Phytophthora Blight, a New Disease of Macadamia

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ABSTRACT

Phytophthora blight of macadamia, caused by *Phytophthora nicotianae* var. *parasitica* and *Phytophthora palmivora,* is a new disease which has been observed following periods of prolonged rain at three locations on the island of Hawaii. Under these conditions, a majority of the racemes and nuts were destroyed. *Phytophthora nicotianae* var. *parasitica* but not *P. palmivora* survives in the dead racemes which remain attached to the branches between flowering periods, and serve as the source of inoculum for new infections. *Phytophthora nicotianae* var. *parasitica* is more virulent than *P. palmivora.* Phytopathology 61:1130-1134.

Macadamia (*Macadamia integrifolia* Maiden & Betche) is a large tree which produces a delicious nut. The acreage planted to macadamia has expanded more rapidly than for any other crop in Hawaii, and currently includes over 9,000 acres, most of which are on the island of Hawaii. The tree is native to subtropical eastern Australia, but has been grown in Hawaii since 1890. There are a few plantings in California. Macadamia recently has been grown in trial plantings in several subtropical or tropical areas in Central America and Africa.

The crop is affected by only a few diseases. *Phytophthora cinnamomi* causes a trunk canker of macadamia in California (8) and Hawaii (4), but has not become a serious pathogen of the crop. Trunks develop cankers only when the fungus is introduced through a wound; roots apparently are quite resistant. Botrytis blight, caused by *Botrytis cinerea,* is sometimes a serious disease of macadamia racemes in Hawaii, but it occurs only sporadically (5, 6) and does not affect young racemes (J. Hunter, *unpublished data*). This paper presents information on a new disease of macadamia which is characterized by blighting of immature racemes and nuts by *Phytophthora* spp., and therefore has been designated Phytophthora blight. The disease was observed on the island of Hawaii in December 1967, at Waiakea-Uka, and in 1968 at the same location and in two large orchards at Honokaa and Keaau.

Phytophthora blight has occurred only in a few scattered trees at Keaau, but occasionally has caused extensive losses at Waiakea-Uka and in the higher elevations at Honokaa during periods of prolonged wet weather. A survey made at Honokaa during one epidemic revealed that 63 of 75 trees had infected racemes, and an average of 57% of the racemes was destroyed. Similar losses have been observed at Waiakea-Uka.

Macadamia racemes are susceptible to Phytophthora blight at all stages of development. The primary site of infection usually is the rachis. The pathogen then rapidly invades the buds and kills them within a few days (Fig. 1, 2). Dead racemes remain attached to the branches for many months. Nuts also are susceptible to Phytophthora blight at all stages of development. Infection occurs first on the husk of the nut, and causes a brownish-black discoloration. Beneath the husk is an extremely hard shell which forms after 3-4 months when the nuts are more than half mature. If this shell has not developed at the time of infection, the fungus rapidly penetrates and destroys the kernel (Fig. 3). Mature nuts are only slightly affected. Diseased nuts fall from the branches a few days after infection occurs. Leaves apparently are not affected.

MATERIALS AND METHODS.—Isolations from necrotic racemes were made on a selective medium containing 50 ml V-8 juice, 40 g agar, 10 ppm pentachloronitrobenzene, 50 ppm nystatin, and 100 ppm vancomycin in 1 liter of water (7). A typical isolate was selected from each of three locations (Waiakea-Uka, Honokaa, and Keaau) and used in all experiments. These are referred to collectively throughout the paper as the three isolates. Inoculum for pathogenicity studies was prepared from cultures grown on V-8 agar (200 ml V-8 juice, 20 g agar, and 2 g $CaCO_3$ in 1 liter of water) at 20 C for 5-8 days under continuous fluorescent lighting (2). The cultures were flooded with sterile distilled water, scraped with a scalpel, and incubated at 24 C for 1.5 hr to induce formation of zoospores. The zoospore suspension was filtered twice through a single layer of tissue paper to eliminate most of the sporangia and mycelia. Zoospore concentrations were adjusted with a Howard Mold Counter. Inoculations of immature racemes were made in the field by spraying them with zoospore suspensions by means of an atomizer, or dipping them in zoospore suspensions contained in test tubes. Racemes treated similarly with water served as controls. In all field experiments, inoculated racemes were enclosed in plastic bags to maintain a high relative humidity.

