# Internal Yellowing, a Bacterial Disease of Papaya Fruits Caused by *Enterobacter cloacae*

KATE A. NISHIJIMA and H. MELVIN COUEY, USDA, ARS, Tropical Fruit and Vegetable Research Laboratory, Hilo, HI 96720, and ANNE M. ALVAREZ, University of Hawaii, Honolulu 96822

**ABSTRACT**

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A disease of papaya fruits was observed in Hawaii in fully ripe fruits that was characterized by soft, yellow, discolored flesh with diffuse margins and an offensive odor. The causal bacterium was isolated repeatedly from diseased fruit, hot-water treatment tanks, papaya flowers, and the crop and stomach of the oriental fruit fly (*Dacus dorsalis*). On the basis of biochemical and physiological characteristics, the bacterium was identified as *Enterobacter cloacae*. Fruits became increasingly susceptible to *E. cloacae* as they ripened. Hot-water-treated fruits had lower disease incidence than untreated fruits.

In 1984, a new quarantine system was developed to disinfest Hawaii-grown papayas (*Carica papaya* L. 'Kapoho Solo') of fruit flies (4). In this system, fruits were selected on the basis of fruit color to minimize fruit fly infestation and treated with hot water in two stages, 42 C for 30 min and 49 C for 20 min. To monitor the effects of the system on fruit quality and fruit fly survival, we regularly sampled commercial fruit and ripened and dissected them in the laboratory. Diseased tissue was observed frequently in the area surrounding the seed cavity. Symptoms included yellow, discolored flesh with diffuse margins and an offensive odor. No external symptoms were observed. The symptoms were similar to the later stages of the purple-stain disease of papaya caused by *Erwinia herbicola* (16). In the latter disease, internal vascular and parenchyma tissue are purple. The stain eventually fades, and flesh in the discolored area becomes soft and yellow and has an abnormal odor and flavor. In the present internal yellowing disease, however, purple stain was not observed in naturally infected fruit. The bacterium that was isolated did not produce purple stain in culture or in inoculated fruit. This paper reports a study of the causal organism of the internal yellowing disease of papaya.

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## MATERIALS AND METHODS

**Culture methods.** Initial isolations were streaked or plated on nutrient agar (NA), tetrazolium chloride agar (TZC) (10), Miller-Schroth agar (MS) (15), or peptone-yeast extract agar (PY: 10 g peptone, 5 g yeast extract, 5 g NaCl, 15 g agar, and 1 L distilled water) (5). At least three successive single-colony isolations were made to ensure purity. Cultures

were incubated at 30 C and maintained on yeast extract-dextrose-calcium carbonate agar (YDC) (24) or King's medium B (KMB) (11) and stored at room temperature in sterile distilled water (SDW) in tubes.

**Isolation methods.** Vascular or parenchyma tissues from papaya fruits that showed purple or yellow discoloration were aseptically excised, dipped in 0.5% sodium hypochlorite, drained on a clean paper towel, and rinsed in SDW. Tissues were crushed in about 5 ml of SDW with sterile mortar and pestle and streaked on agar media.

**Sources of inoculum.** Isolations were made from water tanks used in processing papayas, washes of papaya fruits, leaves, and flowers, and the crop and stomach of the oriental fruit fly (*Dacus dorsalis* Hendel), one of the major insect pests of papaya (22).

Water samples were collected from the hot-water tanks at four papaya packinghouses and from the cold-water culling tank at one packinghouse on the island of Hawaii. Samples were filtered through a 3-$\mu$m pore size polycarbonate membrane (Nucleopore Corp., Pleasanton, CA), collected in sterile test tubes, diluted to several dilution concentrations, and plated on YDC and MS media.

Plant parts for washes were collected from a papaya orchard in Keaau, HI, and washed separately in sterile beakers (2 L) with 50–100 ml of SDW. The liquid was filtered, then streaked on PY, TZC, and MS media.

Specimens of oriental fruit fly (*D. dorsalis*) were collected from papaya fields in Keaau, HI, and dissected under aseptic conditions. The crop and stomach of each fly were excised, surface-sterilized separately in 0.25% sodium hypochlorite, rinsed in SDW, and dissected into a drop of SDW. Loopfuls of this suspension were streaked on PY.

