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# BACTERIAL LEAF SPOT OF CAPE GOOSEBERRY IN QUEENSLAND

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## SUMMARY

**Bacterial leaf spot disease of cape gooseberry** (Physalis peruviana) **and a wild gooseberry** (P. virginiana **f.** macrophysa) **occur in south-eastern Queensland. The bacteria consistently isolated from diseased tissue were shown to belong to the genus** Xanthomonas **Dowson, 1939.**

**In comparative morphological and biochemical tests, the two gooseberry organisms generally resembled each other and** X. vesicatoria **(Doidge) Dowson, 1939, a common pathogen of tomato and capsicum in Queensland. They also generally resembled the published descriptions of** X. physalidicola **Goto and Okabe, 1958, and** X. physalidis **Srinivasan, Patel and Thirumalachar, 1962. In pathogenicity tests on eight solanaceous plants and radish, the two gooseberry organisms had some resemblances to and some differences from all these three species of** Xanthomonas. **On the basis of priority, it is proposed that the two gooseberry bacteria be considered strains of** X. vesicatoria.

## I. INTRODUCTION

An unidentified bacterial leaf spot of cape gooseberry (*Physalis peruviana* L.) was recorded by T. McKnight in 1954 near Kingaroy, south-eastern Queensland (Simmonds 1966). A disease which may be the same has since been found by the authors on cape gooseberry at five localities near Nambour and Brisbane, and on a wild gooseberry (*P. virginiana* Mill. f. *macrophysa* (Rydb.) Waterfall) near Kingaroy.

In the field the disease is characterized by yellowish green pustular spots which eventually become tan-coloured lesions surrounded by a yellowish green halo. Spots may coalesce to form large yellow areas and, eventually, tan-coloured areas surrounded by a band of yellow tissue. Occasionally there is a narrow marginal leaf scorch separated from the rest of the leaf by a band of yellow tissue. Severely spotted leaves develop a general chlorosis and are readily dislodged by wind or handling, causing premature defoliation of affected plants. Lesions on stems have not been observed.

When young leaf lesions are placed in a drop of water, cut through, and examined microscopically, numerous motile bacteria ooze from the cut surface. In the present paper, these bacteria are shown to belong to the genus *Xanthomonas*.

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A review of the literature revealed that three species of *Xanthomonas* have been reported to cause leaf spots of species of *Physalis* in nature, viz. *X. physalidicola* Goto and Okabe, 1958, in *Physalis alkekengi* L. var. *francheti* (Masters) Makino; and *X. vesicatoria* (Doidge, 1920) Dowson, 1939 and *X. physalidis* Srinivasan, Patel and Thirumalachar, 1962, in *Physalis minima* L. (Goto and Okabe 1958, Srinivasan *et al.* 1962). *X. vesicatoria* is a common pathogen of tomato (*Lycopersicon esculentum* Mill.) and capsicum (*Capsicum frutescens* L.) in Queensland. Work was started, therefore, to determine the relationship of the cape gooseberry and wild gooseberry bacteria to these three species.

## II. ISOLATION AND PATHOGENICITY

Infected leaves were washed in tap water, followed by a wash in distilled water, and then gently wiped with 70% alcohol. A small section of leaf tissue was taken from the margin of a lesion and was macerated in a few drops of sterile nutrient (beef extract) broth (see Moffet 1966 for composition); the resulting suspension was streaked onto potato dextrose agar (P.D.A.). After incubation for 3 days at 27°C, a yellow water-insoluble pigmented mucoid bacterial growth was commonly present on the plates.

