Dispersal of Phytophthora cinnamomi on the Island of Hawaii

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ABSTRACT

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Phytophthora cinnamomi was recovered by lupine baiting from soil particles carried on boots of humans, tires of vehicles, and hoofs of feral pigs. It existed in soil as chlamydospores and unknown propagules in organic matter. Zoospores of *P. cinnamomi* that were released when soil was submerged in water were dispersed by rain splash or runoff water. The fungus was trapped by lupine from streams and recovered from dead ohia leaves on the forest floor. It was isolated from the roots of 20 endemic, two indigenous, and five introduced plant species in 22 plant families. All isolates tested were of the A^2 mating type.

Additional key words: epidemiology, soil-borne pathogens.

Phytophthora cinnamomi Rands has been isolated from a wide range of plant species from many parts of the world (15) and is associated with many native plant species in Australian forest communities (11). During our studies of ohia (*Metrosideros collina* subsp. *polymorpha*) decline on the island of Hawaii, *P. cinnamomi* was found at widely distributed locations (6). In this study we investigated the possible means of dispersal of the fungus on this island.

MATERIALS AND METHODS

The lupine (*Lupinus angustifolius* L.) baiting technique (1) was used to detect *P. cinnamomi* in soil and water samples. Lupine seeds were soaked in water overnight, then held 48 hours on moist filter paper in petri plates before use. One part of the soil sample was mixed with 10 parts of distilled water in a beaker before baiting. Lupine seedlings with 2- to 3-cm-long radicles were suspended above samples immersed in water, or added directly. After 48 hours lupine roots were excised, washed in 0.5% NaOCl, and plated on a selective medium (7). Identification of *P. cinnamomi* was based on the presence of swollen vesicles on young hyphae. Reliability of this method was confirmed previously (5).

Unless otherwise stated, water samples or soil-water mixtures were passed through nested sieves with pore sizes of 44 and 20 μm. Liquids passing through the 20-μm sieve were collected. Most *P. cinnamomi* propagules other than zoospores are retained by the 44-μm sieve (7); only zoospores can pass the 20-μm sieve. Materials retained on sieves were placed on the surface of selective medium flooded with 10 ml of water and incubated 12-18 hours at 24 C. Plates were then washed under a slow stream of tap water to remove materials other than germinated propagules and the number and origin of colonies were determined microscopically. Filtrates were plated directly on selective medium using 10 ml/plate. All filtrate was tested in each experiment.

Roots of endemic plant species in ohia forests with decline symptoms and other indigenous and exotic species commonly associated with ohia forests were collected. All sites, which ranged from near sea level to 1500 m elevation, had declining ohia trees in the immediate vicinity. No attempt was made to sample all endemic species, but those sampled were the common associates of ohia trees in native forests. Collected roots were washed overnight in running tap water, surface-sterilized in 1% NaOCl, and then placed on selective medium. The number of plants of each species sampled varied, but at least three plants of a species were sampled (10 root pieces on each of five plates for each plant) from one location. If *P. cinnamomi* was recovered, additional plants at other locations were sampled to verify the isolation. The scientific and common names of flowering plants are those as listed by St. John (12), of tree ferns as listed by Joe (4), and of *Lycopodium* and false staghorn fern as listed by Neal (9).

Mating type of *P. cinnamomi* isolates was determined by pairing each isolate with an A^1 mating type (UCR-97 supplied by G. A. Zentmyer) and with an ohia isolate (56F) determined by G. A. Zentmyer as A^2. The isolates were paired on 20% V-8 juice agar and incubated with light at 24 C. Plates were observed for oospores 7-8 days later.

RESULTS

Dispersal in soil particles.—Soil particles were scraped

from boots of persons who had walked through areas of ohia forests known to contain *P. cinnamomi*. Five to 20 g of soil were baited with suspended lupine on each of five different occasions. *Phytophthora cinnamomi* was recovered each time. On one occasion, 5 g of soil scraped from boots was mixed with 1 liter of sandy loam soil in a 1.5 liter container and five ohia seedlings (4-6 cm in height, grown from seeds sown 8 months previously) were planted to the mixture. One month later, one seedling was dead and *P. cinnamomi* was isolated from roots of all five seedlings. Five control seedlings transplanted to sandy loam soil remained healthy and *P. cinnamomi* was not detected in their roots.