**Identification tests.** Based on initial tests (20,21), preliminary results on API 20E strips (Analytab Products, Plainview, NY), and descriptive information in *Bergey's Manual of Systemic Bacteriology* (12,17), we concluded that the bacteria associated with internal yellowing disease were members of the Enterobacteriaceae but were not in the genus *Erwinia*. To further identify the pathogen, eight strains, PV-5, YP-16, WT-2, WT-3, Dd-18, Dd-23, Wa-10, and Wa-23, selected from various sources, were compared with purple-stain strains PP-1 and PP-2 and *Erwinia herbicola* strain Eh-1, from A. M. Alvarez, and the type strain of *Enterobacter cloacae* (Jordan) Hormaeche & Edwards, ATCC 13047. The phenylalanine deaminase test was done according to the methods of Ewing et al (6). Acid production from cellobiose, lactose, raffinose, and $\alpha$-methyl-D-glucoside tests were done according to methods of Schroth and Hildebrand (21) and pectate degradation according to Hildebrand (9). The API Analytical Profile Index and API 20E strips were used according to manufacturer's instructions. Strips were incubated at 30 C for 18–24 hr. All tests were done at least twice. Cell measurements were made under a light microscope from Gram-stained cells, and flagella were stained with uranyl acetate and observed with an electron microscope (Hitachi HS-8-1).

**Pathogenicity tests.** Healthy fruits, about one-fourth to one-half ripe (based on external yellow color), were selected from field collection bins at several packinghouses and were inoculated with bacterial suspensions or SDW (for control). Bacterial strains were grown on YDC or PY at 30 C for 24–48 hr, and a bacterial inoculum of about $10^9$ colony-forming units (cfu) per milliliter was prepared for each strain. Fruits were wiped with 70% ethyl alcohol before inoculation. Each fruit was injected with about 0.5 ml of the bacterial inoculum or SDW at separate inoculation sites, about 5 cm apart, with a sterile tuberculin syringe (3 cc) fitted with a 25-gauge needle. Inoculation sites were covered with heat-resistant tape. Fruits were incubated in papaya cartons at room temperature until ripe (5–7 days), then cut and checked for disease. Several strains that produced internal yellowing symptoms were reisolated, purified, streaked on TZC and MS plates, and inoculated on API 20E strips.

**Comparison of bacterial strains from various sources.** Fourteen strains and SDW were inoculated in an incomplete block design (3) among 35 fruits. Each fruit was inoculated at three sites at the blossom end with bacterial suspensions of about $10^7$ cfu/ml or SDW.

**Effect of inoculum concentration on disease.** Two strains, PV-5 and YP-16, were inoculated into papaya fruits at the blossom end with inoculum concentrations ranging from $10^1$ to $10^9$ cfu/ml. Bacterial suspensions were made up in SDW and diluted, then bacterial populations were determined by plating out the two highest dilutions ($10^{-7}$ and $10^{-8}$). There were two sets of 10 fruits for each inoculum concentration. One set was inoculated at separate sites with PV-5 and SDW, and the other set was inoculated with YP-16 and SDW.

**Effect of fruit ripeness on susceptibility to disease.** In one experiment, eight fruits at each of three stages of ripeness (colorbreak, one-fourth ripe, and one-half ripe) were selected for color uniformity. In a second experiment, 40–50 colorbreak fruits were selected similarly. Fruits in both experiments were washed, dried, and analyzed individually for yellow color at the blossom end with a Hunter colorimeter (Hunter Associates Lab., Inc., Reston, VA) (8). Fruits in the first experiment were inoculated near the middle at two sites with strain PV-5 and at a third site with SDW. Fruits were incubated at room temperature until ripe (5–7 days), then examined for disease. In the second experiment, the blossom end of each fruit was inoculated with strain PV-5 (at $10^7$ cfu/ml) at one site and with SDW at a second site. Ten fruits at various stages of ripeness were read individually on the Hunter colorimeter, cut, and examined for internal yellowing disease at 1- to 3-day intervals. Inoculation and pathogenicity tests were done at least four times.

**Surveys.** From December 1984 to April 1986, 100-fruit samples of heat-treated papayas were obtained from each of five packinghouses in the Hilo area at about 2-wk intervals. From October 1985 until January 1986, five surveys included both hot-water-treated and untreated papayas. Untreated papayas were collected at the packinghouses within 24 hr of harvest and were not processed through cold-water culling tanks. All fruits were held at room temperature for

