Role of Mycosphaerella Ascospores in Stem-End Rot of Papaya Fruit

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ABSTRACT

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A stem-end disease of papaya was caused by *Mycosphaerella* sp., the perfect stage of *Ascochyta caricae-papayae*. Perithecia were abundant on necrotic papaya leaves and petioles in the orchard. Ascospores germinated and grew on latex that exuded from stems when fruit was harvested and thereby entered the exposed vascular tissue. The pathogen also invaded the fruit through natural wounds at the stem end. Ascospore release followed a diurnal cycle during rainless periods. Numbers of ascospores in the air rose abruptly within 1 hr after 100% RH was reached, peaked in 2–4 hr (0300-0500 hours) and dropped abruptly. Ascospore numbers were consistently higher during periods of frequent, intermittent showers than during dry periods. Fruits showed initial symptoms in 3–4 days and extensive blackening by 7 days after inoculation. If hot water treatment (48 C for 20 min) was applied within 40 hr of inoculation, no symptoms developed.

Additional key words: Ascochyta caricae, epidemiology.

Papaya (*Carica papaya* L.) is one of the major cash crops grown in Hawaii for local and overseas markets. Stem-end rot is a serious problem in the papaya cultivar Solo and caused about 24% market losses, which ranged from 5 to 42%, according to data collected weekly from September 1977 to June 1978. Fruits were harvested, hot-water treated, and packed by seven commercial packers, then refrigerated for 7 days at 10 C to simulate surface shipment, ripened for 4 days at 22 C, and graded for disease. Market losses represent the average for the industry during the monitoring period.

Ascochyta caricae Pat. is one of the predominant fungal pathogens involved in the stem-end rot complex in Hawaii (4,5), but this widely used taxonomic designation was invalidated as a later homonym (8). *A. caricae* was applied by Rabenhorst in 1851 to an organism obtained from fig, although it was inadequately described. Tarr (8) assigned *A. caricae-papayae* nom. nov. to the papaya pathogen described by Patouillard.

Ascochyta strains isolated in Hawaii were similar both to *A. caricae* described by Chowdhury (3) as the causal agent of a papaya fruit rot in Bangalore, India, and *A. caricae-papayae* Tarr, causal agent of papaya leaf spot in Jorhat, India (9). The disease descriptions differed only in that symptoms appeared in the field on fruits or trunks (3,6) or on leaves (9), whereas in Hawaii, stem-end rot was primarily a postharvest problem.

Mycosphaerella sp. was identified as the perfect stage of *A. caricae-papayae* (9), but pathogenicity studies did not distinguish between the two stages of the fungus, since only mycelial suspensions were used as inoculum. No other report deals with *Mycosphaerella* sp. on papaya or infection by its airborne ascospores.

This article establishes the pathogenicity of *Mycosphaerella* sp. on papaya fruits and reports studies on its epidemiology and control in Hawaii.

MATERIALS AND METHODS

Isolations and pathogenicity tests. For isolations, fruits were surface sterilized by immersion in 0.5% sodium hypochlorite for 10 min. Sections of vascular tissue (1–2 mm^2) were removed aseptically and embedded in vegetable juice agar (VA) containing 10% filtered Campbell V-8 vegetable juice, 0.02% calcium carbonate, and 2% agar (Difco). Chloromycetin (200 ppm; Sigma Chemical Co., St. Louis, MO 63178) was added to reduce bacterial contamination in initial isolations. Cultures, which first formed pycnidia of *A. caricae-papayae* in 3 days, were incubated 3–4 more days under continuous fluorescent light at 24 C for development of perithecia. Single ascospore isolations were made in VA without chloromycetin and incubated 7–8 days. *Mycosphaerella* sp. isolates A001, A004, A005, and A009, obtained from Solo cultivar papaya fruits showing stem-end rot symptoms, and isolate A639, obtained from *M. Aragaki*, were used for pathogenicity studies. Mature green Solo cultivar papaya fruits were immersed for 20 min in hot water at 48 C, cooled, surface sterilized as described, and rinsed with sterile tap water before inoculation.

