Pathogenicity and Further Characterization of Calonectria crotalariae Causing Collar Rot of Papaya

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Hawaii Agricultural Experiment Station Journal Series Paper No. 1508.

We thank E. K. Sobers, Georgia Coastal Plain Experiment Station, Tifton, Georgia, for providing cultures of *Calonectria crotalariae* and *C. ilicicola*.

Accepted for publication 1 November 1972.

ABSTRACT

Papaya seedlings infected by *Calonectria crotalariae* are characterized by stunting, chlorosis or loss of foliage, and rotting of the basal stem as well as the crown roots. The taproot is uninfected in the early stages but as the seedlings die, various saprophytic organisms invade to destroy the root system.

The papaya *Calonectria* causes collar and stem rot of three commercial lines of papaya and *Carica cauliflora* in glasshouse tests. It also causes collar and root rot as well as leaf spots of peanut, three species of *Acacia*, four species of *Crotalaria*, and seven species of *Eucalyptus*.

Phytopathology 63:553-558

Prior to 1970, root rot of papaya (*Carica papaya* L.) and the subsequent rapid decline popularly referred to as "replant problem" was attributed to *Phytophthora palmivora* and *Pythium aphanidermatum* (3, 7, 9, 10, 12). A new seedling disease of papaya first noticed during August 1970, in the Puna district on the island of Hawaii was recently described (4). The causal organism was determined to be *Calonectria* sp. with a *Cylindrocladium* imperfect stage (4, 6). Diseased seedlings were characterized by stunting, chlorosis or loss of leaves and rotting of the basal stem and crown roots, often with numerous red-orange perithecia and eventual death (Fig. 1-C). In many seedlings the root systems were completely destroyed as the result of subsequent invasion by various saprophytic organisms (Fig. 1-C). The disease was especially severe and widespread in fields newly established by clearing virgin ohia forests (Fig. 1-A).

In September 1970, a similar disease was found on seedlings of *Acacia koa* regenerating after a forest fire in the Kipapa Gulch area of central Oahu (1). It was shown that the papaya and koa isolates are morphologically and pathogenically indistinguishable.

A review of the literature showed six species of *Calonectria* with *Cylindrocladium* imperfect stages previously reported. Among these, *Calonectria*

