# First report of pink fruit disease of pineapple in Tanzania

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## First report of pink fruit disease of pineapple in Tanzania.

**Abstract — Introduction**. In Tanzania, an unknown disease affecting pineapple fruits was reported on a large scale plantation in November 1991, after the onset of rains. The etiology of this pineapple fruit disease was therefore investigated. **Materials and methods**. Surveys of the plantation affected were done during January–March in 1992 and 1993. For each sampling, four to six pineapples were collected depending on the farm size. Isolations were done from affected fruit tissue. Several tests to characterize strains were used. In addition, the ability of the strains to utilize different carbon sources was tested using the Biolog system. Pathogenicity tests were carried out on young and mature fruits. **Results**. Incidence of the disease in the plantation studied ranged from 1.2 to 10%. However, in some areas of this plantation, no infected pineapple fruits were recorded. Isolations on culture media yielded, after 48 h of incubation, yellow bacterial colonies. Each of the 15 isolates tested induced symptoms of pink disease within 10 d of inoculation, which resembled those observed in naturally infected plants. Strains were found to have similar characteristics to those of *Erwinia herbicola*. **Discussion**. The relatively high incidence of the disease in the plantation studied indicates that the disease may be economically important under intensive large scale production in Tanzania. However, the disease may be a lesser problem in extensive production systems which are dependent on natural flower induction occurring mostly during the dry season, less favorable to infection. (© Elsevier, Paris)

**Tanzania / *Ananas comosus* / plant diseases / microbiological analysis / bacterioses / *Erwinia herbicola***

## La maladie du « rose » de l'ananas signalée pour la première fois en Tanzanie.

**Résumé — Introduction**. En Tanzanie, une maladie inconnue touchant le fruit fut observée dans une plantation industrielle d'ananas en novembre 1991, après le début des pluies. L'étiologie de cette maladie a alors été étudiée. **Matériel et méthodes**. La plantation touchée a été suivie durant les mois de janvier à mars des années 1992 et 1993. L'échantillonnage a porté sur quatre à six fruits selon la taille de la parcelle. Des isolements ont été faits à partir de tissus malades. Plusieurs tests spécifiques ont été utilisés pour caractériser les souches dont l'aptitude à utiliser différentes sources de carbone a été testée, par ailleurs, en utilisant le système Biolog. La pathogénéicité des souches a été étudiée sur fruits jeunes et fruits mûrs. **Résultats**. Dans la plantation étudiée, la maladie a touché de 1,2 à 10 % des fruits. Cependant, en certains endroits, aucun ananas infecté n'a été détecté. Les isolements faits sur les différents milieux de culture ont donné, après 48 h d'incubation, des colonies bactériennes jaunes. Après 10 d d'inoculation, chacun des 15 isolats testés a induit, dans les fruits inoculés, des symptômes de la maladie du « rose », qui ressemblaient à ceux observés sur les ananas infectés naturellement. Les souches bactériennes isolées ont exprimé les mêmes caractéristiques que celles d'*Erwinia herbicola*. **Discussion**. L'impact relativement élevé de la maladie sur la plantation étudiée conduit à penser qu'elle pourrait avoir des répercussions économiquement importantes pour une production intensive à grande échelle en Tanzanie. Cependant, la maladie pourrait être moins dangereuse dans le cadre de systèmes de production extensifs basés sur l'induction florale naturelle, celle-ci intervenant surtout pendant la saison sèche, moins favorable à l'infection. (© Elsevier, Paris)

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## 1. introduction

In Tanzania, pineapple [*Ananas comosus* (L.) Merr.] is produced by small holder farmers, under extensive cultural systems. Important producing areas are the Dar-es-Salaam, Coast and Morogoro regions. Large scale plantations are very few and only had a recent development in the Coast region, which is situated along the west coast of the Indian ocean.

Production from small holder farmers is sold at local fresh fruit markets, processing plants and neighbouring countries. Large scale plantations have been established with the primary aim of producing fruits for export overseas. In the plantations, intensive cultural techniques including artificial flower induction are adopted in order to produce fruits throughout the year.

