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Title: Two Bacterial Diseases of Solanaceous Crops on Guam: *Pseudomonas solanacearum* and *Xanthomonas vesicatoria*

Approved: _____
Theodore D. Allen, Chairman, Thesis Committee

Two bacterial plant pathogens of Solanaceae were shown to be *Pseudomonas solanacearum* and *Xanthomonas vesicatoria*. Comparative studies of N11, Saturn and Venus varieties of tomato indicated that Saturn and Venus were resistant to *P. solanacearum*, but they were less resistant to *X. vesicatoria* and nematodes and were less productive than N11.

TWO BACTERIAL DISEASES OF SOLANACEOUS CROPS ON GUAM: PSEUDOMONAS SOLANACEARUM AND XANTHOMONAS VESICATORIA

by

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TO THE GRADUATE SCHOOL:

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INTRODUCTION

Cases of bacterial wilt of tomato (Lycopersicon esculentum Miller) and eggplant (Solanum melongena L.) were observed to be common and increasing in number by personnel at the Guam Department of Agriculture. Affected tomato plants showed leaf epinasty and adventitious root formation followed by wilting and death. Eggplants showed loss of turgidity of the leaves followed by wilting and death. Bacterial streaming was observed when the cut stems of wilted plants were immersed in distilled water. These symptoms were similar to those described for the wilt caused by the bacterium Pseudomonas solanacearum E. F. Smith (Kelman, 1953).

Cases of bacterial fruit and leaf spot on tomato and pepper (Capsicum annuum L.) were also observed. These spots reduced the marketable value of the fruits and the efficiency of photosynthesis (Chester, 1959). Infected plants had 1-2 mm diameter brown leaf spots which had narrow chlorotic halos. Tomato fruit had 2-3 mm diameter dark brown spots extending 1-2 mm into the tissue. Bacterial streaming from the fruit and leaf spots were observed under the microscope. The symptoms resembled those caused by Xanthomonas vesicatoria (Doidge) Dows.

The tomato varieties Saturn and Venus were reported

resistant to P. solanacearum (Otis S. Twilley Seed Co., 1973) and, therefore, were tested for their resistance to the strain of P. solanacearum found on Guam. The extent to which Saturn and Venus could be used in areas infected with P. solanacearum would also be dependent upon their ability to grow and produce a crop in the presence of X. vesicatoria and nematodes, a perennial problem on Guam. Therefore, their resistance to X. vesicatoria and nematodes were compared to that of N11, the common commercial variety of tomatoes grown on Guam.

The purposes of this study were to identify the two bacterial plant pathogens isolated, and compare the ability of Saturn, Venus, and N11 varieties of tomato to survive and produce fruit on Guam in the presence of P. solanacearum, X. vesicatoria, and nematodes.

Literature Review

The literature review was conducted to confirm the tentative identification of the bacteria.

P. solanacearum has been shown to be destructive in various parts of the world (Doolittle, 1922; Zehr, 1969; and French and Sequeira, 1970) including sections of southern United States (Kelman, 1953; Kelman and Person, 1961; and Dukes, Morton and Jenkins, 1965). The classification and characteristics of this organism have been widely studied (Starr, 1945; Burkholder and Starr, 1984; Breed, Murray and Smith, 1957; Buddenhagen and

Kelman, 1964; Stanier, Palleroni and Doudoroff, 1966; and Sands, Schroth and Hildebrand, 1970). Buddenhagen, Sequeira and Kelman (1962) have divided P. solanacearum into three races according to virulence and host range. Hayward (1964) has defined four biotypes of P. solanacearum on the basis of carbon utilization.

Numerous techniques have been used to identify and differentiate P. solanacearum. Various selective media (Kelman, 1954; and Karganilla and Buddenhagen, 1972) and specific tests (Thornley, 1960; and Hildebrand, 1971) have been developed to identify and differentiate the organism from other pseudomonads. A tobacco leaf infiltration technique (Klement, 1963) can be used to differentiate between the races (Lozano and Sequeira, 1968, 1970). Characteristics used to differentiate between virulent and non-virulent forms of P. solanacearum were studied by Husain and Kelman (1958), Kelman and Cowling (1965), Ado-El-Dahab and El-Goorani (1969, 1972), Kelman and Hruschka (1970, 1973), and Coplin, Sequeira and Hanson (1972).