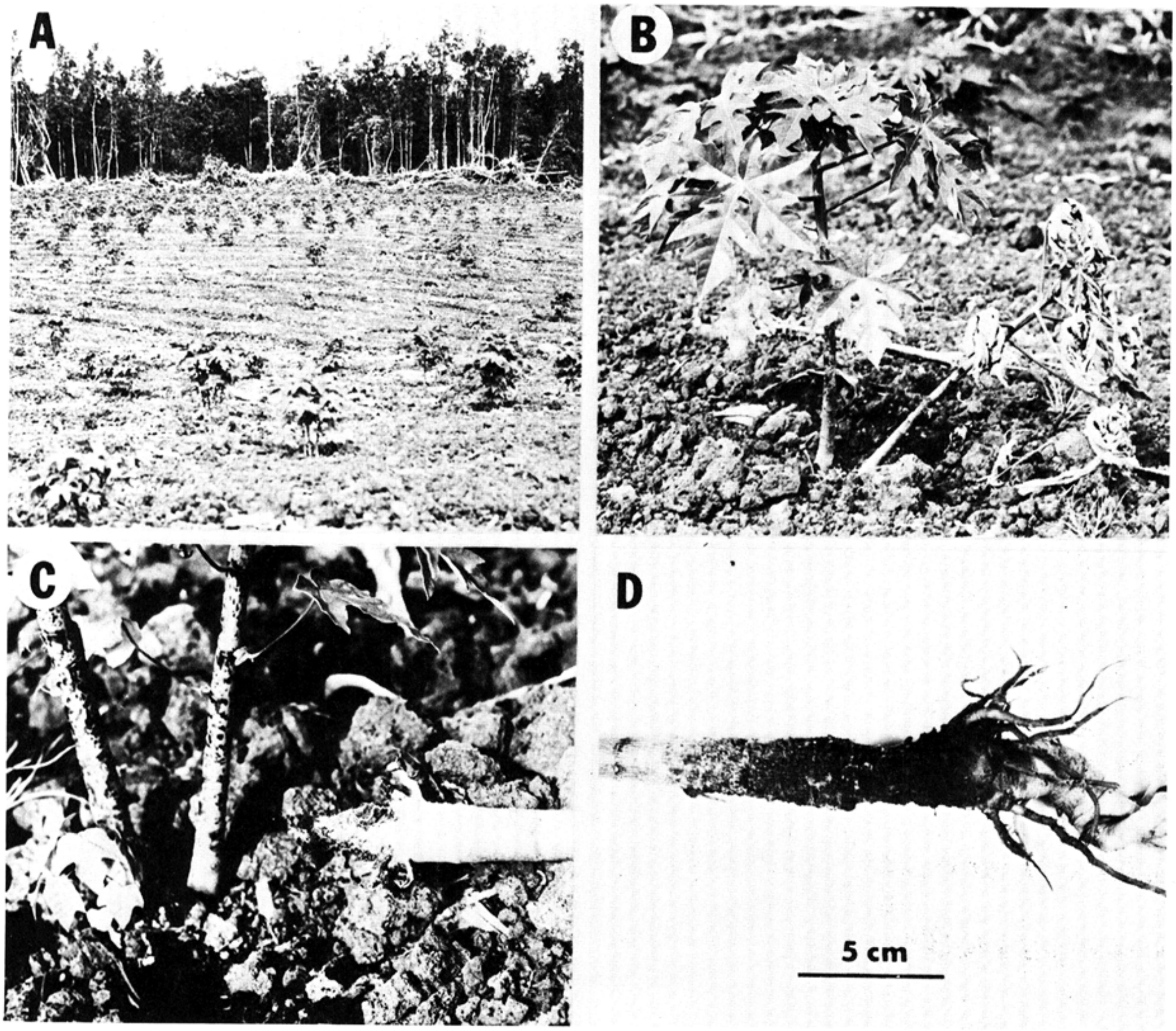


Fig. 1. **A)** Four-month-old papaya field in Puna, Hawaii, established by clearing the virgin ohia [*Metrosideros collina* (Forst) Gray] forest. Note area in center of photo where most seedlings have been killed. **B)** Dead and apparently healthy 4-month-old papaya seedlings occupying same planting hole. **C)** Papaya seedling with most of root system destroyed. **D)** Greenhouse inoculation of 3-month-old papaya seedling with papaya *Calonectria*. Note severe collar rot but uninfected tap root.

crotalariae (Loos) Bell & Sobers, originally described by Loos as *C. theae* var. *crotalariae* causing collar rot of *Crotalaria anagyroides* and *Tephrosia vogelii* (5) resembled the papaya organism. It also causes a leaf spot and necrosis of the pegs, pods, and roots of peanut (*Arachis hypogaea*) (2). Bell & Sobers also reported the pathogenicity of *C. crotalariae* to leaves of 2-month-old seedlings of *Eucalyptus camaldulensis, E. grandis, E. robusta, E. rudis, E. saligna*, and *E. tereticornis*, and to the leaves of *Crotalaria spectabilis*.

MATERIALS AND METHODS.–*Morphology*.–Description of the physical aspects of the pathogen is based on measurements and observations of 12 papaya isolates. From each isolate, 40 perithecia, 40 asci, 100 ascospores, 100 conidia, 25 phialides, and 25 vesicles were measured. The pathogen was grown at room temperature (26 C) in 60-mm diam petri dishes containing 9 ml of potato dextrose agar (PDA), made from the infusion of 200 g fresh potato, 20 g dextrose, and 15 g agar per liter of demineralized water. The growth characteristics of the pathogen were observed on vegetable-juice agar (VJA) made with 100 ml vegetable juice (V-8 juice), 2 g $CaCO_3$, and 15 g agar brought to 1 liter with demineralized water.