Soil adhering to tires of a vehicle driven through decline areas was removed and soil samples ranging from 1.5-9.0 g were baited with suspended lupine. *Phytophthora cinnamomi* was recovered on one of the three different occasions.

Feral pigs were trapped in ohia forests in the Hilo watershed area. Soils adhering to their hoofs (15-20 g/pig) were collected and baited separately. Three of four pigs tested carried soil particles containing *P. cinnamomi*.

Nature of propagules in soil.—Soils were collected from five areas of declining ohia forest and baited with suspended lupine. *Phytophthora cinnamomi* was recovered from lupine radicles even though they were not in direct contact with soil. To determine if *P. cinnamomi* zoospores were present in soils prior to baiting, the same soils were mixed with distilled water and immediately passed through the nested sieves. *Phytophthora cinnamomi* was not recovered by baiting from the filtrates. However, when the supernatants of the soil-water mixtures were passed through the sieves after 2 days, *P. cinnamomi* was recovered from the filtrates. This suggested that *P. cinnamomi* zoospores were not present in field soil, but were released during the process of baiting.

To determine the nature of *P. cinnamomi* propagules in soil, 50 g of soil mixed with 100 ml of distilled water was passed through a 44-μm sieve and materials held by the sieve were plated on six plates of selective medium. Fifty-four colonies of *P. cinnamomi* were obtained from 300 g of soil. Eighteen colonies originated from chlamydospores and the remaining 36 were of undetermined origin, growing from pieces of organic matter.

Dispersal by rain splash.—Puddles in ohia forests, resulting from undrained rainwater over organic matter and solid lava rock, were located. Seven puddles at each of two locations were baited. Ten 2-day-old lupine radicles were added directly to each puddle and then covered with a lid of a 14-cm-diameter petri plate. Most radicles floated on the water surface but a few sank and were in contact with soil. Two days later the radicles were removed, taken to the laboratory, surface sterilized, and placed on selective medium. *Phytophthora cinnamomi* was detected in water of all 14 puddles.

One-liter samples of water from puddles at each of the same two locations were collected with a pipette on each of three occasions. The samples were passed through the nested sieves and filtrates and particles retained on the sieves were baited separately. *Phytophthora cinnamomi* was recovered from both locations on all three occasions but only from the filtrates that passed through the sieves, thus indicating the presence of *P. cinnamomi* zoospores in standing water in the field.

To collect rain splash, a setup similar to that described by Ercolani et al. (2) was used. Plastic 100-ml standard-stem funnels were inserted into 50-ml test tubes and secured with cotton, aluminum foil, and masking tape. The traps then were inserted into the soil at a 70-degree angle next to puddles of water. Six traps, three at each of two puddles, were inserted during periods of heavy rain. At one location, 200 ml of water that splashed into the tubes was baited but *P. cinnamomi* was not recovered. At a second location, 155 ml of water was baited and *P. cinnamomi* was recovered.

Five-hundred-gram samples of field soils naturally infested with *P. cinnamomi* were placed in 1.5-liter containers in the laboratory and flooded with 1 liter of distilled water. Soils from three different locations were tested. After 2 and 4 days, water in a disposable pipette was dropped from a height of 2.7 m over the standing water to simulate raindrops. The resulting splash was collected in another container (65 × 47 × 12 cm) and baited. Three 20-ml replicates of splash were baited from each sample. *Phytophthora cinnamomi* was recovered from all samples. When the splashed water was passed through the nested sieves and baited the fungus was recovered from the filtrate but not from particles held by the sieves, thus indicating the presence of zoospores in splash-droplets.