An unknown disease affecting pineapple fruits, variety Smooth Cayenne, was reported on a large scale plantation at Mlandizi in the Coast region, in November 1991, after the onset of rains. The disease was more severe at the end of the short rains of January–February 1992. Fruits appeared normal at harvest, but, when they were cut, some parts of affected fruits appeared water-soaked as if they were over-ripe. Such fruits lacked the normal aroma, while the immature fruits had no symptoms. About 32 to 35 acres (1 acre = 4047 $m^2$) out of 3000 were affected. Some plots were more affected than others; seriously affected plots were on upland areas where termite hills existed before the establishment of the crop.

The purpose of this study, therefore, was to investigate and establish the etiology of this pineapple fruit disease.

## 2. materials and methods

### 2.1. survey of the plantation

Surveys of the plantation affected and neighbouring fields were done during January–March in 1992 and 1993. The design and diagnosis method of Kirkby and Sperling [1] was used to survey each of the selected pineapple farms. Fruit samples were collected systematically at equal spaced points between the rows in a zigzag pattern covering the entire field. For each sampling, a total of four to six pineapple fruits were collected depending on the farm size. In each survey, fruit samples collected were brought to the laboratory for further investigation. Specimens from fields of neighbouring small holder farmers were also collected for examination. Tissue samples were examined microscopically [2, 3].

### 2.2. isolation

Small pieces of fruit tissue were cut from the inner edge of the brown and normal tissue from affected fruits and surface sterilized in 2.6% sodium hypochlorite for 2 min. The tissues were crushed in sterile distilled water and the resulting suspension was left to settle for 15 min in a laminar air flow chamber. A loopful of the suspension was streaked onto nutrient agar (NA), yeast dextrose carbonate agar (YDC) [2] and medium B of King et al. [4]. Some of the sterilized pieces were blotted dry on sterile filter paper, crushed on sterile glass slide and a loopful of the resulting liquid was also streaked on the same media.

Inoculated plates were incubated at 27 ± 2 °C for 4 d. Single colonies of the consistently recovered bacteria were purified by a series of transfers on YDC and NA. Purified cultures were maintained in 30% glycerol at –10 °C. Working cultures, however, were maintained on NA at 5 to 8 °C. Isolations were also attempted from immature and mature pineapple fruits without internal symptoms using similar procedures.

### 2.3. characterization of bacterial strain

Tests to characterize strains included growth rate, colony colour on YDC and NA, Gram reaction, aeration status, growth on Miller-Schroth (MS) agar [2, 5], growth at 36 °C, nitrate reduction, oxi-

dase and catalase tests [2, 6], tobacco hypersensitivity [7], pathogenicity tests on immature and mature pineapple fruits and on potato tuber pieces [2]. In addition, the ability of the strains to utilize different carbon sources was tested using the Biolog system [8]. All tests were replicated four times and repeated once. The reference strain of *Erwinia herbicola* was kindly provided by Dr. Regina Samson (Inra, Angers, France).

### 2.4. pathogenicity tests

Pathogenicity tests were carried out on young fruits at the dry petal stage (2 month old) [9] and on mature fruits at 2 to 3 weeks before harvest. Fruits were collected from disease free fields and held in water as described by Rohrbach [10] and Kontaxis [11]. Bacterial suspensions were prepared by flooding 48 h-old cultures grown on NA at 27 ± 1 °C with sterile distilled water, and adjusted turbidimetrically to the concentration of about $10^7$ colony forming units (CFU) per mL. Small areas of pineapple fruit were surface sterilized with 2.6% sodium hypochlorite and rinsed with distilled water. About 0.3 mL of the inoculum or sterile water (control) was injected into the fruit to a depth of about 3 cm, using a sterile 25-gauge needle attached to a 5 mL sterile hypodermic syringe. Each needle was used only once. The inoculated area was then sealed with melted candle wax to prevent entry of saprophytic or other pathogenic microbes. Inoculated fruits were maintained at 24–29 °C for 10 d; thereafter they were tested for pink fruit disease using modified procedures of Hine [12]. Fruits were shelled, sliced and placed into test tubes in quadruplicate, boiled for 15 min in water and observed for the occurrence of the pinkbrown pigment. Attempts to re-isolate the pathogen were also made from unboiled tissues using procedures previously described to fulfil Koch's postulate.

## 3. results

### 3.1. plantation survey

Based on the 2-year survey of the plantation, incidence of the disease ranged from 1.2% in some plots to 10% in severely affected plots. However, in some areas of the plantation no infected pineapple fruits were recorded. The mean incidence of the disease in 10 fields of small-holder farmers ranged from 0 to 0.01% (*table I*).

