Unusual Tropical Fruit Diseases with Extended Latent Periods

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Latency or latent period in fungal infections has been defined in terms of the time that lapses between invasion and the establishment of a nutritional or parasitic relationship (17) or, epidemiologically, the production of propagules or inoculum (20). Latency implies a period when the parasitic relationship is dormant or quiescent, since symptom development and inoculum production are not evident. Symptom development and inoculum production, therefore, are the measure of the end point of latency and the result of an aggressive nutritional relationship.

Bacterial pathogens, unlike fungal pathogens, may be present naturally within plant tissues and thus not require a physical penetration (4). In addition, bacteria on the surface of or within the host may have a nutritional relationship without parasitism. Because latency by definition is a temporal phenomenon, survival of the organism through the latent period becomes the critical issue.

The necessity to understand latency is dictated by the need to prevent infection in order to control disease. Historically, control has focused on prevention rather than on therapy. Additionally, certain latent infections are difficult to control because the quiescent propagules are buried within the host and are not accessible to control chemicals.

Latent fungal infections have been studied in the most detail as precursors to postharvest symptom development. Verhoeff (17) discussed latent infections of fruit or floral parts of banana, citrus, mango, papaya, avocado, stone fruits, apple, strawberry, and tomato. The pathogens survive a period of latency as appressoria on the surface of the developing fruit or, in the case of *Botrytis cinerea* Pers. ex Fr., as hyphae in attached senescent or dead floral parts. Hayward (4) discussed latent bacterial infections in tomato, cucumber, pepper, potato, stone and pome fruits, and citrus trees. Of these latent infections, only those in cucumber and apple have been suggested to begin as floral infections.

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Floral infections of tropical fruits by pathogens with extended latent periods have not been addressed until recently. The economic importance of the latent surface infections and the postharvest wound infections (3) has delayed the study of floral infections. Additionally, the sporadic nature of floral infections by pathogens with extended latent periods has made study difficult. The pineapple is exemplary as a host for a floral infection where an extended latent period occurs. The flower of pineapple is the portal for several major pathogens, and the period of latency ranges from 4 to 6 mo (11,16). In papaya, the disease cycle for two newly described diseases may also include floral infections and periods of latency.

Pink Disease of Pineapple

Pink disease of pineapple fruit is characterized by the economically important symptom of a brown pigmentation of the fruit tissue when heated during the canning process (11). The disease may be caused by strains of *Erwinia herbicola* (Lohnis) Dye (*Enterobacter agglomerans* (Beijerinck) Ewing and Fife), *Gluconobacter oxydans* (Henneberg) DeLey, and *Acetobacter aceti* (Pasteur) DeLey and Frateur, hereafter referred to as pink disease bacteria (2,11). Depending on the species and strain of bacteria involved and the severity of infection, browning symptoms may appear in the fruit flesh before cooking (15), or a pinkish discoloration and wilted appearance may be detectable in the whole fruit in the field before harvest. Sensory characteristics of the diseased fruit are not distinguishable from uninfected fruit showing normal postharvest maturity and senescence, with the exception of a "cantaloupelike aroma" with strains of *G. oxydans*. Symptoms are not evident in immature unripened fruit.

Pink disease bacteria are vectored by insects that visit flowers (5). The bacteria have been shown to enter the fruitlet through the opened flower (11,14). Hine (5) has suggested that bacteria invade cracks in the blossom cups at flowering after rainfall on inflorescences that developed under drought stress. In Hawaii, with detached inflorescences held in water (Fig. 1), pink disease bacteria were frequently recovered from nectaries within 6 hr after inoculation and incubation at 18 C and high humidity. Recoveries from nectary gland tissue were consistently higher than those from placental tissue (Table 1). In field tests, flowers were successfully inoculated when plants were not under drought-stressed conditions (14).

Nectar flow has been hypothesized to be involved with bacterial movement into the nectaries. No direct evidence is available, however. Day-night temperature differentials seem to play a major role in nectar flow in pineapple. Hine (5) has suggested that nectar dilution is necessary for bacterial survival and growth in the flower. We believe that high humidity during flowering prevents desiccation of the pink bacteria and nectar concentration by evaporation. Thus, rainfall during flowering would dilute nectar, prevent concentration by evaporation of water, and prevent desiccation of the pink bacteria. Loss of viability with desiccation has been reported (5) and confirmed (*unpublished*).

Once located in the nectary, the bacteria remain quiescent for 4–6 mo until the fruit matures and becomes translucent (cell contents leaking into the intercellular spaces) during the ripening process. Translucency is highly correlated with incidence and severity of pink disease (*unpublished*). In fact, a highly resistant cultivar, 58-1184, is very opaque when ripe and does not develop pink disease when inoculated. However, viable pink disease bacteria at population levels similar to those at inoculation are isolated from nectaries at maturity. Thus, populations of pink disease bacteria remain static or decrease until the fruit begins to ripen (*unpublished*). Whether or not a nutritional relationship between the bacteria and the host exists during fruit development is unknown. The nectary merely appears to provide the conditions for survival of the bacteria.

