The Field Induction of Bacterial Pink Disease in Pineapple Fruit

K. G. Rohrbach and J. B. Pfeiffer

Associate Plant Pathologist and Research Associate, respectively, Department of Plant Pathology, University of Hawaii, Honolulu 96786.

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ABSTRACT

Pink-disease bacteria were applied to a hybrid pineapple cultivar at various stages of inflorescence development. Significant levels of pink disease occurred only when bacteria were applied to open flowers. Inoculum levels of at least 1×10^8 cells per ml were required to induce consistently high levels of disease. Disease levels in two hybrid cultivars varied with the time of the year tested, and were much higher than observed in the standard Smooth Cayenne cultivar.

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Additional key words: bacterial disease, acetic acid bacteria, inoculation techniques, flower infection.

Pink disease of pineapple was first reported in Hawaii by Lyon (4). The disease has been attributed to flower infection by various strains of acetic acid bacteria (1). The disease occurs most frequently in Hawaii during March and April in Smooth Cayenne, the commercial cultivar in Hawaii (Pineapple Research Institute of Hawaii, *unpublished*). However, even then, the occurrence is very sporadic from year to year (5). The erratic occurrence of the disease has prevented the gathering of valid field data. The studies reported here were undertaken to develop field techniques to screen new hybrid cultivars for resistance, and to elucidate the field etiology of the disease.

MATERIALS AND METHODS.—Two hybrid pineapple cultivars [*Ananas comosus* (L) Merr., A and B] having higher natural pink disease susceptibility than Smooth Cayenne were grown according to standard cultural practices (2). The plots in all tests comprised six-to-eight plants and were replicated four times in a randomized complete block design.

Preparation of bacterial inoculum.—A pink-disease bacterial isolate, No. 180, tentatively identified as *Acetomonas oxydans* (Henneberg) Bergey et al. (A. C. Hayward, *unpublished*) was used in all studies. The isolate was cultured for inocula for 48 hours at 29 C on a medium containing 1% yeast extract, 2% dextrose, 2% finely divided calcium carbonate (Mallinckrodt #4052), and 2% agar (w/v). Bacteria were scraped with a rubber policeman, washed from the petri dishes with tap water, filtered through cotton, and diluted to a stock concentration of approximately 1×10^9 cells per ml (0.3 O.D. at 420 nm on a Bausch and Lomb Spectronic 20 colorimeter being 1×10^8). Bacterial suspensions were applied over developing inflorescences in the morning with a compressed air sprayer at the rate of 25 to 50 ml per plant.

Stage of inflorescence development tests.—Plants of cultivar A were forced chemically (3) on 15 July and 19 August to initiate flowering for harvest dates in February and March. For each forcing date, a single inoculation of 1×10^8 cells per ml was applied at different stages of inflorescence development. In standard pineapple terminology these stages are: 1-inch (2.5 cm) open heart, defined as the stage in which the growing point has opened to 1 inch in diameter exposing the emerging inflorescence; early cone, the stage in which one-third of the flower buds are visible; late cone, the stage in which all flower buds are visible; midflower, the stage in which anthesis is completed in the central portion of the inflorescence; and dry petal, the stage in which anthesis is completed for all flowers. These stages for cultivars A and

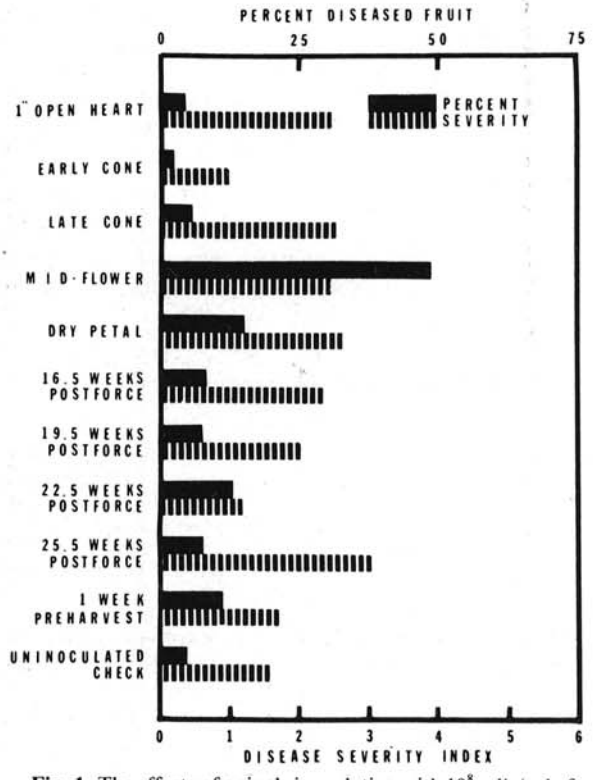

Fig. 1. The effects of a single inoculation with 10^8 cells/ml of a pink-disease bacterial isolate at various stages of pineapple inflorescence development on the percent diseased fruit and severity index scored as 0 = no fruitlets showing symptoms, 1 = 1-2% of the fruitlets with symptoms, 2 = 3-5%, 3 = 6-10%, 4 = 11-25%, 5 = 26-50%, and 6 = 51-100% in cultivar A in pineapple harvested in early March-April.