The bacterial wilt may be spread and increased by nematode infestation of host plants; however, nematode wounds are not required for root infection (Lucas, Sasser and Kelman, 1955; Kelman and Sequeira, 1962, 1965; Libman and Leach, 1962; Pitcher, 1963; Libman, Leach and Adams, 1964; Johnson and Powell, 1969; and

Feldmesser and Goth, 1970).

Resistant strains have been developed. The resistance of Saturn and Venus cultivars of tomato has been found to be polygenic and was not affected by nematodes (Jenkins, 1972).

Unlike P. solanacearum, the taxonomy of X. vesicatoria is less definite. Contradictory results obtained using the common criteria of host specificity (Wernham, 1948; Dye, 1958; Logan, 1960; Schnathorst, 1966; and Stall, Bartz and Cook, 1972) has led to attempts to use other means of classification. Biochemical tests have proven insufficient to differentiate species of Xanthomonas (Colwell and Liston, 1961a, 1961b; Dye, 1962; and Colwell, Moffett and Sutton, 1968). Serological differences have been useful for early detection of pathogenic forms of Xanthomonas which cause leaf spot on Solanaceae. They have not been useful for differentiation of species (Elrod and Braun, 1947; Morton, 1965; Morton, Dukes and Jenkins, 1965; Morton, O'Brien and Manning, 1967; and Charudattan, Stall and Batchelor, 1973). Electrophoresis of bacterial enzymes may prove helpful, though a taxonomic scheme using these techniques is not available at this time (Gill and Khare, 1968; and Taha, El-Sharkawy and Hursingh, 1968).

IDENTIFICATION

Materials and Methods

Isolation: Plant surfaces were disinfected by immersion in 0.5% (w/v) sodium hypochlorate solution for five minutes. Bacteria were isolated by streaking on Bacto plate count (PC) agar (Difco) and stored under mineral oil to maintain virulence (Kelman and Jenson, 1951).

Tobacco Hypersensitivity: This was determined by injecting the leaves of tobacco plants with a suspension containing 10^8 bacterial cells per ml of sterile distilled water.

Koch's Postulates: The bacterium isolated from wilted plants was tested in the following manner. Stems of young tomato plants and eggplants (N11 and BI, respectively, University of Hawaii) were punctured with sterile toothpicks which had been dipped in three day old colonies of the bacterium growing on PC agar. Alternately, the soil in which the plant was potted was inoculated with about 10^9 bacteria per kilogram of soil and a sharp knife was used to injure the plant roots. Wilted plants were tested for bacterial streaming, and the bacteria were isolated and compared to the inoculum.

Bacteria isolated from leaf spots were grown and

checked by injecting pepper and tomato leaves with concentrations from 10^3 to 10^8 bacteria per ml of sterile distilled water. Typical lesions were scored as positive.

Biochemical Tests: The following tests were used for identification: acetyl-methyl-carbinol production by the Voges-Proskauer test (Conn, Jennison and Weeks, 1957), alkaline proteolysis of milk (Dye, 1962), arginine dihydrolase production (Thornley, 1960), brown diffusible pigment formation on PC agar, gelatin hydrolysis on gelatin agar (Conn, Jennison and Weeks, 1957), catalase production (McClung and Lindberg, 1957), flagella stain (Collins and Lyne, 1970), green fluorescent pigment production on King's B medium (Ewing, 1972), indol production by Genezda's method (Conn, Jennison and Weeks, 1957), oxidase production with Key Diagnostic Reagent tablets (Key Scientific Products, Los Angeles, Ca.), oxygen requirements (Dye, 1962), starch hydrolysis on starch agar (Conn, Jennison and Weeks, 1957), sudanophilic granule production by Burdon's method (Conn, Bartholomew and Jennison, 1957), tetrazolium reduction (Kelman, 1954), thiosulfate reduction by Sulkin and Willett's method (Burnett, Pelczar and Conn, 1957), and urease production with Key Diagnostic Reagent tablets. Incubation temperature was 29°C.

Carbon Source Utilization: Solutions (0.5% w/v) of sugars and polyalcohols were made in M9 buffered salts

solution (Davis and Mingioli, 1950) which was modified by omission of citrate and iron. These solutions were sterilized by heating in boiling water for 30 minutes on three consecutive days. Inoculated tubes were incubated at 29°C and observed weekly for four weeks. Fluid from a tube containing bacterial growth was used to inoculate a fresh tube of medium containing the same carbon source. If growth occurred in the second tube the test was scored as positive. Liquid from the second tube was spotted on tetrazolium agar or PC agar; in all cases the resulting colonies were morphologically identical to those obtained with the original inoculum. If no growth occurred with a particular carbon source a fresh tube of that medium was inoculated to confirm the negative result.

