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Evaluating Hawaii-grown Papaya for Resistance to Internal Yellowing Disease Caused by *Enterobacter cloacae*

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Abstract. **Papaya (*Carica papaya* L.) cultivars and breeding lines were evaluated for resistance to *Enterobacter cloacae* (Jordan) Hormaeche & Edwards, the bacterial causal agent of internal yellowing disease (IY), using a range of concentrations of the bacterium. Linear regression analysis was performed and IY incidence was positively correlated with increasing inoculum concentrations for susceptible cultivars Kapoho Solo and Laie Gold but not for resistant cultivars or lines. It was determined that the inoculum concentration of 9 to 10 Log_{10} colony-forming units per milliliter (cfu/mL) was able to reliably differentiate resistant and susceptible papaya germplasm. Red-fleshed cultivars SunUp and Sunrise were the most resistant papaya groups evaluated at this dose concentration. Yellow-fleshed cultivars, Kapoho Solo and Laie Gold, were susceptible to *E. cloacae*. 'Rainbow', an F_1 hybrid between 'SunUp' and 'Kapoho Solo' that is yellow-fleshed, was moderately resistant to *E. cloacae*, exhibiting limited symptoms of the disease. Yellow-fleshed I-Rb F_5/F_6, an advanced inbred line derived from 'Rainbow', is resistant and offers the potential of improving resistance of yellow-fleshed commercial cultivars. A colorimeter was used to objectively measure internal flesh color and distinguish between infected and non-infected tissue in red- and yellow-fleshed papayas using L*C*H* color space analysis. Symptomatic tissue (72.4 and 79.0°) had higher hue angle means than non-symptomatic tissue (62.8 and 75.0°) for all cultivars or lines in red- and yellow-fleshed papayas, respectively. Yellow ("Y") hue color also distinguished infected tissue from non-infected tissue. Symptomatic tissue that had Y hue color resulted in 79 to 81° hue angle means among red- or yellow-fleshed papayas. Our results demonstrated the usefulness of colorimetry to help detect infected papaya tissue. In surveys of naturally infected papaya, high populations (8.57×10^7 cfu/g) of *E. cloacae* were recovered in infected fruit of 'Kapoho Solo' and represent a food safety concern for fresh and processed papaya. In isolations from inoculated fruits, we observed decreases of ≈1 to 2 Log_{10} cfu/g in final bacterial populations when high-dose range inoculum concentrations (9 to 12 Log_{10} cfu/mL) were used. This dose range may represent a saturation range for *E. cloacae* inoculation.**

Although the popularity of convenient, packaged, cut fruit is increasing, there are also concerns over foodborne diseases and spoilage. Fresh-cut and frozen papaya preparations have potential as value-added products of high quality for the papaya industry of Hawaii. However, high coliform bacterial counts have led to unacceptable batches of frozen cube preparations of 'Kapoho Solo' papaya fruit (Nishijima, unpublished data). The source of the high counts was traced to the bacterium *Enterobacter cloacae*, the causal agent of internal yellowing (IY) disease of papaya. Although IY is not discernable on the fruit exterior, internal quality is diminished by fluorescent yellow discolored flesh, tissue softening, and an offensive, rotting odor (Nishijima, 1994; Nishijima et al., 1987).

E. cloacae is widely distributed in the environment (Richard, 1984; Sanders and Sanders, 1997), occurring on or in water, soil, plants, humans, and animals (Richard, 1984). It is also a cross-domain pathogen that causes infections in humans (Sanders and Sanders, 1997) as well as various plant hosts such as elm (Carter, 1945; Murdoch and Campana, 1983), mulberry (Wang et al., 2008), orchid (Takahashi et al., 1997), coconut (George et al., 1976), corn (Rosen, 1922), bulb onion (Bishop and Davis, 1990; Cother and Dowling, 1986), macadamia (Nishijima et al., 2007a), papaya (Nishijima et al., 1987), and mung bean sprouts (Wick et al., 1987). The ability of *E. cloacae* to cause infections in humans and the occurrence of this bacterium in food crops could pose a food safety risk if contaminated products were ingested in high concentrations or by immune-suppressed individuals.

Internal yellowing disease has been present in Hawaii since the 1980s with incidences in 'Kapoho Solo' papaya as high as 43% (Nishijima et al., 1987). Fruit ripeness has been linked to susceptibility to IY, in which disease incidence increases as fruit mature to the full-ripe stage (Nishijima et al., 1987). Disease incidence can also vary with time of year. Surveys conducted from 1985 to 1991 indicated a seasonal variation in IY with higher incidences of disease occurring from October to December (Nishijima, unpublished data). This bacterial disease is an important concern in the food-processing segment of the papaya industry because of the potential risks associated with periods of high IY incidence or seasonal outbreaks and the use of ripe fruit in value-added papaya products. Because coliform bacteria such as *E. cloacae* can jeopardize food safety of value-added products such as minimally processed fresh or frozen papaya cubes, the use of resistant cultivars could reduce or minimize the occurrence of *E. cloacae*-infected fruit by having lower incidences of IY. Limited inoculation studies have identified papaya cultivars that are resistant, susceptible, and intermediate to IY infection when challenged with 0.5 mL of *E. cloacae* at 10^8 to 10^9 colony-forming units per milliliter (cfu/mL) inoculum concentration (Nishijima et al., 2004). However, the effects of varying *E. cloacae* populations on IY response are unknown, including the threshold inoculum concentration necessary to elicit an IY response in susceptible or resistant papaya cultivars.

Papaya cultivars vary in fruit flesh color but can generally be categorized into yellow- or red-fleshed types. Fruit infected with IY have fluorescent yellow discolored tissue that is distinctive in the orange–yellow flesh of 'Kapoho Solo' fruit (Nishijima et al., 2004). However, the yellow discoloration is not as apparent in red-fleshed cultivars such as 'Sunrise' or 'SunUp'. The intensity of the yellow color associated with this disease can also vary among some fruit. A colorimeter was used to measure the intensity of grayness in macadamia kernels with gray kernel disease, also caused by *E. cloacae* (Nishijima et al., 2007a). Similarly, a colorimeter system may be helpful in analyzing IY symptoms of different intensities in yellow- and red-fleshed papaya cultivars. If the colorimeter is more accurate in detecting IY than visual detection, this technology could be applied commercially to screen processed papaya products infected with *E. cloacae*.

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The objectives of this investigation were 1) to determine the effect of various *E. cloacae* inoculum concentrations on IY incidences and responses of papaya germplasm; 2) to evaluate papaya germplasm for resistance to *E. cloacae;* and 3) to quantify IY responses among yellow-fleshed and red-fleshed papaya inoculated with *E. cloacae* using a colorimeter as a method to objectively identify IY-infected papaya tissue.