### 3.2. isolation

Isolations on culture media yielded, after 48 h of incubation, yellow bacterial colonies. These were consistently recovered on all media from pineapple fruits with pink fruit disease symptoms. The colonies were not fluorescent on KB [4] and when observed under both short and long UV-light. No other types of colonies were recovered. Cells of the recovered bacterium were non-motile and Gram-negative, oxidase negative and positive for catalase (*table II*).

### 3.3. pathogenicity tests

Each of the 15 isolates tested induced symptoms of pink disease within 10 d of inoculation. These symptoms resembled those observed in naturally infected

**Table I.**
Incidence of pink fruit disease of pineapple in various farms in the Coast region, Tanzania.

| Location | Farm size (acre)[a] | Incidence (%)[b] | | |
|---|---|---|---|---|
| | | 1992 | 1993 | Mean |
| Mboga | 2.5 | 0.00 | 0.00 | 0.00 |
| Chalinze | 10.0 | 0.01 | 0.01 | 0.01 |
| Ruvu | 15.0 | 0.00 | 0.00 | 0.00 |
| Mdaula | 24.0 | 0.03 | 0.01 | 0.02 |
| Mlandizi | 3 000.0 | 10.00 | 8.50 | 9.25 |
| Picha ya Ndege | 3.5 | 0.00 | 0.00 | 0.00 |
| Msata | 7.0 | 0.00 | 0.02 | 0.01 |
| Chalinze | 9.5 | 0.01 | 0.01 | 0.01 |
| Chalinze | 5.5 | 0.00 | 0.00 | 0.00 |
| Chalinze | 4.0 | 0.00 | 0.00 | 0.00 |

[a] Farm size classification:
less than 1 acre to 23 acres = small farms; more than 24 acres = large farms.

[b] Mean incidence of pink fruit disease is based on a survey conducted for 2 years.

plants. Differences in virulence between strains were not observed. Inoculated fruits appeared water-soaked, unlike the water-treated controls. When artificially inoculated mature fruits with symptoms resembling those of natural infection were shelled, sliced, placed in test tubes and boiled in water for 15 min, a pinkish dark-brown pigment developed; however, pigment development did not occur in artificially inoculated young pineapple fruits or in water-treated mature fruit controls. The pathogen was easily re-isolated 0–5 cm from the inoculation site of mature pineapple fruits. Re-isolated cultures were similar to the bacterial strains used for inoculation. Re-isolation from inoculated young pineapple fruits was only possible from the inoculated area, and at a very low frequency. On tobacco, all tested strains produced a weak hypersensitive reaction in 24 h, which became a strong reaction thereafter.

### 3.4. characterization of strains

Results of physiological, biochemical and nutritional tests are shown in *table II*. All 8 strains isolated from affected pineapple fruits were fast growing and

**Table II.**
Diagnostic tests used to characterize eight strains of *Erwinia herbicola* isolated from diseased pineapple fruits from the Coast region, Tanzania (YDC = yeast dextrose carbonate agar; NA : nutrient agar).

| Diagnostic test[a] | Strain identification number | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9[b] |
| Yellow colonies on YDC/NA | +[c] | + | + | + | + | + | + | + | + |
| Fast growth rate | + | + | + | + | + | + | + | + | + |
| Gram reaction | – | – | – | – | – | – | – | – | – |
| Anaerobic growth | + | ±[d] | – | – | ± | – | – | – | – |
| Growth at 36 °C | + | + | + | + | + | + | + | + | + |
| Growth on MS medium | + | + | + | + | + | + | + | + | + |
| Potato soft rot | – | – | – | – | – | – | – | – | – |
| Nitrate reduction | + | + | ± | + | + | + | + | + | + |
| Growth in 5% NaCl | + | + | + | + | + | + | ± | + | + |
| HR on tobacco | + | + | + | + | + | + | + | + | + |
| Acid production from: | | | | | | | | | |
| Inositol | + | + | + | + | + | + | + | ± | + |
| Mannitol | + | ± | + | ± | + | + | + | + | + |
| Sorbitol | ± | + | + | + | + | ± | + | + | + |
| Starch | + | + | + | + | ± | + | + | + | + |
| Parthogenicity on fruits: | | | | | | | | | |
| Young | – | – | – | – | – | – | – | – | – |
| Mature | + | + | + | + | + | + | + | + | + |
| Biolog: | | | | | | | | | |
| similarity to *Erwinia herbicola* | 0.69 | 0.71 | 0.79 | 0.70 | 0.80 | 0.87 | 0.76 | 0.74 | 0.90 |

[a] All tests were replicated four times and repeated once.
[b] *Erwinia herbicola* reference strain.
[c] + = positive; – = negative.
[d] Variable results between replicates.

produced yellow colonies on YDC and NA. They were Gram-negative rods, positive for nitrate reduction, anaerobic growth, grew at 36 °C and on medium containing 5% NaCl.

