Phytophthora Fruit and Heart Rots of Coconut in Hawaii

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ABSTRACT

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Serious fruit and heart rots of coconut have been found on the major Hawaiian islands of Kauai, Oahu, Hawaii, and Maui. Early symptoms are dark fruit rots and premature loss of young nuts. A *Phytophthora* species is consistently associated with fruit rots. There is a high association of fruit rots and subsequent heart rot followed by tree death. Fruit and heart rots were reproduced by inoculations. The causal organism resembles *Phytophthora katsurae*, but its identification remains undetermined.

Additional keywords: oogonial protuberance, *Phytophthora castaneae, P. heveae*

In Hawaii, coconut palm (*Cocos nucifera* L.) is a popular landscape plant. This palm was relatively free of severe diseases in Hawaii until the 1970s, when declining and dying trees were found on Kauai. During the 1980s, the disease was also found on the islands of Oahu, Hawaii, and Maui, and hundreds of trees have been killed throughout the state. Early disease symptoms were dark fruit rots associated with premature nut drop. Coconut trees with these symptoms gradually declined, initially by the death of the youngest leaf or spear leaf, followed by other young leaves, and then finally the older leaves. Eventually, only leafless trunks similar in appearance to trees affected by lethal yellowing remained (5). This lethal disease has severely reduced the supply of large coconut trees, which are in high demand by landscapers.

This is a report on the etiology of the fruit rot phase of this disease, the association between the fruit and heart rot phases, and pathogen characterization. Preliminary results were published earlier (6).

MATERIALS AND METHODS

Isolations. Isolations were made from fruits, apical meristems, leaves, and trunks of coconut trees from the islands of Kauai, Oahu, Maui, and Hawaii from 1970 to 1990. Diseased leaves were washed thoroughly with mild soap, rinsed in running tap water, and blotted dry with paper towels. Fruits were washed, surface-disinfested with 95% ethyl alcohol, and split open. Microscopic examinations were made of fruit, heart, and trunk rots to identify tissue sections containing possible pathogens. Tissue sections in the transition zone between the healthy and diseased areas of each specimen were surface-disinfested by dipping in 0.05% sodium hypochlorite and drained on a clean paper towel. All specimens were plated on 1.7% water agar or a selective medium containing 2% V8 juice, 1.7% agar, and 3 ppm a.i. benomyl. Benomyl was added from a stock suspension prepared with sterile deionized water and incorporated with V8 juice agar after autoclaving. After 2–3 days at approximately 26 C, mycelia of suspect fungi were transferred to 10% V8 juice agar (VJA) (9) to establish axenic cultures. Bacteria-contaminated *Phytophthora* colonies from isolation plates were purified by placing small pieces of water agar with contaminated mycelia and sporangia in a sterilized petri plate and covering them with a large (3 × 4 cm) piece of solidified water agar. A small (3 × 3 mm) block of VJA was placed on the large water agar piece directly above the small isolation water agar piece containing *Phytophthora* mycelia. The VJA block was transferred after 2–4 days to another VJA plate to establish an axenic culture.

Morphological studies. Eight single zoospore isolates from diseased coconuts from Kauai, Oahu, and Hilo, Hawaii, were used in this study. Three of these, ATCC 76646, ATCC 76647, and ATCC 76648, have been deposited as voucher specimens. Cultures were maintained on VJA at approximately 25 C under continuous cool-white fluorescent irradiation (approximately 2,700 lx) and transferred every 2 wk.

Measurements were made of sporangia from 4- to 5-day-old cultures grown on coconut water agar (CWA) (2% coconut water or liquid endosperm, 2% V8 juice, 0.2% $CaCO_3$, and 1.7% Bacto agar) at 24 C with fluorescent light as described above. The coconut water or liquid endosperm was obtained from large immature fresh fruits. Sporangia for inoculum were produced on 9 ml of CWA in 50-mm-diameter plastic petri plates at 24 C with continuous irradiation for 9 days. Sterile, deionized water (7 ml) was added to each plate after 5 days of growth. Sporangia were also produced by placing 5-day-old VJA culture pieces (about 5 × 5 mm) in a sterile petri plate with 7 ml of sterile water and incubating them for an additional 4 days at 24 C with light. Zoospore production was induced by replacing the water with fresh sterile deionized water (7 ml), then incubating it at 16 C for 20 min followed by 30 min at 25 C. Zoospores were enumerated, and inocula concentrations were adjusted.