Materials and Methods

Papaya cultivars, breeding lines, and fruit preparations. The papaya cultivars and lines selected for evaluation consisted of fruit types exhibiting yellow flesh or red flesh. In addition, some of the evaluated papaya possessed the gene that confers resistance to papaya ringspot virus (PRSV). These genetically engineered (GE) cultivars were Laie Gold F_1 (yellow), Rainbow F_1 (yellow), and SunUp (red). Also evaluated was I-Rb F_5/F_6 (yellow), a mixture of two generations (F_5 and F_6) that were developed as a yellow-fleshed, homozygous PRSV-resistant inbred line derived from 'Rainbow'. The non-GE cultivars were Kapoho Solo (yellow) and Sunrise (red). These six papaya groups were evaluated in dose–response studies that assessed papaya cultivars and lines for *E. cloacae* resistance. Four additional papaya groups (all GE) consisting of red-fleshed and yellow-fleshed segregating lines of 'Rainbow' F_2 and 'Laie Gold' F_2 were used in a separate study in which colorimeter analyses of infected papaya tissue were conducted to determine if the bright yellow coloration characteristic of IY could be quantitatively distinguished from healthy papaya tissue. Fruit surface area with one-third to half yellow skin color, which indicates papaya at one-third to half ripeness, was obtained from the islands of Oahu, Kauai, and Hawaii. The fruit were washed with cloth gloves and tap water to remove soil and residue on the fruit surface and were air-dried. Before inoculation, the entire fruit surface was disinfected by wiping with 70% ethanol. Each fruit was labeled or marked to identify inoculation dose treatment, cultivar, and inoculation sites.

Bacterial strains and fruit inoculations. The *E. cloacae* strains used in dose–response studies consisted of YPV-5B (papaya strain) and ATCC 13047 (type strain from human). Colorimeter analysis studies included two additional strains, B193-3 (ginger strain) and Dd-18 (oriental fruit fly strain). The bacterial strains were grown for 3 to 4 d at 30 °C on PT-M4 agar medium (1.8% bacto-agar, 1% peptone, 0.5% yeast extract, 0.25% sodium chloride, and 0.001% triphenyltetrazolium chloride that was added to sterile, molten agar before pouring) (Nishijima et al., 2004, 2007a). All strains were described or used in previous investigations (Nishijima et al., 1987, 2004, 2007a).

In dose–response studies, inoculum suspensions of *E. cloacae* strains were prepared by scraping cells from the bacterial cultures, mixing into 40 mL sterile distilled water (SDW), and adjusting the suspension to optical density 0.7 to 0.8 ($A_{600\ nm}$) (10^{12} cfu/mL) using a Turner SP-830 spectrophotometer (Barnstead/Thermolyne, Dubuque, IA). The concentrated suspensions were serially diluted (1:10 series) into 36 mL SDW (in flasks) to produce bacterial concentrations ranging from 10^1 to 10^{12} cfu/mL. All dose treatments were spotted (25-µL aliquots) in duplicate onto PT-M4 plates, incubated overnight at 30 °C, and colonies counted to determine actual bacterial populations of each dilution treatment. For each papaya cultivar or line, at least five fruit of each dose concentration range (0, 10^1 to 10^2, 10^3 to 10^4, 10^5 to 10^6, 10^7 to 10^8, 10^9 to 10^{10}, or 10^{11} to 10^{12} cfu/mL) were inoculated in each replicate test. Each test was conducted at least twice. Four sites located along the lengthwise surface of each fruit, one site per corner of an approximate "square grid" at midfruit, were injected (≈1.0-cm depth, 0.5-mL volume) with bacterial inoculum at two sites for each strain or with SDW (control treatment) at all four sites using a sterile 3-mL syringe fitted with a 23-gauge needle. All inoculation sites were covered with tape to prevent cross-contamination from other treatments. Fruit were incubated in fiberboard cartons for 3 d at 26 °C until ripe, dissected at the inoculation sites, and then evaluated for internal yellowing symptoms.

In colorimeter analysis studies of IY responses, fruit were inoculated (0.5 mL) with four *E. cloacae* strains and SDW control at separate sites along the lengthwise surface of the fruit, similar to methods described previously but with the fifth inoculation site located at the center of the "square grid" of inoculation sites. The inoculum for each strain consisted of a 30-mL aqueous cell suspension that was adjusted to optical density of 0.4 to 0.5 ($A_{600\ nm}$) and equivalent to a bacterial concentration of 10^8 to 10^9 cfu/mL (Nishijima et al., 2007a). All inoculation sites on each fruit were covered with tape to prevent cross-contamination from other treatments, and fruit were stored as described previously.

Fruit evaluations. Ripened fruit were cut open with a flame-sterilized knife, evaluated for IY symptoms by examining tissue dissected at the inoculation sites, and IY incidence and severity responses were determined. IY incidence for fruits was determined by the number of fruit having at least one inoculation site positive for IY out of the total number of fruits, whereas IY incidence for sites was determined by the number sites with positive IY reactions out of the total number of inoculated sites per treatment or IY severity response category. Data for IY incidences in colorimeter studies, along with results of a separate but similar inoculation screening study of the same papaya groups, were used in papaya evaluations for IY resistance that were separate from dose–response studies. IY symptoms were rated for severity responses based on the approximate area at the inoculation site that was affected with distinct yellow discoloration with well-defined or diffuse margins and were categorized as follows: IY0 = no yellowing reaction, IY1w = weak or faint, IY1 = slight (discoloration less than 50% of the inoculation area), IY2 = medium (discoloration 50% to 100% of the inoculation area), and IY3 = severe (discoloration spread beyond the inoculation area). In the dose–response studies, response category IY1w was combined with IY1 category and IY response groups were: IY0, IY1, IY2, and IY3, with IY1 describing weak to slight symptoms. IY responses were analyzed in the colorimeter studies using a Minolta CR-300 chromometer (Minolta Corp., Ramsey, NJ). Hue angle (H°), which quantifies color in the L* C* H color space where 0° = red, 90° = yellow, 180° = green, and 270° = blue, was used to analyze the color of IY-positive (IY1w to IY3) or IY-negative (IY0) papaya tissue that represented symptomatic or nonsymptomatic visual observations, respectively.