Growth Rate: Bacteria were inoculated into a solution of 1% peptone and 0.5% sucrose (w/v) in distilled water. Cultures were vigorously agitated on an oscillating shaker at 29°C. Samples were taken from the cultures at 2-hour intervals and dilutions were plated on a medium containing 1% peptone, 0.5% sucrose and 1.5% agar (w/v) and incubated at 29°C.

Results

The following results were obtained for the wilt organism. Grey fluidal colonies were obtained on PC agar; a brown diffusible pigment appeared after 1 to 2 weeks. Colonies on tetrazolium agar were grey, fluidal and

irregular with pink centers. The bacterium was a gram negative rod. Its doubling time was 90 minutes. The bacterium wilted tomato and eggplants within 5 to 14 days. Infection could be obtained through stem puncture or soil inoculation.

Positive results were obtained for the following tests: oxidase production, sudanophilic granule production, thiosulfate reduction and tobacco hypersensitivity. Negative results were obtained for: acetyl-methyl-carbinol production, arginine dihydrolase production, gelatin hydrolysis, green fluorescent pigment production, indol production, starch hydrolysis and urease production. The organism utilized the following carbon sources: dextrose, dulcitol, galactose, glycerol, mannitol, mannose, sorbitol, sucrose and trehalose. It did not grow in adonitol, arabinose, cellobiose, fructose, inositol, inulin, lactose, maltose, melibiose, raffinose, rhamnose or xylose.

The following were the characteristics of the leaf spot organism. Yellow mucoid colonies were obtained on PC agar. The bacterium was a gram negative rod with one polar flagellum. Typical lesions were obtained on N11 tomatoes with a concentration of 10^3 bacteria per ml of sterile distilled water.

Positive results were obtained for the following tests: alkaline proteolysis on milk, catalase production,

gelatin hydrolysis, and tetrazolium reduction. Negative results were obtained for: acetyl-methyl-carbinol production, brown diffusible pigment production, green fluorescent pigment production, indol production, oxidase production, starch hydrolysis, sudanophilic granule production, thiosulfate reduction and urease production. The organism utilized the following carbon sources: cellobiose, dextrose, fructose, galactose, glycerol, maltose, mannose, melibiose, raffinose, sucrose, trehalose, and xylose. It did not grow in adonitol, arabinose, dulcitol, inositol, inulin, lactose, mannitol, rhamnose or sorbitol.

COMPARATIVE STUDY

Materials and Methods

Seeds of Saturn and Venus (Otis S. Twilley Seeds Co.) and seedlings of N11 (Guam Department of Agriculture) were planted in plastic pots which contained about 2 lbs. of the following mixture: 240 lbs. steamed soil, 4 cups bone meal, and 2 cu. ft. peat moss. Forty pots of each variety were planted. Additionally eight seedlings of purple eggplant (BI) and bell pepper, other important solanaceous crops on Guam, were also planted in the mixture.

Six plants of each, Saturn, Venus, N11 tomatoes, bell pepper, and eggplant, were infected by puncturing the stems with sterile toothpicks which had been dipped in three day old colonies of *P. solanacearum*. Two plants of each variety used as controls were punctured with the sterile toothpicks. Wilted plants were checked for bacterial streaming, and exudates were plated on tetrazolium agar. All plants were held at least three weeks.

Three plants of each variety of tomato were injected with one of eight ten-fold dilutions of *X. vesicatoria* ranging from 6×10^8 to 6×10^1 bacteria per ml of sterile distilled water. All leaves of each plant were

injected. Three plants of each variety used as controls were injected with sterile distilled water. Spots were checked for bacterial streaming under a microscope and exudates were plated on PC agar.

Six pots of each of the three varieties of tomato plants were modified by adding chopped roots containing nematode knots from locally grown Marglobe tomatoes to compare the effects of nematode infestation.

Several plants of Saturn and Venus were grown to maturity to check their abilities to produce fruit on Guam. The plants were grown in large planters which contained 2/3 soil and 1/3 peat moss. Ortho Tomato Plant Food and bone meal were used as fertilizers. Two N11 plants were grown in the same planters.