In addition, the strains did not cause soft rot in potato tuber tissue. These characteristics were similar to those of *Erwinia herbicola* (Lohnis) Dye. Strains were confirmed as *E. herbicola* by the Biolog computer program, with similarity indices ranging from 0.69 to 0.87 as compared to that of the reference strain which was 0.90 (*table II*).

## 4. discussion

Four bacterial pathogens have been reported to cause pink fruit disease of pineapple; *E. herbicola*, *Gluconobacter oxydans* (Henneberg) De Ley, *Acetobacter aceti* (Pasteur) Beijerinck (Syn. *A. liquefaciens*) and an unidentified *Erwinia* sp. [7–11, 13–18]. Based on biochemical, physiological and nutritional test results, and symptomatology produced in pathogenicity tests, and in comparison with published reports, the bacterium involved in causing pink fruit disease in the current study was identified as *E. herbicola*. This identification is further supported by the very high similarity indices in Biolog tests. In addition, tissue browning of the fruits affected did not occur until boiled or kept under high temperatures in packed containers, symptoms typical of pink fruit disease caused by *E. herbicola* [11, 16].

Although the epidemiology of the disease and its effect on pineapple production in Tanzania have not been studied, the relatively high incidence of the disease (1.2 to 10%) in the plantation studied indicates that the disease may be economically important under intensive large scale production. However, the low incidence (0 to 0.1%) in small holder plots suggests that the disease may be a lesser problem in extensive production systems (*table I*). High rainfall during the flowering cycle has been reported to be necessary for high pink fruit disease incidence [12]. These conditions agree with observations in the current study where high rainfall in November–December after a long drought period (July–October) resulted in high incidences of pink fruit disease. Artificial flower induction realized soon after the onset of the rains in the pineapple estate at Mlandizi may be a factor that favoured the outbreak of the disease. Production on a small scale being dependent on natural flower induction occurring mostly during the dry season (July–August) is less favourable to infection.

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## La enfermedad "rose" de la piña observada por primera vez en Tanzania.

**Resumen — Introducción**. En Tanzania, en una plantación industrial de piñas, se observó en noviembre de 1991 una enfermedad desconocida que afectaba al fruto tras el comienzo de las lluvias. Se comenzó un estudio etiológico de dicha enfermedad. **Material y métodos**. Se efectuó un seguimiento de la plantación afectada en durante los meses de enero a marzo de 1992 y 1993, utilizándose de cuatro a seis frutos, en función de las dimensiones de la parcela, para el muestreo. Se realizaron una serie de aislamientos a partir de tejidos enfermos. Se utilizaron varias pruebas específicas para caracterizar las cepas, además se utilizó el sistema Biolog para probar la aptitud de éstas para utilizar distintas fuentes de carbono. La patogenicidad de las cepas se estudió en frutos jóvenes y maduros. **Resultados**. En la plantación estudiada, la enfermedad afectó del 1,2 al 10 % de los frutos. No obstante, en algunos lugares no se detectó ninguna piña afectada. Los aislamientos efectuados en los distintos medios de cultivo dieron, tras 48 h de incubación, colonias bacterianas amarillas. Tras 10 h de inoculación, cada uno de los aislados probados ocasionó, en los frutos inoculados, síntomas de la enfermedad "rose", que se parecían a los de las piñas infectadas naturalmente. Las cepas bacterianas aisladas mostraron las mismas características que las de *Erwinia herbicola*. **Discusión**. El impacto relativamente importante de la enfermedad en la plantación estudiada, hace pensar en las importantes repercusiones económicas que se originarían en el caso de una producción intensiva a gran escala. Sin embargo, la enfermedad podría ser menos peligrosa en un contexto de sistemas de producción extensivos basados en la inducción floral natural, que tiene lugar principalmente durante la estación seca y es, por ello, menos propicia a la infección. (© Elsevier, Paris)

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