

Epidemiology of Pink Disease of Pineapple Fruit

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ABSTRACT

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Pink disease of pineapple fruit is caused by strains of acetic acid bacteria. There are no external symptoms but, during the canning process, infected fruit develop a brownish-pink discoloration on heating. In the Philippines, the disease is rare except during August, September and October when disease incidence was 3.9%, 10.2%, and 3.6%, respectively (10-year average). High disease incidence in fruit harvested and canned in September takes place only when flowering in

May and June occurs during wet weather (>25 cm of rain per month) preceded by 3-4 months of dry weather (<9 cm of rain per month). This hypothesis may also apply in Hawaii when dry, summer stress conditions, followed by wet-blooming cycles in November and December, lead to high disease incidence in March.

Additional key words: *Ananas comosus*, *Acetomonas* sp.

Pink disease of pineapple [*Ananas comosus* (L.) Merr.] was first described from Hawaii in 1915 (3) and today is known to occur additionally only in Australia (5) and the Philippines.

Although the nomenclature of the causal bacterium has not been completely resolved, studies in Hawaii (1) and the Philippines (D. K. Kontaxis, Philippine Packing Corporation, Manila, Philippines, *personal communication*) indicate that the disease is most likely caused by strains of acetic acid bacteria belonging to the genus *Acetomonas*.

Bacteria are carried by insects into blossom cups of pineapple during flowering where they utilize ethanol from yeast metabolism as a carbon source (G. G. Dull, Pineapple Research Institute, Honolulu, Hawaii, *personal communication*). Under certain environmental situations, as discussed in this paper, the bacterial pathogen invades the ovary of the fruitlet and then grows into the ripening tissue.

The unique characteristic of pink disease is that infected fruit appear normal until canning, when a light-brown-pink discoloration develops in diseased tissue during the cooking process. Rarely, diseased fruit tissue may show slight discoloration prior to cooking. Diseased tissue may be limited to a single fruitlet, several fruitlets or may involve more than half of the entire cylinder. After removal of the shell, diseased fruit may be detected easily by boiling fruit cylinders 10-15 minutes in water. The discoloration is apparently due to the production of a diketogluconic acid by the bacterium during the infection process (G. G. Dull, Pineapple Research Institute, Honolulu, Hawaii, *personal communication*).

Another interesting characteristic of the disease in the Philippines is that major epidemics occur only during

September of certain years. The Philippines was an ideal location to study this phenomenon because fields were flowering or being harvested on the plantation every month of the year. Plants are sprayed with ethylene at approximately 11 months of age to initiate the flowering and fruiting cycle. About 3 months later, flowering occurs in acropetal sequence with one to several flowers opening each day for 3-4 weeks. The fruit is then harvested about 3 months after the termination of flowering.

In preliminary observations and studies in the Philippines, it was established that high rainfall during the flowering cycle was necessary for high pink disease incidence and that low precipitation during blooming resulted in low pink disease at harvest.

It was also, noted, however, that there were a number of occasions of high rainfall during blooming and low pink disease incidence at harvest. This situation did not necessarily negate the importance of rainfall during the flowering cycle but may have simply indicated the existence of some other limiting factor. This report gives evidence that the limiting factor for high pink disease during the summer epidemic periods in the Philippines is the annual rainfall distribution and that epidemics occur primarily when flowering occurs during sudden wet weather preceded by long, dry, periods.

MATERIALS AND METHODS

Studies were made with the variety Smooth Cayenne in commercial plantation fields at Del Monte, Bukidnon, Philippines. The plantation is located at ~900 m elevation at 9° N latitude on the island of Mindanao. Plantation rainfall records and disease incidence, as determined by cannery records and field tests over a 10-year period, were

utilized to relate environment to disease incidence. Hygrothermographs were used to monitor temperature and humidity continuously for a 2-year period at three locations on the plantation. Environmental conditions prior to, during, and after flowering received special attention. The effect of rainfall on nectar dilution was determined by measuring with a hand refractometer the total soluble solid content of nectar withdrawn from the blossom cups of flowers exposed to varying amounts of rainfall. Over 1,000 samples were taken during a 2-year period. Many inoculations were made in the field under a variety of environmental situations. Inoculum was produced by growing *Acetomonas* as standing cultures at 27 C in fresh pineapple juice collected at the cannery. The juice was filtered through cheesecloth, sterilized by autoclaving and was adjusted to pH 4.5. Log phase cells from this liquid medium were collected by centrifugation at ~5,000 g for 10 minutes. Centrifuged cells were resuspended in sterile distilled water and then adjusted turbidimetrically to contain ~10⁵ colony-forming units per milliliter. For field and greenhouse inoculations, approximately 0.1 ml of the inoculum was placed in the blossom cups of individual fruitlets with a glass syringe. In other studies varying amounts of inoculum were sprayed on the entire fruit.

