Purple Stain of *Carica papaya*

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ABSTRACT

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A new strain of *Erwinia herbicola*, which produces an extracellular, water-soluble, purple pigment in culture, is the causal agent of purple-stain disease of papaya (*Carica papaya*) in Hawaii. Disease incidence was highest during January and February, when the pathogen was readily recovered from symptomless as well as purple-stained fruits. In vitro pigment production was greatest when bacteria were grown in liquid shake culture containing 0.5% sucrose and 1% peptone and buffered at pH 5.5 or lower. The pigment has absorbance peaks at 595, 575, and 580 nm in water, ethanol, and pyridine, respectively.

A bacterial disease of papaya (*Carica papaya*) was first observed in 1976 on the Island of Hawaii (5). Because disease incidence was sporadic, the major papaya packers in Hawaii were surveyed to determine the extent of the disease.

MATERIALS AND METHODS

Isolation. Fruits were surface sterilized in 0.5% sodium hypochlorite for 15 min, air-dried, and cut open. Vascular and parenchymal tissues were separated and crushed in 0.2 ml of sterile distilled water (SDW), and loopfuls were streaked on yeast extract-dextrose-calcium carbonate (YDC) medium (7) and tetrazolium chloride (TZC) medium (3). Colonies were purified by restreaking them three times successively on YDC and were stored in SDW. Leaf tissues of papaya and selected ornamentals were surface-sterilized in 0.5% sodium hypochlorite for 1 min, and isolations were made from necrotic spots showing hydrotic margins.

Bacterial strains and characteristics. The 20 bacterial strains used in this study are listed in Table 1.

Acid production from organic compounds; production of acetoin from glucose; nitrate reduction; oxidase, catalase, and indole production; gelatin liquefaction reducing substances from sucrose; and pectolytic enzyme activity were tested according to Dye (1). Phenylalanine deaminase production was tested by the method of Ewing et al (2). Each test was performed twice.

Pathogenicity tests on papaya and selected hosts. Pathogenicity of the unknown and known *Erwinia* spp. was tested by inoculating papaya fruit and seedlings. The standard inoculum (SI) for all experiments was approximately 10^7 cells/ml of a bacterial suspension in SDW. For fruit inoculations, strains were injected into the vascular tissue of the stem end of papaya fruit with a tuberculin syringe fitted with a 20-gauge needle. Fruits were cut open 3–4 days after inoculation, and isolations were made from tissues. At 2 mo, papaya seedlings were inoculated with 14 pigment-forming *Erwinia* spp. and the known *Erwinia* spp. by severing the tips of the feeder roots and soaking them in SI for 10 min or by wounding the terminal bud with a needle and placing SI on the bud. Seedlings were planted in sterile soil and maintained at 24 or 31 C for 14 days. In a comparative trial, seedlings were kept at 100% relative humidity for 14 days.

Because bacteria related to *E. chrysanthemi* produce rots on the leaves or stems of *Chrysanthemum*, *Philodendron*, or *Dieffenbachia*, strains A300 and A-CO and all known *Erwinia* spp. were infiltrated into the leaves of *Dieffenbachia* sp. and *Philodendron* sp. with a tuberculin syringe fitted with a 25-gauge needle. Polyethylene bags were placed over the inoculated leaves for 24 hr, then removed. The plants were placed in the greenhouse at 24 C for the duration of the experiment. Both stems and leaves of *Chrysanthemum* sp. were infiltrated with each bacterium, placed under intermittent mist with an alternating cycle (30 sec on, 3 min off) for 7 days, and maintained under the same conditions as the other inoculated plants.

Disease survey. One to two hundred fruits per week from five major packing plants on the Island of Hawaii were cut open and examined over a 12-wk period during the summer of 1977. Because purple-stain symptoms were not observed, a more extensive survey of the five major packers was made during January through May 1978. Ten fruits per week from each packer were cut open, and isolations were made from some of the fruits. Fruits were stored at 10 C for 7 days to simulate conditions of surface shipment. They were then ripened at 20–22 C and cut open at the full-yellow stage. A survey also was made of the postharvest hot water treatment tanks, cold water tanks, and fields of the grower where the disease was originally observed.