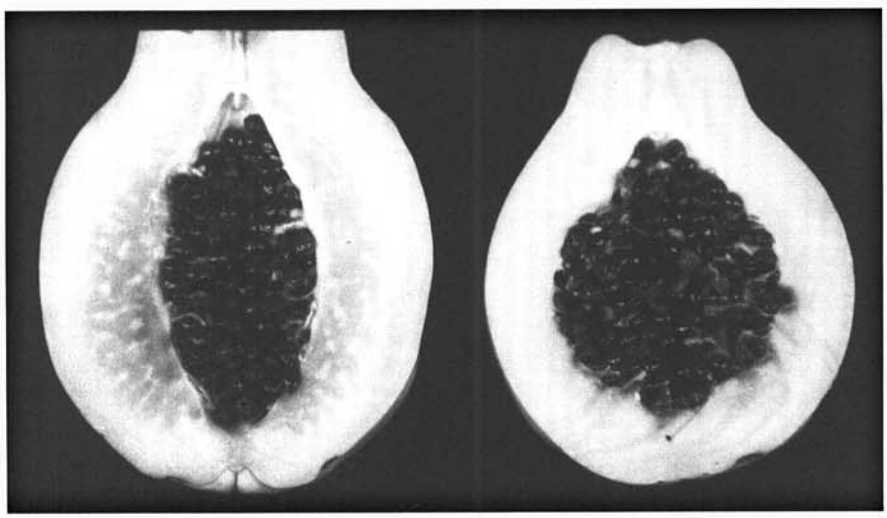

**Fig. 1.** Internal yellowing of papaya caused by *Enterobacter cloacae*. Note translucent appearance of the naturally diseased fruit (left) compared with the healthy fruit (right).

a week, until they were fully ripe, then cut and checked for internal yellowing disease. Isolations were routinely made from diseased fruits.

Water in the hot-water treatment tanks at four papaya packinghouses and the cold-water culling tank at one packinghouse was sampled and analyzed for enteric bacteria three or four times from early January 1985 to the end of March 1985. Water samples (200 ml) were collected in sterile collecting jars before and during papaya processing and were plated on YDC and MS media. Only strains positive (orange) on MS were considered potential members of the Enterobacteriaceae.

Sanitation practices of the water tanks varied for each of the four packinghouses at the time of the surveys. At packinghouse E, calcium hypochlorite (Pittclor, PPG Industries, Inc., Pittsburgh, PA) was added sporadically to the hot-water tanks and chlorine concentration was not monitored. At packinghouse A, calcium hypochlorite was added every 2 hr to the cold-water culling tank and chlorine concentration was maintained at 5–80 ppm throughout papaya processing.

Data were analyzed by analysis of variance and Duncan's multiple range test or by calculation of the G-statistic according to the method of Sokal and Rohlf (23).

## RESULTS

**Symptoms.** Internal yellowing disease was characterized by yellow, discolored tissue with diffuse margins around the seed cavity of infected fruit (Fig. 1). Most infections were present around the blossom end and the middle of the fruit and often included a portion of the seed cavity. A distinctly rotten odor was consistently present. No external symptoms were observed in naturally or artificially infected fruit.

**Isolation.** One type of bacterium was isolated from 12 of 17 papaya fruits with internal yellowing symptoms. Three of six bacterial strains isolated from hot-water treatment tanks, two of 23 strains isolated from five *D. dorsalis* specimens, and three of 22 strains isolated from washes of papaya flowers were the same bacterium. This bacterium was not among 23 strains isolated from washes of papaya fruits and leaves. A different bacterium, tentatively identified as *Pseudomonas aeruginosa*, was one of 17 strains isolated from papaya and one of six strains from hot-water tanks. No fungi were recovered from papaya fruits infected with internal yellowing.

The bacterium that causes purple-stain disease was isolated once from washes of one-half-ripe papaya fruit but was not recovered from water tanks used in papaya processing or from *D. dorsalis* specimens.

**Identification tests.** Eight strains isolated from various sources had the following characters in common: Colonies were mucoid and creamy white on KMB, creamy tan on YDC, orange on MS, and dark pink with translucent margins on TZC. Cells were single, straight rods, 0.3–0.6 × 0.8–2.0 μm, with peritrichous flagella. All eight strains were gram-negative, oxidase-negative, catalase-positive, and were facultative anaerobes.

Biochemical and physiological test results for the eight bacterial strains were consistent with results for the reference strain of *Enterobacter cloacae* (ATCC 13047), except that the two strains isolated from *D. dorsalis* (Dd-18 and Dd-23) were negative for ornithine decarboxylase (Table 1). The eight strains differed from the purple-stain strains and *Erwinia herbicola* Eh-1 in several key biochemical tests: arginine dihydrolase, ornithine decarboxylase, citrate utilization, phenylalanine deaminase, and acid production from several carbohydrates. The results for the purple-stain strains were consistent with results for the *E. herbicola* strain, Eh-1, except that Eh-1 produced acid from sorbitol and failed to produce purple stain on YDC.

