THE SIMULTANEOUS TRANSMISSION OF A PLANT-PATHOGENIC BACTERIUM AND A VIRUS FROM ROSE BY GRAFTING AND MECHANICAL INOCULATION*

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Fulton (1952) reported the transmission of rose mosaic virus (RMV) by mechanical inoculation from *Rosa setigera* Michx. to cucumber and thereafter to other herbaceous plants. This made possible the characterization of the virus, and later, its purification (Fulton 1967). In South Australia we have been unable to transmit viruses from rose leaves, flowers, or roots by mechanical inoculation directly to herbaceous plants. However, we have transmitted several virus isolates by patch-bark grafting rose material to young peach seedlings and then mechanically transmitting virus from young peach leaves to cucumber seedlings (Basit and Francki, unpublished data). From at least one rose plant we have consistently isolated two pathogens via peach seedlings to cucumber seedlings, both of which were at first thought to be viruses but later one of the pathogens was identified as a species of *Pseudomonas*.

Experimental and Results

Young leaves of peach seedlings [*Prunus persica* (L.) Batsch cv. Elberta], patch-bark grafted with diseased material of garden rose cv. Peace, at least 1 month previously, were ground with water in a pestle and mortar. The extract was mechanically inoculated to carborundum-dusted cotyledons of young cucumber (*Cucumis sativus* L. cv. Polaris) seedlings which developed chlorotic and necrotic lesions 3–4 days later and subsequently the plants often collapsed from wilting. No symptoms were produced by inocula prepared from ungrafted control peach seedlings. On mechanical inoculation of *Chenopodium quinoa* L. and *Momordica balsamina* L. with extracts of diseased cucumber cotyledons, young *C. quinoa* leaves developed mosaic symptoms (Fig. 1) whereas *M. balsamina* developed necrotic lesions only on the inoculated leaves (Fig. 2). When the pathogens were reinoculated to cucumber seedlings from each of these plants, the symptoms produced by inocula from the two species were quite distinct (Figs. 3 and 4) and the pathogen recovered from *C. quinoa* was serologically identified as a strain of RMV. The pathogen recovered from *M. balsamina* produced small necrotic lesions with yellow margins

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on cucumber seedlings (Fig. 4) and could easily be differentiated from RMV as shown in the following tabulation:

Characteristic Measured	Pathogen Recovered from	
	C. quinoa	*M. balsamina*
Thermal inactivation point	56°C	54°C
Dilution end point	1 : 100	> 1 : 10,000
Longevity *in vitro* at 25°C	< 24 hr	> 48 hr
Stability in organic solvents	Stable	Unstable
Stable in pH range	6–8	4–9

The pathogen also produced necrotic lesions on guar (*Cyamopsis tetragonoloba* L.) (Fig. 5) and small sunken lesions on cowpea (*Vigna sinensis* Endl.).

At first it was assumed that the pathogen recovered by passage through *M. balsamina* was a virus; however, all attempts to purify it by standard virological techniques failed. It was observed that all infectious material was recovered in the pellet when plant extracts were centrifuged at 1000 *g* for 10 min. These observations cast doubt on our assumption that the pathogen was a virus and the doubt was substantiated when the pathogen failed to pass a bacterial filter with a pore size of 0·22 μm.

Bacteria were isolated on nutrient agar from lesions on cucumber cotyledons and subcultured. These bacterial cultures, when suspended in water and mechanically inoculated to cucumber, guar, cowpea, and *M. balsamina*, produced lesions indistinguishable from those produced by sap from infected cucumber cotyledons. When either cultured bacteria or sap from infected cucumber cotyledons was introduced to cucumber seedlings by stem puncture, the majority of the plants collapsed through wilting within 4 days and the few remaining plants produced characteristic lesions on the cotyledons.

The bacterial culture formed dirty white, flat, smooth colonies on nutrient agar. It utilized glucose oxidatively (Hugh and Leifson 1953), was oxidase negative (Kovacs 1956), 3-ketolactose negative (Bernaerts and DeLey 1963), did not hydrolyse starch, and did not produce fluorescent pigment on King's B medium. Bacteria cultured for 24 hr on nutrient agar were suspended in 0·05% bovine serum albumin, mounted on Formvar-coated electron microscope grids, and stained with 2% phosphotungstic acid, pH 6·7. On examination in a Philips 100B electron microscope, bacilliform cells with bunches of bipolar flagella were observed (Fig. 6). From these observations we tentatively identify the bacterium as a non-fluorescent species of *Pseudomonas*.

Fig. 3.—Symptoms on cucumber (*Cucumis sativus*) produced by rose mosaic virus after passage through *C. quinoa*.

Fig. 4.—Symptoms on cucumber produced by the *Pseudomonas* sp. after passage through *M. balsamina*.

Fig. 5.—Symptoms on guar (*Cyamopsis tetragonoloba*) produced by the *Pseudomonas* sp.

Fig. 6.—Electron micrograph of a *Pseudomonas* sp. cell showing bunches of bipolar flagella.

