

Two Bacterial Diseases of Papaya Trees Caused by *Erwinia* Species in the Northern Mariana Islands

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ABSTRACT

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An *Erwinia* sp. was the cause of blackish, water-soaked, mushy cankers that occurred near or in leaf axils of upper portions of papaya stems in the northern Mariana Islands. This disease was prevalent on wild and commercial papaya trees after near-typhoon storms. The pathogen gained entrance through wounds caused by wind damage to the foliage. A different *Erwinia* sp. was the cause of a systemic blight of papaya resulting in rapid decline of commercial orchards. Symptoms of this disease were dark, angular, greasy, water-soaked lesions on the underside of the leaf causing chlorosis and necrosis of the foliage. Water-soaked lesions on petioles and upper portions of the stems were followed by systemic invasion and rot of the tree top and finally tree death in less than 6 wk. The African snail *Achatina fulica*, a vector of the decline *Erwinia* sp., spread it from diseased to healthy trees in the feeding process. The decline *Erwinia* sp. was recovered from the fresh excreta of snails collected from diseased trees. High resistance to the pathogen was observed in wild papaya trees of the northern Marianas. Commercial varieties tested showed high to low susceptibility. Kapoho Solo, one of the most important commercial lines in Hawaii, was highly susceptible. The two *Erwinia* pathogens do not correspond with any of the defined *Erwinia* species and subspecies. The diseases are referred to as the *Erwinia* mushy canker disease of papaya and the *Erwinia* decline disease of papaya.

Attempts to develop a papaya industry in the northern Mariana Islands to supply the increasing demands of the Japanese tourist trade have failed because of an unknown disease that causes premature death of bearing trees. These islands, a string of volcanic and coral outcroppings located between parallel 10 and the Tropic of Cancer from longitudes 144–146° east, are within the typhoon belt of the northern Pacific. Wind damage from tropical storms was thought to increase the disease. In 1976, personnel at the Office of Agriculture of the Trust Territory of the Pacific Islands (*personal communication*) suggested that a *Botryodiplodia* sp. was associated with the decline and indicated that the symptomatology of the disease was

similar to that of St. Croix decline of the Virgin Islands (1). The Mariana disease is characterized by dark, angular, greasy, water-soaked lesions on the diseased upper portions of the stem; the foliage becomes sparse, rigid, and generally chlorotic, followed by tree death.

In 1931, von Rant (12) described a disease of papaya in Java with symptoms similar to those of St. Croix papaya decline and attributed it to a bacterium, *Bacillus papaya*, subsequently placed in the genus *Erwinia* by Magrou (8). Symptoms of the Java decline began with chlorosis of the foliage with necrotic lesions, especially along midveins. This was followed by infection of petioles and stem and a rapid rot of the apical shoot, leading to death of the tree. Petiole and stem lesions initially appeared as irregular, water-soaked spots typical of those caused by leaf and stem blighting bacteria.

This paper reports the results of an investigation of the causal agent of northern Mariana decline of papaya and factors influencing the disease. We also describe another disease of papaya,

which at first was thought to be part of the decline syndrome, and its causal agent.

MATERIALS AND METHODS

Isolation and identification of the pathogen. Isolations were made from petioles, stem pieces, and water-soaked lesions from diseased papaya, *Carica papaya* L. cv. Kapoho Solo. Representative diseased samples were collected from Saipan, Rota, and Tinian islands. For fungal pathogens, tissue segments from the advancing margin of lesions were cultured on 2% water agar and examined microscopically for fungal growth after 48–72 hr of incubation. Bacterial isolations were made by removing small bits of tissue from the advancing margins of lesions below the epidermal layer. These tissues were placed into 10 ml of sterile distilled water (SDW), agitated for several minutes, and loopfuls of each suspension were then streaked onto Kelman's tetrazolium chloride medium (6), yeast dextrose calcium carbonate medium (3), King's medium B (7), and the Miller-Schroth *Erwinia* medium (MS) (9). Suspected pathogens were purified and stored in SDW at 10 C.