The occurrence of pink disease bacteria (PDB) in the blossom cups of flowers was monitored for 2 years by sampling 500-700 flowers monthly. Flowers (24-48 hours old) were randomly selected and nectar withdrawn with a glass pipette. The nectar was placed into sterile pineapple pulp in test tubes in the field. Samplings were also made in older flowers of the shriveled remains of petals, stamens, and styles.

Identification of the pink disease bacterium.—A selective, simple, reliable technique was used to determine the presence of PDB in plant tissue. Pineapple fruit, about 10 days prior to harvest, were collected in the field, shelled, and cored. The remaining tissue was crushed, adjusted to pH 4.5, placed in test tubes and autoclaved. After inoculation of the pulp with the assay sample, the tubes were incubated 4 days at 27 C and then boiled 20 minutes in water. The occurrence after boiling of a dark-

brown pigment which permeated the pulp was a positive test for PDB. The technique was reliable in qualitatively detecting PDB in soil, plant tissue and on insects. Several cultures of *Acetomonas*, known to induce pink disease when inoculated into ripening fruit, were used initially to check the reliability of the described technique.

RESULTS

Rainfall distribution and incidence of disease.—Incidence of pink disease in the Philippines was high in September, 1957 (16.9%), 1958 (6.7%), 1961 (19.9%), and in 1966 (42.1%). The rainfall distribution pattern for these 4 years was characterized by 3-4 months of dry weather during January-April. These long, dry periods prior to flowering were broken abruptly by heavy rains in May and June. Fruit, flowering during these 2 wet months, were harvested in August and September (Table 1). Incidence of the disease during these 4 years was very low (<1.0%) except during the summer epidemic months.

The most serious epidemic of pink disease ever recorded occurred during summer, 1966. Disease incidence was low in April (0.9%) and May (0.8%), increased in June (3.4%) and July (7.6%), and reached epidemic levels in August (15.7%) and September (42.1%), before declining rapidly in October (3.0%). The peak of the epidemic lasted about 45 days from the latter part of August until the last week in September. Fruit harvested during August and September were exposed to drought conditions prior to blooming (19.5 cm total rainfall during January, February, March, and April) and heavy rainfall (35.5 cm) during the flowering cycle in May and June. Fruit that flowered in July (for October harvest), were exposed to two wet months, May and June, prior to blooming. Disease incidence declined rapidly in October (3.0%).

Pink disease incidence in the Philippines was low in September 1960 (1.4%), 1962 (0.11%), 1963 (1.23%), 1964 (0.54%), and 1965 (1.97%). Rainfall distribution patterns for low disease-September-months differed from high disease-September-months in that rainfall was more equally distributed prior to the blooming cycle (Fig. 1, 2).

TABLE 1. Rainfall distribution and incidence of pink disease of pineapple at Del Monte, The Philippines, 1957-1966

Year	Month												September pink disease (%)
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
1957	6.5 ^a	5.7	12.0	13.7	5.7	27.7	24.2	35.7	30.0	12.5	13.7	3.5	16.9 ^b
1958	2.5	3.0	3.0	6.2	13.5	26.2	35.5	28.2	17.7	17.0	43.5	19.0	6.7
1959	7.0	1.2	9.7	2.2	26.7	34.2	47.2	28.7	30.2	14.2	5.5	7.5	2.1
1960	7.3	13.7	16.5	22.0	17.7	25.7	48.2	28.0	39.7	23.2	29.7	15.2	1.40
1961	29.0	8.0	9.5	9.5	34.2	18.7	39.2	29.7	16.0	22.7	14.5	12.2	19.9
1962	16.5	27.3	18.0	5.0	22.0	26.2	24.2	28.0	36.7	9.7	18.5	16.7	0.1
1963	23.0	17.3	13.7	3.2	11.5	25.7	23.0	31.7	24.0	15.2	4.5	9.5	1.2
1964	5.0	17.7	3.5	34.2	45.7	32.7	22.7	14.2	50.5	21.2	30.7	15.2	0.5
1965	28.7	13.7	17.0	14.5	21.2	34.7	21.0	41.7	24.5	18.5	10.0	8.5	1.9
1966	4.2	3.7	4.5	7.0	35.5	21.5	42.7	24.5	14.5	24.2	19.0	12.0	42.1
Avg	10.8	9.3	8.9	9.8	19.5	22.8	27.3	24.2	23.6	14.8	15.8	9.9	

^aRainfall in centimeters.