Reisolation of E. cloacae *and determination of bacterial populations.* Putative *E. cloacae* strains were re-isolated from infected papaya tissues in dose–response studies to confirm the presence of the bacterium in inoculated fruit of various dose treatments and to determine bacterial populations of various IY reactions in resistant or susceptible cultivars or lines. Selected tissue sections (≈1.0 × 1.5 × 0.5 cm) were aseptically excised with a sterile scalpel, weighed, surface-disinfected in 0.5% sodium hypochlorite (plus one to two drops of liquid detergent) (Contrex AL; Decon Laboratories, Inc., King of Prussia, PA) solution for 30 s, drained on clean laboratory tissue, and macerated in 5 mL SDW in a pulsifier bag (Microbiology International, Frederick, MD). An aliquot (1 mL) of the suspension was serially diluted (1:10 series) in 9 mL of SDW in tubes to obtain dilution concentrations ranging from 10^{-1} to 10^{-9}. All dilution concentrations were spotted (25-µL aliquots in duplicate) onto PT-M4 plates and incubated overnight at 30 °C. Colonies were counted and bacterial populations were calculated as cfu per gram of tissue sample. (Colony-forming units measure viable bacterial cells and are used in microbiology to determine microbial load or concentration.) To confirm the presence of *E. cloacae* in tissue isolations, individual colonies were isolated, sequentially re-streaked on PT-M4 plates to obtain purified strains, and then tested for oxidative and fermentative reactions in tubes of OF media (Difco Laboratories, Detroit, MI) with glucose as the carbohydrate source. Selected facultative anaerobes were identified according to the manufacturer's instructions using API 20E strips (bioMerieux Inc., Durham, NC) incubated at 30 °C for 18 to 24 h.

To fulfill Koch's postulates in the colorimeter analysis studies, selected tissue sections from IY reactions were analyzed for presence of *E. cloacae*. Tissues were dissected aseptically and surface-disinfected as described previously. A 1.0-g portion of tissue was macerated aseptically with a sterile glass rod in a test tube containing 9 mL SDW and a 20-µL aliquot of the suspension was streaked onto PT-M4 plates, which were incubated overnight at 30 °C. Methods to confirm *E. cloacae* among selected purified strains obtained from single colony isolations were performed as described previously.

Naturally occurring internal yellowing disease. Evaluation of IY symptoms also was conducted for naturally occurring infections among 129 fruit in 2005, 140 fruit in 2006, and 314 fruit in 2007 that were collected from packinghouses or fields on the island of Hawaii. Surveyed cultivars consisted of Kapoho Solo, Rainbow, Sunrise, and SunUp. Fruit were ripened, dissected, and data were collected for IY incidence, IY severity responses, colorimeter analysis of IY symptoms, and bacterial populations, as described previously.

Experimental design and statistical analysis. The experimental design for the dose–response studies was a factorial with cultivar, inoculum dose, and bacterial treatment (*E. cloacae* strains ATCC 13047 or YPV-5B, or SDW) factors. A replicate sample consisted of five fruit per inoculum dose treatment, each fruit with four inoculation sites (two per bacterial strain or four SDW). The experiment (all dose treatments) was repeated at least once for each papaya cultivar or line. In colorimeter studies, color analysis was conducted on inoculated fruit with positive (i.e., IY1w, IY1, IY2, or IY3) or negative (IY0) symptoms to quantify visual IY responses in red-fleshed or yellow-fleshed papaya cultivars or lines. The experiment was arranged in a split plot design in which the main plot was fruit flesh color (red or yellow) and the subplots were papaya cultivar or line and inoculation treatment (*E. cloacae* strain YPV-5B, ATCC 13047, B193-3, or Dd-18; or SDW control). A replicate consisted of at least two individual fruit, each inoculated with all four bacterial strains and SDW control. There were at least four replicates per IY response per fruit flesh color category. Data consisted of hue angle (H°) measurements and hue color category (e.g., yellow or yellow–red). All data (percent IY incidence for fruit or inoculation sites; hue angle) were analyzed by the general linear model procedure of SAS Version 9.2 (SAS Institute, Inc., Cary, NC). Means separation (where appropriate) were performed by pairwise comparisons of means by Fisher's protected least significant difference test at $P = 0.05$. Linear regression analysis of percent IY incidence means and dose treatments (Log_{10} cfu/mL) were performed using the regression procedure of SAS (Proc Reg of SAS, Version 9.2).

Results

Inoculum dose–response studies. The effect of various inoculum concentrations (dose treatments) on IY incidences was used to evaluate papaya germplasm for IY resistance. Dose treatments were presented either as continuous data (Log_{10} cfu/mL) (e.g., 0, 1, 2, 3, etc.) or as discrete data (Log_{10} categories cfu/mL) (e.g., 1 to 2, 3 to 4, etc., until 11 to 12). Linear regression analysis indicated that IY incidence (y) could be predicted from inoculum concentration, Log_{10} cfu/mL (x), for 'Kapoho Solo' ($y = 8.825x - 8.785$; $r^2 = 0.779$; n = 24) ($P < 0.0001$) and for 'Laie Gold' ($y = 5.426x - 4.687$; $r^2 = 0.684$; n = 24) ($P < 0.0001$), but not for 'Rainbow', I-Rb F_5/F_6, 'SunUp', or 'Sunrise' (r^2 = 0.067, 0.049, 0.092, 0.028, respectively) (all $P > 0.05$). Also, the relationship of IY incidences and increasing dose treatments of *E. cloacae* was positively correlated for susceptible cultivars Kapoho Solo ($r = 0.883$) ($P < 0.0001$) and Laie Gold ($r = 0.827$) ($P < 0.0001$) but not for resistant cultivars or lines ($P > 0.05$).

Analysis of variance of IY incidence data indicated significant differences among means for cultivar and inoculum dose category (Log_{10} category, cfu/mL) but not for bacterial strain ($P < 0.0001$, $P < 0.0001$, $P = 0.21$, respectively) (Table 1). Interactions were significant only for cultivar × dose category ($P < 0.0001$). 'Kapoho Solo' had the highest IY incidence (44.2%) among six papaya cultivars or lines, whereas dose treatment categories 7 to 8, 9 to 10, and 11 to 12 Log_{10} cfu/mL produced IY incidences of 23.5%, 25.8%, and 30.0%, respectively (Table 1). When data were analyzed by dose treatment category, means for cultivars were significantly different at dose treatment category 9 to 10 Log_{10} ($P = 0.03$) and 11 to 12 Log_{10} ($P = 0.05$) but were not different at the other dose treatment categories (P = 0.33 to 0.46) (analysis not shown). The two most susceptible cultivars were differentiated at dose treatment categories 9 to 10 and 11 to 12 Log_{10} with 'Kapoho Solo' having the highest IY incidences (90.0% and 87.5%, respectively) followed by 'Laie Gold' F_1 (50.0% and 52.5%, respectively) (Table 2). Mean IY incidences among the other four cultivars were no higher than 26.7% (Table 2), indicating some level of resistance to the pathogen. When data were sorted and analyzed by cultivar, the effect for dose treatment category was significant only for 'Kapoho Solo' ($P = 0.006$) and 'Laie Gold' F_1 ($P = 0.013$). The results were similar to findings in the colorimeter papaya screening study in which red- or yellow-fleshed papaya were inoculated with *E. cloacae* strains (ATCC 13047, B193-3, Dd-18, or YPV-5B) at 10^9 cfu/mL inoculum concentration and were evaluated for IY symptoms and color-analyzed with a colorimeter (data shown in Table 3). Papaya groups were categorized as resistant (10% or less IY), moderately resistant (11% to 39% IY), moderately susceptible (40% to 59% IY), or susceptible (60% or greater IY) based on IY incidence data gathered in this study. Using these resistance-susceptible categories and corresponding disease incidence criteria, yellow-fleshed cultivars Kapoho Solo and Laie Gold F_1 were susceptible (81.3% and 65.2% IY, respectively), I-Rb F_5/F_6 and 'Rainbow' F_1 were moderately resistant (16.8% and 33.1% IY, respectively), and red-fleshed cultivars Sunrise and SunUp were resistant (0% and 3.6% IY, respectively) (Table 4).