Conidia and vesicles were obtained from 7-day-old cultures and perithecia and ascospores were obtained from 30-day-old cultures. Only perithecia with extruded ascospores were considered for measurements.

The morphological characteristics of the papaya isolates were compared with those of *Calonectria crotalariae* and *C. ilicicola* Boedijn & Reitsma as reported by Sobers (8).

Pathogenicity tests.—Plants used in the pathogenicity tests are listed in Table 1. All plants were tested for susceptibility of leaves, stems, and roots.

The inoculum for the roots was prepared by comminuting 21-day-old cultures grown on 9 ml of PDA and diluting with three parts of demineralized water. Six ml of this mixture was placed at the base of each seedling and covered with a small amount of soil. Control plants were similarly inoculated with autoclaved fungus cultures and incubated in a separate moist chamber. Three-month-old papaya plants were inoculated in the same manner with 25 ml of the inoculum mixture.

Inoculum for leaves and stems was prepared by flooding 21-day-old cultures grown on 9 ml of PDA and brushing off the mycelium, conidia and perithecia and filtering through four layers of cheesecloth. Trapped perithecia were crushed and the ascospores washed through. The spores were suspended in a 1:2,000 solution of Tween 20 in demineralized water, and diluted to a final spore concentration of 3×10^4 spores/ml. An atomizer was used to spray the inoculum on the upper and lower leaf surfaces, apical shoots and stem. The leaves of control plants were sprayed with a 1:2,000 solution of Tween 20 in demineralized water. The inoculated and control plants were placed in moist chambers for 24 hr, then placed on glasshouse bench.

Spore germination.—The germination of conidia and ascospores was observed on 2.5% water agar plates at 15, 20, 24, 28, 31, and 34 C. Germination was also observed on 2.5% water agar plates buffered at various pH's with a 0.05 M sodium phosphate buffer system.

RESULTS.—*The pathogen.*—The pathogen grows well on PDA and VJA with profuse growth of aerial mycelium. The aerial mycelium is white at first but in the presence of appreciable amounts of glucose it becomes tinted brown. On PDA the medium and the subsurface mycelium are also colored a brown to reddish-brown color. Abundant subsurface chlamydospores are formed in chains or irregularly shaped aggregations on PDA with a lesser amount on VJA.

Single conidium and single ascospore cultures produced both *Cylindrocladium* stage and *Calonectria* perfect stage and these monosporic cultures could not be distinguished from each other nor from cultures started from mass conidia and mass ascospore transfers. The spores usually germinate from either of the two end cells; however, germination from the two middle cells was not uncommon. The germination tubes from the same or different spores anastomose readily.

Sterile stipes arise perpendicular from hyphal strands (Fig. 2-A). They taper distally ending in a globose vesicle averaging 11.0 μ in diam; this vesicular cell average 17.4 μ in length with a range of 11.4 - 28.6 μ (Fig. 2-C). The stipe is septate and averages 4.0 μ in diam at the base and 290 μ in length. Dichotomously and trichotomously branched conidiophores are usually borne laterally from this stipe. Frequently, the spore-bearing structure is the main axis from which one to four laterally borne stipes averaging 102 μ in length with vesicles averaging 6.4 μ in diam and 11.8 μ in length are produced (Fig. 2-B). The stipes of the papaya isolates usually form before the development of conidiophores and conidia. However, conidial heads often form before the complete development of the stipe, or stipes may not be present in the conidial head at all.