^bIncidence of pink disease in the cannery based on a sample of at least 2,000 fruit per day.

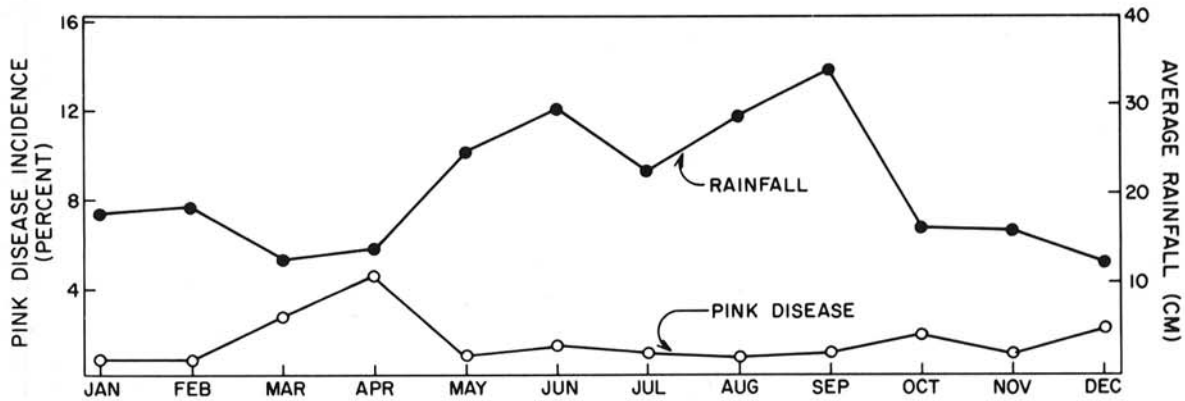


Fig. 1. Distribution of rainfall and incidence of pink disease of pineapple fruit at Del Monte, The Philippines. Data are the average monthly rainfall and incidence of disease during 1962, 1963, 1964, and 1965 when disease incidence during the summer epidemic period was low.

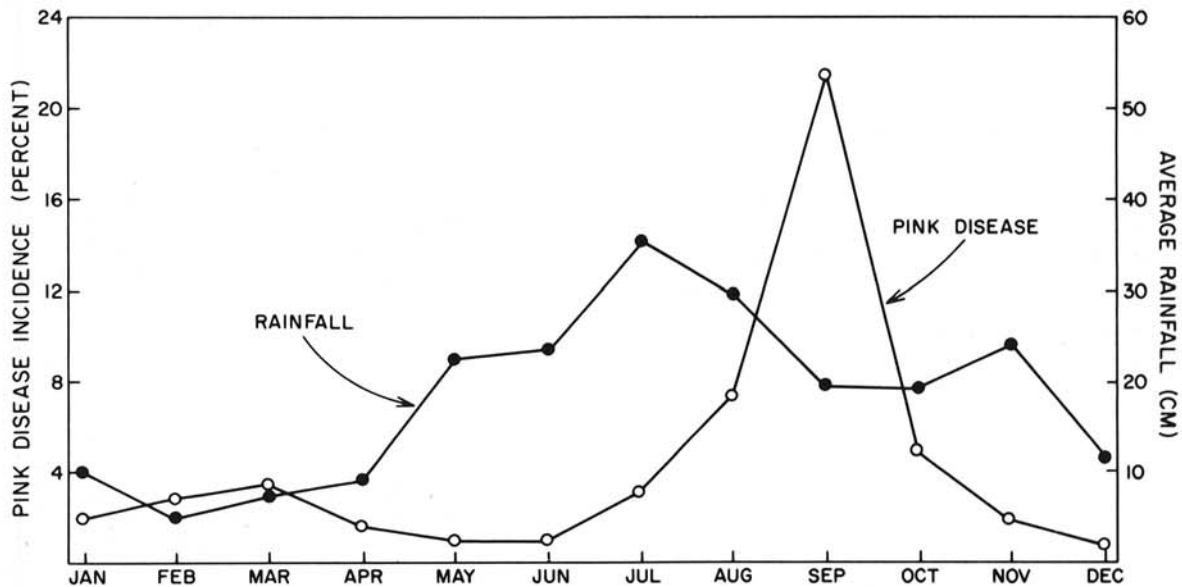


Fig. 2. Distribution of rainfall and incidence of pink disease of pineapple fruit at Del Monte, The Philippines. Data are the average monthly rainfall and incidence of disease during 1957, 1958, 1961, and 1966 when disease incidence during the summer epidemic period was high.