Temperature of the inflorescence and developing fruit may effect survival of the bacteria causing pink disease. Incidence of pink disease is extremely seasonal and sporadic in Hawaii as well as in other pineapple production areas of the world. The disease occurs only in fruits that develop during the cool weather. Several years can pass without economically important levels of disease. During an epidemic, incidence may be 30–50% in one week's harvest and drop to negligible levels the next. Maximum recoveries of pathogenic pink disease bacteria were obtained at infection temperatures of 18 C. Postinfection temperatures of 29 or 35 C reduced recoveries from an average of 86% at 18 C to 24 and 14%, respectively (12). The

lack of disease in fruit developing during the summer may be associated with failure of the bacteria to survive in fruit at higher temperatures (12). The absence of pink disease in the lowland tropics also tends to confirm this theory.

Marbling Disease of Pineapple

Marbling disease is characterized by a brown appearance (11) and granular consistency of the infected tissue and is caused by strains of *A. peroxydans* Visser 't Hooft, other *Acetobacter* species, and *E. h.* var. *ananas* (Serrano) Dye (11). The etiology of marbling disease is similar to that of pink disease. Bacteria invade through the open flower (13). Symptoms develop in the mature unripened fruit 1 mo earlier than with pink disease, however (*unpublished*). In Taiwan, Yow (19) suggested that bacteria enter through growth cracks in the fruit. Midafternoon rainfall rapidly lowered fruit temperature and resulted in reduced air pressure inside the fruit. Thus, bacteria were drawn into the fruit through growth cracks. In Hawaii, fruit were inoculated with marbling bacteria 6 wk before harvest, allowed to dry, and sprinkled midday with cold (4.4 C) water until temperatures on the side of the fruit exposed to the sun dropped from 37.8 to 35 C. The inoculations were not successful (*unpublished*).

Bacteria responsible for marbling disease established through flowers are not as sensitive to postinfection temperatures or humidity as are the pink disease bacteria, as reflected by recovery of bacteria from infected flowers (Tables 2 and 3). The limiting factor for marbling disease appears to be the entrance of the bacteria into the flower rather than their subsequent survival. Application of a surfactant such as 2% Ortho X-77 (Chevron Chemical Co., Richmond, CA), with or without inoculation, increased infections without apparent phytotoxicity (Table 4). The strain causing disease in the uninoculated plants plus surfactant differed from the inoculated strain. Where conditions are conducive for marbling disease (e.g., the lowland tropics), the disease is far less sporadic than pink disease.

Table 1. Relative infections of nectary gland and placental tissues of detached pineapple inflorescences with pink disease bacteria (*Gluconobacter oxydans*) and marbling bacteria (*Acetobacter aceti*) when exposed to 18 C and high humidity for 48 hr

Tissue	Percentage infection		Bacterial index[z]/flower	
	Pink	Marbling	Pink	Marbling
Nectary gland	77	100	2.2	2.3
Placental	44	94	2.0	1.5

[z]0 = No growth, 1 = very light growth, 2 = moderate growth, 3 = heavy growth, and 4 = very heavy growth.

Table 2. Effects of five temperatures on infection of nectaries of detached pineapple inflorescences by pink disease bacteria (*Gluconobacter oxydans*) and marbling bacteria (*Acetobacter aceti*) under high humidity

Temperature (C)	Percentage infection		Bacterial index[z]/flower	
	Pink	Marbling	Pink	Marbling
13	100	98	2.6	2.3
18	97	100	2.6	2.6
24	75	100	1.7	2.6
29	25	100	1.1	2.6
35	6	100	1.0	2.0

[z]0 = No growth, 1 = very light growth, 2 = moderate growth, 3 = heavy growth, and 4 = very heavy growth.

Fig. 1. Inoculation of flowers of a detached pineapple inflorescence with pink disease bacteria.

Pineapple Fruit Collapse

Pineapple fruit collapse is caused by *Erwinia chrysanthemi* Burkholder et al and is the most serious disease of pineapple in Malaysia. Symptoms initially are a slight olivaceous green discoloration with some juice exudation, followed by complete collapse of the internal fruit tissues. On rare occasions, inflorescences and developing fruit become infected (8). The bacteria have been shown to enter the open flower and remain quiescent until 2–3 wk before maturity, when symptoms first appear (7). Cultivar differences in susceptibility have been reported; cv. Smooth Cayenne appears to be resistant (8). As with pink disease, the causal bacteria are thought to be carried to the flowers by insects, particularly ants, which feed on rotting fruit as well as on nectar on flowers (6).