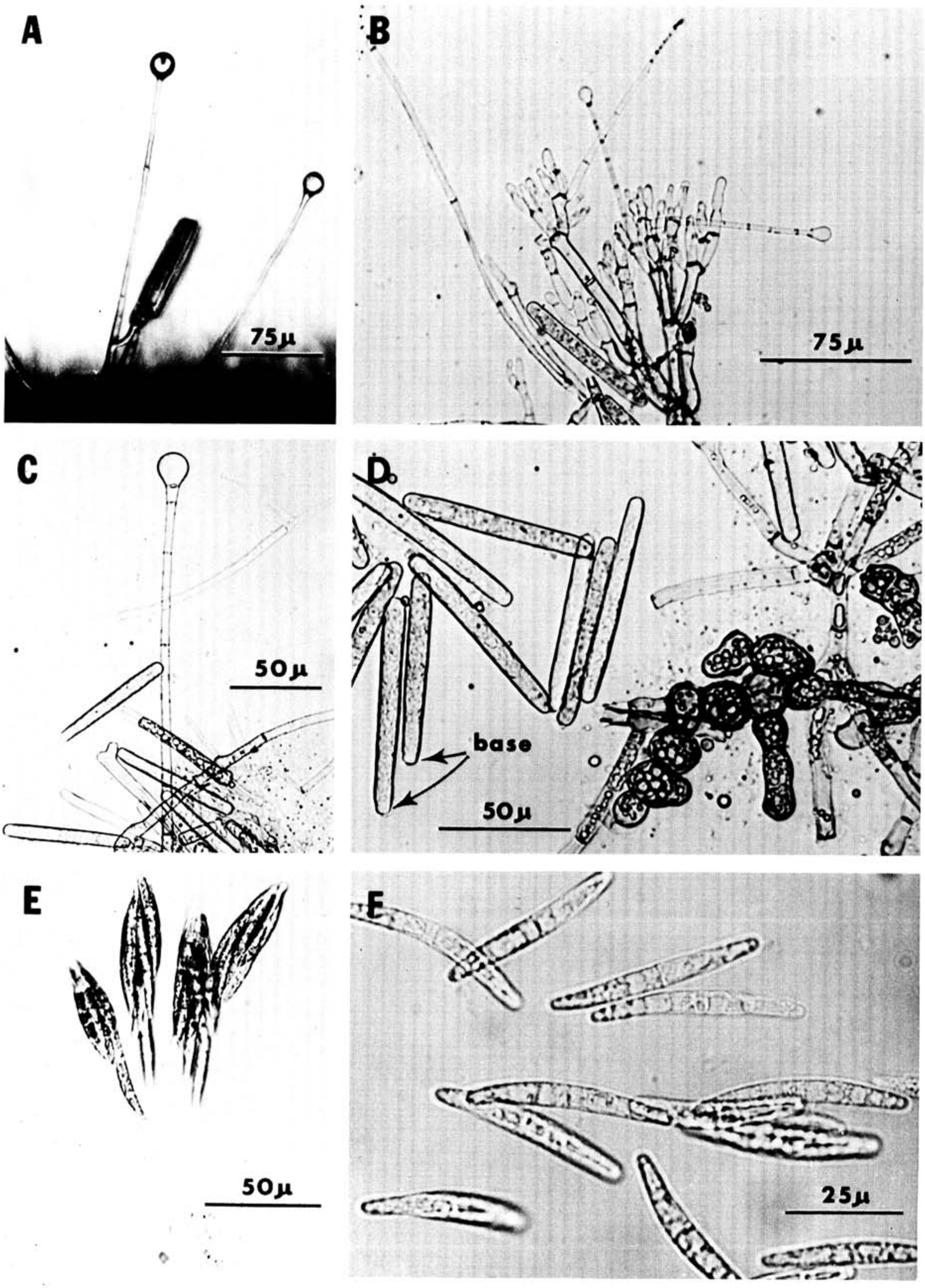
The phialides are reniform to doliform, hyaline and average 3.8 μ in diam and 11.7 μ in length. Undisturbed conidia are massed in a palisade-like arrangement (Fig. 2-A). The conidia are hyaline, nearly cylindrical, slightly tapered towards the basal end, both ends rounded and one to three septate, mostly three (Fig. 2-D). They average 6.3 μ in diam and 74.0 μ in length. The conidia of the papaya isolate are narrower at the base (Fig. 2-D) than at the

TABLE 1. Summary of pathogenicity of *Calonectria crotalariae* isolated from *Carica papaya*