Gametangial measurement. Gametangia and oospores produced on VJA at 24 C in darkness within 15 days and those formed in naturally infected fruits were measured. Means and standard deviations were derived for all measurements.

Pathogenicity studies. Coconut fruits were inoculated by spraying entire detached clusters of large green or small immature fruits with a zoospore suspension adjusted to 10^3 or 10^4 zoospores per milliliter or by placing glass fiber assay disks covered with mycelia from a 3-day-old colony grown on 2% VJA close to the stem end of detached fruits. Fruits were incubated for 24 hr at moisture saturation in humidity chambers, then kept in the laboratory and observed for symptom development.

Young coconut seedlings approximately 1.5 m tall were used for stem and foliar inoculations. Stems were exposed by removing the three oldest leaves and inoculated by drenching with 2.5×10^5 zoospores per plant. Three potted plants were inoculated, and three others served as controls. In other tests, unwounded leaves and shoots were sprayed with a zoospore suspension and incubated for 24 or 48 hr at moisture saturation. Leaves wounded with a sterile needle were inoculated by placing an agar piece containing actively growing *Phytophthora* on the wound and were incubated for 24 hr. All plants were maintained in a glasshouse and observed weekly. All tests were repeated at least once.

RESULTS

Symptomatology. Lesions on field-collected, diseased fruits were large

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brown to black elliptical areas with gray centers. Irregular, mottled patterns formed as dark brown areas expanded and surrounded circular to subcircular islands of green tissue (Fig. 1A). Smaller irregular patches of darker green, water-soaked tissue also occurred. Infections were common at the stem end and on fruit tissue covered by the sepals. The disease involved much more of the internal tissue than was externally apparent. Diseased husk (exocarp) was reddish brown (Fig. 1B), and the infected endosperm was white, off-white, cream-colored, or slightly brown. Oospores were abundant in the husk tissue (Fig. 1C and D), with one to six per host cell. Gray to black husk tissue contained chlamydospores of *Thielaviopsis paradoxa* de Seynes and *Phytophthora* oospores and gametangia.

Severe bud or heart rot was associated with trees producing infected fruit and dying leaves. On Kauai, at least 70 trees producing infected fruits eventually developed heart rot and died. Wilted young leaves indicated death of the apical meristem. Longitudinal sections through such trees revealed severe rancid heart rot with high populations of bacteria and yeast. The interphase tissue below the totally necrotic heart was variously colored with cream or tan, grayish brown, reddish brown, or dark brown. The apical meristem and adjacent tissue were soft, and infected trunk sections were fibrous and firm. Thin tissue sections taken from throughout the trunk and tip revealed *Phytophthora* in pockets of reddish brown tissue. However, most of the tissue did not contain gametangia or oospores of *Phytophthora*, and the fungal gametangia were difficult to find microscopically.