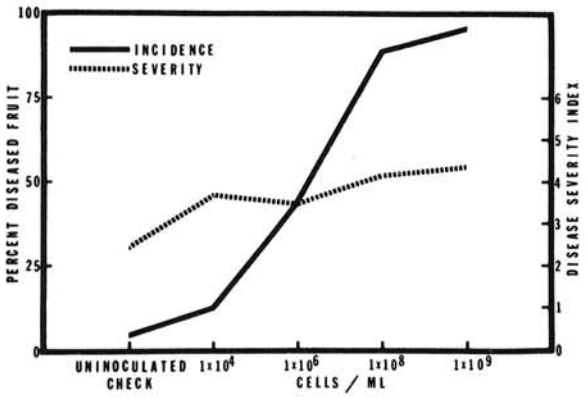

Fig. 2. Effects of four inoculum levels of a pink-disease bacterial isolate applied five to seven times during flowering on the percent diseased fruit and disease severity index in cultivar A in early April harvested fruit [asterisk denotes significance difference (P = 0.05) from the uninoculated check]. Disease severity index was scored as 0 = no fruitlets showing symptoms, 1 = 1-2% of the fruitlets with symptoms, 2 = 3-5%, 3 = 6-10%, 4 = 11-25%, 5 = 26-50%, and 6 = 51-100% in cultivar A in pineapple harvested in early March-April.

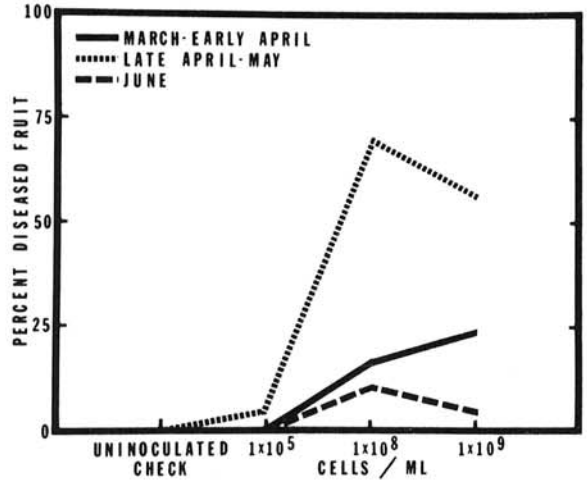

Fig. 3. The effects of three inoculum levels of a pink-disease bacterial isolate applied twice during midflower on the percent diseased fruit in cultivar B for three different harvest periods [asterisk denotes significant difference (P = 0.05) from the uninoculated check].

B represent approximate times from forcing of 7 weeks for 1-inch open heart, 8 weeks for early cone, 9.5 weeks for late cone, 10.5 weeks for midflower, and 13.5 weeks for dry petal. Additional inoculations were made at 16.5, 19.5, 22.5, and 25 weeks post-force, and, 1 week preharvest.

Inoculum-level tests.—Plants of cultivar A were forced on 7 and 28 August to result in harvest dates in February-early March and late March-Aptril. Five to seven semi-weekly inoculations were made with bacterial suspensions of 1×10^9, 1×10^8, 1×10^6, and 1×10^4 cells per ml.

Plants of cultivar B were forced on 21 August, 21 September, and 25 October to give harvest dates of March-April, April-May, and June, respectively. Treatments consisted of two inoculations during midflower stage at 1×10^9, 1×10^8, and 1×10^5 cells per ml. An uninoculated check was included in all tests for comparison.

Evaluation of disease.—Fruit were harvested when approximately 50 to 100% of the fruitlets were yellow. Disease was evaluated by removing the fruit shell and autoclaving both the shell and cylinder for 20 to 25 minutes at 1.97 kg-force/cm^2 (28 psi) steam pressure. Dark-brown to black discoloration following heating is the economically damaging symptom of pink disease. Incidence was recorded as percent diseased fruit and severity of this discoloration was scored as: 0 = no fruitlets showing symptoms; 1 = 1-2% of the fruitlets with symptoms; 2 = 3-5%; 3 = 6-10%; 4 = 11-25%; 5 = 26-50%; and 6 = 51-100%. Severity does not distinguish between multiple fruitlet symptoms developing from a single fruitlet invasion and multiple fruitlet invasions. Incidence data were analyzed using an analysis of variance and Duncan's multiple range test for significance between the means.

