Tissue Necrosis in Tobacco Caused by a Saprophylic Bacterium

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ABSTRACT

Suspensions containing 10^8 cells/ml of *Pseudomonas fluorescens* caused necrosis when injected into tobacco leaf tissue. The injected tobacco was incubated in the dark for 3 days in a moist chamber at 24 C and subsequently transferred for 1 day to a relative humidity of 25-35%. It was concluded that the saprophyte *P. fluorescens* has the potential to cause tissue necrosis in tobacco leaf tissue similar to that caused by many pathogenic bacteria. Phytopathology 60:1279-1280.

In compatible and a great number of incompatible host-parasite combinations, bacteria multiply and cause tissue necrosis in tobacco leaves (2). Saprophytic bacteria such as *Pseudomonas fluorescens* Migula are nutritionally more versatile and biochemically more active than the pathogenic pseudomonads (3, 4). Nevertheless, under normal greenhouse conditions the saprophytic bacteria neither multiply nor induce symptoms in tobacco (2). In the present study, conditions are described under which *P. fluorescens* can multiply and cause tissue necrosis in tobacco.

Nicotiana tabacum L. 'Samsun NN' plants were grown under controlled conditions (24 C, 8-hr day at light intensity of 1,500 ft-c) in vermiculite and irrigated twice daily, once with Hoagland's solution and once with water to keep the vermiculite at saturation. Plants with 4 to 6 expanded leaves were used in all experiments. The treated plants were incubated at 24 C in the dark or for 8 hr daily in the light at 1,500 ft-c.

Cells of *P. fluorescens* (ATCC No. 13525) were grown in nutrient broth for 24 hr at 24 C in shake culture. After incubation, the bacteria were washed, suspended in distilled water at a concn of 10^8 cells/ml, and injected into tobacco leaves (1). After injection, plants were placed in an environment of 25-35% relative humidity until water congestion disappeared. This equilibration of infiltrated water with the atmosphere was accomplished in the dark for those treat-