Results

All bell pepper, eggplant and N11 tomatoes artificially infected with P. solanacearum wilted within two weeks. One Saturn also wilted but with this exception, Saturn and Venus varieties remained free of symptoms. All wilted plants were positive for bacterial streaming and the bacteria that grew on tetrazolium agar resembled that of virulent P. solanacearum. Wilted plants are shown in Figures 1 and 2.

The following was the typical plant reaction to leaf spot caused by X. vesicatoria. The bacterium invaded the tissue through openings such as stomata or wounds. A

Figure 1. Control and experimental pepper plants one week after infection with P. solanacearum.

Figure 2. Control and experimental eggplants one week after infection with P. solanacearum.

spot which turned yellow and then brown developed at the site of infection. A halo of yellow surrounded the brown dead tissue of the plant and indicated advancement of the disease. Eventually the whole leaf turned yellow and fell. At this time the bacterium had advanced to produce spots on other parts of the plant (stems, leaves, and petioles).

The following were reactions of the three varieties of tomato to various concentrations of X. vesicatoria two weeks after infection.

The leaves of N11 showed about 3-4 spots when injected with a concentration of 6×10^1 bacteria per ml of sterile distilled water. New leaves, stems and petioles which had grown above injected leaves showed no symptoms of infection. Saturn showed over 20 spots per leaf with about 50% chlorosis on the injected leaves. New growth above injected leaves showed no symptoms. Venus showed about 10 spots per injected leaf. A few injected leaves had fallen from the plant. New growth above injected leaves had no symptoms.

For the concentration 6×10^2 bacteria per ml, N11 had small areas of necrosis with as many as 15 spots per leaf on injected leaves. New growth had no symptoms of infection. Saturn had identical results to those for the previous concentration. Venus also looked the same as the previous concentration.

For the concentration 6 X 10^3 bacteria per ml, N11 showed necrosis with yellow halos where the leaves were injected as well as about 20 spots per leaf. New growth showed no symptoms of infection. Saturn showed the same pattern of infection on injected leaves as in the first concentration, but new growth had a few spots. Venus had the same pattern of infection as the previous concentration.

For the concentration 6 X 10^4 bacteria per ml, N11 showed about 30 spots and 20% chlorosis on injected leaves. New growth had no symptoms. Saturn was the same as the previous concentration. On Venus many injected leaves had fallen from the plants and new growth had a few spots.

For the concentration 6 X 10^5 bacteria per ml, N11 had large necrotic areas with 20% chlorosis on injected leaves. New growth had a few spots. On Saturn most of the injected leaves had wilted and fallen from the plants. Some new growth had as much as 20% chlorosis. On Venus most of the injected leaves had fallen from the plants; those that remained had large necrotic areas with 50% chlorosis. New growth had a few spots per leaf.

For the concentration 6 X 10^6 bacteria per ml, N11 had less than 50% chlorosis on the injected leaves. New growth was the same as the previous concentration. Saturn was the same as for the previous concentration.

Venus had few of the injected leaves remaining on the plants. Those that did remain had large necrotic areas. New growth had less than 5 spots per leaf.

The concentrations of 6×10^7 and 6×10^8 bacteria per ml produced the same results as had the concentration of 6×10^6 bacteria per ml for each variety.

The controls did not appear to be infected.

The spots that were selected and checked for bacterial streaming and yellow growth on PC agar were all positive. Leaf spots are shown in Figure 3.

Immediately following the reading of the results, a period of high winds and heavy rains occurred. The plants were checked again after an additional week. Controls and other plants close to the Xanthomonas infected plants had developed spots on their leaves and stems.

Addition of nematode nodules resulted in reduction of growth of tomato plants. Plant heights (Table 1) were measured for three consecutive weeks and compared with heights of plants grown in soil without nematodes.

The root systems of N11 with and without nematodes were identical and no root nodules were found on the roots of the plants grown in the presence of nematodes. The root systems of Saturn and Venus on the other hand were visibly affected by the nematodes. The plants of these two varieties produced only one or two main roots

with few branches, and these roots showed large nodule formation. The plants grown without nematodes produced several main roots with many branches and no nodules.

Six mature plants of Saturn and Venus produced few fruit, while two N11 plants each produced several tomatoes under the same conditions. The flowers of Saturn and Venus appeared to be immature upon blooming. In some cases the blossoms aborted; in other cases parts of the flowers did not develop, pistils or petals. All the plants appeared healthy.

Figure 3. N11 leaf infected with X. vesicatoria.

