Infection and Colonization Potential of Sporangia, Zoospores, and Chlamydospores of Phytophthora palmivora in Soil

W. H. Ko and Mary J. Chan

Associate Professor and Technician, respectively, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo 96720.

Journal Series Paper No. 1725 of Hawaii Agricultural Experiment Station.

We thank D. Sumida for assistance in statistical analysis.

Accepted for publication 9 May 1974.

ABSTRACT

Among the three spore types of *Phytophthora palmivora* tested, sporangia were the most infective to papaya seedlings, chlamydospore infection potential was intermediate, while zoospores were the least infective. Infection potential of a sporangium was higher than that of the 16 zoospores released from it. Of the three spore types, sporangia also were the most effective in colonizing autoclaved papaya stems. However, the colonization potential of zoospores and chlamydospores was about the same. When zoospore concn was increased to 16 times of sporangium concn, percentage of papaya stems colonized by sporangia was about the same as nonmotile zoospores, but was higher than motile zoospores.

Phytopathology 64:1307-1309.

Additional key words: Carica papaya.

In Hawaii, *Phytophthora palmivora* Butler infects fruits and the upper portions of the trunk of papaya (*Carica papaya* L.) during rainy periods. Diseased fruits, covered with numerous sporangia on the surface and thick-walled chlamydospores inside the tissue, fall to the ground and become the source of inoculum that causes root rot and death of seedlings when papaya seeds are subsequently replanted in the same field (2, 3, 6). Since very little is known about the fate of this fungus in soil, we compared the ability of sporangia, zoospores, and chlamydospores to induce disease and to colonize substrates when they reach the soil. Oospores were not included in these studies because they were not found in diseased fruits or roots collected from the fields.

MATERIALS AND METHODS.—Suspensions of sporangia and zoospores of *P. palmivora* (18F-2P, ATCC26008) were prepared as described previously (4). The number of zoospores released per sporangium was determined by observing 10 individual sporangia directly under the microscope and by calculating from 10 observations the ratio of zoospores to sporangia in a suspension. To obtain nonmotile zoospores for comparison, spore motility was terminated by agitating the zoospore suspension in a test tube for 1.0-1.5 min with a Vortex mixer (4). Chlamydospores of *P. palmivora* were produced by growing the fungus in papaya juice medium for 1 mo at room temp (3). They were detached from mycelia by grinding the washed mycelial mats suspended in water at 3,800 rpm for 1 min with an Omni-Mixer. The resulting suspension was filtered through two layers of cheesecloth, and chlamydospores in the filtrate were separated from the mycelial fragments by sedimentation in a test tube. Spore concns were determined by the microsyringe method (7).

For studying the effect of spore type on disease incidence, 25 papaya seeds were planted in a sandy loam soil in a 2-liter plastic container. The soil used was collected from a noninfested papaya production area. After 1 mo, seedling roots were inoculated by evenly distributing 50 ml of spore suspension over the soil surface. Seedlings were watered, and wilted seedlings were removed twice daily. Three replicates were used for each treatment.

For determining the optimum incubation time for substrate colonization, 50 ml of spore suspension was thoroughly mixed with 200 ml of soil in a 400-ml beaker. The final moisture content of soil was approximately 45% water-holding capacity. Fifty sections (2×10 mm) of autoclaved papaya stems from 2 to 3-mo-old seedlings were distributed in the inoculated soil. Beakers were covered with petri dish lids. After incubation at 24 C for 0, 12, 24, 48, and 74 h, stem sections were removed from soil, washed in running tap water for 30 min, and placed on a selective medium (9). The presence of *P. palmivora* was indicated by the production of sporangia typical of this fungus on the medium around stem sections after incubation at 24 C under light for 3 days.

All experiments were done at least twice. Analysis of variance was applied to appropriate data, and differences between means were determined using Duncan's multiple range test.

RESULTS.—*Comparison of infection potential of sporangia, zoospores and chlamydospores.*—Among the three spore types of *P. palmivora,* sporangia had the highest and zoospores the lowest infection potential (Table 1). At the concn of 24×10^3 spores/container, the percentage of seedlings killed by sporangia, zoospores, and chlamydospores was 98, 16, and 49, respectively.

TABLE 1. Comparison of infection and colonization potential of sporangia, zoospores and chlamydospores of *Phytophthora palmivora*

Type of propagule	Disease severity[a] (% mortality)	Substrate colonization[a] (%)
Sporangia	98 A[b]	86 A
Zoospores (motile)	16 B	4 B
Chlamydospores	49 C	8 B

[a]The spore concns used were 24×10^3 and 20×10^3 spores per container for the studies of disease severity and substrate colonization, respectively.

[b]Average of 45 papaya seedlings or 50 autoclaved papaya stems per treatment. Means followed by the same letter in the same column are not significantly different, $P = 0.05$.

TABLE 2. Comparison of infection and colonization potential of sporangia and zoospores released from the same number of sporangia of *Phytophthora palmivora*

Type of propagule	Disease severity[a] (% mortality)	Substrate colonization[b] (%)
Sporangia	100 A[c]	50 A
Motile zoospores	44 B	14 B
Nonmotile zoospores	8 C	58 A

[a]The spore concns used were 5×10^3 and 83×10^3 spores per container for sporangia and zoospores, respectively.

[b]The spore concns used were 16×10^3 and 250×10^3 spores per container for sporangia and zoospores, respectively.

[c]Average of 45 papaya seedlings or 50 autoclaved papaya stems per treatment. Means followed by the same letter in the same column are not significantly different, $P = 0.05$.