Purple-Stain of Papaya

The etiology of purple-stain of papaya (1) is not well documented. However, pink disease of pineapple and purple-stain of papaya both occur in winter fruit, are very sporadic, and may be caused by strains of *E. herbicola*. The bacteria are present in both symptomless and purple-stained fruit (9) but are not recovered from postharvest dip tanks (hot or cold). Floral infections are not observed, and inoculations of flowers do not produce disease in fruit at maturity. Nevertheless, symptoms develop throughout the vascular tissue and latex ducts of naturally infected fruit. Infections appear to occur early in fruit rather than after harvest (A. M. Alvarez, *personal communications*).

Internal Yellowing of Papaya

Internal yellowing of papaya fruit (1) was recently studied by Nishijima et al (10). The disease is caused by a strain of *Enterobacter cloacae* (Jordan) Hormaeche and Edwards, which is closely related taxonomically to *E.*

herbicola (Enterobacter agglomerans), one of the three strains responsible for pink disease. Other unidentified strains of bacteria also appear capable of causing the disease. Floral infections may occur, since symptoms are present in the calyx end or middle of the fruit and the bacteria are found in flowers. Additionally, causal bacteria have been recovered from fruit flies (*Dacus dorsalis* Hendel) suspected of dispersing the pathogen.

Table 3. Effects of two temperatures at high and low relative humidity for 48 hr on infection of nectaries of detached pineapple inflorescences by pink disease bacteria (*Gluconobacter oxydans*) and marbling bacteria (*Acetobacter aceti*)

Temperature (C)	Treatment	Percentage infection		Bacterial index[z]/flower	
		Pink	Marbling	Pink	Marbling
18	In polyethylene bag	92	100	1.9	2.4
	Not bagged	23	95	1.0	2.4
29	In polyethylene bag	47	98	1.3	2.4
	Not bagged	14	88	1.1	1.6

[z]0 = No growth, 1 = very light growth, 2 = moderate growth, 3 = heavy growth, and 4 = very heavy growth.

Table 4. Effects of application of 2% Ortho X-77 on infection of pineapple by inoculated and naturally occurring marbling bacteria (*Acetobacter aceti*) as indicated by percentage of diseased fruit and severity index[y]

Treatment	Test 1		Test 2	
	Percentage diseased fruit	Severity index	Percentage diseased fruit	Severity index
Inoculated 5 wk preflower	0 a[z]	0.0	0 a	0.0
Inoculated semiweekly throughout flowering	0 a	0.0	17 ab	1.0
Inoculated semiweekly throughout flowering + 2% Ortho X-77	48 b	2.0	42 b	1.3
Uninoculated	0 a	0.0	6 a	1.0
Uninoculated + 2% Ortho X-77	75 b	3.4	59 b	2.5

[y]Severity index: 0 = no fruitlets showing symptoms, 1 = 1–2% of fruitlets showing symptoms, 2 = 3–5%, 3 = 6–10%, 4 = 11–25%, 5 = 26–50%, 6 = 51–100%.
[z]Means in a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Waller-Duncan multiple range test.

Conclusions

The bacterial diseases discussed are unique in that the flower appears to be the site for infection, a highly variable microflora is capable of entering the flower, the causal agent remains quiescent in the developing fruit, and symptoms develop when the fruits ripen. Additionally, these diseases are extremely sporadic under natural conditions. The unpredictable occurrence of these diseases and the length of the latency period have made etiological work very difficult and time-consuming. In pineapple, the etiology of pink and marbling diseases has been partially elucidated by using susceptible cultivars, artificial inoculations, and duplicate tests at varying times. Disease incidence appears to depend on contamination of flowers with bacteria, movement of causal populations into the flower, and survival in the flower for an extended latent period. Thus, environmental conditions must be favorable during at least three different phases of the etiology. With pink disease, bacterial populations do not appear to increase until the fruit ripens. Whether or not a nutritional relationship is established between the time the bacteria enter the nectary and the point when the population increases in the ripening fruit remains to be determined. The assumption is that without population increase during the quiescent period, no nutritional relationship exists and the main function of the nectary is a location for the bacteria to enter the developing fruit for survival until such time as fruit tissue becomes susceptible to bacterial movement in the fruit and population increase.

These diseases also represent a continuum in dysfunction with fruit maturity. Woltz (18) describes pathogens that are nonparasitic, or exopathogens. Certain strains of the bacteria responsible for pink disease cause no detectable symptoms in the ripe or overripe fruit but cause distinct symptoms in the cooked fruit tissue. Thus, a dysfunction in the fruit is not apparent, and the bacteria would not be considered pathogens or exopathogens. In contrast to no dysfunction in the ripe fruit, internal yellowing of papaya appears only in the fully ripe to overripe fruit, with complete collapse of the fruit structure. Fruit collapse of pineapple is intermediate in the development and ripening spectrum in that complete collapse can occur in mature unripened fruit. With marbling disease, symptom development occurs during fruit development before ripening.

The ecological significance of the various strains and multiple species involved in the development of these floral infections is essentially unknown. With the use of monoclonal antibody technologies, it should be possible to better follow the natural movement of the bacteria involved in these diseases.

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