Test Plants	Leaves[a]	Collar[b]	Roots
Caricaceae			
Carica cauliflora	–	18/20	1/20
C. goudotiana	–	0/20	0/20
C. monoica	–	1/20	0/20
C. papaya			
Kapoho Solo	–	0/20	0/20
Line 8	–	8/20	2/20
Sunrise Solo	–	17/20	5/20
Waimanalo	–	10/20	2/20
Leguminosae			
Acacia confusa	+	7/20	5/20
A. koa	+	40/40	36/40
A. melanoxylon	+	32/32	30/32
Arachis hypogaea	+	16/16	16/16
Crotalaria incana	+	20/20	18/20
C. mucronata	+	20/20	13/20
C. retusa	+	20/20	15/20
C. spectabilis	+	20/20	19/20
Samanea saman	–	0/15	0/15
Myrtaceae			
Eucalyptus camaldulensis	+	6/20	14/20
E. citriodora	+	3/32	3/32
E. punctata	+	5/20	9/20
E. robusta	+	15/20	17/20
E. saligna	+	2/22	7/22
E. sideroxylon	+	29/29	29/29
E. tereticornis	+	19/20	19/20
Melaleuca leucadendra	–	0/15	0/15

[a] + = infected; – = not infected.

[b] Numerator is the number of infected seedlings; denominator is the total number inoculated.


A
75µ
B
75µ
C
50µ
D
base
50µ
E
50µ
F
25µ

apex which is contrary to that reported for *C. crotalariae* (2). However, an examination of *C. crotalariae* (ATCC-18904) obtained from Sobers revealed that its conidia are also narrower at the base than at the apex.

The perithecia are scattered to gregarious, subglobose to ovate, orange to red and average 405 μ in diam and 455 μ long. On the host, they form on the surface and on PDA on and beneath the surface of the agar. The perithecia begin to form in 10 to 15 days. With maturity the ascospores are forcibly discharged or extruded in a yellowish spore mass.

The asci are hyaline, and clavate with eight ascospores. The asci are thin-walled and average 15 μ in diam at their widest point and 129 μ in length. Upon maturation the ascospores congregate at the distal end of the ascus (Fig. 2-E). The ascospores are hyaline, curved, fusoid with mostly rounded ends, one to three septate and average 5.0 μ in diam and 45.5 μ in length (Fig. 2-F).

Spore germination.–Optimum temperature for conidial and ascospore germination was between 24 to 28 C. The optimum pH for ascospore germination was pH 6.1, whereas conidia germinated well over a broad range of 2.5 to 8.2.

Pathogenicity.–Pathogenicity tests with *Calonectria* isolated from papaya, on four commercial lines of papaya showed different degrees of susceptibility to the pathogen (Table 1). Young papaya seedlings usually remained symptomless until sudden and permanent wilt of the seedlings. The collar area of the seedlings completely collapsed with the root system still intact and unaffected by the pathogen.

Three-month-old seedlings of Sunrise Solo were also susceptible to the pathogen. Symptoms on these older plants were latex ooze from the collar area, stunting, and chlorosis. The seedlings were killed 4 to 5 wk after inoculation with extensive rotting of the collar area and development of numerous perithecia. The upper lateral roots were also infected but the main taproot remained unaffected by the pathogen (Fig. 1-D).

Crotalaria incana L., *C. mucronata* Desv., *C. retusa* L., and *C. spectabilis* Roth seedlings were killed between 1 to 10 wk after inoculation with a severe rot of the collar region and to a lessor degree, of the roots. Abundant perithecia were formed around the collar region of those plants killed more than 6 wk after inoculation.

Sixteen 4-week-old peanut seedlings developed a few tan to black circular lesions 1 to 4 mm diam on the leaves. None of the 16 plants inoculated was killed 9 wk after inoculation, and except for slightly reduced growth looked normal above ground. However, after removing and washing the plants and peanut pods, a severe black rot with numerous lesions on roots, pods and in the cortical tissue of the collar area was evident.

Acacia koa Gray and *A. melanoxylon* R. Br. in the cotyledonous stage were extremely susceptible to the pathogen. All of the 40 *A. koa* seedlings were killed within 2 to 6 wk after inoculation. All of the 32 *A. melanoxylon* seedlings were killed within 1 to 6 weeks after inoculations. Of 20 *A. confusa* Merr. seedlings inoculated, seven were killed within 6 to 10 wk after inoculation with extensive rot of the roots and collar.