Pathogenicity tests with the two gooseberry bacteria and *X. vesicatoria* were carried out using 2-day-old nutrient broth cultures made from single colonies grown on P.D.A. Young plants of cape gooseberry, *Physalis virginiana* f. *macrophysa, P. minima,* tobacco (*Nicotiana tabacum* L.), egg plant (*Solanum melongena* L.), *S. nigrum* L., capsicum, tomato and radish (*Raphanus sativus* L.) were inoculated by spraying the cultures onto the leaves, using a hand atomizer, or by pricking a drop of a 2-day-old culture into the stems or fruit. Control plants were treated in the same manner, using sterile nutrient broth. The plants were transferred to a moist chamber for 2 days and then placed in a glass-house.

Six isolates from naturally infected cape gooseberry leaves were proved pathogenic to cape gooseberry and the organism was re-isolated. The lesions produced (Figures 1 and 2) were very similar to those produced by natural infection. Symptoms in cape gooseberry produced by the wild gooseberry bacterium resembled those caused by the cape gooseberry bacterium, whereas *X. vesicatoria* was considerably less virulent in this host. However, *X. vesicatoria* infected tomato whereas the two gooseberry bacteria did not. A comparison of the symptoms produced in the inoculated plants is given in Table 1. The organisms were re-isolated in all cases where positive symptoms are recorded.

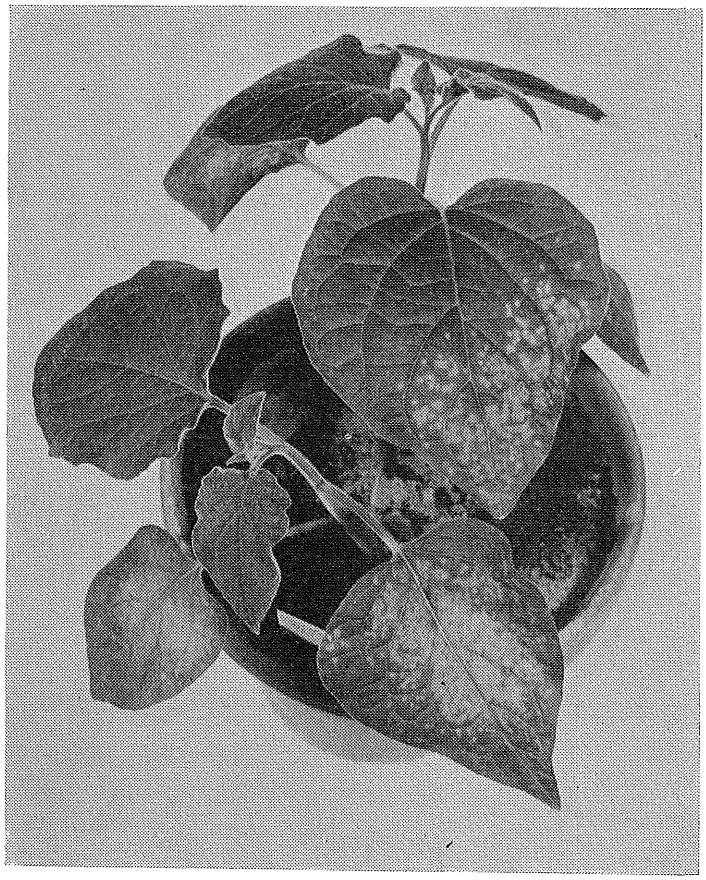

Fig. 1.—Cape gooseberry plant spray-inoculated with *Xanthomonas* sp. (cape gooseberry).

## III. MORPHOLOGICAL AND BIOCHEMICAL DESCRIPTION

The procedures used to determine the morphology and biochemical reactions of the organisms were those described by Moffett (1966). In the following description the isolates from cape gooseberry leaves collected on Stradbroke Island (near Brisbane) will be referred to as G1, G2, G3 and G4, the isolates from Brookfield (near Brisbane) as G5 and G6, and the isolate from the wild gooseberry collected near Kingaroy as GW. A re-isolate of G1 from inoculated cape gooseberry leaves will be called G1 re-isolate, the *X. vesicatoria* (tomato) T1, and the *X. vesicatoria* (capsicum) C1, C2 and C3. All the *X. vesicatoria* isolates were collected in the Ormiston district (near Brisbane).