The lowest dose category tested for 'Kapoho Solo' and 'Laie Gold' F_1 was at 3 to 4 Log_{10} cfu/mL, which elicited 12.5% and 6% IY incidence, respectively, and represents an approximate minimum bacterial population necessary for symptom expression in the most susceptible cultivars (Table 2). For 'Laie Gold' F_1, dose treatment categories greater than 7 to 8 Log_{10} cfu/mL had an apparent "plateau effect" in which, despite increasing inoculum concentrations, mean percent IY incidence stabilized at 50.0% to 52.5% (Table 2), indicating that this cultivar was less susceptible than 'Kapoho Solo'. Minimum inoculum concentrations necessary to elicit an IY response varied for the other cultivars with resistant cultivar Sunrise producing IY symptoms only at 9 to 10 Log_{10} cfu/mL, whereas moderately resistant to resistant papayas I-Rb F_5/F_6, 'Rainbow' F_1, and 'SunUp' produced IY symptoms at doses 1 to 2 and 3 to 4 Log_{10} cfu/mL (Table 2).

In evaluations of IY severity responses resulting from papaya inoculations at various dose treatment categories, 'Kapoho Solo' was the only cultivar among the evaluated cultivars and lines that exhibited increasing incidences of severe IY3 reactions with increasing inoculum concentrations (Fig. 1). There were also proportionately greater incidences of IY3 response (57%) than the less

Table 1. Effects of papaya cultivar or line, *Enterobacter cloacae* strain (ATCC 13047 or YPV-5B), and inoculum concentration (Log_{10} category cfu/mL) on mean percent internal yellowing (IY) incidence of inoculated sites.

Effect	No. of reps.	IY incidence (%)[z]
Cultivar or line (flesh color)		
Kapoho Solo (yellow)	24	44.2 a
Laie Gold F_1 (yellow)	24	25.8 b
Rainbow F_1 (yellow)	21	15.7 c
SunUp (red)	36	11.9 c
I-Rb F_5/F_6 (yellow)	36	3.6 d
Sunrise (red)	24	0.4 d
Bacterial strain		
ATCC 13047	84	17.9
YPV-5B	81	13.3
Dose treatment (Log_{10} category, cfu/mL)		
11 to 12	24	30.0 a
9 to 10	24	25.8 a
7 to 8	23	23.5 ab
5 to 6	27	18.1 b
3 to 4	26	5.8 c
1 to 2	12	5.0 c
None (sterile distilled water)	29	0.0 c
Significance of effect		
Cultivar (C)		***
Bacterial strain (B)		NS
Dose treatment (D)		***
C*B		NS
C*D		***
B*D		NS
C*B*D		NS

[z]Means in column, by effect, followed by the same letter are not significantly different according to the least significant difference test of percent IY incidence at $P = 0.05$.

NS, ***Non-significant at $P \leq 0.05$, or significant $P \leq 0.001$.

Table 2. Mean percent internal yellowing (IY) incidence in yellow- or red-fleshed papaya cultivars or lines inoculated with *E. cloacae* strains (ATCC 13047 or YPV-5B) at various inoculum concentrations.

		IY incidence (%)						
		Dose treatment category (Log_{10} cfu/mL)						
Cultivar or line	Flesh color	0	1 to 2	3 to 4	5 to 6	7 to 8	9 to 10	11 to 12
Kapoho Solo	Yellow	0.0	—[z]	12.5	28.0	62.5	90.0 a[y]	87.5 a
Laie Gold F_1	Yellow	0.0	—	6.0	21.7	50.0	50.0 b	52.5 b
Rainbow F_1	Yellow	0.0	0.0	10.0	30.0	30.0	26.7 c	7.5 c
I-Rb F_5/F_6	Yellow	0.0	3.3	0.0	12.0	4.3	4.0 d	—
SunUp	Red	0.0	10.0	12.5	25.0	25.0	2.5 d	16.2 c
Sunrise	Red	0.0	—	0.0	0.0	0.0	3.3 d	0.0 c

[z]— = No data for these cultivars or lines.
[y]Means within columns followed by the same letter are not significantly different according to least significant difference test at $P = 0.05$.

Table 3. Effect of cultivar or line, inoculation treatment, internal yellowing (IY) response, and hue color on hue angle values of red- or yellow-fleshed papaya cultivars or lines inoculated with *Enterobacter cloacae* strains (ATCC 13047, B193-3, Dd-18, or YPV-5B) or sterile distilled water (SDW).

	Red-fleshed papaya		Yellow-fleshed papaya	
Effect	No. observations	Mean[z] hue angle (H°)	No. observations	Mean[z] hue angle (H°)
Cultivar or line				
Laie Gold F_2–segregant	109	72.10 a	176	77.08
Rainbow F_2–segregant	55	64.66 b	57	78.83
SunUp	223	61.56 c	N/A	N/A
Sunrise	114	59.44 d	N/A	N/A
Rainbow F_1	N/A	N/A	106	77.51
I-Rb F_5/F_6	N/A	N/A	106	76.17
Laie Gold F_1	N/A	N/A	93	75.92
Kapoho Solo	N/A	N/A	242	75.58
Inoculation treatment				
E. cloacae strain				
ATCC 13047	91	64.80	133	77.72
B193-3	82	64.14	129	77.47
Dd-18	78	63.93	132	77.44
YPV-5B	87	64.20	136	77.11
SDW control	163	62.52	250	74.65
IY severity response[y]				
IY3	4	68.43	21	80.08 b
IY2	21	73.34	88	80.81 a
IY1	12	75.05	116	78.94 c
IY1w	11	69.27	72	76.58 d
IY0	453	62.79	483	75.03 e
Hue color				
Y (yellow)	18	80.14 a	194	80.74 a
YR (yellow–red)	483	63.10 b	586	75.15 b
Significance of effect				
Cultivar		**		NS
Inoculation treatment		NS		NS
IY severity response		NS		***
Hue color		*		***

[z]Means in columns, by effect, followed by the same letter are not significantly different according to the least significant difference test of hue angle values at $P = 0.05$.
[y]Internal yellowing (IY) severity responses were evaluated and rated as none (IY0), weak or faint (IY1w), slight (IY1), medium (IY2), or severe (IY3).
NS, *, **, ***Non-significant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.
N/A = Not applicable; cultivar or line does not occur in this flesh color group.

severe IY1 and IY2 responses (10% and 23%, respectively) when inoculated at concentration 9 to 10 Log_{10} cfu/mL (Fig. 1). There were no occurrences of IY3 responses in the other papaya groups at this inoculation concentration (data not shown). These results confirmed that 'Kapoho Solo' is the most susceptible cultivar and inoculum concentration 9 to 10 Log_{10} cfu/mL is optimal for evaluating papaya for resistance to *E. cloacae* (Table 2).

