

Epidemiology of Botrytis Blight of Macadamia Racemes

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ABSTRACT

Incidence of Botrytis blight of macadamia varied greatly depending upon meteorological conditions and abundance of inoculum and susceptible flowers. *Botrytis cinerea* developed only on senescent flower parts, with no evidence of infection of immature buds. This was attributed to lack of nutrient exudates, which are required for spore germination, on the surface of immature racemes. Exudates from mature and senescent racemes stimulated spore germination.

Botrytis cinerea incidence was correlated with the number of hours per week of temperatures between 18 and 22 C, relative humidity of 95-100%, and the presence of water as detected with a leaf-wetness recorder.

Additional key words: *Macadamia integrifolia*.

Temperatures above 22 C had a significant negative correlation with the incidence of Botrytis blight. Absence of Botrytis blight during some periods of cool, wet weather was attributed to the heavy tropical rains stripping the senescent flower parts from the racemes. Normally, these are the main source of nutrients for the fungus. Heavy rains, which may exceed 1 inch in 1-2 hr, also suppress blight development by removing spores from racemes and the atmosphere. Detection of inoculum increase in the orchard was best determined by direct observation of senescent racemes, rather than by examination of Hirst spore trap slides for spores.

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Botrytis blight of macadamia (*Macadamia integrifolia* Maiden & Betche) racemes is caused by *Botrytis cinerea* Pers. ex Fr. The disease, originally named by Holtzmann (3) as raceme blight, has also been referred to as blossom blight (5). Botrytis blight, however, has become the preferred name to distinguish this disease from Phytophthora blight of macadamia racemes, a disease recently reported by Hunter et al. (4). The first reported outbreak of Botrytis blight occurred on the island of Hawaii in several macadamia plantings in early 1960. A severe epidemic followed in 1961. From 1962 through 1965, the disease was not severe except in one isolated planting in a wet area. Major epidemics occurred again in early 1966 and also in 1967. Almost no nuts were set during certain flowering periods of both years. The disease has not been serious again since 1967.

Attempts to control Botrytis blight with fungicides during these outbreaks were not consistently successful. In 1967, a laboratory bioassay technique which facilitated rapid screening of fungicides for control of *B. cinerea* was developed by Rohrbach et al. (8). Difolatan (N-[(1,1,2,2-tetrachloroethyl)sulfonyl]-cis-4-cyclohexene-1,2-dicarboximide) and benomyl were selected as the most promising materials. In field tests, excellent control was obtained with benomyl; and good control, with Difolatan.

Concurrently with these fungicide investigations, we also studied the biology of the disease as a prerequisite to developing a safe, effective, and economical fungicide control program. Several factors responsible for the sporadic occurrence of the disease were determined. This information provides a rational basis for timing fungicide applications.

RESULTS.—*Susceptibility of racemes in relation*

to maturity.—Experiments were conducted on macadamia trees of cultivar 246 to determine the stage at which racemes become susceptible and the length of time required for infection to occur. Racemes were inoculated in the field a few days prior to anthesis or just after anthesis (hereafter referred to as the expanded-bud and mature-flower stage, respectively) by spraying them with a suspension of 300,000 spores/ml of water. In all experiments, racemes were enclosed in water-atomized, plastic bags to maintain a high humidity conducive to development of *B. cinerea*. The bags were removed 0, 24, 48, or 96 hr after inoculation, and the racemes were surface sterilized by quickly dipping them in a 0.5% soap solution followed by a 2-min dip in a 0.26% sodium hypochlorite-1% ethanol solution. Racemes were then rinsed with distilled water and again enclosed in plastic bags attached to the branches. Observations for development of *B. cinerea* were made several days later. Controls were established to insure that (i) the sterilization procedure would destroy spores on the surface of the flowers, which remained healthy unless invasion of the tissue had occurred prior to treatment; and (ii) that spores placed on unuseptible tissue would remain viable and would be capable of infecting mature racemes several days later.

Botrytis cinerea spores suspended in water did not infect immature racemes regardless of the length of time they were in contact with the tissue. However, the spores remained viable and infected the racemes when they reached the mature, white-flower stage, except when the flowers were surface sterilized prior to maturation. Spores sprayed on the surface of the mature flowers infected these tissues within 24 hr, as indicated by failure to prevent infection when surface sterilization was delayed 24 hr.

Stimulation of spore germination by exudates from flowers.—The ability of spores of *B. cinerea* to germinate on racemes at various stages of maturity was compared. Water suspensions of spores were sprayed on racemes still attached to the branches, and 24 hr later the spores were removed by washing the racemes. The percentage of spores that had germinated on immature, mature, or senescent racemes was 0, 48, and 53, respectively.