Table 1. Mean height in cm of N11, Saturn and Venus varieties of tomatoes grown in the presence (N) and absence (C) of nematodes. Each value is an average of 6 plants.

Week	N11		Saturn		Veuns	
	N	C	N	C	N	C
1	29.0	35.5	9.0	18.0	7.0	14.5
2	34.5	46.5	13.0	30.5	14.0	24.0
3	34.0	54.0	15.5	36.0	14.0	28.0

DISCUSSION

The wilt bacterium was tenatively identified as P. solanacearum on the basis of the symptoms produced in eggplants and tomatoes (Kelman, 1953). Sands, Schroth, and Hildebrand (1970) used an Adensonian system to classify the phytopathogenic pseudomonads. The bacterium described here falls into their group III classification because it is sudanophilic, slow growing, and does not produce a green fluorescent pigment. Group III contains P. solanacearum. The colonial appearance on tetrazolium medium resembled those of virulent P. solanacearum (Kelman, 1954). According to Buddenhagen, Sequeira and Kelman's classification (1962) the P. solanacearum infecting tomatoes belonged to race 1.

Hayward (1964) divided P. solanacearum into four biotypes based on sugar and polyalcohol utilization using acid production in a peptone-salts medium. In the method used in this study, growth matched acid production in Hayward's medium whereas no growth matched no acid production. The bacterium described in this report had the characteristics of Hayward's biotype 4 which were no growth from maltose, lactose and cellobiose and growth from dulcitol, mannitol and sorbitol. On the basis of these data it was concluded that the bacterium was P.

solanacearum race 1, biotype 4.

The leaf spot bacterium fit well in Dye's classification (1962) of the genus Xanthomonas because it produced yellow colonies, had one polar flagellum, liquified gelatin, produced alkaline proteolysis on milk, used glucose, mannose, galactose, trehalose and cellobiose, though it did not utilize arabinose, and was urease negative. Since the species of this genus are currently classified by host specificity it was concluded that the bacterium was X. vesicatoria.

The results obtained after inoculating with P. solanacearum indicated that bell pepper, eggplant, and N11 were susceptible to the bacterium. Saturn and Venus were not susceptible. The natural route of infection is through the root system. The method used in the experiment did not simulate field conditions but rather ensured a high inoculum in the stem of the host plant. Plant reactions to the two routes of infection may differ, although the literature does not indicate that it would. Preliminary results from field studies conducted at the Guam Department of Agriculture verify the results for N11, Saturn and Venus.

Of the varieties of tomato (N11, Saturn and Venus) injected with X. vesicatoria, N11 appeared to be the most resistant. Higher concentrations of the bacterium were needed to produce typical lesions. For a given

concentration the symptoms of necrosis, chlorosis and defoliation were less severe for the N11 than the Saturn and Venus. Symptoms produced by injecting a concentration of 6×10^4 bacteria per ml into N11 appeared to coincide with symptoms produced by injecting concentrations of 6×10^1 to 6×10^2 bacteria per ml into Saturn and Venus.

The observation following rain and high winds of X. vesicatoria leaf spots on plants which had not been injected with bacteria indicated that some plant to plant infection had taken place. Within two weeks of the storm the symptoms observed on the non-injected plants were restricted to typical lesions.

The concentrations of bacteria injected were arbitrarily selected and only simulated natural bacterial levels. The artifical inoculation method used may affect the plants' defense mechanisms. Therefore, field tests in naturally infected fields are needed to determine the defoliation effect on Saturn and Venus.

Saturn and Venus appeared to grow poorly in the presence of nematodes. The concentration of nematodes used in the experiment were probably higher than that normally expected in field conditions, but in the field the numbers of nematodes would be expected to increase rapidly if susceptable plants were used for a number of seasons. Nematodes, however, can be controlled

chemically whereas P. solanacearum cannot be. Studies are needed to determine if treatment for nematodes would be sufficiently effective and economical to allow the use of Saturn and Venus. N11, while it was resistant to nematodes, showed reduced growth in their presence.

Mature Saturn and Venus looked normal and healthy but produced few fruits. In contrast N11 grown in the same environment produced large healthy fruit. From these results it seems that Saturn and Venus are unsuitable for use on Guam. Preliminary results from the Guam Department of Agriculture verify these findings. They found that Saturn and Venus set fruit later, had fewer fruits per plant and were shorter than N11. Possible explanations of reduced productivity of Saturn and Venus are: high light intensity, temperature and nitrogen level, and insufficient daily excursions of temperature.

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