Identification was made according to standard determinative and various nutritional tests (2,5,10). Early in the study, two different sets of symptoms were noted on diseased papaya, and two distinct *Erwinia* sp. were isolated. Hereafter, the two sets of symptoms (as described under Results) and the associated *Erwinia* sp. are referred to as type MC (mushy canker) and type D (decline).

Pathogenicity tests. Injured and uninjured leaves of 6-wk-old Kapoho Solo papaya seedlings were inoculated in the field and greenhouse by misting with a hand atomizer that contained a bacterial suspension of approximately 10^7 colony-forming units (CFU) per milliliter of test

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isolates from MC or D groups. Wounds were inoculated by rubbing leaves with corundum or by pricking leaves with sterile needles. The apical shoots of some plants were pricked with needles laden with bacteria from 48-hr-old cultures. Inoculated plants were incubated in moist chambers for 24 hr at near 100% relative humidity (RH) and 28 C. Thereafter, plants were grown at ambient temperature and RH. Because the RH in the Marianas is about 100% between 1900 and 0700 hours, field inoculations were done after 1800 hours. Seventeen papaya cultivars provided by R. A. Hamilton, Department of Horticulture, University of Hawaii at Manoa, were inoculated with the D pathogen in this manner.

Host specificity. Four and six strains of MC and D, respectively, were inoculated into young chrysanthemum plants by pricking the growing shoots with a needle laden with bacteria. Potato slices, cultivar Netted Gem, were also inoculated by swabbing the slices with bacteria. Seedlings of papaya cultivar Sunrise Solo were inoculated with two strains each of *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* and with four strains of *E. chrysanthemi* pv. *chrysanthemi* by wounding as described previously. MC and D strains were tested for ability to produce the hypersensitive reaction using tobacco cultivar Glurk (10).

Vector studies. African snails (*Achotina fulica* Bowditch) collected from diseased trees were transferred to healthy, greenhouse-grown, 3-mo-old seedlings and healthy 1-yr-old grown trees and allowed to feed overnight. Excreta from snails collected from diseased trees were diluted 1:1,000 by weight in SDW, and loopfuls of the suspension were streaked on MS medium. Suspected pathogens were isolated and inoculated into 6-wk-old Kapoho Solo seedlings as previously described.

RESULTS

Symptoms and identification of the pathogen. Two distinct types of disease symptoms of papaya were recognized. Type MC-infected trees exhibited blackish, mushy cankers that developed in or near leaf axils in the fleshy, young portions of the stem, especially after severe wind damage by near-typhoon winds (Fig. 1). Type D-infected trees exhibited a general decline accompanied by chlorosis and necrosis of the foliage and dark, angular, greasy, water-soaked lesions on petioles and stem (Fig. 2A-F). Disease occurrence was not always associated with wind damage.

No fungal pathogens were isolated from type MC trees or from the more than 20 type D trees showing symptoms of decline. Bacteria, however, were consistently isolated, along with saprophytic fungi in the genera *Alternaria*, *Cladosporium*, or *Fusarium*. Microscopic

examination of freehand sections of tissues from the advancing margin of cankers and lesions also revealed abundant bacterial ooze and no fungal hyphae.

All bacteria isolated from diseased papaya trees—both type MC and type D—were identified as *Erwinia* species on the basis of physiologic and biochemical tests. Key tests for their identification and differentiation from related soft rot species and subspecies are listed in Table 1. Colonies were orange in color on MS medium, mucoid with pink centers on TZC, creamy white on King's medium B, and creamy brown on yeast dextrose calcium carbonate medium YDC. Colonies of strains MC were generally larger in diameter than colonies of strains D on all media. Cells of MC and D were single, straight rods, 0.5–1.0 × 1.0–3.0 μm, motile by peritrichous flagella, and Gram negative. They were facultative anaerobes, oxidase negative but positive for catalase production. Neither group rotted potato slices.

Pathogenicity tests. Strains D but not MC caused symptoms typical of decline when sprayed onto 6-wk-old seedlings (Fig. 2G). The lower surfaces of leaves of 10 of 10 Kapoho Solo seedlings exhibited water-soaked specks 48 hr after inoculation (Fig. 2G). Seventy-eight hours after inoculations, symptoms were typical of field infections. The lower surfaces of leaves contained many dark, angular, greasy, water-soaked spots. On the upper surfaces, they appeared as dark brown to black discolored blotches. Seven days after inoculation, the veins in the affected area turned black and the entire lamina became necrotic and collapsed at the point of attachment to the petiole, hanging at a 90° angle (Fig.