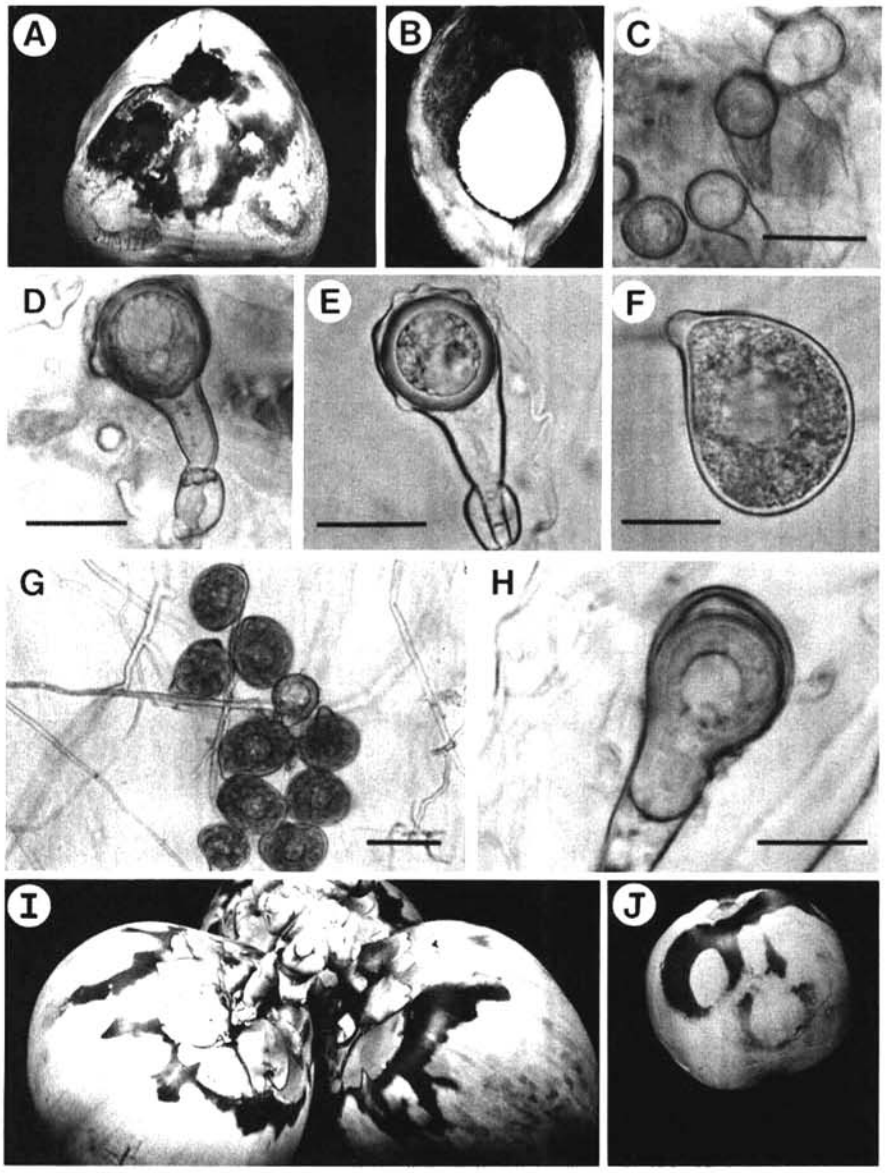

Fig. 1. Photographs and photomicrographs of infected *Cocos nucifera* and *Phytophthora* sp. pathogenic to coconut. **(A)** Naturally infected coconut fruit; **(B)** internal necrosis of naturally infected fruit; **(C)** gametangia and oospores of coconut *Phytophthora* in naturally infected fruit husk tissue (bar = 30 μm); **(D)** *Phytophthora* oogonium with protuberances and long tapered base in host tissue (bar = 20 μm); **(E)** gametangia in axenic culture (bar = 20 μm); **(F)** sporangium produced on agar culture (bar = 20 μm); **(G)** sporangial mass produced on agar culture (bar = 40 μm); **(H)** elongate *Phytophthora* oospore found in naturally infected fruit (bar = 17.5 μm); **(I)** early fruit rot symptoms following inoculation; **(J)** green circular mottling on inoculated fruit with calyx removed.

Isolation. Isolations from fruits and reddish brown leaf lesions readily produced a *Phytophthora* species that formed numerous sporangia on water agar or selective medium in 3 days, and oogonia with distinctive protuberances in 10 days on VJA (Fig. 1E). *Fusarium* spp., *T. paradoxa*, and other fungi were also recovered from older sections of some fruit lesions on water agar.

Isolating *Phytophthora* from the rancid diseased heart and trunk tissue was difficult. It was only recovered from specimen pieces containing oospores or gametangia. The fungus was not recovered from older, completely necrotic parts of the heart rot or from borings made into dead or dying trees.