## TABLE 1

RESULTS OF PATHOGENICITY TESTS OF *Xanthomonas* SP. FROM CAPE GOOSEBERRY AND WILD GOOSEBERRY AND *X. vesicatoria* FROM TOMATO AND CAPSICUM ON EIGHT SOLANACEOUS HOSTS AND RADISH*

| Plant inoculated | Symptoms | | | |
|---|---|---|---|---|
| | *Xanthomonas* sp. (cape gooseberry) | *Xanthomonas* sp. (wild gooseberry) | *X. vesicatoria* (tomato) | *X. vesicatoria* (capsicum) |
| Cape gooseberry— | | | | |
| Leaves .. .. .. | Watersoaked circular pustules surrounded by yellowish haloes, becoming dry, tan-coloured lesions and frequently coalescing to form large necrotic areas. | As for the cape gooseberry isolates. | Small discrete cream-white circular lesions, the centres darkening to a pale tan-colour. | As for *X. vesicatoria* (tomato). |
| Stem .. .. .. | Spray inoculated—nil. Prick inoculated—vascular discoloration above and below point of inoculation. Above point of inoculation, vascular discoloration continued into the leaf petioles. | As for the cape gooseberry isolates. | Spray inoculated—nil. Prick inoculated—vascular discoloration for a short distance (approx. 2 mm) above and below point of inoculation. | As for *X. vesicatoria* (tomato). |
| Tomato— | | | | |
| Green fruit approaching maturity. Q2 (derived from Grosse Lisse) | Nil | Nil | Two weeks after inoculation dark brown to tan sunken lesions up to 7 mm in diameter. | Nil |
| Leaves. Q2 and Rouge de Marmande | Nil | Nil | Irregularly circular water-soaked lesions becoming tan in colour with dark tan margins frequently surrounded by narrow haloes. | As for *X. vesicatoria* (tomato). |
| Stem. Q2 .. .. .. | Nil | Nil | Vascular discoloration occurred. | Vascular discoloration occurred. |

| | | | | |
|---|---|---|---|---|
| Capsicum— | | | | |
| Leaves .. .. .. | Pin-point cream spots becoming tan-coloured surrounded by pale haloes. | Nil | As for the cape gooseberry isolates. | As for the cape gooseberry isolates, but the lesions frequently continued to increase in size, coalescing to form large necrotic areas. |
| *Solanum nigrum*— | | | | |
| Leaves .. .. .. | Pale green circular lesions developing into tan pin-point spots surrounded by wide pale green haloes. | Tan lesions with darker tan margins and wide green-yellow haloes. | As for the cape gooseberry isolates. | As for the cape gooseberry isolates. |
| Tobacco— | | | | |
| Leaves .. .. .. | White-grey circular sunken parchment-like lesions. Isolates were weakly pathogenic. | As for the cape gooseberry isolates. | As for the cape gooseberry isolates. | As for the cape gooseberry isolates. |
| *Physalis virginiana*— | | | | |
| Leaves .. .. .. | Pale creamy-white pustules with watersoaked centres later becoming tan in colour. Pustules developed on the veins. | As for the cape gooseberry isolates. | As for the cape gooseberry isolates. | As for the cape gooseberry isolates. |
| Stems .. .. .. | Vascular discoloration followed by collapse of the plant. | Vascular discoloration and stunting of the plant. | Nil | Nil |
| *Physalis minima*— | | | | |
| Leaves .. .. .. | Circular watersoaked lesions with bacterial ooze drying to a crust. | Watersoaked circular lesions after 9 days, drying and becoming tan-coloured. | Circular light tan to white-coloured lesions. | As for *X. vesicatoria* (tomato). |
| Egg plant— | | | | |
| Leaves .. .. .. | Nil | Nil | Nil | Nil |
| Radish— | | | | |
| Leaves .. .. .. | Nil | Nil | Nil | Nil |

* Results were recorded at 5–7 days except where noted.

