Relationship of Isolate Source to Virulence of Pseudomonas syringae on Phaseolus vulgaris

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ABSTRACT

Virulence of 30 *Pseudomonas syringae* isolates from 11 hosts and six geographic locations was compared on bean cultivar Tenderwhite in the greenhouse using inoculum levels of 10^3 and 10^8 cells/ml. All bean and lima bean isolates tested at both inoculum levels induced typical olive-green, water-soaked lesions within 3 to 4 days at 22 C. These lesions soon became necrotic with marginal chlorosis. Isolates from pear, apple, sour cherry, lilac, plum, peach, walnut, and sorghum produced only tiny dark necrotic flecks within 24-48 hr with inoculum containing 10^8 cells/ml, but no macroscopic symptoms were obtained with the lower inoculum level. The logarithmic growth in bean pods was similar for *P. phaseolicola* race 1 and isolates of *P. syringae* from bean, pear, lilac, and sorghum. However, between 2 and 4 days after inoculation, the population of *P. phaseolicola* and the bean isolate continued to increase, but at a slower rate, whereas the populations of the other isolates declined. These studies indicate a positive relationship of virulence to bean with *P. syringae* isolate from snapbean and lima bean only. Phytopathology 62:678-680.

Induction of the hypersensitive reaction is a characteristic shared by many phytopathogenic bacteria. The main characteristics of the bacterially induced hypersensitive reaction in the host are that the cells of the tissues containing the bacteria lose their turgor; this is followed by a rapid cellular collapse and subsequent tissue necrosis (5). The establishment of a true host-parasite relation leads to disease development only if a pathogen is able to multiply in the live host without the induction of a hypersensitive reaction.

This paper reports results of studies on the host-parasite interaction in the compatible and incompatible combinations when bean (*Phaseolus vulgaris* L.) plants were exposed to virulent and avirulent strains of *Pseudomonas syringae*. A preliminary report has been given (8).

The bacterial isolates included in this study were stored on nutrient agar glycerol (NAG) medium containing 0.8% nutrient broth, 2.0% glycerol, and 2.0% agar in slant tubes at 4 C. Inoculum was prepared by growing the bacteria on NAG medium for 24 hr at 28 C. Bacteria were washed from the medium with sterile distilled water and diluted to the desired concentration at 600 nm using a Bausch and Lomb Spectronic 20 colorimeter.

Bean seeds of the cultivar Tenderwhite were sown in vermiculite, then transplanted (one/pot) into 3:1 soil-sand mixture in 5-inch clay pots and grown in a greenhouse at 22 ± 1 C.

Bacterial suspensions of 10^8 and 10^3 cells/ml were atomized (using a De Vilbis atomizer under pressure of 15 psi and from a distance of 10 inches) onto both surfaces of the first set of young trifoliate leaves when they were one-third expanded. In one set of trials, plants were placed in a moist chamber and intermittently misted for 24 hr prior to and after inoculation. No moist chamber treatment was used in

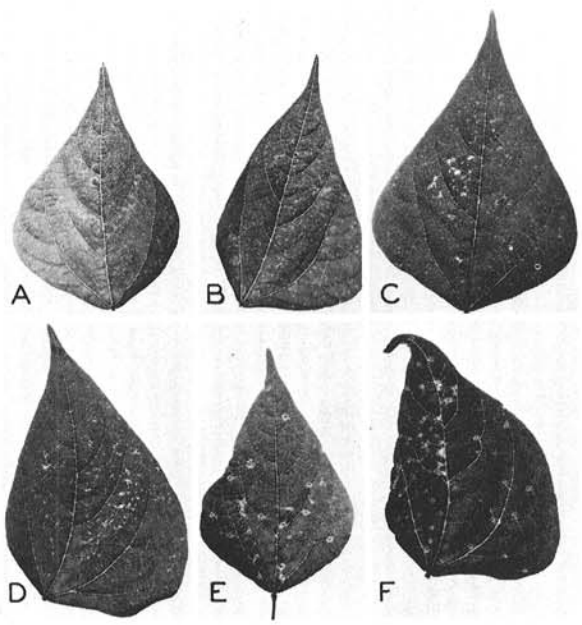

Fig. 1. Tenderwhite bean leaflets showing symptoms of flecks and typical lesions 8 days after inoculation with six isolates of *Pseudomonas syringae* at a concentration of 10^8 cells/ml from various sources: A = sour cherry; B = lilac; C = peach; D = pear; E = lima bean; and F = snapbean.

the other trials. Each trial consisted of 150 plants with each treatment replicated 5 times.

