

# Use of the Root-Dipping Inoculation Method in Testing Tomatoes for Wilt Resistance

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## INTRODUCTION

The wilt disease caused by *Pseudomonas solanacearum* E.F.S. is at present the most important bacterial disease of tomatoes in Puerto Rico, especially in the humid sections of the Island.

The search for a variety of tomato resistant to bacterial wilt under our conditions has not been successful up to now in Puerto Rico (1, 2, 3, 4, 5)<sup>2</sup>, although the potential value of a resistant variety was recognized in the earliest papers concerned with the control of bacterial wilt. It is to be noted that the work in North Carolina (6) on this problem was more hopeful.

Work is in progress at this Experiment Station to develop tomato (*Lycopersicon esculentum* Mill.) lines resistant to bacterial wilt. Greenhouse tests have been used for the screening of lines in the search for resistance. In this work use has been made of the inoculation techniques for determining resistance as described by Winstead and Kelman (6).

These techniques included inoculation with pure cultures by: 1, Forcing a sharp needle into the stem through a drop of bacterial suspension placed in the axil of the second or third expanded leaf below the stem apex; 2, cutting the lateral roots with a scalpel (root injury) along one side of the plant to a depth of approximately 4 cm., and pouring 10 ml. of the bacterial suspension over the severed roots; or 3, dipping the roots in a bacterial suspension.

On the basis of their studies Winstead and Kelman concluded that, although stem inoculation consistently produced higher wilt readings in resistant plants, the greatest differentiation between resistant and susceptible plants was obtained by root inoculation following the root-injury technique.

Preliminary inoculation trials<sup>3</sup> to determine the effectiveness of the root-injury technique were conducted using the highly susceptible Marglobe variety. For undetermined reasons poor results were consistently obtained.

## PROCEDURE

Much better results, however, were secured when the roots were dipped in the bacterial suspension and the seedlings planted after treatment. The

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<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 118.

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following technique was used: Seed of tomato variety Marglobe were planted in enameled basins  $9\frac{3}{4}$  inches in diameter filled with steam-sterilized river sand. The seed germinated well and the young plants were kept in vigorous condition by drenching with one application of a proprietary nutritive solution (Nutrileaf), 1 tablespoonful in 1 gallon of water. When the plants were 4 weeks old they were gently pulled from the sand and dipped for 10 minutes in a suspension of *Pseudomonas solanacearum* E.F.S., isolate No. 65, a virulent isolate obtained locally from a bacterial wilt-diseased tomato plant. This isolate has been kept in the laboratory under oil for more than a year.

The inoculum was obtained from 4 to 5 day-old cultures grown on potato-dextrose agar in Blake bottles. The bacterial suspension was made following the method of Winstead and Kelman (6) by adding 100 ml. of sterile dis-

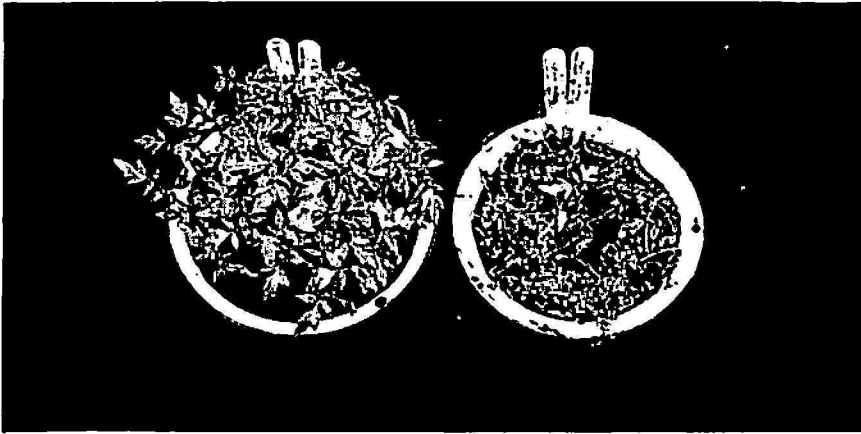


FIG. 1.—A, Control and B, inoculated tomato seedlings.

tilled water to each bottle and then diluting the suspension from each bottle by the addition of 300 ml. of water. In this case a slight deviation from the Kelman and Winstead technique was used in that sterile distilled water was employed instead of tapwater.

After roots of the plants had stayed in the suspension for 10 minutes they were planted immediately in the sand, 100 to 150 plants per basin, and placed in a screened house. The basins were covered with glass jars to maintain high humidity. Uninoculated plants which had been pulled out and replanted, and otherwise kept under the same condition were grown in basins beside the inoculated plants as controls.

### RESULTS

The experiments were repeated six times at different times of the year and in each test more than 90 percent of the tomato seedlings consistently wilted and died (fig. 1) from 7 to 10 days after inoculation. The pronounced stunting and cessation of growth of uninoculated controls accompanying the root-dipping technique which, according to Winstead and Kelman, made

them discontinue its use, was negligible or not observed at all in our experiments.

In view of the above-mentioned experiences we believe that the use of the root-dipping inoculation method might be of value in testing for resistance to bacterial wilt of tomatoes under our conditions.

#### SUMMARY

This paper presents some experiments dealing with the effectiveness of the root-dipping method of inoculation in testing tomatoes for resistance to bacterial wilt. In the several trials performed more than 90 percent of the inoculated seedlings wilted and died when root-dipped in the suspension of the bacterial pathogen. We did not observe the pronounced stunting of growth of seedlings which, according to other investigators who have used this method, precludes the employment of this technique of inoculation. In our opinion the root-dipping method of inoculation should be a valuable adjunct in testing for resistance to bacterial wilt.

#### RESUMEN

Este estudio presenta los resultados de pruebas en las cuales se usó el método de inoculación por las raíces para determinar la resistencia del tomate al germen causante de la marchitez bacteriana. Los resultados obtenidos de los distintos ensayos demostraron que sucumbió más del 90 por ciento de las plántulas inoculadas con el patógeno por este método. No pudimos observar en ninguno de los ensayos el notable retardamiento en el desarrollo de las plántulas inoculadas y lo cual, de acuerdo con otros investigadores, descarta el uso de este método de inoculación. Creemos a la luz de nuestra experiencia que el método de inoculación por las raíces es un medio eficaz para determinar la resistencia del tomate a la marchitez bacteriana.

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