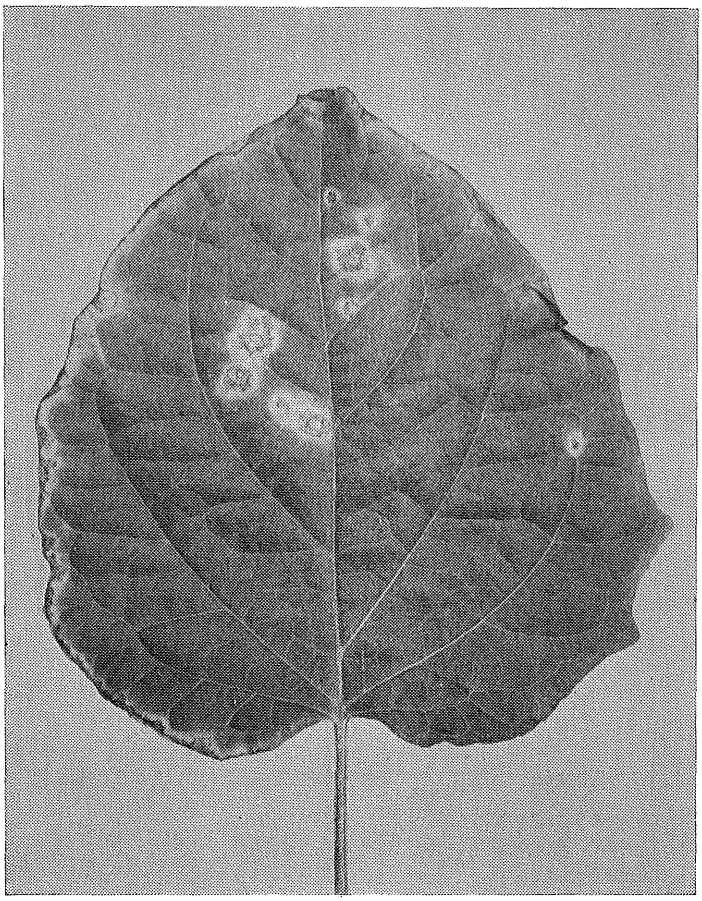

Fig. 2.—Cape gooseberry leaf 3 weeks after spray-inoculation with *Xanthomonas* sp. (cape gooseberry). Note marginal infection.

*Morphology.*—All cultures were Gram-negative, rod-shaped cells with rounded ends, occurring singly or in pairs. They were non-sporing and non-capsulated and ranged in size from 1·2 to 2·4$\mu$ x 0·6–0·9$\mu$.

*Motility.*—All organisms were observed to be motile in hanging-drop preparations. Flagella of all isolates were stained using Rhodes' (1958) modification of Fontana's silver-plating method. Further, the flagella of the cape gooseberry isolates, *X. vesicatoria* (tomato) and *X. vesicatoria* (capsicum) were examined with an electron microscope after negative staining with phosphotungstic acid or shadowing with platinum-palladium. All isolates had a single polar flagellum (Figure 3).

*Cultural characteristics.*—After 2 days, all nutrient broth cultures were uniformly turbid. A pellicle was not formed but as the cultures aged a ring of yellow slimy growth was formed on the side of the tubes at the broth surface.

