated differences between treatments were due to water matric potential and not temperature, except for treatments at -1,500 J/kg ψ_m , which showed a significantly higher number of CFU (zoospores) surviving in soil at 30°C compared with soil at 20°C (Table 1, Fig. 3). Using the linear regression formulas of zoospore viability at -1,500 J/kg ψ_m and assuming $y = 0.001\%$ germination, one can predict that *P. colocasiae* zoospores can survive a maximum of 113, 137, 121, and 150 days in field soils at 20, 24, 28, and 30°C, respectively.

DISCUSSION

P. colocasiae zoospores, which are produced abundantly in nature on the surface of taro leaf blight lesions, were found to remain viable up to 107 days in soil at -1,500 J/kg, 63 days in soil at -20 J/kg, and less than 41 days in soils at -3 J/kg ψ_m . These results do not agree with results by Golliher et al. (6), who reported less than 15 days survival. The rapid decline of zoospore viability in the first 6 days of zoospore incorporation to moistened field soil followed a similar trend reported by Golliher et al. in 1979. The different length of time that *P. colocasiae* is able to survive in soils at different matric potentials independent of temperature corroborates Turner's 1965 report that zoospores of *P. palmivora* in soil at 100% moisture holding (-3 to -10 J/kg ψ_m) capacity maintained their viability for 12 days, while in soils at 50% (-1,500 to -2,000 J/kg ψ_m), the zoospores remained viable up to 2 years. Soils at -1,500 J/kg ψ_m are at wilting point (2); at this matric potential, there is no available water in the soil pores and the zoospores may not germinate, promoting extended viability. It

Fig. 3. Linear regression analysis of *Phytophthora colocasiae* zoospore viability in Makiki clay loam at -1,500, -20, and -3 J/kg ψ_m incubated at 20, 24, 28, and 30°C for 63 days.

is apparent that zoosporangia of foliar *Phytophthora* spp. do not survive on air-dried leaves for more than 2 h (12), and in wet soil they tend to germinate readily by release of zoospores that do not survive very long. The inability of Gollifer et al. (6) to recover *P. colocasiae* from infested soil beyond 16 days may be attributed to water saturation of soil common in baiting techniques, which could have promoted the germination of the majority of the zoosporangia in a relatively short time. This factor alone can account for the accelerated decrease in viability. The chlamydospores produced by *P. colocasiae* in soil in our studies had a cell wall thickness of 1.8 μ m or less, unlike the chlamydospores produced in culture by Butler and Kulgarni (4), whose thickness was 3 μ m. Thin-walled chlamydospores are more prone to lysis by soil microorganisms.

Although our results are preliminary, the ability of *P. colocasiae* zoosporangia to thicken their cell walls when in field soil at $-1,500$ J/kg ψ_m enables the pathogen to persist in soil for more than 3 months, far longer than previously reported (5,6). Resting zoosporangia and chlamydospores are important survival propagules of this fungus. However, because this *Phytophthora* sp. is so highly specialized in its pathogenicity, in the absence of the host this pathogen would not be expected to survive in soil for more than 5 months. One can predict that the use of host-free periods longer than 1 year would be an effective method to eradicate the pathogen from oceanic island nations. The second author observed the epidemics of taro leaf blight in Rota when the pathogen was introduced to that island from Guam in 1967. The crop was completely destroyed that year, and the Rota farmers abandoned taro from 1968 to 1981, when the crop was reestablished. The disease has not been

observed on the island since taro production resumed.

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