2C). These necrotic dry leaves, hanging from a declining tree, are a principal diagnostic symptom of the disease. Systemic symptoms developed, and water-soaked lesions became visible in the petiole and upper portion of the stem within 4–7 days of inoculation (Fig. 2B, E, F). Wilting and necrosis of the shoot occurred within 2 wk. Infection did not occur in 10 of 10 Kapoho Solo seedlings spray inoculated with MC strains (Fig. 2G).

MC strains, when inoculated by wounding the plant, caused discrete, slimy cankers within 72 hr followed by a blackish rot and an offensive rotting odor, typical of the disease in the field. The bacteria did not become systemic, and only the apical shoot was affected. Apical shoots that were wound inoculated with MC strains died within 72 hr, and the bacteria continued to invade the plant. Water-soaked lesions along the entire stem were visible in less than 5 days, and seedling collapse and rot occurred in 7 days.

Varietal susceptibility. Seventeen commercial papaya cultivars were inoculated with strain D either by misting foliage with a suspension of approximately 10⁷ CFU/ml or by wounding with a needle laden with bacteria. Although the cultivars appeared to vary in susceptibility when spray inoculated, all were highly susceptible when wound inoculated (Table 2). In the spray inoculation experiments, resistance occurred with some varieties because invasion of the laminae and petioles was followed by a general necrosis-rot of the petioles, causing them to dehiscence before the bacteria could systemically invade the stem. In very susceptible varieties, the leaves did not dehiscence; rather, they

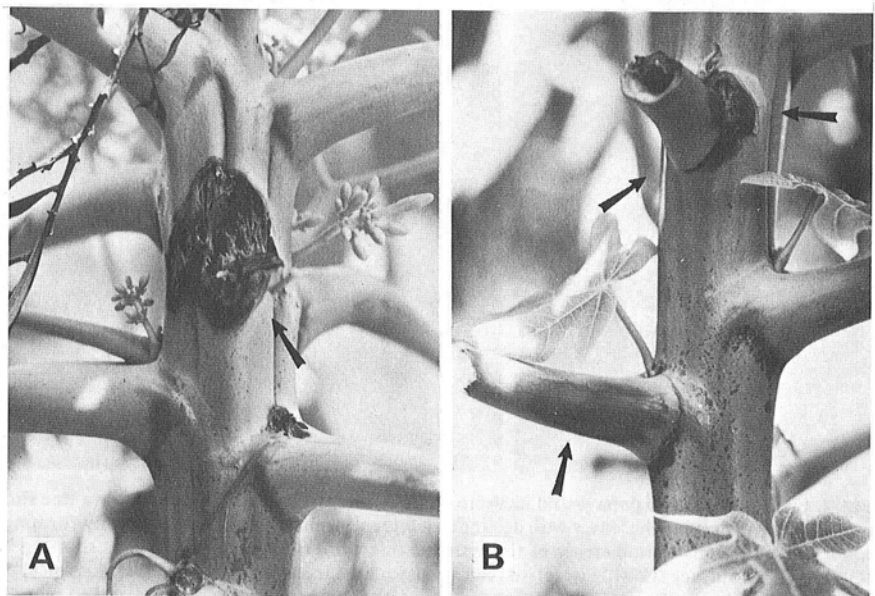


Fig. 1. Northern Marianas papaya stem canker caused by the mushy canker *Erwinia* (MC strains) after severe wind damage by a near-typhoon storm. (A) Canker in the fleshy apical portion of a wild male tree showing typical water-soaked, mushy rot. (B) Initial canker in the leaf axil of a storm-damaged petiole (above) and broken petiole showing dark, water-soaked rot (below).