Colorimeter studies. For the 10 papaya groups that were evaluated, hue angle (H°) means between papaya of red or yellow flesh color were significantly different ($P = 0.003$) and data were sorted into separate flesh color data sets for further analysis. Analysis of variance for red-fleshed papaya indicated significant differences among hue angle means for cultivar or line ($P = 0.005$) and hue color category ($P = 0.040$). Yellow-fleshed papaya had significant differences in means for IY response ($P = 0.0004$) and hue color category ($P < 0.0001$). Among four red-fleshed cultivars or lines, a red-fleshed segregant of 'Laie Gold' F_2 had the highest hue angle mean (72.1°), whereas 'Sunrise' was lowest (59.4°) (Table 3). Among yellow-fleshed cultivars or lines, hue angle means were 75.6 to 78.8° and were not significantly different ($P = 0.695$). Interestingly, hue angle means for yellow-fleshed segregants of 'Laie Gold' F_2 and 'Rainbow' F_2 were similar in values to their yellow-fleshed F_1 counterparts. However, hue angle mean of red-fleshed segregant of 'Laie Gold' F_2 was significantly higher (72.1°) than means for the other red-fleshed papaya groups (59.4, 61.6, 64.7°) and indicates more "yellowness" in this papaya group (Table 3).

Among IY responses, hue angle means of symptomatic IY reactions (IY1w to IY3) were significantly higher than non-symptomatic reaction (IY0) for yellow-fleshed papaya groups (Table 3) and indicated that infected tissue can be distinguished from non-infected tissue. A similar but not significant ($P = 0.089$) trend was observed for red-fleshed papayas. When data for symptomatic responses were analyzed by individual papaya groups, red-fleshed papaya 'Sun-Up', 'Sunrise', and 'Rainbow' F_2-red segregant had the lowest hue angle (H°) means (66.2, 69.7, and 74.7°, respectively), whereas all other red- or yellow-fleshed papaya groups had hue angle means of 77 to 81° (data not shown). The same red-fleshed cultivars also had the lowest hue angle means (61.1, 59.4, and 63.4°, respectively) in non-symptomatic responses (data not shown). Hue angle means among IY severity responses for papaya groups were also evaluated. When data were sorted by individual cultivar or line, only yellow-fleshed 'Kapoho Solo' had hue angle means (73.6, 76.1, 78.5, 79.4, and 80.9°) that were significantly different ($P = 0.039$) as well as positively related to increasing IY severity responses (IY0, IY1w, IY1, IY2, and IY3, respectively) (Table 5). Other yellow-fleshed papaya groups with hue angle means that were significantly different among IY severity responses were 'Rainbow' F_1 ($P = 0.017$) and 'Laie Gold' F_2-yellow segregant ($P = 0.035$) (Table 5).

Colorimeter readings were recorded as hue angle values and the corresponding hue color category (Y = yellow; YR = yellow–red) that is assigned by the colorimeter. The hue angle means were significantly higher for Y (80.1 and 80.7°) than YR (63.1 and 75.2°) in both papaya flesh groups (red and yellow, respectively) (Table 3), cultivars, or lines (data not shown) and all IY response categories (IY0, IY1w, IY1, IY2, and IY3) (data not shown). Additionally, the hue angle means (80° and higher) for inoculation reactions of Y hue color were not significantly different ($P > 0.05$) between red- and yellow-fleshed papaya groups or between cultivars or lines, indicating that infected papaya with hue angle values 80° or greater and designated as Y hue color denote a positive IY reaction in red- or yellow-fleshed papaya. This criterion would be useful in distinguishing infected and non-infected papaya tissue in a breeding program.

To further analyze the accuracy of using Y hue color designation as an indicator of IY infection, the percent incidence of Y hue color among symptomatic or non-symptomatic IY

Table 4. Evaluation of red- or yellow-fleshed papaya cultivars and lines for resistance to internal yellowing (IY) infection in fruits inoculated with *Enterobacter cloacae* strains (ATCC 13047, B193-3, Dd-18, or YPV-5B) at 10^9 cfu/mL.

IY resistance category[z]	Cultivar or line	GE[y] or non-GE	Flesh color	No. fruits	Percent IY[x] incidence
Resistant	Sunrise	Non-GE	Red	23	0.0
Resistant	SunUp	GE	Red	141	3.6
Moderately resistant	Rainbow F_2–red	GE	Red	254	16.5
Moderately resistant	I-Rb F_5/F_6	GE	Yellow	291	16.8
Moderately resistant	Laie Gold F_2–red	GE	Red	52	21.2
Moderately resistant	Rainbow F_1	GE	Yellow	133	33.1
Moderately susceptible	Laie Gold F_2–yellow	GE	Yellow	211	41.2
Moderately susceptible	Rainbow F_2–yellow	GE	Yellow	1030	56.6
Susceptible	Laie Gold F_1	GE	Yellow	201	65.2
Susceptible	Kapoho Solo	Non-GE	Yellow	112	81.3

[z]Internal yellowing resistance categories were based on IY-positive incidences (resistant = 10% or less IY, moderately resistant = 11% to 39% IY, moderately susceptible = 40% to 59% IY, susceptible = 60% or greater IY).

[y]Genetically engineered or not genetically engineered for resistance to papaya ringspot virus (PRSV). SunUp is the genetically transformed Sunset that was modified for PRSV resistance, Rainbow F_1 is a hybrid of SunUp × Kapoho Solo, Laie Gold F_1 is a hybrid of Kamiya × Rainbow F_2, Rainbow F_2 and Laie Gold F_2 are progeny (seeds) of their respective F_1 generation that segregated into red- or yellow-fleshed papaya. I-Rb F_5/F_6 is a mixture of F_5 and F_6 generations of a PRSV-resistant, inbred line derived from Rainbow.

[x]Percent IY was calculated by number of fruit with at least one positive infection at inoculated sites on each fruit out of a total number of fruit evaluated.