**Pathogenicity tests.** Twelve strains of *E. cloacae*, isolated from papaya fruits with internal yellowing symptoms, caused the same discolored flesh and offensive odor as in naturally infected fruits. Lesions surrounding the area of inoculation varied in size and spread from localized "pockets" of discolored flesh (about 1.5 × 1.5 cm) to soft, spreading discolored flesh (about 3–5 cm in diameter). Symptom expression varied with fruit ripeness at the time of examination, and the second type of lesion was more common in riper fruits.

**Table 1.** Comparison of physiological and biochemical characteristics of internal yellowing strains with type culture of *Enterobacter cloacae* (ATCC 13047), purple-stain strains PP-1 and PP-2, and *Erwinia herbicola* Eh-1

| Characteristic | Internal yellowing strains[a] | *E. cloacae* | Purple-stain strains | *E. herbicola* |
|---|---|---|---|---|
| API 20E tests | | | | |
| β-Galactosidase | +[b] | + | + | + |
| Arginine dihydrolase | + | + | − | − |
| Lysine decarboxylase | − | − | − | − |
| Ornithine decarboxylase | v[c] | + | − | − |
| Citrate | + | + | − | − |
| Hydrogen sulfide | − | − | − | − |
| Urease | − | − | − | − |
| Tryptophan deaminase | − | − | − | − |
| Indole | − | − | − | − |
| Voges-Proskauer | + | + | + | + |
| Gelatin liquefaction | − | − | − | − |
| Acid from | | | | |
| Glucose | + | + | + | + |
| Mannitol | + | + | + | + |
| Inositol | − | − | − | − |
| Sorbitol | + | + | − | + |
| Rhamnose | + | + | + | + |
| Sucrose | + | + | + | + |
| Melibiose | + | + | − | − |
| Amygdalin | + | + | + | + |
| Arabinose | + | + | + | + |
| Oxidase | − | − | − | − |
| Nitrate reduction | + | + | + | + |
| Catalase | + | + | + | + |
| Other tests | | | | |
| Phenylalanine deaminase[d] | − | − | + | + |
| Acid from[e] | | | | |
| Cellobiose | + | + | +[f] | +[f] |
| Lactose | + | + | − | − |
| Raffinose | + | + | − | − |
| α-methyl-D-glucoside | + | + | − | − |
| Pectate degradation[g] | − | − | − | − |
| Purple stain on YDC[h] | − | − | + | − |
| Yellow pigment on KMB[i] | − | − | + | + |

[a] Eight strains, isolated from various sources, were used in all tests: PV-5, YP-16, WT-2, WT-3, Dd-18, Dd-23, Wa-10, and Wa-23.
[b] + = Positive, − = negative, and v = variable.
[c] Two strains, Dd-18 and Dd-23, were negative; the other strains were positive.
[d] By the method of Ewing et al (6); incubated at 30 C, read after 3 days.
[e] By the method of Schroth and Hildebrand (21); incubated at 30 C, read after 2, 4, 7, 14, 21 days.
[f] Positive 7–14 days after inoculation.
[g] By the method of Hildebrand (9); incubated at 30 C.
[h] Yeast extract, dextrose, calcium carbonate agar (24).
[i] King's medium B (11).

Of five strains that were not *E. cloacae*, only one caused symptoms similar to internal yellowing disease. When five papaya strains were compared with nine strains from other sources (hot-water tanks, papaya flowers, and *D. dorsalis*), typical internal yellowing symptoms were produced by 11 *E. cloacae* strains including the type strain (ATCC 13047) (Table 2). The two strains of *P. aeruginosa* elicited symptoms that were distinctly different from those produced by *E. cloacae*. One *E. cloacae* strain, WT-5, caused only slight internal yellowing symptoms.

Colony morphology on TZC and MS media and biochemical characteristics of several reisolated strains were identical to that of the original strains. Two strains (Dd-18 and Dd-23), isolated originally from oriental fruit fly, remained negative for ornithine decarboxylase.

**Effect of inoculum concentration on disease.** Incidence of internal yellowing increased with increasing inoculum concentrations of strains PV-5 and YP-16, and no significant difference was found between the two strains (Fig. 2). Natural infection or lesion spread from an adjacent inoculation site, produced disease symptoms in 4% of sites inoculated with SDW.

**Effect of fruit ripeness on susceptibility to disease.** When internal yellowing strain PV-5 was inoculated into papaya at three stages of ripeness, disease incidence at the time of grading (fruits fully ripe) was highest in fruits that were ripest (one-half ripe) when inoculated (Table 3). When colorbreak fruits (average Hunter b reading of 16.0) were inoculated with PV-5 and examined for internal yellowing disease at different stages of ripeness, fully ripe fruits had the highest percentage of infection (38%) (Table 4).