The foliage and young shoots were especially susceptible in all *Eucalyptus* species. Killing of the apical stems resulted in stimulation of axillary buds. The severely infected seedlings of all species wilted and died within a week after inoculation. The roots and collar area of slow-growing species were more susceptible to the pathogen than those of fast-growing species. *E. sideroxylon* (A. Cunn.) Benth. and *E. tereticornis* J.E.Sm. were most susceptible, the seedlings of which were killed 1 to 4 wk after inoculation. *E. saligna* J.E.Sm., *E. citriodora* Hook, and *E. punctata* DC. were the most resistant although a few seedlings were killed.

Pathogenicity of an isolate of *Calonectria crotalariae* (Sobers' culture) to Sunrise Solo papaya, *Acacia koa*, *A. melanoxylon*, *A. confusa*, and *Eucalyptus sideroxylon* was indistinguishable from that of a papaya isolate of *Calonectria* to the above hosts. *C. ilicicola* (also provided by Sobers) proved nonpathogenic when inoculated on Sunrise Solo papaya and *Acacia koa*. Negative results obtained are not conclusive due to the poor sporulating nature of the isolate.

DISCUSSION.–*Calonectria crotalariae* (ATCC-24024) obtained from papaya is indistinguishable pathogenically or morphologically from an isolate (ATCC-18904) obtained from peanut furnished by Sobers. Further, we observed that, frequently, the central stalk continues as a conidiophore, with stipe and terminal vesicle developing laterally from it. These laterally borne stipes may also arise from secondary and tertiary branches of the conidiophores. We also observed that the cylindrical conidia are slightly wider at the apex than at the base which differs from Bell & Sobers' (2) observation "slightly wider at the base than at the apex".

The hosts of *Calonectria crotalariae* are predominantly in the Leguminosae. Included are the genera *Acacia*, *Arachis*, *Crotalaria*, and *Tephrosia*. *Eucalyptus* in the Myrtaceae also included confirmed hosts of the pathogen (2). *Carica* is the only genus in

Fig. 2. A) Undisturbed conidia, massed in a palisade-like arrangement, borne on a conidiophore with stipe as the main axis. The vesicles are covered with water droplets. **B)** Wet mount of conidiophore, resulting in a loose or open appearance. Stipes arising as lateral branches from conidiophore. **C)** Typical globose vesicle of papaya *Calonectria*. **D)** Three-septate conidia and chlamydospores. Basal end of conidia (identified by attachment scar) is narrower than apex. **E)** Ascospores aggregating at distal end of asci. **F)** Curved, fusoid 1-3 septate ascospores with mostly rounded ends.

the Caricaceae that has been reported to be susceptible to any of the species of *Calonectria* or *Cylindrocladium.*

The occurrences of papaya collar rot have so far been reported only from the Puna district, which suggest that a limiting factor may be high rainfall. Pahoa, located in Puna, has a fairly well distributed rainfall of 144 inches a year (11). Most of the papaya growing areas outside of Puna are much drier and require irrigation which usually eliminates extremes in water availability. No papaya collar rot has been reported from the Island of Oahu where papaya is grown in areas with 60 to 70 inches of rain a year. However, infected *Acacia koa* seedlings were found in an area on Oahu where rainfall is about 135 inches a year.

Kapoho Solo is the commercial papaya variety selected and grown in the Puna area on the Island of Hawaii and is apparently adapted to wet climatic conditions as it is grown in areas with more than 100 inches of rainfall a year (12). Kapoho Solo, although relatively tolerant in greenhouse inoculations, is apparently vulnerable to the disease in Puna as all occurrences of *Calonectria* collar rot has been on this line of papaya. Observations of collar rot have not yet been made of the other three papaya lines under field conditions, although it is likely that they may be even more susceptible to *Calonectria* collar rot than Kapoho Solo if planted in Puna.

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