Morphological studies. All *Phytophthora* isolates recovered from diseased coconut produced nondeciduous, papillate, ovoid, asymmetric (bilaterally symmetrical) sporangia in axenic culture (Fig. 1F and G). Sporangia formed on VJA and CWA at 24 C with light. Sporangia of representative isolate ATCC 76648 averaged 31.5 ± 5.0 μm in breadth with a range of 22.8–41.8 μm on CWA, and averaged 40.0 ± 4.0 μm in length with a range of 30.8–49.0 μm. Isolates ATCC 76647 and ATCC 76646 had similar sporangial dimensions.

For pathogenicity tests, large numbers of sporangia were produced in flooded CWA or on young colony pieces in water. Six of the isolates commonly produced 10^3–10^4 zoospores per milliliter.

All single zoospore isolates produced oospores in unpaired cultures. Oospores were abundant on VJA and formed with or without light. Oogonia were distinctive, with varying numbers of irregular protuberances on the surface and tapered, funnel-shaped bases (base of oospores to top of antheridia) averaging 9.8 μm long (Fig. 1E). Oogonial diameters ranged from 22.1 μm to 30.8 μm with a mean of 27.0 ± 2.2 μm, and lengths averaged 36.8 ± 2.5 μm for isolate ATCC 76648. Oospores averaged 24.0 ± 1.8 μm in diameter and were generally spherical and aplerotic. Antheridia were amphigynous, with means of 9.8 ± 0.6 μm in diameter and 10.5 ± 1.3 μm in length. Fertilization tubes were readily observed. Similar dimensions were obtained for ATCC 76646 and ATCC 76647; however, the tapered oogonial base averaged 11.8 ± 3.0 μm for ATCC 76647.

Gametangial morphology varied in host tissue. Tapered oogonial bases averaged 11.2 ± 2.8 μm in length but were occasionally short (4.9–7.4 μm) or extremely long (14.8–20.2 μm). In

different fruits, only 5–29% of the oogonia had characteristic protuberances. Furthermore, when frequencies of ornamented oogonia were low, approximately half had only one or two visible protuberances (Fig. 1D). Oogonia averaged 21.6 ± 2.7 μm in diameter, and the oospores measured 20.5 ± 2.8 μm in diameter. Oospore length ranged from 17.2 μm to 32.0 μm with a mean of 22.8 ± 3.5 μm. Considerable variation in oospore shape was observed in fruit samples, in which obovoid or clavate oospores were present. In a few oogonia, the oospore extended into the elongate, funnel-like oogonial base (Fig. 1H). Antheridia averaged 9.1 ± 0.9 μm in diameter and 11.1 ± 1.5 μm in length.

Pathogenicity test results. Fruit rot symptoms on large green coconuts appeared 3–5 days following artificial inoculation. Numerous elongate, water-soaked lesions appeared on the fruit surface sprayed with zoospores. Large, dark, necrotic, water-soaked lesions, typically 7 cm long by 1.5 cm in diameter, began at the stem end near the calyx (Fig. 1I) and expanded to near the blossom end of the fruit 7 days after inoculation. Irregular expansion of necrotic or dark brown areas produced mottled brown-green patterns and circular patches of green tissue, characteristic of field samples (Fig. 1J). As much as half of the external fruit surface was necrotic in 3 wk. Internally, husks were reddish brown to brown, the endocarp was blackened, and gametangia with oospores were present 2–3 wk after inoculation. Sporangia were observed at the stem end of inoculated fruits 7 days following inoculation. Single-site inoculations of the fruits near the calyx resulted in similar disease development, but disease progress was slower.

Small immature fruits, approximately 5 cm long, were similarly diseased following spray inoculations with zoospores. The rate of necrotic spread was rapid, and green, mottled patterns did not develop. Small fruits were completely necrotic in 3 wk in some cases. The pathogen was readily recovered from fruits in reisolations.

Heart rots were reproduced on stem-inoculated plants. All inoculated seedlings died in 6–10 wk, and the *Phytophthora* was reisolated.

Mature leaves, petioles, and exposed shoots were difficult to infect. Water-soaked lesions, measuring 1 × 2 mm, developed on the petioles but did not develop into large spots or rots. A few small flecks also developed on leaf blades but failed to expand. Limited lesion development occurred on wound-inoculated leaf sites but expanded slowly. The fungus was reisolated after 3 wk from the lesions developing at wound sites, as well as from petiole flecks, but was not recovered from other small leaf lesions formed without wounding.