**Surveys.** Internal yellowing disease was observed in fruits sampled from each

**Table 2.** Internal yellowing symptoms in papaya fruits inoculated[a] with bacterial strains from various sources

| Source of strain | Species identification[b] | Symptom expression | Inoculation sites infected (%) |
|---|---|---|---|
| Fruits with internal yellowing | | | |
| PV-2 | *P. aeruginosa* | Atypical[c] | 17.1 |
| PV-5 | *E. cloacae* | Typical | 71.4 |
| PV-6 | *E. cloacae* | Typical | 54.3 |
| PV-7 | *E. cloacae* | Typical | 65.7 |
| YP-16 | *E. cloacae* | Typical | 80.0 |
| Hot-water tanks | | | |
| WT-2 | *E. cloacae* | Typical | 77.1 |
| WT-3 | *E. cloacae* | Typical | 80.0 |
| WT-4 | *P. aeruginosa* | Atypical | 11.4 |
| WT-5 | *E. cloacae* | Slight | 48.6 |
| Papaya flowers | | | |
| Wa-10 | *E. cloacae* | Typical | 85.7 |
| Wa-23 | *E. cloacae* | Typical | 68.6 |
| *Dacus dorsalis*, crop and stomach | | | |
| Dd-18 | *E. cloacae* | Typical | 68.6 |
| Dd-23 | *E. cloacae* | Typical | 48.7 |
| Type culture | | | |
| ATCC-13047 | *E. cloacae* | Typical | 71.4 |
| Control[d] | | Slight | 5.7 |

| Source | df | MS |
|---|---|---|
| Replicates | 4 | 6.153*[e] |
| Strains | 14 | 16.711** |
| Among *E. cloacae* strains | 11 | 3.958* |
| Among sources of *E. cloacae* | 4 | 2.545 |
| Within sources of *E. cloacae* | 7 | 4.764* |
| *E. cloacae* vs. others | 1 | 118.813** |
| Among others | 2 | 1.600 |
| Error | 56 | 1.275 |

[a] Fourteen strains and sterile distilled water (SDW) were inoculated separately into three sites per fruit in an incomplete block design (3) among 35 fruits. The experiment was repeated four times.
[b] Strains were identified as *Pseudomonas aeruginosa* or *Enterobacter cloacae* based on results of bacteriological tests on API 20E strips.
[c] *P. aeruginosa* produced symptoms that were distinctly different from those produced by *E. cloacae*.
[d] Control consisted of SDW.
[e] * = Significant at $P < 0.05$ and ** = significant at $P < 0.01$.

**Table 3.** Number of papaya fruits developing disease at either zero, one, or two sites as a result of inoculation[a] of fruits at three stages of ripeness

| Visual ripeness | Av. Hunter b reading at blossom end | No. positive infections/fruit | | |
|---|---|---|---|---|
| | | Zero sites | One site | Two sites |
| Colorbreak | 16.5 | 21 | 8 | 3 |
| One-fourth ripe | 23.0 | 14 | 8 | 10 |
| One-half ripe | 25.9 | 5 | 10 | 17 |
| Total | | 40 | 26 | 30 |
| Calculated from binomial | | 29.3 | 47.4 | 19.3 |

| Source | df | G |
|---|---|---|
| Among ripeness | 2 | 21.75**[b] |
| Goodness of fit to binomial | 2 | 20.29*** |

[a] Each fruit was inoculated at two sites with strain PV-5 (at $10^9$ cfu/ml), identified as *Enterobacter cloacae*, and at one site with sterile distilled water (SDW). Fruits were incubated at room temperature for 5–7 days, then examined for disease. Fewer than 2% of the sites inoculated with SDW produced internal yellowing.
[b] ** = Significant at $P < 0.01$ and *** = significant at $P < 0.005$.