Inoculations were performed by allowing ascospores to discharge directly onto the exposed peduncles or fruit surfaces. Agar blocks (2.5 cm^2) of 8-day-old cultures containing perithecia grown on VA at 24 C were inverted on top of a 0.5-cm plastic tube section (10 mm inside diameter) and placed over the peduncle or fruit surfaces to be inoculated. Twenty fruits were used in each of the following inoculation treatments: (i) broken peduncle, no free water, (ii) broken peduncle, 0.1 ml of water applied to peduncle surface before inoculation, (iii) peduncle wrapped with parafilm, unwounded stem-end surface exposed, (iv) as (iii) plus 0.1 ml water, (v) unwounded surface of fruit body, (vi) surface of fruit body wounded by needle pricks, and (vii) uninoculated control. Half of the fruits of each trial were incubated in sealed humidity chambers (100% RH), and the remainder were placed in chambers regulated to deliver a continuous fresh air supply at 100% RH. Incubation temperature was 22–26 C for all fruits. Agar blocks containing the inoculum were removed after 1 day and fruits were observed daily for 8 days thereafter. Pathogenicity tests were conducted with isolates A001, A004, A005, A009, and A639. Ten fruits were inoculated as described and each isolate treatment was applied as in (i). Infection rate was recorded as numbers of fruits developing symptoms per day.

Morphological characteristics, germination, and growth rates. Ascospores were collected by inverting a perithecial culture over a clean microscope slide for 1 hr. Spores were stained with a solution containing 2 parts lactophenol and 1 part acid fuchsin and measured at ×400 with a calibrated ocular micrometer in a compound light microscope. Ascospore size was determined from measurements of 300 ascospores from about 20 perithecia. Perithecia then were removed from duplicate VA plates and measured at ×100. They were subsequently crushed on microscope slides and the free ascospores were stained. Only data from

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perithecia containing mature, stained ascospores were used to compute average dimensions of perithecia. Rates of ascospore germination and germ tube elongation were determined on sterile dry microscope slides or slides coated with VA, 2% water agar (WA), or papaya latex and incubated at 24 C and 100% RH. Data were recorded at hourly intervals. In papaya latex, slides were incubated for 5 days until pycnidia formed.

Ascospore trapping. Fresh and dry papaya leaves were collected from trees and from the ground in papaya orchards in Puna and Keaau, Hawaii. Leaves were placed in plastic bags and moistened, and air was circulated through the bags to an outlet over which a vaseline-coated glass slide was mounted. After 2 hr, airborne spores that were captured on the slide were either stained or transferred to VA and incubated 7 days for identification. Small pieces of the collected leaves also were attached to the inside cover of VA plates, and spores that settled on the agar were identified.

Water samples (250 ml) were collected from hot and cold water tanks at papaya packing plants. Samples were centrifuged for 20 min at 17,300 *g*. The upper half of the supernatant was discarded, the debris from two tubes was combined, and the process was repeated 3 times to collect spores. Five subsamples of each sample were examined microscopically.

A Hirst spore trap (Casella and Co. Ltd., London, England) was operated continuously for 2 wk in a papaya packing plant. A second trap was operated for 2–3 days every week during a 3-mo period in an orchard in Keaau. The trap's orifice was located 1.2 m above the ground. Vaseline-coated slides with 24-hr air spora samples were stained and observed microscopically. Spores with the shape and dimensions of *Mycosphaerella* sp. were recorded at hourly intervals. Viability of ascospores was determined by incubating unstained slides at 100% RH and recording percent germination after 8 hr. A rain gauge, leaf wetness gauge, and hygrothermo- graph (Belfort Instrument Co., Baltimore, MD 21224) were located 6 m from the spore trap. During 12 days of spore trap operation, 10 fruits were picked from trees near the trap in order to see whether spore showers correlated with disease incidence. Five fruits were sealed in cartons immediately after harvest; the remainder were exposed to orchard air overnight and sealed the following day. Fruits were refrigerated (10 C) for 7 days, removed to room temperature (22–26 C), and examined daily for infection by *Mycosphaerella* sp.