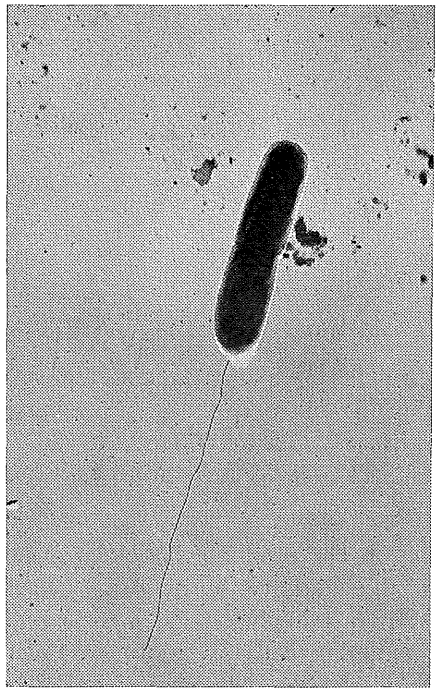

Fig. 3.—Electron micrograph of *Xanthomonas* sp. (cape gooseberry). Platinum-palladium shadowing.

Colonies on beef extract agar, P.D.A., peptone yeast extract agar and peptone sucrose agar (Hayward 1960) were circular, smooth, shining, margin entire, low convex to convex, opaque, mucoid, 1-4 mm in diameter, all possessing a yellow water-insoluble pigment. The colonies varied in colour; comparison with the colour standards of Ridgway (1912) showed they were barium yellow or citron yellow on beef extract agar, amber yellow to barium yellow on P.D.A., naphthalene yellow to primrose yellow on peptone yeast agar and barium yellow on sucrose peptone agar. The amount of slime produced on the different media was variable. Growth on P.D.A. and sucrose peptone agar was very slimy, whereas very little slime was produced on beef extract agar and peptone yeast agar.

On cooked potato slopes, growth was mucoid, smooth, shining, margin entire and amber yellow, empire yellow or wax yellow in colour, extending over the entire plug, which was rapidly digested.

*Absorption spectrum of the pigment.*—The peaks and shoulders obtained from spectra of the pigment extracted from cultures G1 and GW are given in Table 2. These isolates possess a carotenoid pigment with absorption maxima close to the 418, 437 and 463 mμ (petroleum ether) found characteristic of the genus *Xanthomonas* by Starr and Stephens (1964).

**TABLE 2**

ABSORPTION MAXIMA (mμ) OF THE CAROTENOID PIGMENT EXTRACTED FROM CULTURES G1 AND GW IN THE SOLVENTS METHANOL, BENZENE AND PETROLEUM ETHER

| Solvent | First Shoulder | | First Peak | | Second Shoulder or Peak | |
|---|---|---|---|---|---|---|
| | G1 | GW | G1 | GW | G1 | GW |
| Methanol .. .. .. | (420) | (420) | 444 | 445 | (465) | (469) |
| Benzene .. .. .. | (430) | (432) | 456 | 455 | 486 | 482 |
| Petroleum ether .. .. | (419) | (418) | 442 | 441 | 472 | 468 |

Parentheses= shoulder

*Temperature range.*—The optimum temperatures for all isolates growing in nutrient broth were within the range 25-29°C. The maximum temperatures for isolates G1-G6, G1 re-isolate, GW and T1 were 33-35°C, and for C1-C3, 37-38°C. The isolates G2, T1 and C3 grew at 10°C, whereas the others grew at 15°C but not at 10°C.

*Biochemical reactions.*—The results of the biochemical tests were as follows:

All isolates produced acid but no gas from glucose, fructose, mannose, galactose, sucrose and starch. Acid and gas were not produced from lactose, rhamnose, ribose, salicin, inulin, sorbitol, erythritol, dulcitol and inositol. The carbohydrate reactions which were variable are given in Table 3.

**TABLE 3**

BIOCHEMICAL REACTIONS OF *Xanthomonas* SP. (CAPE GOOSEBERRY), *Xanthomonas* SP. (WILD GOOSEBERRY) AND *X. vesicatoria* IN NINE CARBOHYDRATES