Flowering during wet weather, if not preceded by stress conditions caused by low rainfall, resulted in low pink disease incidence. For example, September (10-year average, 23.6 cm) is one of the three wettest months of the year but incidence of pink disease is low during December (1.21%).

The same situation exists for plants flowering during July and August, the two wettest months of the year. Incidence of disease is low (<2% - 10-year average) in fruit harvested in October and November. Incidence of disease is also low to nonexistent in plants flowering during the dry months of December, January, February, March and April. Relative humidity and temperature prior to,

during, or after flowering were not related to the incidence of disease.

Although pink disease is less important commercially in Hawaii, a situation similar to that in the Philippines exists regarding rainfall distribution and incidence of disease. Serious epidemics have been reported only during winter months and primarily in March. Dry summer stress conditions, followed by wet-blooming cycles in the fall lead to high winter disease incidence. For example, the most serious epidemic of pink disease in Hawaii occurred in March, 1964. Rainfall prior to blooming was: August 1.0 cm, September 5.7 cm, October 4.0 cm, and November 8.5 cm. Rainfall during

the flowering cycle in December totaled 21.7 cm. Fruit which flowered during this first wet month were harvested in March, when incidence of disease was 30-40% in many fields.

Seasonal occurrence of pink disease bacteria.—Pink disease bacteria were common in blossom cups and flower parts throughout the year. Positive recoveries ranged from 1.9% to 88.9%. There was no correlation between occurrence of PDB in flowers during the flowering cycle and disease at harvest. During the first two wet months of 1966 (May, June), 47% of 1802 flowers sampled contained PDB. Fruit flowering during this period, as previously discussed, had a very high incidence of disease in September (42.1%). Similar studies during August and September revealed that 40% of the flowers were infested with PDB. August and September rainfall was high (24.5, 14.5 cm, respectively) but disease incidence in November and December was low (1%).

Effect of moisture stress prior to flowering and of rainfall during flowering on invasion of ovaries by PDB.—Uniform plants of similar age were dug from plantation fields, transplanted into 20-liter containers, and placed in the greenhouse. Plants were stressed for water, or not, depending on the treatment, for 2 months prior to blooming by withholding irrigation until collapse of cells in the water-storage tissue of leaves. This technique had been used previously to demonstrate drought conditions in pineapple (2). During the flowering cycle plants were inoculated through the flowers as previously discussed and then exposed to a "wet" or "dry" treatment. The "wet" treatment consisted of misting plants for 15 minutes, every 2 hours for 4 weeks; whereas plants in the "dry" treatment were watered only from the base. The misting device delivered the equivalent of ~5 mm rainfall per hour. Ovaries of individual fruitlets were removed 4 weeks after inoculation to assay for PDB. Table 2 shows the effect of moisture stress prior to blooming on invasion of ovaries by PDB. The only significant invasion, 39% of 265 inoculated flowers, occurred in the "dry-vegetative, wet-flowering" treatment.

TABLE 2. Effect of moisture stress prior to flowering as a predisposing factor for infection of pineapple fruit by pink disease bacteria (PDB)

Treatments		Number of ovaries sampled	Recovery of PDB (%) ^c
Vegetative	Flowering		
Dry ^a	Wet	265	39.0
Wet	Wet	208	2.4
Dry	Dry	163	1.5
Wet ^b	Dry	212	0.9

^aWater withheld for 2 months prior to flowering, followed by water sprays of 15 minute duration during flowering every 2 hours for 4 weeks.

^bPlants watered as necessary prior to flowering, then only watered from base.

^cOvaries removed 4 weeks after infestation of blossom cups and PDB determined by use of the pineapple-pulp technique.

When fast green dye (1%, w/v) was introduced into the blossom cups of flowers from plants in the four treatments (Table 2) there was rapid dye movement into ovaries through cracks at the base of the blossom cup only in the "dry-vegetative, wet-flowering" treatment.