RESULTS

Pathogenicity tests. Papaya fruits inoculated with ascospores on the broken peduncle surface showed initial watersoaked symptoms in 3–4 days. This was followed by blackening, which progressively developed at the stem-end until the eighth or ninth day, when 25–33% of the fruit area had a black, firm rot, very similar to symptoms described for *A. caricae* (4–6). Fruits that were cut open on the 10th day had dark brown to black flesh, and discoloration of the vascular tissue often extended into the seed cavity. Mycelium and pycnidia could be recovered from dark areas, and cultures developed perithecia within 4 days after transfer of diseased tissue to VA plates. Presence of free water at the inoculation site was not necessary and did not increase the rate of infection or the total numbers of fruits infected. If the peduncle was severed immediately before inoculation, 100% of the fruits were infected after 10 days. If the peduncle was wrapped with parafilm and ascospores fell on cracks at the stem-end surface, however, only 66% of the fruits became infected after 10 days. No symptoms developed on the fruit body unless the epidermis was wounded by needle pricks. Fruits incubated with a constant fresh air supply (100% RH) ripened and developed symptoms more rapidly than fruits incubated with the same humidity in a closed system, but there was no difference in the total number of fruits infected by the seventh day. Fruits that were treated with hot water (48 C for 20 min) within 40 hr after inoculation showed no signs of infection even after 10 days. All isolates used showed the same degree of pathogenicity and symptoms were similar.

Morphological characteristics, germination, and growth rates. Perithecia were dark brown, flask-shaped to slightly oval, with the following dimensions: 100–180 × 70–200 μm (mean, 129 × 127 μm). Asci measured 28.6–52.8 × 6.6–13.2 μm (mean, 36.1 × 9.4 μm). Ascospores were hyaline, septate, constricted in the middle, straight or slightly curved, broadly rounded at the end of the larger cell, and narrowly rounded at the end of the smaller cell. Ascospore size ranged from 7.5–15.0 × 3.3–5.0 μm (mean, 11.6 × 4.8 μm). The ascospore germination rates on VA and WA were similar, as shown by nearly parallel slopes in Fig. 1. Germination first was detected after 2 hr, and 100% of the ascospores germinated by the fifth hour. Ascospores collected in water droplets germinated 1 hr sooner than those collected on a dry slide that was incubated under 100% RH, but later the germination rates were similar (Fig. 1). Germ tubes elongated quickly, and within 9 hr some exceeded 94 μm. The mean length of 100 germ tubes was 38.5 μm.

Ascospores germinated, and the pathogen grew and produced pycnidia and perithecia on papaya latex, but germination and mycelial growth was slower in latex than VA. Pycnidia formed sooner when latex was placed on dry slides incubated at 100% RH than when latex was partially immersed in water.

Ascospore trapping. No symptoms of *Mycosphaerella* infection (9) were observed on papaya leaves. When air at 100% RH was blown over live papaya leaves and petioles, no ascospores were recovered. The dead leaves and petioles discharged ascospores, most of which germinated on slides under 100% RH and grew in VA. These ascospores took longer to develop sporulating colonies than did ascospores obtained from VA cultures. Fewer than three ascospores per cubic meter of air were trapped per hour during the 2-wk sampling in each of two packing plants. No spores were found in hot water from tanks of three packing plants or in cold water from tanks with or without 100 ppm chlorine.

February 1978 was an unusually dry month with only 36.4 mm of rain. On the 10 rainless days during this month, RH increased gradually soon after sundown and remained between 90 and 100% RH from 2200 hours until sunrise, after which RH decreased rapidly, reaching about 65% around 1200 hours. Data of two typical 24-hr periods are represented in Fig 2. On all rainless days the number of ascospores trapped in the orchard increased sharply within 1 hr after RH reached 100%. A peak occurred 2–4 hr after the initial rise, followed by a sharp decline. Very few spores were collected during the day. Means of spore counts, temperature, and humidity were calculated for all rainless days during the 3-mo sampling period. Ascospore numbers in the air reached a peak