| *Xanthomonas* isolate | L-Arabinose | Xylose | Raffinose | Maltose | Melibiose | Trehalose | Dextrin | Glycerol | Mannitol |
|---|---|---|---|---|---|---|---|---|---|
| G1 (cape gooseberry) .. .. | + | + | − | + | + | + | − | + | − |
| G2 (cape gooseberry) .. .. | − | + | − | + | − | + | − | − | − |
| G3 (cape gooseberry) .. .. | − | + | − | + | − | + | − | − | − |
| G4 (cape gooseberry) .. .. | − | + | − | + | − | + | − | − | − |
| G5 (cape gooseberry) .. .. | − | + | + | + | + | + | − | + | − |
| G6 (cape gooseberry) .. .. | − | + | − | + | + | + | − | + | − |
| G1 re-isolate .. .. .. | + | + | − | + | − | + | − | − | − |
| GW (wild gooseberry) .. .. | − | + | + | + | − | + | − | − | − |
| T1 (tomato) .. .. .. | + | − | − | + | + | + | + (slight) | + | + |
| C1 (capsicum) .. .. .. | + | − | + | − | − | − | − | + | − |
| C2 (capsicum) .. .. .. | + | − | − | − | − | + | − | − | − |
| C3 (capsicum) .. .. .. | + | − | + | − | + | + | − | − | − |

+ = Acid but no gas

− = No acid and gas

Starch was hydrolysed by all isolates except those from capsicum. Gelatin, 1% tributyrin, sodium hippurate and malonate were hydrolysed, whereas alginate and pectate were not. Ammonia was produced from peptone. Urease was not produced. Metabolism of glucose was oxidative. Asparagine was not utilized as a sole carbon and nitrogen source. The sodium salts of the organic acids citric, succinic and acetic were utilized by all isolates but tartaric and oxalic were not; growth was inhibited in benzoic; the isolates C1, C2, C3 and GW utilized lactic and all isolates except G1 and G1 re-isolate utilized formic. The methyl red test was negative. Acetoin was not produced in a glucose-phosphate-peptone water after 4 days. All isolates except G1, G4 and G6 were oxidase-positive. All isolates were catalase-positive. A fluorescent pigment was not produced in the medium of Georgia and Poe (1931). The only isolates which produced tyrosinase (Starr 1943) were G1, G3 and G1 re-isolate. Growth could not be detected in nutrient broth containing 4% sodium chloride.

## IV. DISCUSSION

The morphological, cultural and biochemical characteristics of the cape gooseberry organism clearly place it in the genus *Xanthomonas* Dowson, 1939, as described in the 7th edition of Bergey's Manual of of Determinative Bacteriology Breed, Murray, and Smith 1957). The specific identity of the organism is, however, less clear.

The cape gooseberry bacterium must be compared with the three species of *Xanthomonas* which have been recorded occurring naturally on species of *Physalis.* These are *X. vesicatoria,* the only xanthomonad previously recorded on members of the Solanaceae (tomato and capsicum) in Queensland; *X. physalidicola* on *Physalis alkekengi* in Japan, which was less virulent than *X. vesicatoria* on tomato, capsicum and potato (Goto and Okabe 1958); and *X. physalidis* on *P. minima* in India, which was non-pathogenic to tomato and capsicum (Srinivasan, Patel, and Thirumalachar 1962). The major morphological, biochemical and pathogenic characteristics of the four organisms as determined above or recorded in the literature are given in Table 4.

The morpohological characteristics of the four organisms were similar, except for flagellation and the presence of capsules. All these organisms except *X. physalidicola* were motile with a single polar flagellum. However, the strain of *X. physalidicola* studied by Goto and Okabe (1958) could have been a non-flagellate strain within a generally motile species. The cape gooseberry organism and *X. vesicatoria* differed from *X. physalidicola* and *X. physalidis* in being non-capsulated. This character is variable and cannot be considered of importance in differentiation of species.