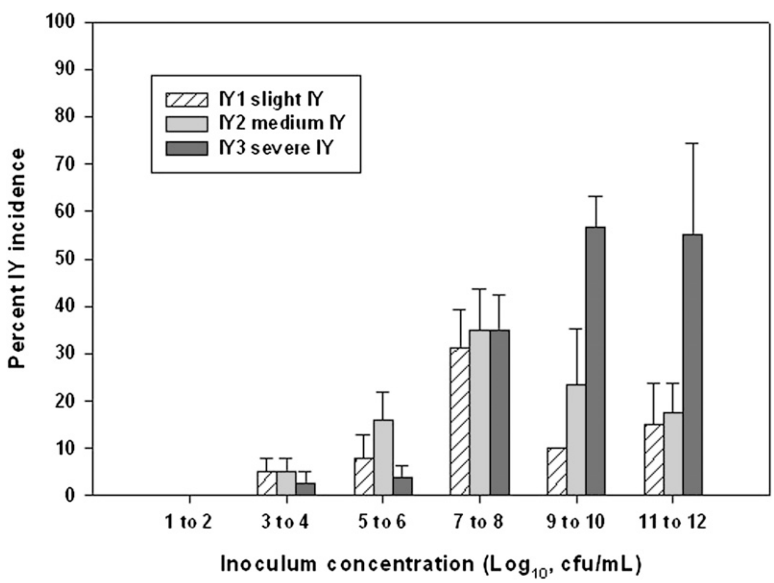

Fig. 1. Incidence of internal yellowing (IY) severity responses of 'Kapoho Solo' papayas inoculated with *Enterobacter cloacae* strains (ATCC 13047 or YPV-5B) at varying inoculum concentrations (range Log_{10}, cfu/mL). Vertical bars represent SEM.

responses was determined and used to compare visual designation of IY positive or negative reactions, respectively, to colorimeter analysis (Y hue color). For symptomatic reactions, red-fleshed papaya inoculated with *E. cloacae* strains resulted in lower percent incidences of IY occurrence among inoculated sites than yellow-fleshed papaya and ranged from 1.3% ('Sunrise') to 25.7% ('Laie Gold' F_2-red segregant) compared with 31.9% ('Rainbow' F_1) to 82.1% ('Rainbow' F_2-yellow segregant), respectively (data presented as number of occurrences; Table 6). Symptomatic IY that was confirmed by Y hue color among red-fleshed papaya was in red segregants of 'Laie Gold' F_2 and 'Rainbow' F_2, which had Y hue color occurrences of 79.0% and 33.3%, respectively (data presented as number of occurrences; Table 6). The 67% visual IY positive that was not Y hue color (i.e., "false" IY positive) that was encountered in 'Rainbow' F_2-red segregant exemplifies the occasional difficulty of visual designation of IY in red-fleshed papayas. Among yellow-fleshed papaya, 'Rainbow' F_1 and yellow segregant of 'Laie Gold' F_2 had the highest incidences of symptomatic IY responses that were confirmed by Y hue color, 91.3% and 86.2%, respectively (data presented as number of occurrences; Table 6). Among papaya with non-symptomatic IY responses, the percent incidence of Y hue color was 0% or at most 14.3%, which was in the yellow segregant of 'Rainbow' F_2 (data presented as number of occurrences; Table 6). Colorimeter analysis possibly detected positive IY responses that were not visibly observed in the yellow-fleshed segregant of 'Rainbow' F_2, which had among the highest H° means (and therefore "yellower" flesh) among the papaya cultivars or lines (Table 6). The accuracy of Y hue color relative to visually positive IY reaction was also evaluated by determining the percent incidence of symptomatic IY response among Y hue color designations. Results were similar for red- and yellow-fleshed papaya in which 91% to 100% of bacteria-inoculated sites that were Y hue color by colorimeter also were visually IY positive (Table 6). Hue angle means for papaya groups with Y hue color designations and visually symptomatic tissue ranged from 80 to 82°, except for 78.6° in a red-fleshed papaya ('Rainbow' F_2-red segregant) (Table 6). These results demonstrate the usefulness of Y hue color in confirming IY-positive reactions in red- or yellow-fleshed papaya.

Reisolation of E. cloacae *and determination of bacterial populations.* IY severity response categories were not correlated with final bacterial concentration (data not shown). However, when individual dose treatments were sorted into two categories (10^1 to 10^8 or 10^9 to 10^{12} cfu/mL) for data analysis, inoculum concentration affected final bacterial concentration of overall IY responses. Final bacterial populations (ranging from 10^6 to 10^9 cfu/mL) increased by ≈3 to 5 Log_{10} cfu/g when low to medium doses (10^1 to 10^8 cfu/mL) were used to inoculate five cultivars (Kapoho Solo, Laie Gold F_1, Rainbow F_1, Sunrise, SunUp) or one line (I-Rb F_5/F_6) in dose–response studies (data not shown). In contrast, final bacterial populations (ranging from 10^7 to 10^{10} cfu/mL) decreased by ≈1 to 2 Log_{10} cfu/g when high dose inoculum treatments (10^9 to 10^{12} cfu/mL) were used (data not shown). The high dose range may represent a saturation range for *E. cloacae* inoculation. Re-isolated bacterial strains from each papaya cultivar or line in dose–response studies and colorimeter studies were repeatedly identified as *E. cloacae* according to procedures described earlier, thus fulfilling Koch's postulates (data not shown). *E. cloacae* was also confirmed in red- and yellow-fleshed papaya with symptomatic or weak or non-symptomatic IY responses that were Y hue color.

Naturally occurring internal yellowing. Fruit evaluated for IY from collections on Hawaii island during 2005 to 2007 showed naturally occurring IY of severity categories ranging from IY1 to IY3 in six fruit of 'Rainbow' F_1, six fruit of 'Kapoho Solo', and two fruit of 'Laie Gold' F_2 (data not shown). When infected tissues were analyzed using the colorimeter, all tissues were Y hue color with a mean H° of 81.9. These results were comparable to those for inoculated yellow-fleshed papaya (n = 194) in which the mean H° was 80.7 for tissue of Y hue color (Table 3). Naturally occurring IY incidences varied for the four surveyed cultivars and ranged from 2.6% IY in 'Rainbow' F_1 (seven of 269 fruit) to 12.7% IY in 'Kapoho Solo' (30 of 237 fruit). There was no incidence of IY among surveyed fruit of 'Sunrise' (39 fruit) or 'SunUp' (38 fruit). 'Kapoho Solo' fruit evaluated with IY symptoms were confirmed to be

Table 5. Mean hue angle values of internal yellowing (IY) responses of red- or yellow-fleshed papaya cultivars or lines inoculated with *Enterobacter cloacae* strains (ATCC 13047, B193-3, Dd-18, or YPV-5B) or sterile distilled water (SDW).