The virulence of the three isolates was compared by spraying racemes with a suspension of 30,000 zoospores/ml, followed by surface sterilization after 0, 1.5, 3, 6, 12, 24, and 48 hr. Surface sterilization consisted of briefly dipping racemes in a 0.5% detergent solution, submerging them for 2 min in a 5% Clorox solution (0.26% sodium hypochlorite) containing 1% ethyl alcohol, then thoroughly rinsing them with distilled water. This treatment was not phytotoxic, and was adequate to kill the surface-borne inoculum. To

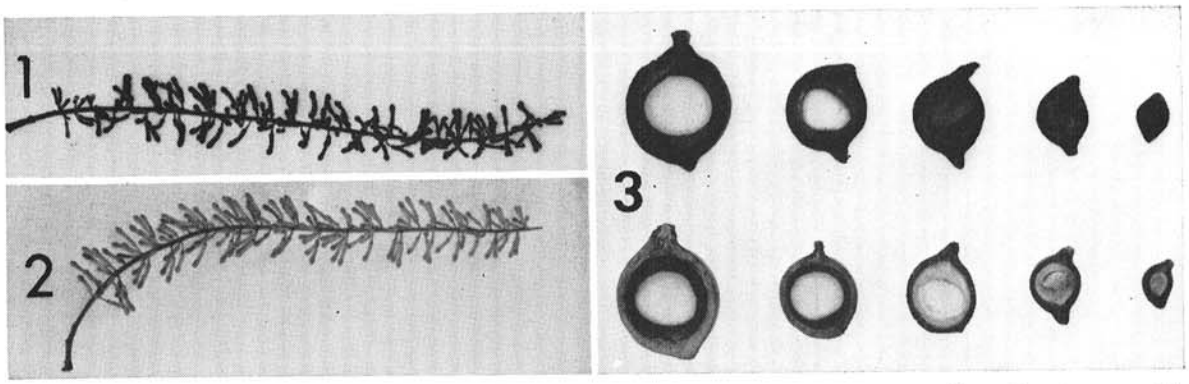

Fig. 1-3. 1) Immature macadamia racemes killed by *Phytophthora nicotianae* var. *parasitica*. Symptom consists of raceme turning brown. 2) Healthy raceme with normal green color. 3) Longitudinal sections of infected nuts (upper row) and healthy nuts (lower row) at various stages of development. The infected tissues are discolored. Note that the kernel does not become infected when the shell has developed between the outer husk and kernel at the time of infection.

determine the ability of the three isolates to infect macadamia roots, sporulating 7-day-old agar cultures were minced, suspended in water, and poured around the roots of 4-month-old seedlings. The plants had been grown from surface-sterilized nuts and were transplanted to pasteurized soil at the time of inoculation.

Rates of mycelial growth of the three isolates in relation to temperature (4, 8, 12, 16, 20, 24, 28, 32, 36 C) were determined on 5% V-8 agar. Plates were inoculated by placing an agar plug, cut with a cork-borer from the edge of a fungal colony, directly in the center of the plate. The radius of the colony was determined after 5 days. Three replicates were used for each temperature.

The effects of several factors on sporangial production were determined. The effect of light was studied by growing the fungi on 20% V-8 agar at 24 C under continuous fluorescent lighting (200 ft-c), continuous darkness, or alternating 12-hr periods of light and darkness. Fruits were inoculated by inserting an agar plug from a culture into a hole in the fruits, then sealing the hole with Scotch tape. The effect of temperature on sporangial production was determined by growing the cultures on 5% and 20% V-8 juice agar and under continuous fluorescent lighting for 5 days at 12, 16, 20, 24, 28, or 32 C. Oatflake, yeast starch, Czapek-Dox, Sabouraud dextrose, white rice, cornmeal, potato-dextrose, and 5 and 20% V-8 juice agar media (1, 3) were used to compare mycelial development and sporulation. Cultures were observed after 4 days' incubation at 24 C.