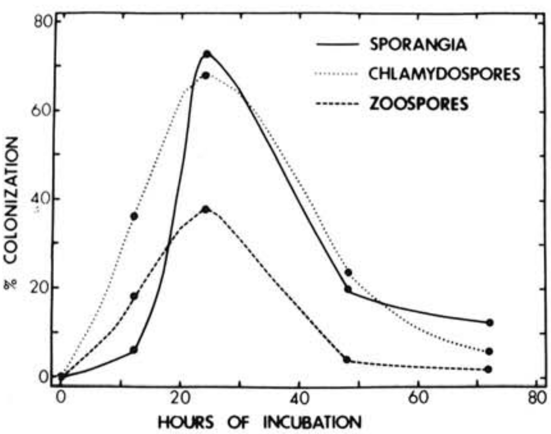

Fig. 1. Relationship between time of incubation and percentage of autoclaved papaya stems colonized by sporangia, zoospores, and chlamydospores of *Phytophthora palmivora*.

One sporangium of *P. palmivora* released an average of 16 zoospores. When the disease-inducing ability of sporangia was compared with zoospores at a 16 times greater concn, the former were still more effective in infecting papaya seedlings than the latter (Table 2). The percentage of seedlings killed by sporangia, motile zoospores, and nonmotile zoospores was 100, 44, and 8%, respectively.

Comparison of colonization potential by sporangia, zoospores, and chlamydospores.—The shortest time for maximum colonization of papaya stems by sporangia, zoospores, and chlamydospores of *P. palmivora* was 24 h (Fig. 1). Therefore, an incubation period of 24 h was used in this study. Sporangia were more effective in colonizing substrate than zoospores or chlamydospores, and the ability of zoospores and chlamydospores to colonize substrate was about the same (Table 1). At the concn of 20×10^3 spores per container, the percentage of autoclaved papaya stems colonized by sporangia, zoospores, and chlamydospores was 86, 4, and 8, respectively. When the ratio of sporangia to zoospores was about 1:16, the ability of sporangia to colonize substrate was greater than motile zoospores, but was about the same as nonmotile zoospores (Table 2). The percentage of papaya stems colonized by sporangia, motile zoospores, and nonmotile zoospores was 50, 14, and 58%, respectively.

DISCUSSION.—There have been very few studies comparing infection and colonization potential of different spore types of the same species, probably because of difficulty in obtaining large quantity of certain spore types. Green (1) reported that the minimum number of conidia of *Verticillium albo-atrum* for 100% infection of tomato was much higher than that of microsclerotia. Our results showed that sporangia of *P. palmivora* were the most infective to papaya seedlings. These were followed by chlamydospores, and zoospores were the least infective of the three spore types. Since energy must be consumed for the activity such as cell membrane synthesis and flagellum formation during the conversion of sporangia to zoospores, energy in one sporangium is greater than the total energy in 16 zoospores released from that sporangium. Under natural conditions soil does not contain available nutrients for spore germination (8). In *P. palmivora,* sporangia (just like zoospores) germinate by producing germ tubes in soil in the vicinity of papaya roots (5). This may explain why zoospore concns 16 times that of sporangium concn caused less mortality of papaya seedlings than the sporangia. Motile zoospores were more infective than nonmotile zoospores because the former exhibited positive tropism toward papaya roots (4). Apparently, the advantage of motility and tactic response of zoospores did not compensate for energy loss when they were released from sporangia.

Among the three spore types of *P. palmivora* studied, sporangia also were most effective in colonizing substrate. Colonization potential of zoospores was about the same as that of chlamydospores. The size of chlamydospores is more than 10 times that of zoospores. Therefore, when spore mass is taken into consideration, chlamydospores are relatively ineffective in colonizing substrate. When zoospore concn was increased to 16 times that of a sporangium concn, the percentage of papaya stems colonized by sporangia was about the same as that for nonmotile zoöspores, but was higher than that for motile zoospores. Nonmotile zoospores are more effective than motile zoospores in substrate colonization, because of difference in speed of germination (4). Zoospore germination occurs only after encystment. Apparently, fewer zoospores were required for colonization of one papaya stem than for killing one seedling. Therefore, in substrate colonization increase in number of zoospores compensated for loss of energy when they were released from sporangia. During the colonization process, the percentage of papaya stems with *P. palmivora* decreased after 24 h (Fig. 1). This suggests that the ability of certain fungi to colonize certain substrates may not be correlated with their ability to survive in soil.

LITERATURE CITED

1. GREEN, R. J., JR. 1969. Survival and inoculum potential of conidia and microsclerotia of Verticillium albo-atrum in soil. Phytopathology 59:874-876.
2. HUNTER, J. E. 1970. Dissemination of Phytophthora sporangia from papaya fruits. Phytopathology 60:1534 (Abstr.).
3. KADOOKA, J. Y., and W. H. KO. 1973. Production of

chlamydospores by Phytophthora palmivora in culture media. Phytopathology 63:559-562.

4. KLIEJUNAS, J. T., and W. H. KO. 1974. Effect of motility of Phytophthora palmivora zoospores on disease severity and substrate colonization in soil. Phytopathology 64:426-428.
5. KO, W. H. 1971. Direct observation of fungal activities on soil. Phytopathology 61:437-438.
6. KO, W. H. 1971. Biological control of seedling root rot of papaya caused by Phytophthora palmivora. Phytopathology 61:780-782.
7. KO, W. H., L. L. CHASE, and R. K. KUNIMOTO. 1973. A microsyringe method for determining concentration of fungal propagules. Phytopathology 63:1206-1207.
8. KO, W. H., and J. L. LOCKWOOD. 1967. Soil fungistasis: relation to fungal spore nutrition. Phytopathology 57:894-901.
9. MC CAIN, A. H., O. V. HOLTZMANN, and E. E. TRUJILLO. 1967. Concentration of Phytophthora cinnamomi chlamydospores by soil sieving. Phytopathology 57:1134-1135.