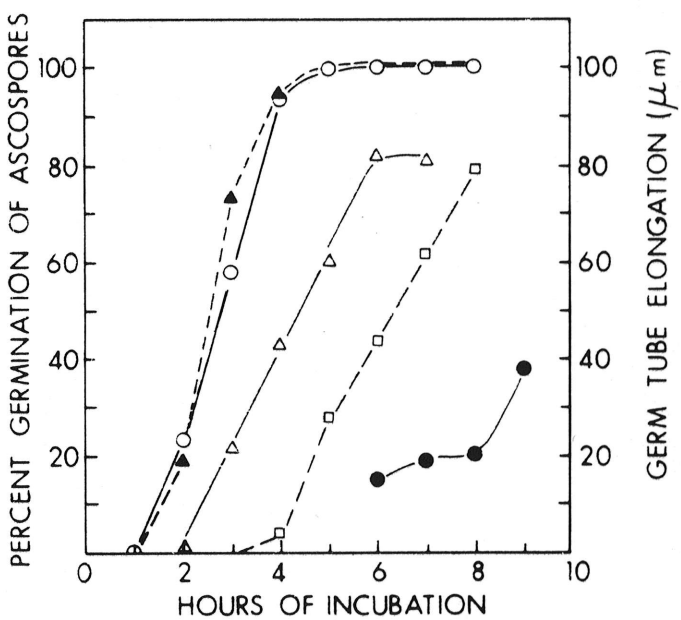

Fig. 1. Ascospore germination and germ tube elongation of *Mycosphaerella* sp. as a function of time on various substrates. ▲---▲ 2% water agar; o—o vegetable agar; △ —— △ wet slide; □ - - - □ dry slide; • – • germ tube elongation.

between 0300 and 0500 hours, which coincided with the rise in RH, and dropped abruptly.

March and April had total precipitations of 68.3 and 62.9 mm, respectively. The mean number of spores collected per day during these months was 4,708/m^3 of air on rainy days as compared with 314/m^3 of air collected on dry days. Ascospore numbers increased immediately after rain started, reached a peak within 1 hr, and abruptly declined (Fig 3A). A new peak occurred with each successive rain (Fig 3B), but ascospore numbers decreased and did not rise again if the shower was heavy and lasted several hours (Fig 3C).

Temperature showed no relationship to air spora samples except as related to increased RH. Fruits sealed in cartons in the orchard immediately after harvest developed as much disease (52%) as those left exposed in the orchard overnight (54%). *Mycosphaerella* sp. was recovered from diseased tissues in both cases.