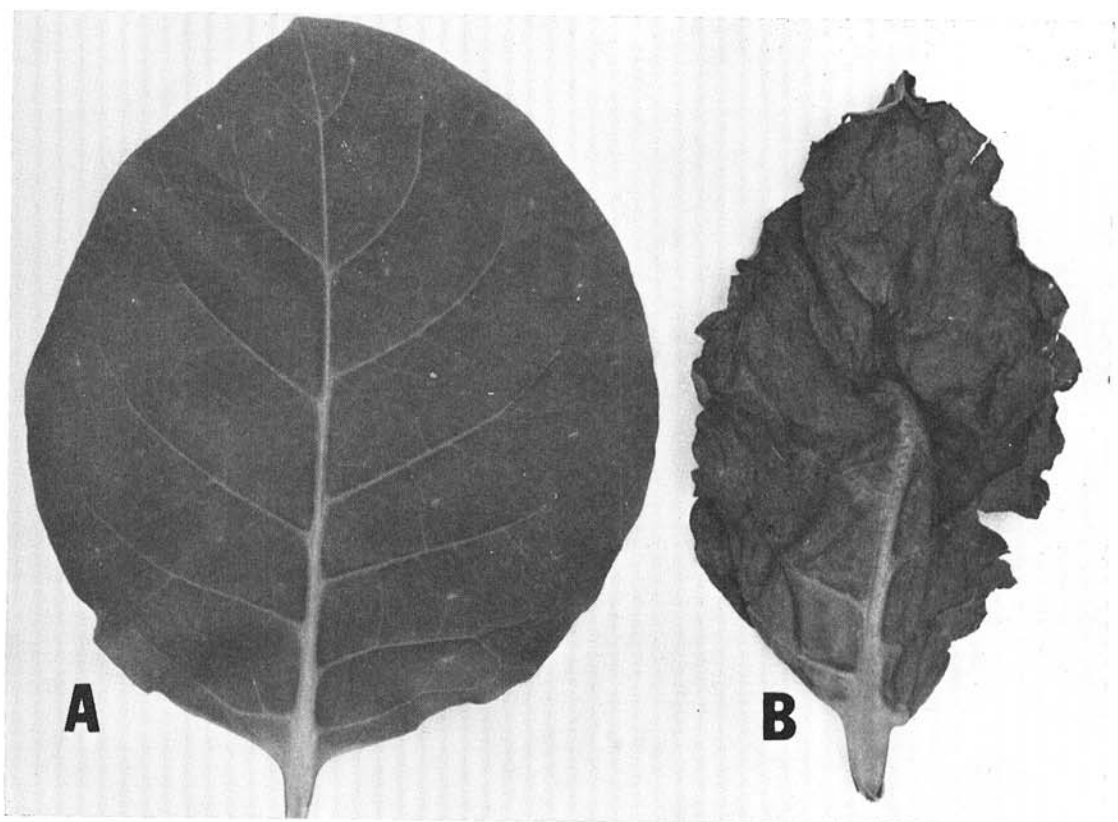

Fig. 1. Tobacco leaves injected with 10^8 cells/ml of *Pseudomonas fluorescens*. **A)** Leaf incubated for 4 days at 25-35% relative humidity (RH). **B)** Leaf incubated for 3 days at 100% RH, then for 1 day at 25-35% RH. The leaves were maintained at 24 C in the dark.

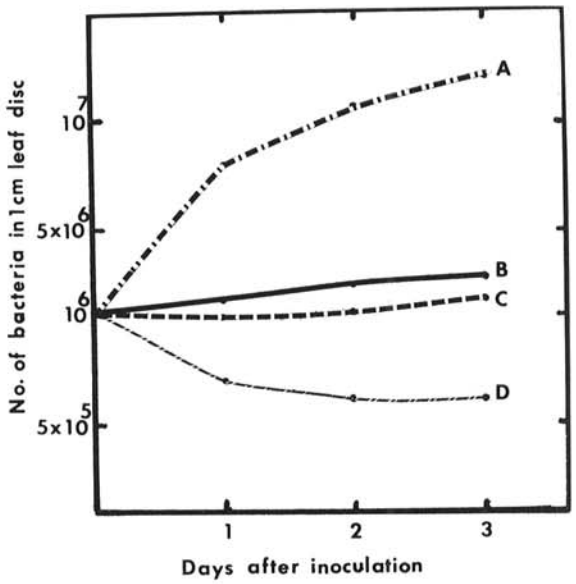

Fig. 2. Multiplication of *Pseudomonas fluorescens* at 24 C in tobacco leaf tissues. **A)** Leaves maintained in the dark at 100% relative humidity (RH). **B)** Leaves maintained in the dark at 25-35% RH. **C)** Leaves maintained in 1,500 ft-c for 8 hr daily at 100% RH. **D)** Leaves maintained in 1,500 ft-c for 8 hr daily at 25-35% RH.

ments which were inoculated and kept in darkness, and in light for those inoculated and kept in an 8-hr day cycle.

Tobacco leaves inoculated with *P. fluorescens* developed total necrosis when incubated in the dark for 3 days in a moist chamber, then transferred for 1 day to a relative humidity of 25-35% in either darkness or light (Fig. 1). The necrosis did not appear in inoculated leaves during the 3-day incubation in the darkened moist chamber. However, it gradually developed as the leaves lost water at 25 to 35% relative humidity. Necrosis never developed in inoculated leaves kept in a moist chamber for 3 days in the light (8-hr day), then transferred to 25-35% relative humidity (RH) for one or more days in either light or darkness. Necrosis also failed to appear in inoculated leaves maintained continuously at 25-35% RH in either light or dark regimes. Finally, control leaves injected with water under each of the experimental conditions did not develop necrosis.

Multiplication of bacteria in tobacco leaf tissue incubated under different conditions is presented in Fig. 2. Multiplication was determined by the dilution-plate technique. These data revealed that development of tissue necrosis in inoculated tobacco leaves was associated with the multiplication of bacteria.

We concluded from this study that when the saprophytic *P. fluorescens* bacteria multiply in tobacco leaves, they cause necrosis similar to that induced either by *P. tabaci,* the causal agent of the wildfire disease, or by numerous bacterial species which induce the hypersensitive reaction. The specific conditions which favor symptom production by *P. fluorescens* in tobacco leaves at 24 C are: 3-day exposure of the inoculated plant to 100% RH in the dark, followed by 24-hr incubation at 25-35% RH.

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