It is evident from Table 4 that the cape gooseberry organism closely resembles *X. physalidicola, X. physalidis* and *X. vesicatoria* in biochemical characteristics. As any differences were small and since variation occurred between the individual isolates of the cape gooseberry organism and between the

**TABLE 4**

SUMMARY OF A NUMBER OF MORPHOLOGICAL, BIOCHEMICAL AND PATHOLOGICAL CHARACTERS OF *Xanthomonas* sp. (CAPE GOOSEBERRY), *X. physalidicola*, *X. physalidis* AND *X. vesicatoria**

| Characteristic | *Xanthomonas* sp. (cape gooseberry) | *X. physalidicola* | *X. physalidis* | *X. vesicatoria* |
|---|---|---|---|---|
| Single polar flagellum | + | − | + | + |
| Capsule | − | + | + | − |
| Gelatin liquefaction | + | + | + | + |
| Nitrite from nitrate | − | − | − | − |
| Indole production | − | − | − | − |
| Hydrogen sulphide production | + | + | + | + |
| Fermentation of carbohydrates— | | | | |
| Glucose | + | + | + | + |
| Mannose | + | + | | + |
| Galactose | + | + | | + |
| Xylose | + | + | | − |
| Sucrose | + | + | | + |
| Fructose | + | + | | + |
| Maltose | + | + | + | V |
| Starch | + | + | | + |
| Dextrin | − | + | | V |
| Raffinose | V | | + | V |
| Glycerol | V | V | | V |
| Lactose | − | − | + | − |
| Dulcitol | − | | + | − |
| Mannitol | − | − | + | V |
| Salicin | − | | − | − |
| Starch hydrolysis | + | + | + | V |
| Optimum temperature | 26–29°C | 28–30°C | 27–30°C | 25–29°C |
| Maximum temperature | 33–35°C | 38°C | | 35–38°C |
| Methyl red test | − | | − | − |
| Voges-Proskauer reaction (acetoin production) | − | | − | − |
| Peptonization and litmus reduction in litmus milk | + | + | + | + |
| Ammonia from peptone | + | + | + | + |
| Pathogenicity to: | | | | |
| Cape gooseberry | + | | + | + |
| *Physalis minima* | + | | + | + |
| *P. alkekengi* | | + | | |
| *P. virginiana* | + | | | + |
| Tomato | − | + | − | + |
| Capsicum | + | + | − | + |
| Potato | | − | | |
| Tobacco | + | | − | + |
| *Solanum nigrum* | + | | | + |
| *S. melongena* | − | − | − | − |
| *Nicandra physalodes* | | | − | |
| Radish | − | − | | − |

+ = Present, positive reaction or acid production
− = Absent, negative reaction or no acid production
V = Variable

* Data on *Xanthomonas* sp. (cape gooseberry) and *X. vesicatoria* were taken from the writers' work; data on *X. physalidicola* and *X. physalidis* were taken from Goto and Okabe (1958) and Srinivasan *et al.* (1962), respectively.

isolates of *X. vesicatoria* (Table 3), these biochemical differences are an inadequate basis for speciation. Indeed, the work of Dye (1962) indicates clearly that some variation in biochemical characters within species is to be expected, and that the characteristics of the presently recognized species overlap considerably. A somewhat similar conclusion was obtained by Colwell and Liston (1961) following their Adansonian analysis of a number of species of *Xanthomonas*. However, Colwell and Liston (1961) found that the species studied could be placed in two related groups, and that *X. physalidicola* and *X. vesicatoria* fell in different groups. These results were not confirmed by R. A. Lelliott and D. W. Dye (Dye, private communication 1965), who in a computer analysis found that *X. physalidicola, X. vesicatoria* and *X. campestris* fell into one uniform group.

The natural host and in a few instances the host range have been used as determinative characters in distinguishing species of *Xanthomonas* (Wernham 1948; Patel, Srinivasan, and Thirumalachar 1965). However, Dye, by serial transfers into bean plants of a number of species of *Xanthomonas* originally isolated from different hosts, was able to show that host specificity may not be a stable character. For the species under discussion the main differences found in the present work or reported in the literature (Table 4) were that the cape gooseberry organism did not infect tomato, whereas *X. physalidicola* and *X. vesicatoria* did; the cape gooseberry organism infected capsicum and tobacco, whereas *X. physalidis* did not; and the cape gooseberry organism was more virulent in cape gooseberry than *X. vesicatoria,* whereas in capsicum *X. vesicatoria* (capsicum) was more virulent than the cape gooseberry organism or *X. vesicatoria* (tomato) (Figure 4).