RESULTS.—Pink disease symptoms developed at significant levels only when inoculations were made at the midflower stage (Fig. 1). Disease incidence in fruit harvested in early March-April was higher than in fruit harvested in February-early March, but the susceptible cases.

In the inoculum-level tests with cultivar A where inoculum was applied five to seven times during flowering, the highest disease incidence occurred with the highest concentrations of inoculum, 10^8 or 10^9 cells per ml. With 10^6 cells per ml, disease incidence was only one-half that of the higher inoculum levels (Fig. 2). Disease incidence and severity were similar in fruit harvested during both of the February-March periods. In cultivar B, tests in which inoculum was applied only twice during midflower, 10^8 and 10^9 cells per ml induced significant levels of disease in fruit harvested in late March-early April and late April-May, but not in fruit harvested in June (Fig. 3). Disease severity was similar at inoculum concentrations where disease occurred, but varied between harvest periods (Fig. 4).

DISCUSSION.—The study of pink disease of pineapple in Hawaii has been severely limited in the past by the inability to predict disease occurrences and to artificially induce disease in the field. Although injection of pink-disease bacteria into maturing fruit produces symptoms, the technique does not distinguish apparent strain differences (1).

Preliminary work [(1) and also the *unpublished* Pineapple Research Institute of Hawaii Report by J. B. Smith and I. W. Buddenhagen], as well as work on detached pineapple inflorescences (5), has indicated that flowering is the stage of development when pink-disease bacteria enter the internal nectary and placental regions of the flower. The results of this study substantiate that the open flowers are the site and stage of entry of inoculum that later results in pink disease.

High levels of pink disease were induced under field conditions by a single spray inoculation at flowering.

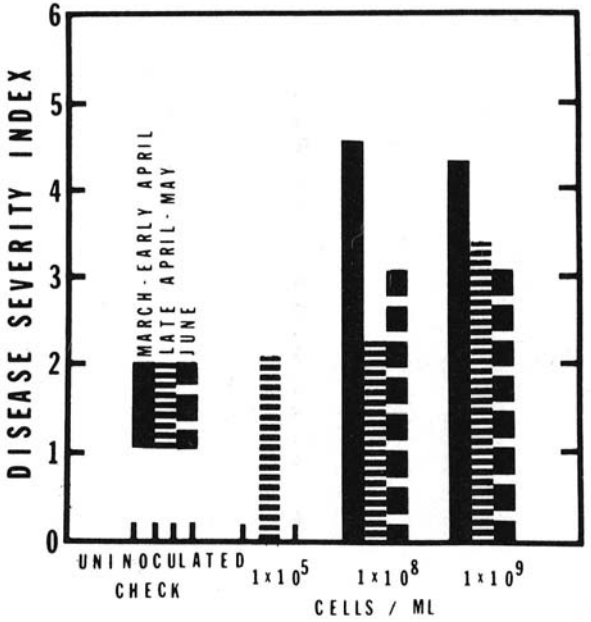

Fig. 4. The effects of three inoculum levels of a pink-disease bacterial isolate applied twice during midflower on the disease severity index in cultivar B for three different harvest periods. The disease severity index was scored as 0 = no fruitlets showing symptoms, 1 = 1-2% of the fruitlets with symptoms, 2 = 3-5%, 3 = 6-10%, 4= 11-25%, 5 = 26-50%, and 6 = 51-100% in cultivar A in pineapple harvested in early March-April.

Although single and multiple inoculations were not compared in the same test, multiple inoculation during the flowering period induced a much higher disease incidence. Normally, only one whorl of flowers, 5-10, opens on any given day, and approximately 21 days are required for the 100-200 flowers per fruit. Thus, multiple inoculations probably increased disease incidence by increasing the number of flowers exposed to inoculum.

Disease incidence also was closely related to inoculum level. In contrast, disease severity was not related to the number of flowers exposed or to the inoculum level, but was affected by harvest period.

In conclusion, the ability to induce pink disease under field conditions has led to work now underway on screening of new hybrid cultivars for resistance and on studies on the etiology of the disease.

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ERRATUM, VOLUME 65

Page 804, column 2, sixth line missing. Second sentence should read: "Disease incidence in fruit harvested in early March-April was higher than in fruit harvested in February-early March, but the susceptible stage of inflorescence development was the same in both cases."