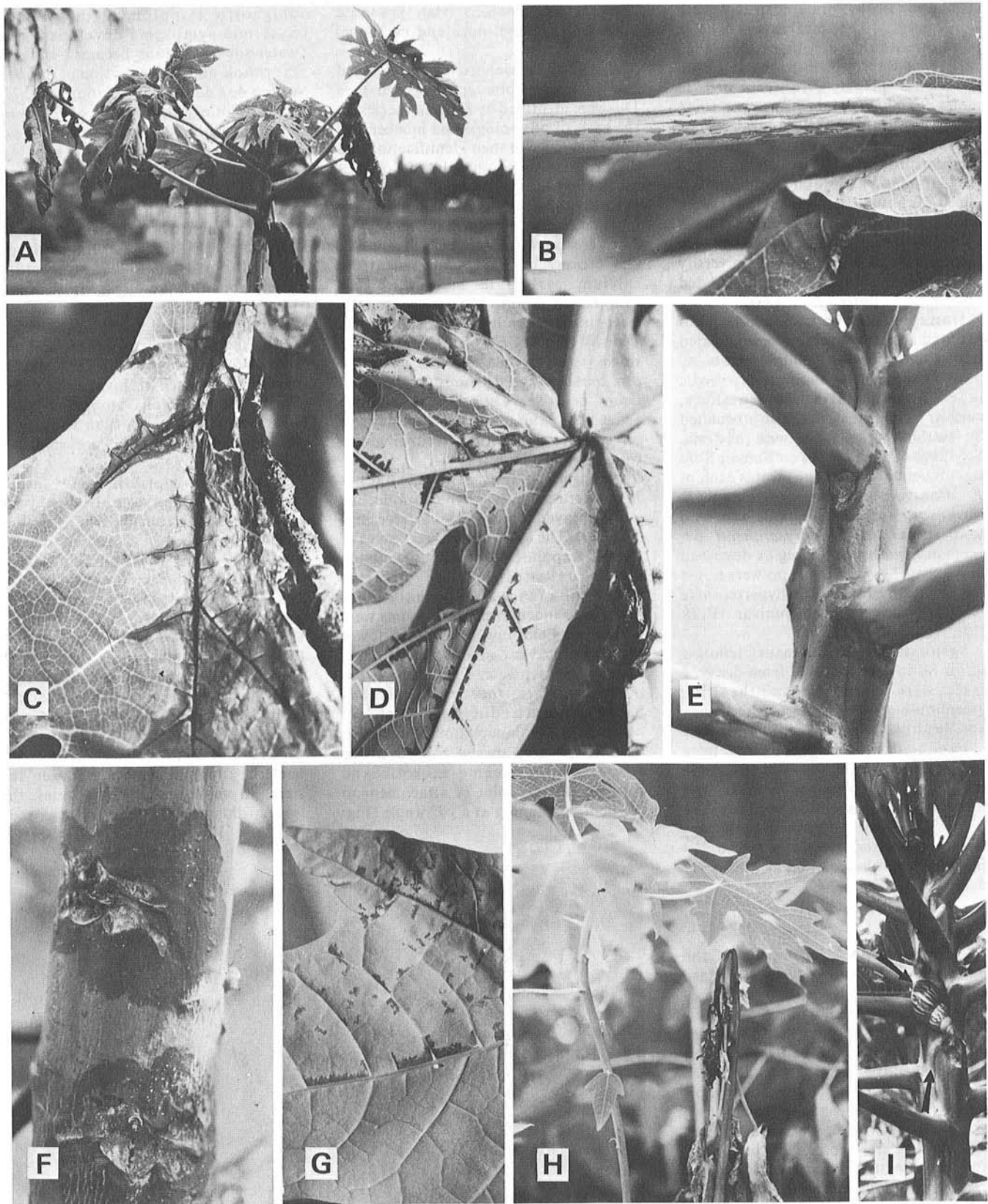


Fig. 2. *Erwinia* decline of papaya and its vector. (A) One-year-old Kapoho Solo papaya tree showing chlorosis and necrosis of the foliage 1 wk after inoculation by spraying the leaves with decline *Erwinia* sp. strain (D strain) at 10^7 colony-forming units (CFU) per milliliter. (B) Petiole showing dark, water-soaked lesions characteristic of the systemic infection. (C) Upper leaf surface showing blotchy irregular, dark lesions and blackened veins. (D) Lower leaf surface showing water-soaked, greasy, angular lesions close to veins. (E) Apical portion of the stem showing water-soaked lesions and initial stem cankers. (F) Advanced stages of canker development showing water-soaked margins. (G) Initial symptoms on 6-wk-old Kapoho Solo seedlings showing water-soaked lesions on the lower leaf surface 48 hr after inoculation with mushy canker *Erwinia* sp. (MC strain) at 10^7 CFU/ml. (H) Six-week-old Kapoho Solo seedlings 11 days after spraying foliage with MC (left) and D strains (right) at 10^7 CFU/ml. Note the severe necrosis and shoot dieback caused by the D strain and the healthy appearance of the seedling inoculated with the MC strain. (I) *Achetina fulica*, the vector of the D strain, feeding on wild papaya trees in the northern Marianas.

became necrotic and dry, and the bacterium entered the vascular system of the plant by invading through the petioles. The most resistant cultivars were Saipan Red, Dwarf Solo No. 7355, and Waimanalo Solo. Kapoho Solo, Taiping, and accession 7836 showed the greatest susceptibility (Table 2). The wild papaya

now indigenous to the Mariana Islands showed the highest resistance. Trees only succumbed to the disease when bacteria were inoculated by wounding the stem.

Specificity. D strains from papaya but not MC caused a wilting reaction on chrysanthemum shoots, followed by death in 4 days. None of the strains from

other *Erwinia* groups caused a systemic reaction similar to D strains when inoculated into papaya. However, two strains of *E. chrysanthemi* pv. *chrysanthemi* and one strain of *E. carotovora* subsp. *atroseptica* caused a systemic reaction 2 cm in length. All D strains but not MC strains caused a hypersensitive reaction in tobacco.