Growth characteristics of several *P. syringae* isolates from different hosts and one isolate of *P. phaseolicola* race 1 were studied in bean pods. Detached bean pods of the cultivar Tenderwhite were sterilized in 1% sodium hypochlorite (Klenzade) for 5 min, rinsed in sterile distilled water, and injected in six places with a hypodermic syringe at the rate of ca. 0.01 ml/injection with inoculum containing 10^7 cells/ml. Ten injected bean pods/isolate were placed in 15-cm petri plates with moist filter paper and incubated at room temperature. The measurement of bacterial growth in vivo was accomplished by taking 5-mm-diam discs from the center of the injected area with a sterile cork borer at 0, 1, 2, 3, 4, and 7 days after injection. Ten discs were macerated in a mortar containing 10 ml sterile distilled water. A tenfold dilution series was prepared from the supernatant liquid, and 1-ml samples from the appropriate dilution of the suspension were transferred into petri plates and mixed with 15-ml crystal violet medium containing 2.3% nutrient agar, 5.0% sucrose, and 2 ml crystal violet (0.1% crystal violet in 95% ethanol) at 45 C. Plates were dried in an oven for 30 min at 40 C and incubated at room temperature for 3 days, after which bacterial colonies were counted.

The bacterial isolates included in this study differed considerably in their virulence on bean. All bean and lima bean (*Phaseolus lunatus* L.) isolates tested at 10^3 and 10^8 cells/ml induced typical olive-green, water-soaked lesions within 3 to 4 days after inoculation. These lesions soon became necrotic, with marginal chlorosis (Fig. 1-E, F). More lesions were produced by the higher than by the lower concentration of inoculum. The isolates of *P. syringae* from pear (*Pyrus communis* L.); apple (*Malus sylvestris* Mill.); peach (*Prunus persica* Batsch); sour cherry (*Prunus cerasus* L.); plum (*Prunus cerasifera* Ehrh.); lilac (*Syringa vulgaris* L.); walnut (*Juglans californica* Wats.); and sorghum (*Sorghum vulgare* Pers.) produced only tiny dark necrotic flecks within 24-48 hr with inoculum containing 10^8 cells/ml (Fig. 1-A, B, C, D). No macroscopic symptoms were obtained with the lower level of inoculum. Three isolates from sweet cherry (*Prunus avium* L.) tested at both levels never induced any symptoms. Different isolates from the same host showed only minor differences in the size and number of lesions produced. There was no significant difference between misting the plants prior to and after inoculation for a period of 24 hr and "inoculation in situ", in regard to the time of appearance of symptoms and the number of lesions produced by the different compatible *P. syringae* isolates at both inoculum levels. However, misting the plants delayed the appearance of the fleck necrotic type of lesions.

The logarithmic growth in bean pods was similar for *P. phaseolicola* and isolates of *P. syringae* from bean, pear, lilac, and sorghum. However, between 2 and 4 days after inoculation, the population of *P. phaseolicola* (H-49) and the bean isolate (H-14) continued to increase, but at a slower rate, whereas the populations of the other isolates declined. The bean isolates and *P. phaseolicola* induced water-soaked lesions without the formation of any brown necrosis, whereas the other isolates caused atypical symptoms which consisted of light or dark brown, sunken, necrotic areas that fluoresced when exposed to ultraviolet light.

The hypersensitive reaction in bacterial diseases originally described by Klement (3) appears now to be a general response in leaves of nonhost plants injected with phytopathogenic bacteria (4, 6). In our experiments, rapid formation of the atypical symptoms in bean pods and young trifoliate leaves and the abrupt termination of the logarithmic growth phase suggest that the incompatible isolates of *P. syringae* induce a rapid reaction in the host. This is good evidence of the hypersensitive host response, a defense mechanism which operates in the incompatible host-parasite relationship (2, 5, 6).

We have found a striking positive association of virulence to bean with specific source plants. These results are somewhat in agreement with Crosse & Garrett (1), who found that inoculation of cherry leaf scars with cherry strains of *P. morsprunorum* resulted in severe disease, whereas plum strains were ineffective. In our experiments, the isolates from pear, sour cherry, lilac, walnut, plum, peach, apple, and sorghum produced the fleck type symptoms only at the high inoculum level of 10^8 cells/ml. Although bean plants in the field are exposed to both virulent and avirulent strains of *P. syringae*, the fleck

symptom is rarely, if ever, seen because the natural inoculum concentration probably is less than 10^8 cells/ml. However, more research on this aspect of epidemiology is sorely needed.

Bean pods proved to be a very suitable medium for obtaining added evidence for the existence of the hypersensitive response induced by *P. syringae* (7). All avirulent strains of *P. syringae* studied induced the formation of light or dark brown sunken necrosis at the site of inoculation. This discoloration and the accompanying fluorescence in ultraviolet light could be caused by the formations of phenolic compounds.

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