Recovery from runoff water.—Streams containing runoff water from ohia forests were baited for *P. cinnamomi*. For direct baiting, 2-day-old germinated lupine and 1-cm^3 pieces of green avocado fruit were used. Ten of each bait were wrapped in a single layer of cheesecloth, tied with string, and placed in the stream. The bags were suspended with the string so as to be immersed in the water but not touching the stream bottom. Five bags of each bait were placed in areas of slowly moving water at about 10- to 15-m intervals on each of two streams. After 2 days, the baits were collected, surface sterilized, and placed on selective medium. The experiment was repeated.

Avocado was not a satisfactory bait for *P. cinnamomi*. The plates were overgrown with fast-growing *Pythium* spp., and no colony of *P. cinnamomi* was observed. However, the fungus was recovered from two and four of the 50 lupine seedlings suspended at each stream on both occasions.

Five liters of runoff water were collected from streams at four locations in ohia forests during periods of heavy rain by immersing 1-liter flasks into the flowing water. When 1-liter samples were baited directly, *P. cinnamomi* was recovered from all four locations. One to five of 30 lupine seedlings from each stream were infected. The remaining 4 liters from each stream were passed through the nested sieves. *Phytophthora cinnamomi* was not recovered from particles retained by the sieves. It was recovered from filtrates passing through the sieves from two of the four streams. When the experiment was repeated 1 month later, *P. cinnamomi* was again recovered only from filtrates, this time from one of the four streams.

To insure that chlamydospores were not present in runoff water, 100 liters of runoff water from each of two streams was passed through 144-μm and then 44-μm

sieves directly in the field and particles retained by the 44-μm sieve were baited. *Phytophthora cinnamomi* was not recovered by baiting from particles retained by the 44-μm sieve at either location, nor could colonies of *P. cinnamomi* be observed when particles were placed directly on selective medium. As a check, 500-ml samples from each stream were collected at the same time, passed through the 20-μm sieve, and baited. *Phytophthora cinnamomi* was recovered from the filtrate from each stream. Apparently only *P. cinnamomi* zoospores were present in runoff water.

Recovery from organic matter.—To determine if *P. cinnamomi* had an aerial phase or was colonizing dead plant tissue above ground, dead or dying leaves of staghorn fern, Sadleria fern, and ohia trees near the ground were collected from two areas of declining forest known to contain *P. cinnamomi* in the soil. The collected tissues (600 g from each plant) were washed under running tap water, ground in a Waring Blendor at high speed, and then either added directly to selective medium (5 g tissue/plate) or baited with lupine (200 g tissue/250 ml beaker). The fungus was not observed directly on media and it was not recovered from the lupine baits.

To determine if *P. cinnamomi* colonized dead ohia leaves on the ground following dispersal by rainsplash, leaves were collected at two different times from each of three locations. Seven-hundred grams of leaves from each location were washed in running tap water, surface-sterilized in 1% NaOCl, and then ground in a Waring Blendor. One-hundred grams of ground tissue was either added to selective medium (5 g/plate) and incubated, or added, flooded with distilled water for 24 hours, washed, and then incubated. The remaining 600 g of ground tissue was added to 200 ml of distilled water (200 g/400 ml beaker) and baited.

The fungus was not recovered from the baited samples. However, four colonies of *P. cinnamomi* were observed on selective medium, originating from tissues without flooding from one location.

Recovery from roots of vegetation.—*Phytophthora cinnamomi* was isolated from the roots of 20 endemic, two indigenous, and five introduced species in 22 plant families (Table 1). All were of the A^2 mating type, forming oospores when paired with the A^1 mating type. Furthermore, eight additional isolates from ohia roots and 12 isolates obtained from soils by baiting at locations throughout the island were also A^2. The endemic species included three species of tree fern, one sedge, three shrubs, and 13 tree species associated with declining ohia forests. The tree ferns are the second most common component of mature ohia forests. Of the indigenous and exotic plant species associated with *P. cinnamomi, Lycopodium,* false staghorn fern, yellow strawberry guava, and in some areas, the thimbleberry, are common invaders of ohia forests. Avocado and the pin-cushion protea were plants introduced to cleared lands that formerly supported ohia forests.