African snail vector. The African snail, introduced during the Japanese occupation of the Marianas, is a very serious threat to agriculture in the islands. Snails feed on all sorts of green vegetation, and it is not uncommon to find more than 20 snails on a papaya tree (Fig. 21). Strains of D were recovered from the excreta of 10 of 10 snails collected from diseased trees and plated on MS medium. Snails collected from diseased trees and transferred to healthy, mature Sunrise Solo trees and 3-mo-old, greenhouse-grown trees transmitted the disease to 4 of 4 and 5 of 5 trees, respectively, while control trees that were protected with a barrier of snail bait remained healthy. Symptoms in the field-grown trees appeared 1 wk after snail transfer, while in greenhouse trees, water-soaked lesions were observed on the petioles and stems 5 days after transfer.

Table 1. Comparison of biochemical, physiologic, and cultural characters of two *Erwinia* pathogens of papaya with related *Erwinia* spp.^a

Characteristic	MC		<i>E. carotovora</i>	<i>E. chrysanthemi</i>	<i>E. cypripedii</i>	<i>E. rhapontici</i>
	D		subsp. <i>carotovora</i>			
Growth at 36 C	+ ^b	+	v ^b	+	+	- ^b
Pectate degradation ^c	+	+	+	+	-	-
Sucrose reduction	-	+	v	v	-	+
Pink diffusible pigment	-	-	-	-	-	+
Acetoin	-	+	+	+	-	+
Gelatin liquefaction	-	-	+	+	-	-
Phenylalanine deamination	-	-	-	-	+	v
Indole	-	-	-	+	-	-
Gas from glucose	-	-	v	+	+	-
Casein hydrolysis	-	-	v	v	-	-
Blue pigment	-	+	-	+	-	-
Growth in 5% sodium chloride	+	-	+	-	+	+
Lecithinase	-	-	-	+	-	-
Phosphatase	-	+	-	+	+	+
Sensitivity to erythromycin (50 µg)	-	+	-	+	+	+
Acid production from						
cellobiose	+	-	+	+	+	+
citrate	+	-	+	+	+	+
dulcitol	-	+	-	-	-	+
galacturonate	+	+	v	v	+	-
DL-lactate	+	-	+	+	+	+
α-lactose	+	-	+	-	-	+
malonate	+	-	-	+	v	+
maltose	+	-	v	-	-	+
melezitose	-	-	-	-	-	+
proline	+	-	-	-	+	+
raffinose	+	-	+	v	-	+
rhamnose	+	-	+	+	+	+
salicin	+	-	+	+	+	+
xylitol	-	-	-	-	-	+
xylose	+	+	+	+	+	-

^a Six and 10 strains of MC and D, respectively, were used in the tests; data for the other *Erwinia* spp. were taken from Bergey's manual (2). MC and D strains are the cause of the mushy canker and decline diseases of papaya, respectively.