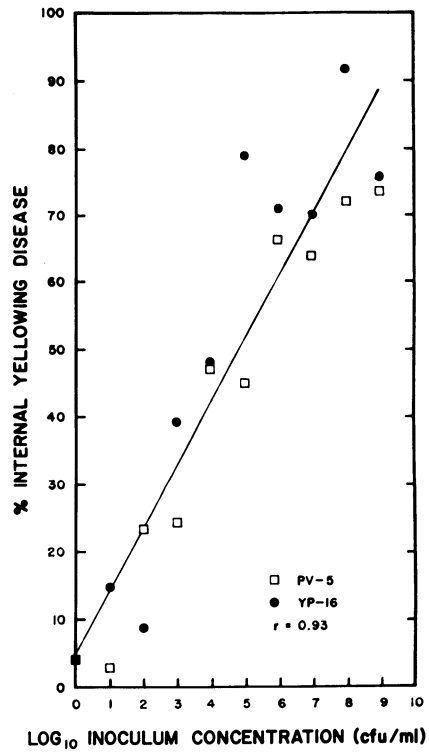


**Fig. 2.** Internal yellowing disease in papaya fruits inoculated with varying concentrations of *Enterobacter cloacae* strains PV-5 and YP-16. Two sets of 10 fruits per inoculum concentration were inoculated: one set with PV-5 and SDW, the other with YP-16 and SDW. Fruits were incubated for 5–7 days until ripe, then cut and examined for disease. Data were based on the average of four tests.

of the five packinghouses surveyed, but the incidence was slightly higher among fruits from packinghouse A (Table 5). Less internal yellowing disease was observed in hot-water-treated fruits than in untreated fruits from four of the five packinghouses (Table 6). Overall, for the period October 1985 to January 1986, the incidence of internal yellowing among hot-water-treated and untreated fruits was 3.8 and 15.6%, respectively. Among the five packinghouses, fruits from A had the highest incidence of internal yellowing, 8.6 and 43.4% for those hot-water-treated and untreated, respectively.

Infrequent recovery of enteric bacteria from the culling tank at packinghouse A was probably due to the regular addition of calcium hypochlorite to the unheated water. One of four samplings before papaya processing contained enteric bacteria, and no enteric bacteria were recovered in four samplings after processing. In contrast, enteric bacteria were recovered readily from the hot-water treatment tanks. Before papaya processing, enteric bacteria were recovered from 11 of 12 samples and from 5 of 13 samples from the 42 C tank and 49 C tank, respectively. Once fruit processing was under way, enteric bacteria were recovered from all water samples from both hot-water tanks at all packinghouses, including packinghouse E, where calcium hypochlorite was added sporadically to the hot-water tanks. Disease incidence among fruit from packinghouse E was not significantly lower than that among fruit from the other packinghouses (Table 6).

## DISCUSSION

The bacterium isolated from diseased papaya fruits and demonstrated to cause the internal yellowing disease was identified as *Enterobacter cloacae* on the basis of biochemical and physiological tests and by comparison with the type strain of *E. cloacae*, ATCC 13047. This pathogen differed from two strains (PP-1 and PP-2) of *Erwinia herbicola* isolated from fruits with the purple-stain disease. Strains of *E. cloacae*, but not *E. herbicola*, were recovered from hot-water treatment tanks, papaya flowers, and the oriental fruit fly. The internal yellowing disease was found to be more prevalent and widespread than the purple-stain disease. Purple-stain disease, however, was observed occasionally during surveys, and strains isolated from diseased fruit conformed to physiological and biochemical characteristics of *E. herbicola*, confirming a previous report (16).

There are five other reports of bacterial diseases of papaya caused by *Erwinia* spp. (7,13,25–27). *Bacillus papaya* was reported to cause a disease of papaya in Java (26), but Magrou (14) placed this bacterium in the genus *Erwinia*. Leu et al (13) reported that *Erwinia cypripedii* caused a black rot of papaya trees that also affected fruit in Taiwan. Trujillo and Schroth (25) reported two bacterial diseases of papaya trees caused by *Erwinia* spp. Webb (27) reported that an *Erwinia* sp. caused a bacterial canker of papaya trees in St. Croix, U.S. Virgin Islands, whereas Frossard et al (7) reported that an *Erwinia* sp., probably in the group *E. amylovora*, caused a decline of papaya trees in the French Antilles.

The isolation of plant-pathogenic strains of *E. cloacae* is rare but not unique. *Erwinia dissolvens* (originally *Pseudomonas dissolvens*) and *Erwinia nimipressuralis*, both placed in *E. cloacae* (17), were reported to cause stalk rot of corn (19) and wetwood of elm trees (2), respectively.

The susceptibility of papaya fruits to internal yellowing increased as the fruits ripened. When fruits at different stages of ripeness were artificially inoculated with strain PV-5, the ripest fruits inoculated were most susceptible to disease (Table 3). Similarly, when fruits were inoculated at colorbreak stage and checked for internal yellowing at different stages of ripeness, disease incidence increased with fruit ripeness (Table 4). We used the dual inoculation technique in the first experiment to demonstrate that the fruit as a whole shifts from a resistant to a susceptible state as it ripens. If all fruits were equally susceptible to internal yellowing, lesions would be distributed randomly among the inoculation sites and a binomial distribution of fruits into the three infection classes would be expected. However, the proportion of fruits with either no disease or diseases at both inoculation sites is much greater than predicted by the binomial distribution (Table 3).