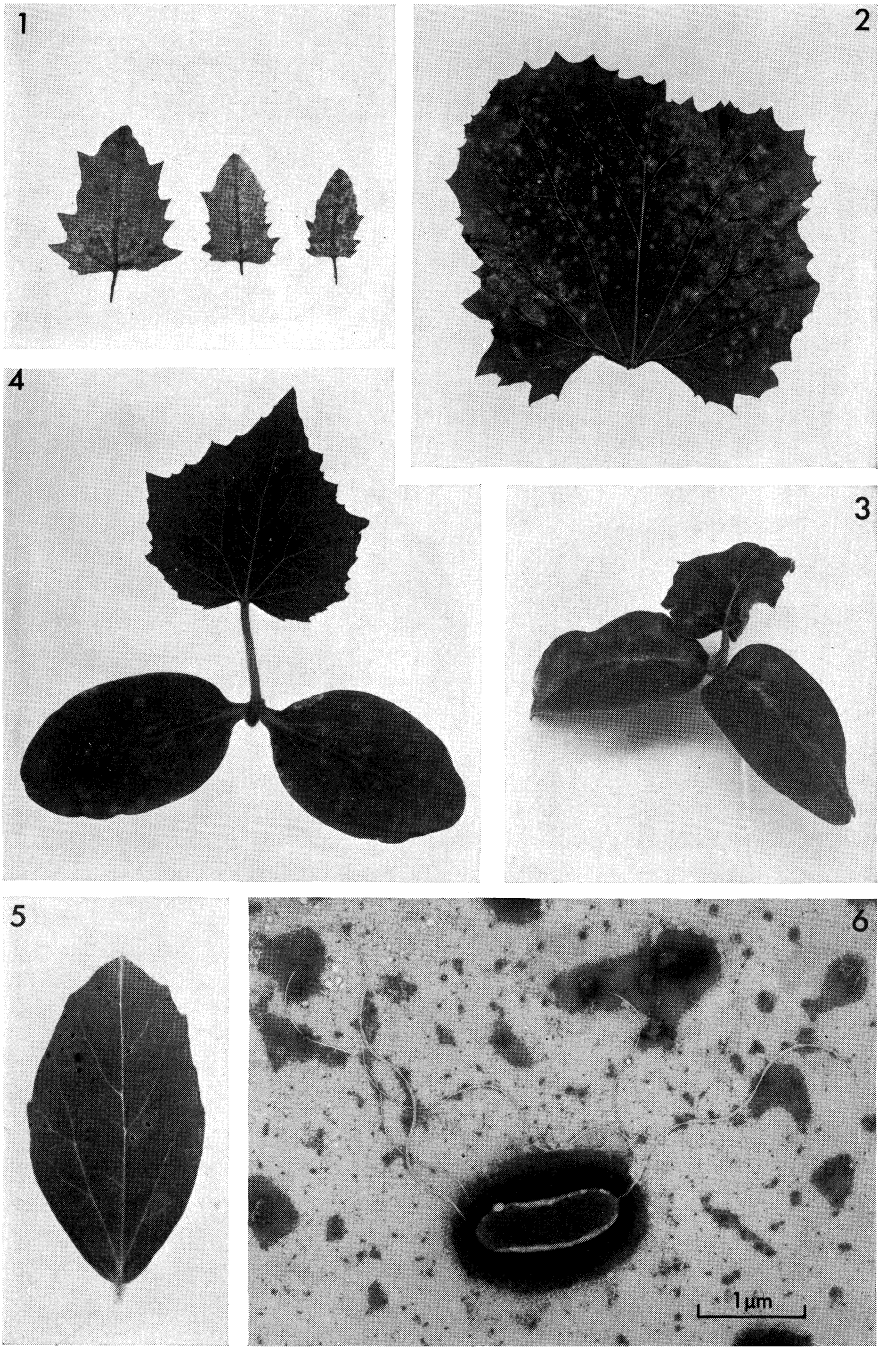

Fig. 1.—Symptoms on *Chenopodium quinoa* systemically infected with one of the pathogens (rose mosaic virus) isolated from garden rose cv. Peace.

Fig. 2.—Symptoms on a *Momordica balsamina* leaf inoculated with one of the pathogens (*Pseudomonas* sp.) isolated from garden rose cv. Peace.

Discussion

The above observations illustrate the dangers of identifying a pathogen as a virus on the basis of virus-like symptoms produced on test plants inoculated by standard virological techniques. The confusion of a bacterium with a virus as a plant pathogen has previously been reported by Yarwood *et al.* (1961). They demonstrated that the disease initially described as beet latent virus (Smith 1951) was actually caused by *Pseudomonas aptata.* A similar confusion has been reported where *P. aeruginosa* was found to form virus-like plaques in a human tissue culture system (Ludovici and Christian 1969).

The *Pseudomonas* sp. which we have isolated from rose produces symptoms on guar very similar to those reported for RMV (Fulton 1952); also the symptoms on *M. balsamina* are similar to those produced by a pathogen isolated from sour cherry infected by recurrent necrotic ringspot and yellows (Fulton 1957). Recently, Fulton (1968) has shown that RMV and cherry necrotic ringspot virus are serologically related. The strain of RMV which we have isolated from rose in South Australia does not produce any symptoms on either guar or *M. balsamina.*

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