^b + = positive; - = negative; v = variable.

^c Strains MC and D were positive on Hildebrand's media A and C but not B (10).

Table 2. Susceptibility of different papaya cultivars to *Erwinia* strains D^a

Cultivar	No. of dead plants/no. inoculated	
	Spray inoculated	Wound inoculated
Sunrise × El Salvador	4/5	4/4
Bentong	2/4	4/4
Higgins Solo	4/5	4/4
Sunflower Leaf	3/5	4/4
7836	7/7	4/4
La Chola Roja	5/8	4/4
Wilder Solo	5/6	4/4
Taiping	5/5	4/4
Migvar Solo	5/6	4/4
Dwarf Solo No. 7355	1/7	4/4
Waimanalo Low Bearing Solo	1/3	4/4
Kapoho Solo	5/5	4/4
Izalco	3/4	4/4
El Salvador	7/8	4/4
Kaekdum	5/6	4/4
Sunrise Solo	4/4	4/4
Saipan Red	3/13	4/4

^a Infected leaves on surviving plants abscised before systemic infection reached the stem. Plants were either spray inoculated with 10⁷ colony-forming units per milliliter or wounded on apical shoots with a needle laden with the pathogen.

DISCUSSION

The northern Mariana decline of papaya is caused by an *Erwinia* sp., and it is probably the same disease described by von Rant in Java (12). The bacterium may also be the cause of the St. Croix decline, which has been attributed to *Corynespora cassicola* and a *Botryodiplodia* sp. The described symptoms and color photographs of the decline provided to us by Kenneth Hibbard after a trip to the Virgin Islands in 1978 support this speculation. Furthermore, the fungal pathogens associated with the St. Croix decline occur in the northern Marianas (11) and are not the cause of decline.

The physiologic and biochemical characteristics of the decline bacterium are dissimilar from any of the *Erwinia* groupings (2,4), although they somewhat resemble those of *E. chrysanthemi*. The *Erwinia* that caused water-soaked mushy cankers is also different from all the described *Erwinia* species and subgroups, including subspecies *carotovora*, which it somewhat resembles. Because neither bacterium has the characteristics of a defined *Erwinia* species, the pathovar system cannot be used unless the description of a species is amended to include the distinctive characteristics of these two bacteria. Furthermore, the pathovar system in our opinion has distinct limitations, especially when the host range is not clearly defined or when pathogenicity is equivocal. Many soft rot *Erwinia* have the potential to cause an assortment of reactions when plants are wound inoculated and grown in the

greenhouse with a high RH. Thus, the findings that some *Erwinia* strains produced a reaction in papaya seedlings in a greenhouse or that the papaya D isolates caused a reaction on chrysanthemums when wound inoculated should not be interpreted as evidence of their capacity to infect and cause disease in the field.

The two *Erwinia* pathogens from papaya will probably be given a species ranking upon further study. Until then, we refer to these two diseases as the *Erwinia* decline of papaya (D strains) and the *Erwinia* mushy canker disease of papaya (MC strains).

The decline disease can be somewhat controlled by use of a snail bait against *A. fulica*. However, the best possibility for an economic control may come from the development of more resistant varieties. The primitive conditions where the study was made did not enable an extensive study of susceptibility with large sampling numbers, but the cultivars appeared to vary in susceptibility. A

breeding program should thus receive serious consideration.

Added in galley: During a subsequent trip to the Virgin Islands by M. N. Schroth, a bacterium was isolated from declining papayas that caused a rapid decline of seedlings similar to the Mariana decline. However, the bacterium was not the same as the one that caused Mariana decline. It remains unidentified.

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