Exudate solutions were prepared from immature, mature, and senescent detached racemes by submerging them in water for 3 min or 24 hr. In addition, immature racemes still attached to the branches were submerged in water for 24 hr. *Botrytis cinerea* spores were added to these exudate solutions after they had been sterilized by filtering, and the percentage of germinated spores was determined after incubation at 24 C for 12 hr.

A high percentage of the spores germinated when incubated for 12 hr in water in which detached, mature, or senescent racemes were dipped for either 3 min or 24 hr. Spores did not germinate when incubated in water used to soak detached, immature racemes for only 3 min, but when the soaking was continued for 24 hr, most of them germinated. However, when the immature racemes were still attached to branches when they were soaked for 24 hr, only 5% of the spores germinated.

Flowering pattern of two cultivars.—An understanding of the biology of *Botrytis* blight, especially as related to disease forecasting and timing of fungicide applications, presupposes knowledge of the flowering habit. A study was therefore conducted to evaluate a data collection method for quantifying flower production, and to compare the flowering pattern of two common cultivars, 246 and Honokaa Special. Five trees of each cultivar were surveyed at several locations during the flowering season for 2 years. A triangular segment of each tree, from the trunk to two points ca. 6 ft apart on the perimeter of the canopy, was delineated with engineering tape, and the number of racemes in this segment with fully expanded buds and mature flowers was recorded weekly.

Data collected on cultivars 246 and Honokaa Special, and observations on several other cultivars, indicated extensive variation in flowering habit of macadamia. Approximately 6 weeks are required for maturation of racemes, but initiation of buds usually occurs over an extended period of time; thus, racemes at all stages of development frequently are present at the same time. However, most cultivars do exhibit one or more periods of extensive flower production. Although Honokaa Special bears a few racemes almost continuously throughout the year, one very distinct flower peak lasting 2 weeks occurred with this cultivar (Fig. 1). In contrast, cultivar 246 produced flowers over a period of ca. 4 months, with most of the production usually concentrated in one main peak and one or two smaller peaks.

Incidence of B. cinerea in relation to meteorological conditions.—Observation stations were established in three orchards containing cultivar 246,

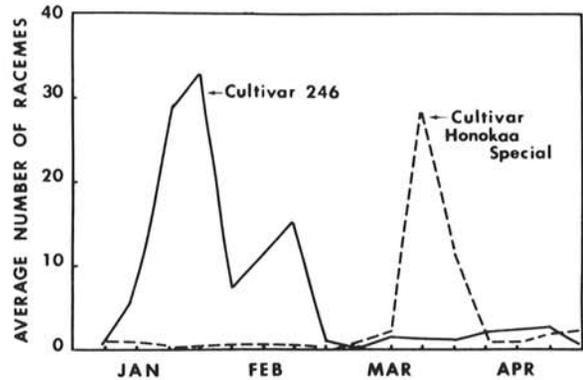


Fig. 1. Flowering pattern of macadamia cultivars 246 and Honokaa Special, based upon the average number of racemes counted weekly in a triangular segment of five trees. Heavy flowering of Honokaa Special was confined to one short period, although a few racemes were produced throughout most of the year. Cultivar 246 produced flowers over ca. 4 months with one major flower peak and, usually, 1-2 smaller peaks.

and at three elevations (800, 1,100, and 1,400 ft) in one orchard containing the Honokaa Special cultivar. An increase in elevation was associated with an increase in cool, wet weather at this location. A recording hygrothermograph, rain gauge, and leaf-wetness recorder were placed at each station. Hirst spore traps were used at two stations. Throughout the blossom period, *B. cinerea* incidence was recorded weekly at each station on 100 recently matured racemes. Macadamia flowers are not susceptible until maturity, so this served as a weekly measure of new infections. The amount of *B. cinerea* on each raceme was recorded as 0, 1, 2, and 3 for nil, slight, moderate, and severe infections, respectively. These data were collected for 2 years, and further observations were made during 2 subsequent years. Temperature data were converted to the number of hours per week below 18 C, between 18-22 C, and above 22 C. Leaf wetness and relative humidity data were converted to the number of hours per week when water was present on the sensing element, and the number of hours per week of 95% or higher relative humidity. Correlation coefficients of weather factors in relation to incidence of *B. cinerea* were determined for the six stations.

Disease incidence varied greatly in relation to weather conditions. During some years, the orchards were almost entirely free of *Botrytis* blight, whereas during other years, most racemes were diseased. Abrupt changes in disease incidence frequently occurred within 1 or 2 weeks in response to changes in the weather.