Red-fleshed papaya

IY response[z]	Rain. F_2-red segregant: No. observations	Rain. F_2-red segregant: Hue angle (H°)	LG F_2-red segregant: No observations	LG F_2-red segregant: Hue angle (H°)	Sunrise: No. observations	Sunrise: Hue angle (H°)	SunUp: No. observations	SunUp: Hue angle (H°)
IY3	0	—[y]	1	80.80	1	69.70	2	61.60
IY2	1	73.00	8	80.59	0	—	12	68.54
IY1	5	75.02	5	80.36	0	—	2	61.85
IY1w	0	—	5	75.16	0	—	6	64.37
IY0	49	63.43	90	70.61	113	59.35	201	61.06
Significance		—		NS		—		NS

Yellow-fleshed papaya

IY response[z]	Rain. F_2–yellow segregant: No. observations	Rain. F_2–yellow segregant: Hue angle (H°)	LG F_2–yellow segregant: No. observations	LG F_2–yellow segregant: Hue angle (H°)	Laie Gold F_1: No. observations	Laie Gold F_1: Hue angle (H°)	Rainbow F_1: No. observations	Rainbow F_1: Hue angle (H°)	I-Rb F_5/F_6: No. observations	I-Rb F_5/F_6: Hue angle (H°)	Kapoho Solo: No. observations	Kapoho Solo: Hue angle (H°)
IY3	0	—	5	81.60 a[x]	8	78.60	0	—	1	78.30	7	80.93 a
IY2	4	83.93	37	82.13 a	11	80.20	5	80.54 a	9	78.24	22	79.42 b
IY1	20	80.72	8	79.80 b	19	77.70	14	80.43 a	13	77.17	42	78.53 c
IY1w	8	77.38	8	77.21 c	1	74.30	5	78.44 b	10	76.88	40	76.05 d
IY0	25	76.97	118	75.11 d	54	74.06	82	76.77 c	73	75.61	131	73.56 e
Significance		NS		*		NS		*		NS		*

[z]Internal yellowing severity responses were rated as none (IY0), weak or faint (IYw), slight (IY1), medium (IY2), or severe (IY3).

[y]— = No data as a result of "0" observations for the IY response or data could not be analyzed.

[x]Means in columns, by cultivar or line, followed by the same letter are not significantly different according to the least significant difference test of hue angle values at $P = 0.05$.

NS, *Non-significant or significant at $P \leq 0.05$.

infected with *E. cloacae* with bacterial populations ranging from 7.4×10^3 to 8.57×10^7 cfu/g; however, *E. cloacae* was not isolated from 'Rainbow' F_1 fruit with IY symptoms.

Discussion

Internal yellowing, caused by *E. cloacae*, is a limiting factor for production of value-added products such as fresh or frozen papaya cubes. High coliform counts and reduced quality as a result of yellow discoloration and tissue softening are factors that can lead to product rejection. Identifying or developing IY-resistant papaya cultivars can help reduce the risk of bacterial contamination and ensure compliance with food safety regulations. We evaluated papaya cultivars and lines for IY resistance and identified an optimal inoculum concentration of 9 to 10 Log_{10} cfu/mL that differentiated resistant and susceptible germplasm. Based on IY incidence ranges as criteria, the six evaluated cultivars or lines were ranked for resistance at this dose concentration as follows: 'Sunrise', 'SunUp', and I-Rb F_5/F_6 (resistant, 10% or less IY); 'Rainbow' F_1 (moderately resistant, 11% to 39% IY); 'Laie Gold' F_1 (moderately susceptible, 40% to 59% IY); and 'Kapoho Solo' (susceptible, 60% or greater IY). These six cultivars or lines were similarly ranked in colorimeter studies in which positive IY incidence data were used. Two resistant cultivars (Sunrise and SunUp, both red-fleshed papaya), four moderately resistant cultivars or lines (red-fleshed segregants of 'Rainbow' F_2 and 'Laie Gold' F_2 and yellow-fleshed I-Rb F_5/F_6 and 'Rainbow' F_1). and two moderately susceptible lines (yellow-fleshed segregants of 'Laie Gold' F_2 and 'Rainbow' F_2) were identified (Table 4). The highly susceptible nature of 'Kapoho Solo' (Nishijima et al., 2004) was confirmed (Table 4). 'Laie Gold' F_1 and I-Rb F_5/F_6 had higher IY incidences in the colorimeter studies than in dose–response studies and were determined susceptible and moderately resistant, respectively (Table 4), rather than moderately susceptible and resistant.

Four of the five most resistant papaya groups evaluated were red-fleshed papaya (Table 4) and indicate a possible natural resistance to IY in this papaya flesh color group. Similarly, evaluation of onion bulb cultivars indicated that red onion bulb cultivars were more resistant than white and yellow cultivars when inoculated with *E. cloacae* (Schroeder et al., 2010) and imply that disease resistance factors such as pigments or phenolic substances may be associated with red-pigmented plants (Gandikota et al., 2001; Link, et al., 1929; Link and Walker, 1933). The most resistant yellow-fleshed papaya group was I-Rb F_5/F_6, which represents the best potential for development of resistant yellow-fleshed papayas and merits further evaluation. Disease resistance factors in this papaya line may be attributed to its red flesh genetic heritage through its 'SunUp' lineage.

Our studies also determined that the bright yellow coloration of IY-infected fruit

Table 6. Comparison of visual designations with colorimeter analysis for hue Y (yellow) color for non-symptomatic or symptomatic internal yellowing (IY) responses of red- or yellow-fleshed papaya cultivars or lines inoculated with *Enterobacter cloacae* strains (ATCC 13047, B193-3, Dd-18, or YPV-5B).[z]

	IY response—visual: non-symptomatic (NonS)[y]					IY response—visual: symptomatic (Sym)[y]				Colorimeter: Hue Y	
Cultivar or line	No. inoc. sites	No. NonS response	H° NonS response	No. Hue Y	H° Hue Y	No. Sym response	H° Sym response	No. Hue Y	H° Hue Y	% Sym response	% NonS response
Red-fleshed papaya											
Laie Gold F_2– red segregant	74	55	70.87 e[x]	1	79.40	19	79.11	15	80.39	93.8	6.2
Rainbow F_2– red segregant	36	30	64.76 f	0	—[w]	6	74.68	2	78.60	100.0	0.0
SunUp	152	130	60.97 g	0	—	22	66.16	0	—	0.0	0.0
Sunrise	76	75	59.81 g	0	—	1	69.70	0	—	0.0	0.0
Flesh group total or mean	338	290	62.94 A[v]	1	79.40	48	72.43 A	17	80.18	94.4	5.6
Yellow-fleshed papaya											
Laie Gold F_2– yellow segregant	120	62	75.55 c	2	79.70	58	81.09	50	81.82	96.2	3.8
Rainbow F_2– yellow segregant	39	7	77.80 a	1	85.20	32	80.28	19	82.49	95.0	5.0
Rainbow F_1	72	49	76.97 ab	2	80.25	23	79.96	21	80.35	91.3	8.7
I-Rb F_5/F_6	75	45	75.71 bc	0	—	30	77.45	12	80.16	100.0	0.0
Laie Gold F_1	62	23	74.40 cd	0	—	39	78.50	27	80.00	100.0	0.0
Kapoho Solo	162	55	74.05 d	0	—	107	78.05	52	80.05	100.0	0.0
Flesh group total or mean	530	241	75.48 B	5	81.02	289	79.06 A	181	80.83	97.3	2.7
Significance			***				NS				

[z]Number of sites with non-symptomatic or symptomatic IY responses are compared with number of sites designated hue Y by colorimeter followed by respective mean hue angle values (H°). Colorimeter designation of hue Y that matched symptomatic IY response is indicated by percent incidence. Results for sterile distilled water inoculations are not shown.