Rainfall and nectar dilution.—Since preliminary studies (R. B. Hine, *unpublished*) indicated no reproduction of PDB in the high sugar concentrations found in nectar and that rainfall during flowering seemed essential for infection, the effect of rainfall on nectar dilution was investigated. Over 1,000 samples of nectar were taken on several occasions from the blossom cups of recently opened flowers. In one study total soluble solids in 80% of 250 samples from flowers opening in the absence of rain ranged from 20-26% (equivalent to about 25-30% sucrose, w/v) with no readings below 18%. Rainfall, either simulated in the greenhouse or in the field greatly reduced these values. In a field sampling, typical of several, nectar collected from 250 newly opened flowers exposed to 2 cm of rain in 24 hours had total soluble solids ranging from 1.7 - 12.3 with an average of 6.9%.

DISCUSSION

When these studies were initiated a number of interacting factors were candidate explanations for the erratic epidemics of pink disease of pineapple fruit: (i) inoculum potential, (ii) vector populations, (iii) environment during infection, and (iv) host susceptibility. Since the first two were high throughout the year, they were considered unimportant as factors limiting disease incidence. However, considerable information indicated that infection took place primarily through flowers when wet weather occurred during flowering if preceded by moisture-stress. This did not preclude infection during other stages of fruit development but simply emphasized that flowers were the most common and most important entry site for bacterial invasion and that environmental conditions prior to flowering were important predisposing factors. Proof that PDB invaded fruit primarily through flowers was also based on data collected from inoculations of fruit of varying ages. Fruit sprayed with PDB after flowering rarely became diseased regardless of rainfall or temperature patterns.

Also supporting the theory that flowers are the major portal of entry, is limited experimentation, (R. B. Hine, *unpublished*), that demonstrated control of pink disease if insects were prevented from visiting flowers during the flowering cycle by the use of insect proof meshes.

Rainfall is a critical factor in the infection process because optimum growth of PDB occurs in nectar diluted to 5-10% total soluble solids and no growth occurs in undiluted nectar. Also, rainfall is important because PDB are susceptible to desiccation (R. B. Hine, *unpublished*). Pink disease bacteria, introduced into blossom cups during dry weather, could not be recovered after 4-5 weeks as assayed by the pineapple-pulp technique. The effect of desiccation on viability was corroborated in laboratory studies where PDB could not be recovered from glass slides after exposure to a relative humidity of 75% at 27 C after 4 weeks.

Rainfall, however, without the predisposing factor of moisture-stress prior to flowering, is not a significant

factor in infection. This is convincingly demonstrated by noting the low incidence of disease in fruit flowering during the three wettest months of the year, July, August and September, even in the presence of high inoculum potentials.

Successful invasion of the ovaries by PDB is apparently due to the development of cracks in the base of blossom cups under "dry-vegetative, wet-flowering" sequences. Studies in Hawaii (4) indicated that blossom cups during flowering follow a pattern of lignification, suberization, and the formation of a periderm-like layer. Since the lining of the blossom cup is rigid, any rapid growth of internal tissue causes small cracks of various orientation to appear in the base of the cup (4). This phenomenon occurs when plants are subjected to low moisture condition followed abruptly by high moisture levels. It should be noted, also, that fruit increases in weight from about 170 g during flowering to about 2,150 g at maturity (6).

Although stylar canals and nectary ducts occur in

pineapple flowers, their role as possible portals of entry for PDB into the ovary needs elucidation.

LITERATURE CITED

1. BUDDENHAGEN, I. W., and G. G. DULL. 1967. Pink disease of pineapple fruit caused by strains of acetic acid bacteria. *Phytopathology* 57:806 (Abstr.).
2. KRAUSS, B. H. 1949. Anatomy of the vegetative organs of the pineapple, *Ananas comosus* (L.) Merr. II. The leaf. *Bot. Gaz.* 110:333-404.
3. LYON, H. L. 1915. A survey of the pineapple problems. *Hawaii. Plant. Rec.* 13:125-139.
4. OKIMOTO, M. C. 1948. Anatomy and histology of the pineapple inflorescence and fruit. *Bot. Gaz.* 110:217-231.
5. OXENHAM, B. L. 1957. Diseases of the pineapple. *Queensl. Agric. J.* 83:13-26.
6. SINGLETON, V. L. 1965. Chemical and physical development of the pineapple fruit. I. Weight per fruitlet and other physical attributes. *J. Food Sci.* 30:98-104.