Pigment characteristics and conditions for production. The effect of temperature on pigment production was determined by growing strain A300 on YDC plates and incubating them at 10, 25, 31, and 47 C. Two plates were streaked per treatment. The presence or absence of the pigment was recorded at 12 and 24 hr.

The effect of pH on pigment production was determined by growing strain A300 in a medium buffered at 0.5 intervals from pH 4.5 to 8.0 and containing 1% peptone and 0.5% sucrose. The medium was buffered with dibasic sodium citrate and monobasic potassium phosphate. The sucrose was added after the buffered peptone was autoclaved and the pH was checked. The culture was placed on a rotary shaker and checked for the presence of pigment after 12 and 24 hr.

To determine the role of oxygen in pigment production, strain A300 was streaked on YDC plates and either placed immediately in a desiccator, which was evacuated and checked for anaerobiosis (BBL Gas Pak Anerobic System, Cockeysville, MD 21030), or covered with sterile mineral oil. Plates were incubated at 23 C and observed every 6 hr for 24 hr.

The absorption spectrum of the purple pigment in 95% ethanol was determined. Strain A300 was grown on YDC plates by incubating them at 28 C for 12 hr. Bacteria were removed, and the pigmented agar was cubed and placed in a flask with 95% alcohol (about 50 ml of ethanol for every 50 cm^2 of agar). Flasks were stored overnight at 17 C. The pigmented ethanol was then decanted and centrifuged at 27,000 *g* for 30 min, and the absorbance spectrum of the supernatant was determined. This method was repeated using pyridine as the solvent.

The absorbance spectrum of the pigment in water was determined by growing the bacterium in liquid shake culture (pH 5.5) containing 1% peptone and 0.5% sucrose. The culture was shaken on a rotary shaker (about 130 rpm) for 12

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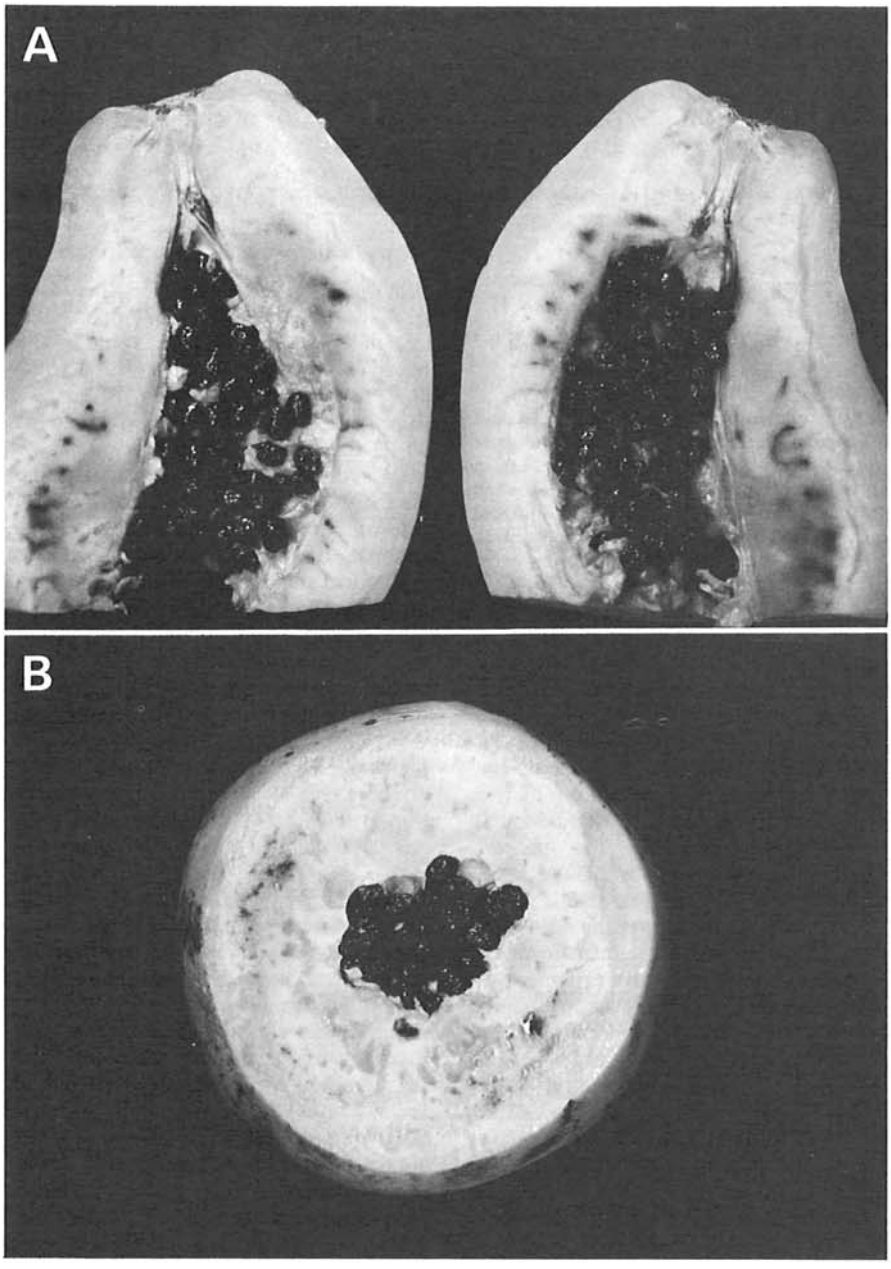