[y]Internal yellowing non-symptomatic responses were evaluated as none or no symptoms (IY0), whereas symptomatic responses were evaluated as weak or faint (IYw), slight (IY1), medium (IY2), or severe (IY3) symptoms.

[x]Means in column of NonS IY response followed by the same lowercase letter are not significantly different according to the least significant difference test of hue angle values at $P = 0.05$.

[w]— = No data as a result of "0" sites with hue Y for cultivar or line.

[v]Means in row of red or yellow flesh group followed by the same uppercase letter are not significantly different according to the least significant difference test of hue angle values at $P = 0.05$. Means (H° values) for hue Y were either not significantly different ($P > 0.05$) or could not be analyzed.

NS, ***Non-significant at $P \leq 0.05$ or significant at $P \leq 0.001$.

can be measured with a colorimeter and that infected tissue can be distinguished from healthy papaya tissue in red- or yellow-fleshed fruit. Infected tissue that was visibly symptomatic usually had Y hue color with 79.1 to 81.1° hue angle means among red- or yellow-fleshed papayas. Additionally, the incidence of inoculation sites with Y hue color that were also symptomatic for IY ranged from 91% ('Rainbow' F_1) to 100% ('Rainbow' F_2-red segregant, I-Rb F_5/F_6, 'Laie Gold' F_1, and 'Kapoho Solo') and demonstrates the accuracy of this color analysis element in identifying infected tissues. In comparison, YR hue color occurred at 86.7% incidence in non-symptomatic tissue, resulted in significantly lower ($P = 0.002$) hue angle means for red-fleshed papaya compared with yellow-fleshed papaya (63.1° and 75.1°, respectively), and indicates an association with non-infected tissue. These results demonstrate the capability of color analysis in papaya breeding programs for evaluating resistance to IY or in commercial operations for sorting infected from non-infected fruit and fruit products.

Although fruit inoculations with the optimal dose treatment of 9 to 10 Log_{10} cfu/mL was effective in evaluating papaya cultivars for resistance to *E. cloacae* infection, the varying dose–response protocol used in this study also was valuable in producing dose–response profiles for cultivars or lines and demonstrated the unpredictable nature of cultivar evaluations. The dose–response profile for 'SunUp' revealed unexpected results for IY incidences at different dose treatments (e.g., 5 to 6 and 7 to 8 Log_{10} cfu/mL) (Table 2) and contradicted an earlier evaluation of this cultivar as immune (Nishijima et al., 2004).

Bacterial contamination in papaya by *E. cloacae* (Nishijima et al., 1987) or *E. sakazakii* (= *Cronobacter sakazakii*) (Keith et al., 2008) from naturally occurring infections in papaya fruit poses a food safety risk and represents an obstacle to the development of fresh-cut or frozen papaya products. Our studies indicated that *E. cloacae* populations can occur as high as 8.57×10^7 cfu/g in naturally infected papaya, which is higher than a previously reported population of 4.17×10^5 cfu/g (Nishijima et al., 2007b). Both populations, occurring in 'Kapoho Solo' fruit, exceed the food safety guideline limit for coliforms of less than 100 cfu/g (Sodexho Supplier Code of Practice, 2007) that is being used by individuals in the papaya industry. Although the minimum *E. cloacae* population required to elicit infection in humans is not known, it is possible that this cross-domain (i.e., plant and human) pathogen can pose a risk to susceptible individuals through contaminated vegetables and fruit (Neto et al., 2003).

In our surveys of papaya with naturally occurring IY, we were not able to isolate *E. cloacae* from 'Rainbow' F_1 fruit. The occasional non-culturable nature of this bacterium was observed previously with gray kernel disease of macadamia (Nishijima et al., 2007a).

Specific food technology methods and techniques are needed to treat papaya and papaya products to ensure a safe and high-quality commodity. Non-thermal technologies (e.g., high hydrostatic pressure, pulsed electric fields, ionizing radiation, ultrasonication) (Ross et al., 2003), vacuum and steam technology (Kozempel et al., 2002), and traditional and alternative antimicrobial treatments (Raybaudi-Massilia et al., 2009) are examples of technologies used to control pathogenic and spoilage microorganisms in fresh-cut fruits and vegetables. Promising technologies for preserving quality and preventing deterioration of fresh-cut papaya or frozen papaya cubes include applications of chitosan coating (Gonzalez-Aguilar et al., 2009) or combination treatments of ozone wash and heat that reduce *E. cloacae* microbial counts and enhance papaya quality and flavor (Yonemura, 2009). The baseline data of natural populations of *E. cloacae* in papaya that were generated in this investigation could be useful in dose–response or kinetics profiles of the various food treatment systems.

The role of fruit flies and other insect foragers in the transmission of human diseases through contaminated food is an emerging area of interest (Janisiewicz et al., 1999; Sela et al., 2005) that may provide insight into the possible mode of entry of *E. cloacae* in papaya fruit. *E. cloacae* occurs in the stomach and gut of the oriental fruit fly (*Bactrocera dorsalis* Hendel) (Jang and Nishijima, 1990), and papaya is a host of tephritid fruit flies, including *B. dorsalis* (Liquido et al., 1989). The occurrence of *E. cloacae* in internal yellowing-diseased papaya fruit and in washes of papaya flowers supports the hypothesis that fruit flies could transmit this bacterial plant pathogen to papayas (Nishijima et al., 1987). The preferential status of different papaya cultivars for fruit fly insect pests is an area that may be worthy of investigation as a way to select papaya germplasm that are less susceptible to fruit fly infestation and have less potential for high bacterial populations in fruit.

In conclusion, our protocol for treating papaya cultivars with various inoculum concentrations produced incidence and severity response profiles relative to bacterial concentration that were useful in evaluations for IY resistance. Minimum, maximum, and optimum dose concentrations were identified and used to rank papaya cultivars and lines for resistance. Naturally occurring populations of *E. cloacae* in 'Kapoho Solo' were identified that were higher than previous reports and underscored the importance of finding food processing or horticultural methods (e.g., breeding for resistance) to minimize microbial contamination in papaya. The use of a colorimeter to identify infected papaya fruit was demonstrated in which hue angle ranges and Y hue color characterized IY-infected tissue in red- and yellow-fleshed papayas. The application of this technology as well as other food processing technologies and the use of resistant papaya cultivars would help encourage market growth in fresh-cut or frozen papaya cube products by ensuring a safe food product.

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