Epidemiological studies were conducted at three locations with the aid of recording hygrothermographs and rain gauges. Disease incidence was observed weekly. In related studies, racemes were inoculated and observed after 10 days to compare the ability of the three isolates to sporulate under natural field conditions. Sporangia were recovered by shaking racemes for 30 min in water in an Erlenmeyer flask. Survival of the pathogen in naturally infected racemes that remain attached to the branches between flowering seasons was determined monthly. Dead racemes were collected from Honokaa and Waiakea-Uka and soaked in water for 24-48 hr, after which florets or pieces of the rachis were embedded in papaya fruits or selective V-8 agar medium.

RESULTS.—*Isolation and pathogenicity tests.*—*Phytophthora* spp. were recovered consistently from necrotic racemes and nuts. Symptoms developed on racemes and nuts within 3 days after inoculation with a suspension of 8,000 zoospores/ml; ca. 6 days were required with inoculum containing only 1,000 zoospores/ml. Most initial infections on inoculated racemes occur on the flower buds. Diseased racemes and nuts were surface-sterilized with 15% Clorox, rinsed in sterile distilled water, and plated on the selective V-8 medium. All three *Phytophthora* isolates were reisolated consistently. The Keaau isolate was not so infectious as were the other two isolates. The average number of buds infected per raceme (average of six racemes) after 6-hr incubation was 57 and 75 for the Waiakea-Uka and Honokaa isolates, respectively, and only 6 for the Keaau isolates. Nearly all the infections occurred within 12 hr. Relative virulence of the isolates also was confirmed in a simplified experiment by dipping racemes in zoospore suspensions at a concentration of 30,000/ml. After 5 days, the average number of infected buds per raceme for the Waiakea-Uka, Honokaa, and Keaau isolates was 139, 104, and 65, respectively.

Roots of seedlings did not become infected by these isolates. There were no symptoms 6 months after inoculation, and attempts to recover the isolate from the roots were unsuccessful.

Physiological tests.—Minimum temperature for mycelial growth of the Honokaa and Waiakea-Uka isolates was 8 C, and 16 C for the Keaau isolate. Maximum temperature was 35-36 C for all isolates, but the Keaau isolate grew slightly better at 36 C. Optimum temperature was 32 C for the Honokaa and Waiakea-Uka isolates, and 28 C for the Keaau isolate. Optimum temperature for sporulation was 20-24 C for all three isolates. Light to moderate sporulation occurred at 16 and 28 C. No sporulation occurred at 12 C. At 32 C, only a few sporangia were produced by the Honokaa and Waiakea-Uka isolates, whereas

the Keaau isolate sporulated slightly better at this temperature. More sporulation occurred on the 20% than on the 5% V-8 juice agar medium. Continuous lighting was essential for maximum sporulation of all three isolates. Approximately half as many sporangia were produced under alternating 12-hr periods of light and darkness. Almost no sporulation occurred under continuous darkness with the Honokaa and Waiakea-Uka isolates, and only a few spores were produced by the Keaau isolate. Many of these spores were spherical and without detectable papillae, although a few were typical lemon-shaped sporangia. All three isolates produced sporangia on papaya fruits held under continuous light, but the Keaau isolate produced about 15 times more sporangia than did the other two isolates. No spores were produced by the Honokaa and Waiakea-Uka isolates on fruits held in continuous darkness. A few spherical spores were produced in darkness by the Keaau isolate. The Keaau isolate could be differentiated from the other isolates by the appearance and extent of mycelial development and amount of sporulation on oatflake, yeast starch, Sabouraud dextrose, potato-dextrose, and V-8 agar media. In general, the Honokaa and Waiakea-Uka isolates produced more mycelial growth, whereas the Keaau isolate produced more sporangia.