Fig. 1. Purple-stain symptoms in papaya fruit: **(A)** Naturally infected fruit with characteristic reddish purple pigment in vascular tissue and latex ducts. **(B)** Cross section through diseased fruits, showing pigment in latex ducts. The pigment has faded in some ducts, leaving translucent yellow areas surrounding the seed cavity.

Table 1. *Erwinia* spp. used in study of purple-stain disease of *Carica papaya*

Erwinia spp.	Original host	Pigment produced*	Source
A300	*Carica papaya*	Purple	Purple-stained fruits, 1976
A239	*C. papaya*	Purple	Purple-stained fruits, 1976
A333	*Syngonium podophyllum*	Purple	Leaf spot, 1977
A-CO	*Chrysalidocarpus lutescens*	Purple	Leaf spot, 1977
J3, J6, J7, J8, J9	*C. papaya*	Purple	Symptomless fruits, January 1978
F1, F2, F3, F6, F7	*C. papaya*	Purple	Symptomless fruits, February 1978
PP022, PP023	*C. papaya*	None	Symptomless fruits, 1978
E. herbicola #103	Apple	None	ICPB M. P. Starr, Davis, CA
E. amylovora #22	Pear	None	UCBPP M. N. Schroth, Berkeley, CA
E. carotovora #78	Unknown	None	UCBPP M. N. Schroth, Berkeley, CA
E. chrysanthemi #422	Carnation	Blue	R. S. Dickey, Ithaca, NY

*Grown on YDC.

hr, then centrifuged, and the absorbance spectrum of the supernatant was determined. Noninoculated controls were used in all experiments, and each experiment was performed three times.

RESULTS

Natural symptoms. The diagnostic characteristic of the disease was a reddish purple coloration of the entire vascular and latex tissue (Fig. 1). Immediately after fruits were cut open, the flesh was speckled bright purple, but the pigment faded after several hours, leaving a greenish yellow color in vessels, ducts, and surrounding parenchymal tissue, uncharacteristic of papaya fruit. This tissue became soft and translucent and rotted as the fruit ripened. Microscopic examination of diseased vascular tissue and latex ducts showed bacteria but no fungi.

Bacterial characteristics. One predominant colony type was consistently isolated from fruit with purple-stain symptoms. Yellow-brown mucoid colonies formed on YDC, and white mucoid colonies with red centers formed on TZC medium. The purple pigment appeared 7 hr after cultures were streaked on TZC or YDC.