The incidence of internal yellowing was lower among hot-water-treated

**Table 4.** Effect of ripeness on incidence of internal yellowing disease in papayas inoculated[a] at colorbreak stage

| Visual ripeness when examined | Av. Hunter b reading at blossom end[b] | No. fruits | Disease[c] (%) |
|---|---|---|---|
| Colorbreak | 14.8 | 7 | 0 |
| One-fourth ripe | 21.2 | 51 | 0 |
| One-half ripe | 27.8 | 57 | 1.8 |
| Three-fourths ripe | 30.8 | 43 | 16.3 |
| Full ripe | 32.3 | 110 | 38.2 |

[a] Forty to 50 colorbreak fruits were inoculated with *Enterobacter cloacae* strain PV-5 (at $10^7$ cfu/ml) at one site and with sterile distilled water (SDW) at a second site. After inoculation, fruits were cut and examined for internal yellowing disease at 1- to 3-day intervals. The experiment was repeated five times.
[b] Readings at time of examination for disease. Average of initial readings for all fruits was 16.0.
[c] Only sites inoculated with PV-5 resulted in disease. Sites inoculated with SDW had no disease.

**Table 5.** Incidence of internal yellowing disease in hot-water-treated papayas[a]

| Packinghouse | Disease incidence classes[b] (no.)[c] | | | | Overall disease incidence (%) |
|---|---|---|---|---|---|
| | 0 | 1–10% | 11–20% | >21% | |
| A | 11 | 11 | 4 | 8 | 9.7 |
| B | 16 | 16 | 2 | 0 | 2.5 |
| C | 15 | 15 | 3 | 1 | 3.7 |
| D | 15 | 14 | 4 | 1 | 3.7 |
| E | 15 | 15 | 2 | 2 | 4.9 |

[a] Fruits were treated at 42 C for 30 min, then at 49 C for 20 min.
[b] Classes are based on percentages of fruits showing symptoms of internal yellowing.
[c] Values based on 34 samplings from December 1984 to April 1986 of 100-fruit samples from each of five packinghouses in the Hilo area. Fruits were ripened for 8 days after treatment, then examined for disease.

**Table 6.** Incidence of internal yellowing disease in hot-water-treated[x] and untreated papayas sampled[y] from five packinghouses

| Packinghouse | Heat-treated (%) | Non-heat-treated (%) |
|---|---|---|
| A | 8.6 bcd[z] | 43.4 a |
| B | 3.3 de | 17.7 ab |
| C | 3.5 de | 3.9 cde |
| D | 1.6 e | 12.0 bc |
| E | 4.6 cde | 26.1 ab |
| Av. | 3.8 f | 15.6 g |

[x] Fruits were treated at 42 C for 30 min, then at 49 C for 20 min.
[y] One hundred treated and 100 untreated fruits were samped from each packinghouse in each of five samplings from October 1985 to January 1986. Fruits were ripened (7–8 days) at room temperature, then examined for disease.
[z] Geometric means within a group not followed by the same letter are significantly different ($P = 0.05$) by analysis of variance and Duncan's multiple range test.

fruits than among untreated fruits. Hot-water treatment of papaya has been used to control postharvest fungal diseases of papaya (1), and its apparent activity against a bacterial disease of papaya enhances the usefulness of this method of control.

Based on our observation of internal yellowing symptoms occurring primarily at the blossom end and middle of the fruit, and the recovery of *E. cloacae* from papaya flowers, we suggest that the flower may have been the site of infection. The pink disease of pineapple (18) is another bacterial disease in which the flower is the site of entry for the pathogen. Because *E. cloacae* also was recovered from fruit flies, we suspect that fruit flies, and possibly other insects, may aid in the dispersal of the pathogen. If the pathogen enters the fruit through the flowers, it remains dormant during flowering to fruit development. Physiological changes that accompany fruit ripening then permit full development of the bacterial disease.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**