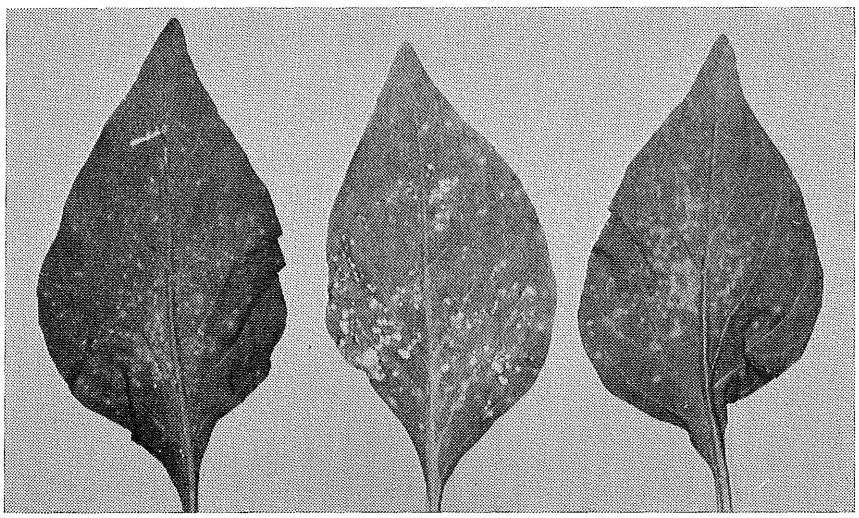

Fig. 4.—Capsicum leaves spray-inoculated with, from left to right, *Xanthomonas vesicatoria* (tomato), *X. vesicatoria* (capsicum), and *Xanthomonas* sp. (cape gooseberry). *X. vesicatoria* (capsicum) produced greater necrosis than the other two organisms.

Since the cape gooseberry organism was similar to, but slightly different from, each of the three species of *Xanthomonas,* pathogenicity also appears to be a doubtful basis on which to determine to which species the cape gooseberry organism belongs. Further, the cape gooseberry and wild gooseberry bacteria were similar in almost all their characteristics, except that the wild gooseberry bacterium did not infect capsicum. In this regard the wild gooseberry bacterium resembled *X. physalidis,* but it differed from that organism in its ability to infect tobacco. Possibly a further collection of isolates from species of *Physalis* might have revealed an even greater range of pathogenicity.

Other characters have been used in attempts to determine relationships between species of *Xanthomonas.* The results of deoxyribonucleic acid (DNA) studies also support the evidence that species of *Xanthomonas* form a uniform group. The DNA base compositions of a number of species were similar (De Ley and Van Muylem 1963). Further, DNA hybridization was accomplished between *X. pelargonii* and nine species of *Xanthomonas* (Friedman and De Ley 1965; De Ley and Friedman 1965). It was proposed that the nine species be considered to belong to one genospecies known as *X. campestris,* with the species names possibly being retained as labels or varieties of *X. campestris. X. vesicatoria* was among the nine species studied, but *X. physalidicola and X. physalidis* were not.

It is likely that in the future the above and other characters will be used in attempts to distinguish species in the genus *Xanthomonas.* Such work either will support the suggestion that two or more groups exist within the genus, or will indicate that the genus constitutes a very uniform group with differences of subspecific rank only. Until such information is available, it is suggested on the basis of priority that the cape gooseberry and the wild gooseberry organisms be considered to be pathogenically distinct strains of *Xanthomonas vesicatoria.*

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