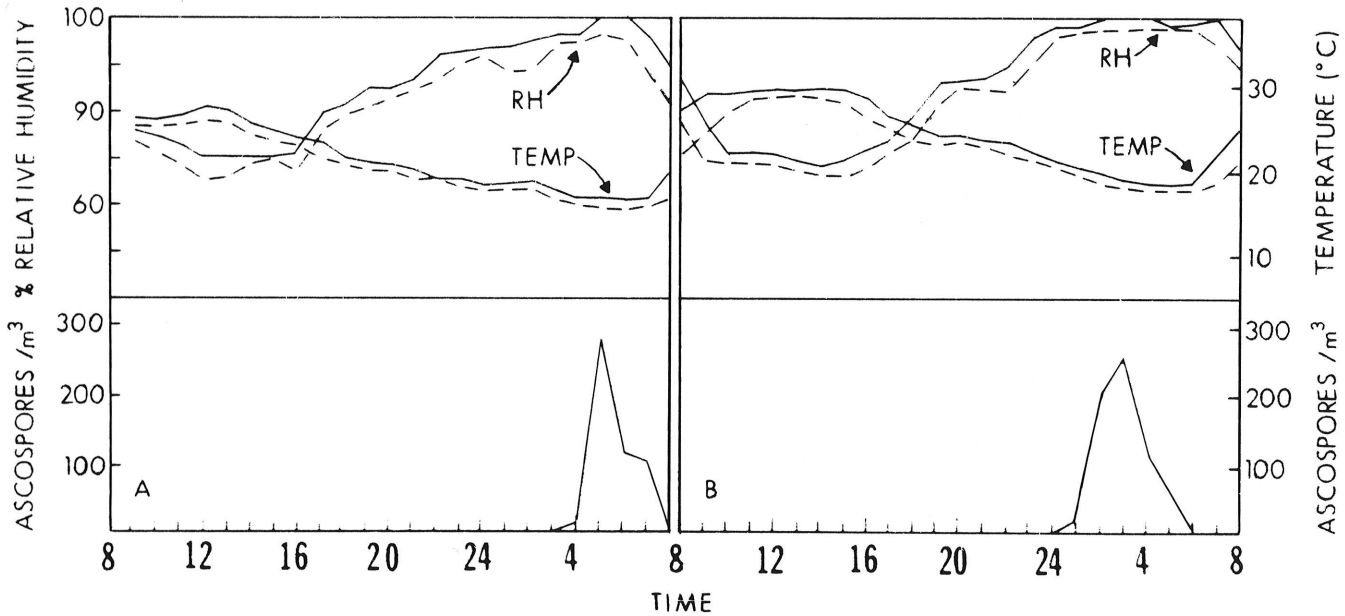

Fig.2. Diurnal release of ascospores on two typical dry days, showing relationship to fluctuations in temperature and relative humidity. Solid lines represent maxima, dashed lines represent minima for daily readings. A = March 2 and 3, 1978; B = April 20 and 21, 1978.