The pathogen is a gram-negative, single rod, 0.6–1 μm × 1–2 μm (average, 0.7 μm × 1.5 μm) with peritrichous flagella and has the following characteristics of the genus *Erwinia*: facultative anaerobe, negative for oxidase and indole production, positive for catalase and acetoin production. Acid was produced from arabinose, ribose, glucose, rhamnose, sucrose, maltose, mannitol, inositol, α-methyl glucoside, and dextrin but not from xylose, raffinose, lactose, melibiose, cellobiose, or dulcitol. Gas was not produced from glucose, sucrose, or rhamnose. Key biochemical tests distinguishing this *Erwinia* from other *Erwinia* spp. are listed in Table 2.

Pathogenicity tests on papaya and selected hosts. All strains of *Erwinia* that produced purple pigment in culture also caused a purple coloration of the vascular tissue in papaya fruit. Fruit inoculated in the laboratory developed purple coloration in the vascular tissue of the stem end and seed cavity, but pigmentation throughout the latex ducts was less striking than that observed in naturally infected fruits. Nonpigment-producing strains of *E. herbicola* isolated from apple and papaya produced no symptoms in papaya fruit; neither did other species of *Erwinia*. The papaya pathogen did not cause symptoms on papaya seedlings and did not affect the other inoculated plants.

Disease survey. Fruits exhibiting disease symptoms were obtained only from grower A, who originally reported the disease. Purple-stained fruits frequently appeared in sampled lots from selected fields during January and February, but no symptoms were ob-

served in later months. In a random survey of all growers in January, only 3 of 400 fruits showed symptoms, but the pathogen was recovered from 23 of 397 symptomless fruits, all from grower A. In February, the pathogen was recovered from 32 of 320 symptomless fruits; in March, from 10 of 160; in April, from 2 of 160; and in May, from 2 of 80, all from grower A. The pathogen was also isolated from 2 of 80 symptomless fruits sampled directly from one field of grower A. It was not isolated from the hot water tank used in postharvest treatments or from the cold water transportation duct leading to the packing line.

Pigment characteristics. Pigment production was greatest when bacteria were grown at 25 or 31 C in liquid shake culture containing 0.5% sucrose and 1% peptone and buffered at pH 5.5 or lower. Pigment produced above pH 5.5 was unstable, and the liquid shake culture solution was colorless after 24 hr. Neither growth nor pigment production occurred at 47 C, and only slight growth and no pigment production occurred at 10 C. Bacteria grew well at 37 C but did not produce the pigment; when cultures were restreaked and incubated at 25 or 31 C, pigment was produced. The absorbance peaks of the pigment in water, 95% ethanol, and pyridine were 595, 575, and 580 nm, respectively.

Table 2. Key biochemical tests comparing purple-pigment-producing *Erwinia* spp. with known *Erwinia* spp.

	Purple-pigment-producing *Erwinia* spp.	*E. herbicola*	*E. amylovora*	*E. carotovora*	*E. chrysanthemi*
No. of isolates	13	1	1	1	1
Production of reducing substances from sucrose	+	+	+	−	−
Phenylalanine deaminase	+	+	−	−	−
Nitrate reduction	+	+	−	+	+
Pectolytic enzymes	−	−	−	+	+

DISCUSSION

The unusual aspect of this disease is its causal agent, which has bacteriological characteristics of *E. herbicola*, a species not normally considered a plant pathogen. Unlike *E. herbicola*, the papaya pathogen produces a purple to dark-blue pigment within papaya fruits and in culture. In our opinion, however, pigment production alone would not warrant exclusion of the papaya strains from the *E. herbicola* spp. *E. chrysanthemi* is the only *Erwinia* reported to produce a dark-blue, water-insoluble pigment, indigoidine (6). The pigment produced by the papaya pathogen differs from indigoidine in its solubility properties and absorbance spectra in pyridine (4). The difference in color between the two pigments is readily recognized when *E. chrysanthemi* and the papaya strains are grown on YDC.

Although purple-stain disease occurs sporadically, it is potentially important during winter months. Because the disease causes no external symptoms, the incidence is particularly difficult to assess, and infected fruit could easily reach the consumer. Fruits with off-colored greenish yellow flesh, in the market before fully ripe, may reflect occurrence of the disease, even though the purple pigment is not detected.

There may be a foliar phase on papaya, since the pathogen was also recovered from leaf spots on ornamentals. The pathogen was not recovered from papaya leaves in this study, however.

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