1. Akamine, E. K., and Arisumi, T. 1953. Control of postharvest storage decay of fruits of papaya (*Carica papaya* L.) with special reference to the effect of hot water. Proc. Am. Soc. Hortic. Sci. 61:270-274.
2. Carter, J. C. 1945. Wetwood of elms. Ill. Nat. Hist. Surv. Bull. 23:407-448.
3. Cochran, W. G., and Cox, G. M. 1957. Experimental Designs. 2nd ed. John Wiley & Sons, New York. 611 pp.
4. Couey, H. M., and Hayes, C. F. 1986. Quarantine procedure for Hawaiian papaya using fruit selection and a two-stage hot-water immersion. J. Econ. Entomol. 79:1307-1314.
5. Drew, R. A. I., Courtice, A. C., and Teakle, D. S. 1983. Bacteria as a natural source of food for adult fruit flies (Diptera: Tephritidae). Oecolgoia (Berlin) 60:279-284.
6. Ewing, W. H., Davis, B. R., and Reavis, R. W. 1957. Phenylalanine and malonate media and their use in enteric bacteriology. Public Health Lab. 15:153-167.
7. Frossard, P., Hugon, R., and Verniere, Ch. 1985. Un dépérissement du papayer aux Antilles françaises associé à un *Erwinia* sp. du groupe *amylovora*. Fruits 40 (9):583-595.
8. Glazener, J. A., Couey, H. M., and Alvarez, A. 1984. Effect of postharvest treatments on Stemphylium rot of papaya. Plant Dis. 68:986-988.
9. Hildebrand, D. C. 1971. Pectate and pectin gels for differentiation of *Pseudomonas* sp. and other bacterial plant pathogens. Phytopathology 61:1430-1436.
10. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
11. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
12. Lelliott, R. A., and Dickey, R. S. 1984. Genus VII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith, 1920, 209. Pages 469-476 in: Bergey's Manual of Systemic Bacteriology, Vol. 1. N. R. Krieg and J. G. Holt, eds. Williams & Wilkins, Baltimore. 964 pp.
13. Leu, L. S., Lee, C. C., and Huang, T. C. 1980. Papaya black rot caused by *Erwinia cypripedii*. Plant Prot. Bull. Taiwan 22 (4):377-384.
14. Magrou, J. 1937. Genre *Erwinia*. Pages 214-215 in: Dictionnaire des bactéries pathogènes. P. Hauduroy, G. Ehringer, A. Urbain, G. Guillot, J. Magrou, eds. Masson et Cie, Paris.
15. Miller, T. D., and Schroth, M. N. 1972. Monitoring the epiphytic population of *Erwinia amylovora* on pear with a selective medium. Phytopathology 62:1175-1182.
16. Nelson, M., and Alvarez, A. 1980. Purple stain of *Carica papaya*. Plant Dis. 64:93-95.
17. Richard, C. 1984. Genus VI. *Enterobacter* Hormaeche and Edwards 1960, 72; Nom. Cons. Opin. 28, Jud. Comm. 1963, 38. Pages 465-469 in: Bergey's Manual of Systemic Bacteriology. Vol. 1. N. R. Krieg and J. G. Holt, eds. Williams & Wilkins, Baltimore. 964 pp.
18. Rohrbach, K. G., and Pfeiffer, J. B. 1975. The field induction of bacterial pink disease in pineapple fruit. Phytopathology 65:803-805.
19. Rosen, H. R. 1922. The bacterial pathogen of corn stalk rot. Phytopathology 12:497-499.
20. Schaad, N. W. 1980. Initial identification of common genera. Pages 1-11 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 72 pp.
21. Schroth, M. N., and Hildebrand, D. C. 1980. *Erwinia* I. *E. amylovora* or true erwiniae group. Pages 26-30 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 72 pp.
22. Seo, S. T., Farias, G. J., and Harris, E. J. 1982. Oriental fruit fly: Ripening of fruit and its effect on index of infestation of Hawaiian papayas. J. Econ. Entomol. 75:173-178.
23. Sokal, R. R., and Rohlf, F. J. 1969. Biometry. W. H. Freeman, San Francisco. 776 pp.
24. Sutton, D. D., Ark, P. A., and Starr, M. P. 1960. The causal agent of bacterial brown rot of Cypripedium orchids. Phytopathology 50:182-186.
25. Trujillo, E. E., and Schroth, M. N. 1982. Two bacterial diseases of papaya trees caused by *Erwinia* species in the northern Mariana Islands. Plant Dis. 66:116-120.
26. von Rant, A. 1931. Über eine Bakterienkrankheit bei dem Melonenbaume (*Carica papaya* Linn.) auf Java. Zentralbl. Bakteriol. Parasitenkd. 84:481-487.
27. Webb, R. R. 1985. Epidemiology and control of bacterial canker of papaya caused by an *Erwinia* sp. on St. Croix, U.S. Virgin Islands. Plant Dis. 69:305-309.