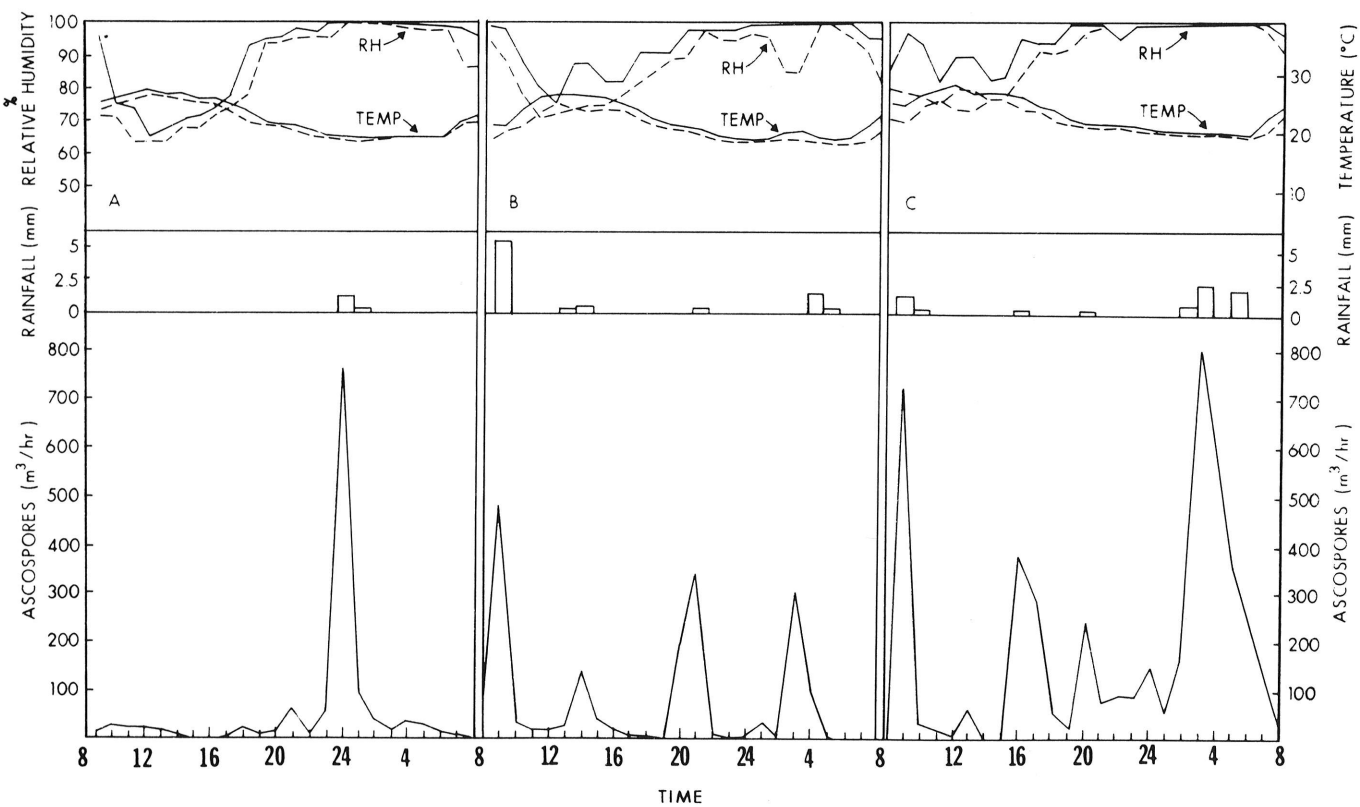

Fig. 3. Relationship of ascospore release to rainfall patterns over a 24-hr period on three typical wet days. A = March 9 and 10; B = March 15 and 16; C = April 27 and 28, 1978.

DISCUSSION

Pathogenicity of *Mycosphaerella* sp. on papaya fruits has not been reported previously. However, *Mycosphaerella* sp. was associated with *A. caricae-papayae* in papaya leaf lesions, and the ascigerous stage was produced from single pycniospore cultures (9). Our measurements of perithecia, asci, and ascospores closely resembled those reported for *Mycosphaerella caricae* Syd. (7,9). However, previous inoculations of mature papaya fruits were unsuccessful in the laboratory and field, and the disease was reported only as a leaf spot (9). In contrast, the leaf spot phase was not observed in Hawaii, but fruits were readily infected.

A fruit rot of papaya, attributed to *A. caricae* (3) was serious both as an orchard and a postharvest disease, but a perfect stage was not mentioned. Ascospore infections may have been neglected because pycnidia of the imperfect stage are so easily recovered from diseased fruits. Pycniospores are also pathogenic (3,5,6), and several additional days are needed before perithecia develop from pycnidial cultures in vitro.

Several considerations are pertinent to the potential role of *Mycosphaerella* sp. in stem-end rot disease: (i) ascospores are airborne and are present in large numbers in the orchard, (ii) ascospores germinate quickly at 100% RH and germ tubes elongate rapidly in the next 9 hr, (iii) rain is not required for ascospore release, germination, or infection, but sporadic rain showers increase spore levels in the orchard, and (iv) ascospores grow and sporulate in papaya latex.

Papayas are commonly harvested between 0600 and1200 hours in Hawaii's orchards. On dry days this period immediately follows the diurnal peak in ascospore release (0300–0500 hours). Ascospores landing on fruit surfaces, particularly on broken peduncles that exude sticky latex, then can invade the vascular tissue of the papaya fruit within a few hours. No evidence of infection would be visible for 3–4 days after fruits are packed and exported. Ascospores of *Mycosphaerella citri* were viable for limited periods (49 hr) after discharge, even when RH dropped (30–90%), and they germinated when conditions again became favorable (10). If the papaya pathogen behaves similarly, ascospores shaken from dry papaya leaf and petiole sufaces during harvest would still be able to infect detached fruits.

The implication that *Mycosphaerella* stem-end rot is initiated in the orchard at or before harvest was partially demonstrated when fruits sealed in cartons immediately after the noonday harvest developed as much disease as fruits left uncovered in the field overnight. In addition, ascospores were abundant in the orchard, whereas very few ascospores were recovered from air at the packing plants and none from hot or cold water tanks. It was previously shown that a biweekly orchard spray program reduced stem-end rot by 24–43% over a 50-wk test period (2), also providing indirect evidence for the orchard origin of the postharvest disease.

Despite our observation that *Mycosphaerella* infections were completely controlled by the standard postharvest hot water treatment (1), stem-end rot accounts for large fruit losses in the markets. It is very likely that fruits are inadequately heated during commercial operations. On the other hand, inoculum levels in papaya orchards might be so high during rainy weather that postharvest treatment alone provides insufficient control.

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