TABLE 1. Plant species from which *Phytophthora cinnamomi* has been isolated on the island of Hawaii[a]

Scientific name	Common name	Family
Endemic		
Acacia koa Gray	koa	Leguminosae
Antidesma pulvinatum Hbd.	mehame	Euphorbiaceae
Broussaisia pellucida (Gaud.) Fosb.	puahanui	Sagifragaceae
Cheirodendron trigynum (Gaud.) Heller	olapa	Araliaceae
Cibotium chamissoi Kuulf.	hapuu-ii, tree fern	Dicksoniaceae
Cibotium glaucum (J. E. Smith) Hook. & Arn.	hapuu, tree fern	Dicksoniaceae
Cibotium splendens (Gaud.) Krajina	hapuu-pulu, tree fern	Dicksoniaceae
Cladium leptostachyum Ness & Meyen	uki	Cyperaceae
Coprosma ernodeoides Gray	kukaenene	Rubiaceae
Coprosma mensiesii Gray	pilo	Rubiaceae
Freycinetia arborea Gaud.	ieie	Pandanaceae
Ilex anomala H. & A. F. *sandwicensis* (Endl.) Loess.	kawau, Hawaiian holly	Aquifoliaceae
Metrosideros collina (Forst.) Gray subsp. *polymorpha* (Gaud.) Rock	ohia-lehua	Myrtaceae
Myrsine lessertiana A. DC.	kolea-lau-nui	Myrsinaceae
Pandanus sp.	hala, screw pine	Pandanaceae
Perrottetia sandwicensis Gray	olomea	Celastraceae
Pipturus albidus (H. & A.) Gray	mamake	Urticaceae
Psychotria hawaiiensis (Gray) Fosb.	kopiko	Rubiaceae
Sophora chrysophylla (Salisb.) Seem.	mamane	Leguminosae
Styphelia tameiameiae (Chem.) F. Muell.	pukiawe	Epacridaceae
Indigenous		
Dicranopteris linearis (Burm.) Underw.	uluhe, false staghorn fern	Gleicheniaceae
Lycopodium sp.	wawae-iole, club moss	Lycopodiaceae
Exotic		
Melastoma malabathricum L.	malabar	Melastomaceae
Leucospermum cordifolium (Knight) Fourc.	nodding pin cushion	Proteaceae
Persea americana Mill.	avocado	Lauraceae
Psidium cattleianum Sabine f. *lucidum* Deg.	yellow strawberry guava	Myrtaceae
Rubus rosaefolius Sm.	thimbleberry	Rosaceae

[a]Isolations of *P. cinnamomi* were made by plating field-collected, surface sterilized roots, on a selective medium.

DISCUSSION

Little was known about the means of dispersal of *P. cinnamomi* and the type of propagules dispersed in nature (10). The soils we studied were high in organic matter and nearly always wet, and consequently were sticky and adhered readily. Therefore, *P. cinnamomi* on this island can be dispersed by essentially any moving object that contacts the soil. The origins of *P. cinnamomi* colonies on soil-dilution plates were free chlamydospores and organic matter. The type of propagules present in organic matter were undetermined, although chlamydospores and oospores of *P. cinnamomi* have been observed in diseased root tissues (5, 8).

Zoospores but not chalmycospores of *P. cinnamomi* were found in puddles, droplets of rainsplash, and runoff water in streams in ohia forests. These results agree with those of Thomson and Allen (13) who also found only zoospores of *Phytophthora* spp. in irrigation water. Dispersed zoospores of *P. cinnamomi* probably can establish the fungus at a new location by initiating colonization of plant roots or organic matter because the fungus has been isolated from some dead ohia leaves and roots of many plant species in ohia forests. Zentmyer and Mircetich (14) reported appreciable invasion by *P. cinnamomi* of dead avocado roots and wheat straw under conditions of high soil moisture.

Galindo and Zentmyer (3) isolated the A^1 mating type of *P. cinnamomi* from a cankered macadamia tree on the island of Hawaii, but all isolates we tested from native forests were of the A^2 mating type.

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