DISCUSSION

A trunk rot of chestnut, *Castanea crenata* Siebold & Zucc., caused by a *Phytophthora* sp. was first reported by K. Uchida in Japan (10). The causal organism was subsequently described and named *P. castaneae* Katsura & Uchida (2). Ko and Chang observed that the binomial was invalid as a later homonym, and renamed the fungus *P. katsurae* (3). *P. katsurae* was reported to cause trunk rot of chestnut in Japan (2), was isolated from cacao (*Theobroma cacao* L.) in the Ivory Coast (4), and also was collected from soil in Taiwan (3).

A disease syndrome on coconut, similar to the one reported herein, has been reported from the Ivory Coast and has been attributed to *P. heveae* (7,8). Both *P. heveae* and the *Phytophthora* from coconut in Hawaii are homothallic species, which produce oogonia having tapered bases and oospores of similar sizes. The coconut *Phytophthora* in Hawaii and *P. katsurae* are readily distinguished from *P. heveae* by oogonial protuberances or ornamentation and nondeciduous sporangia. Oogonial protuberances are common in agar cultures of the coconut *Phytophthora* but are less obvious in host tissue. It is possible that the *Phytophthora* reported on coconut from the Ivory Coast is the same as the coconut pathogen in Hawaii. Isozyme analysis have shown similarities between the Hawaii and Ivory Coast isolates (M. Coffey, *personal communication*).

The coconut *Phytophthora* collected in Hawaii shares some morphological characteristics with *P. katsurae*. Both have asymmetrical, nondeciduous, papillate sporangia; oogonial wall protuberances; and tapered oogonial bases. Both are homothallic, producing large numbers of oospores without pairing, and oospore diameters are similar. Yet, they also differ in other sporangial and gametangial characteristics. The average sporangial length (39.8 μm) of the coconut *Phytophthora* is much longer than described for *P. katsurae* (27.5 μm) (2), although the mean for the coconut *Phytophthora* is within the range given by Katsura (10–42.5 μm) and Ko and Chang (25.1–47.4 μm) (3). Additional differences are in oogonial wall protuberances, which are prominent and numerous on *P. katsurae* (2,3) but few on the coconut *Phytophthora* isolates. The number of protuberances of the coconut *Phytophthora* increased at lower temperatures but were still less than those for *P. katsurae*.

Long, tapered, oogonial bases are common in host tissue for the coconut *Phytophthora*. These are shorter in culture, but oogonial length still exceeds the oogonial length described for *P. katsurae*. Except for a single diagram in Katsura's work, all other oogonia appear to have tapered bases about 5–8.7 μm long, in measurements from diagrams. Likewise, tapered base lengths of oogonia in other photomicrographs are approximately 5.5 μm (3). These are very short compared to the average tapered base length of oogonia produced by the coconut pathogen, which ranged from 9.2 to 11.8 μm for three isolates.

Physiological differences are also apparent. *P. katsurae* reportedly requires water and cold treatment to produce sporangia, whereas the coconut isolates form sporangia on VJA or CWA at 24 C with irradiation. The identity of the coconut *Phytophthora* remains in doubt, and nomenclatural studies using cultures from Africa, Taiwan, and Hawaii will be continued.

Inoculations demonstrated that the coconut *Phytophthora* from Hawaii is highly pathogenic to coconut fruits, causing rapid, severe rots. There also was rapid development of sporangia, gametangia, and oospores upon and within these infected fruits. The heart rot phase was reproduced on seedlings in a glasshouse. Leaves removed from the seedling exposed susceptible tissues, which were readily infected. Leaf removal procedures simulate common tree-trimming operations in Hawaii. Poor disease development following leaf inoculations of young plants may be due to the high temperature of the glasshouse (close to 35 C during the summer months), low relative humidity, or relative resistance of green foliage.

The mode of dissemination of this disease is not known. Until more is known, exclusion, eradication, and sanitation principles must be applied to reduce the spread of this lethal disease.

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