Botrytis blight usually was much more severe during periods of cool, wet weather. Disease incidence at all six stations was positively correlated with the number of hours per week of (i) temperatures between 18-22 C; (ii) 95-100% relative humidity; and (iii) leaf wetness. Correlations were significant (.05) at two stations for 18-22 C, and at five stations, for relative humidity and leaf wetness.

TABLE 1. Correlation of duration of weather factors and incidence of *Botrytis cinerea* on macadamia racemes

Cv. 246 Station	Correlation coefficients (r)				
	< 18 C	18-22 C	> 22 C	No. hr/week RH > 95%	Leaf wetness
Keaau	-.085	+5.560* ^a	-.611*	+.364	+.515
Kaneshiro	+.048	+.422	-.713*	+.710*	+.882*
Honokaa - 2	-.090	+.144	-.556*	+.764*	+.820*
Cv. Honokaa Special Station					
Honokaa 1	-.460	+.792*	-.640*	+.633*	+.802*
Honokaa 2	+.034	+.256	-.621*	+.775*	+.741*
Honokaa 3	+.175	+.332	-.602*	+.753*	+.853*

^a * = significance at .05 level.

^b RH = relative humidity.

TABLE 2. Correlation of various weather factors with leaf wetness at five macadamia orchards

Station	Correlation coefficients (r)			
	> 95% RH ^a	< 18 C	18-22 C	> 22 C
Keaau	+.735* ^b	-.204	+.618*	-.574
Kaneshiro	+.784*	+.111	+.380	-.761*
Honokaa 1	+.709*	-.437	+.659*	-.542
Honokaa 2	+.884*	-.139	+.463	-.407
Honokaa 3	+.886*	+.082	+.507	-.713

^a RH = relative humidity.

^b * = significance at .05 level.

In contrast, disease incidence was negatively correlated with higher temperatures at all stations (Table 1).

Hours of leaf wetness were inversely correlated with temperatures above 22 C, whereas there was a positive correlation with the number of hours between 18-22 C, the arbitrarily chosen temperature range which was positively correlated with development of *B. cinerea* (Table 2).

An increase in the amount of *B. cinerea* spores in the orchard could not be detected sooner on Hirst spore trap slides than by direct examination of senescent racemes. Field observations of clumps of *B. cinerea* conidia and mycelia on senescent flowers were as sensitive an indicator of inoculum increase as were observations of spore trap slides, and field observations were much simpler.

DISCUSSION.—*Botrytis cinerea* is an ubiquitous organism causing diseases of many crops during periods of cool, wet weather. Botrytis blight of macadamia, however, is a sporadic disease in Hawaii, and does not always occur during this type of weather, particularly when severe tropical rains are frequent. During several blossom periods in subsequent years, Botrytis blight was very rare even though the weather was cool and wet. Repeated observations convincingly demonstrated that prolonged periods of extremely heavy rain are not conducive to the development of *B. cinerea* on

macadamia racemes. Rainfall in some macadamia-growing areas of Hawaii may average 6-12 inches/week during rainy periods, and it is not uncommon for 1 inch of rain to fall in 1-2 hr. These heavy rains strip nearly 100% of the senescent flower parts from the racemes, and these rapidly decompose on the ground. Further inoculum production is therefore limited. In addition, spores are removed from flower parts by these heavy rains. Likewise, it is known that heavy rains cause "rain-scrubbing" or "wash-out" of spores suspended in the air (2). These effects of severe tropical rains thus account for the enigma of a low incidence of Botrytis blight in Hawaii during certain periods of cool, wet weather.

Apparently, spore germination and infection are associated with the presence of nutrient exudates on racemes. This may explain why immature racemes, which did not stimulate spore germination, remained healthy unless nutrients were added to the spore suspension. A similar phenomenon was reported by Orellana & Thomas (7), who noted stimulation of growth of *B. cinerea* and also subsequent infection of host tissue as a result of nutrients on the surface of castorbean capsules. High, moderate, and low susceptibility of capsules was associated with high, moderate, and low amounts of leachable sugar. Further evidence of the role of plant exudates in stimulating *B. cinerea* conidia has been presented by Barash et al. (1) and Kosuge & Hewitt (6).

Onset of senescence of flowers, which coincides with an increase in susceptibility, occurs within a few days after anthesis. A very conspicuous, expanded-bud stage precedes anthesis by ca. 7-10 days. A large increase in the number of racemes reaching this stage of development could serve as a useful indicator of when to initiate fungicide sprays, assuming that fungicides are sprayed on a regular schedule during blossom periods rather than when an epidemic is predicted only.

Emphasis of future research will be on integrating relevant parameters such as inoculum levels and abundance of susceptible flowers with meteorological values. This will provide a basis for formulating a practical disease-forecasting system for this extremely sporadic disease.

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