Identification of the isolates.—Isolates from Honokaa and Waiakea-Uka appear to be nearly identical in morphology, physiology, and virulence, whereas the one from Keaau was consistently different in nearly all respects. Isolates from Waiakea-Uka and Keaau were identified by D. Jean Stamps of the Commonwealth Mycological Institute, England, as most closely resembling *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterh. (IMI Specimen No. 134070) and *Phytophthora palmivora* (Butl.) Butl. (IMI Specimen No. 149123), respectively.

Epidemiological studies—Onset of Phytophthora blight was always preceded by prolonged periods of nearly continuous rain. The disease was first observed at Waiakea-Uka following 40 inches of rain distributed over a 3-week period. Average minimum and maximum temperatures during this period were 16.6 and 22.2 C, respectively. Disease onset at Honokaa was first observed after 16 days of continuously wet weather that resulted in 16 inches of rain. Infected racemes and nuts often were found only on particular branches or sections of a tree rather than distributed evenly throughout the tree. Both species produced only a few sporangia on the racemes and almost none on the nuts, which may account for this restricted occurrence. Frequently, within the canopy of the tree a pattern of spread from an initial infection on a high branch and radiating outward to other racemes was apparent. As soon as the rains subsided, no further spread was observed. Even with occasional light rains and 100% relative humidity every night, the pathogen did not become active again unless heavy rains occurred for a few days.

The papaya fruit-trapping technique was more successful than the selective medium method for recovering *Phytophthora* cultures from killed racemes. *Phytophthora nicotianae* var. *parasitica* was recovered every month from both locations during the off-flowering season (ca. 8 months) between 1967 and 1968. The onset of flowering at Waiakea-Uka in 1968 coincided with very rainy weather, and new racemes rapidly became diseased, presumably from inoculum present in the racemes killed in the 1967 epidemic. No survival structures were found upon microscopic examination of sections of the racemes.

Survival of *P. palmivora* in necrotic racemes was studied only in inoculated racemes; however, this species could not be recovered from this source after 1 month.

DISCUSSION.—Phytophthora blight of macadamia is probably not a new disease in Hawaii. The senior author recalls seeing immature racemes killed at Honokaa during an extremely rainy period in December 1965. Probably *Phytophthora* was responsible rather than *Botrytis cinerea,* as was thought at that time, because we now know that immature racemes are not susceptible to *B. cinerea.*

Honokaa is over 40 miles from Waiakea-Uka, but *Phytophthora* isolates from these locations appear to be nearly identical, whereas those from Keaau, which is only 6-8 miles from Waiakea-Uka, are another species. Honokaa and Waiakea-Uka, which are similar ecologically, are cooler and receive more rain than does Keaau. Waiakea-Uka is a cool, wet area at 1,100-ft elevation, and the Honokaa Orchard is on a slope ranging from 800 to 1,400 ft; the orchard at Keaau is at a much lower elevation. Phytophthora blight at Honokaa has been observed only at the higher elevations, which tend to be cool and wet. The disease has been much more serious at Waiakea-Uka and Honokaa than at Keaau, but it is not known whether this difference is due to the climate or to the *Phytophthora* spp. involved at the different locations.

Survival of *P. nicotianae* var. *parasitica* in killed racemes that remain attached to the branches, and its occurrence in wetter areas, presumably make this organism a more serious threat than *P. palmivora,* which apparently does not persist in killed racemes, and is less virulent. However, even if the fungus survives in the canopy of the tree, extremely wet weather must prevail for at least 1-2 weeks before Phytophthora blight will develop. Epidemics occurred at Waiakea-Uka and Honokaa in 1967 and 1968, and presumably resulted in the accumulation of a large amount of inoculum in the orchards. However, no new infections were noted in 1969, which was a comparatively dry year.

These studies indicate that Phytophthora blight can cause devastating losses during prolonged rainy periods. Since Botrytis and Phytophthora blights are both "cool, wet-weather diseases", and are the two most important diseases of macadamia, new orchards should only be established in relatively dry areas.

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