

**NOTE: Comparison of Three Laboratory Methods to Evaluate the Pathogenicity and Virulence of Ten *Pseudomonas syringae* pv. *syringae* Strains on Apple, Pear, Cherry and Peach Trees**

T. Thomidis,<sup>\*,1</sup> C. Tsipouridis,<sup>1</sup> E. Exadaktylou<sup>1</sup> and P. Drogoudi<sup>1</sup>

The pathogenicity and virulence of ten Greek *Pseudomonas syringae* pv. *syringae* strains from different hosts (citrus, pear, apple, peach and cherry) were evaluated using three different laboratory methods, which produced results in good agreement. All ten strains were virulent on apple, pear, cherry and peach trees. The extent of tissue colonized varied considerably among strains and cultivars. On excised shoots and twigs of apple and pear, strains BPI 176, BPI 203, PI 2 and PI 14 were the most virulent and strains BPI 689, BPI 992, BPI 4, BPI 20, PI 18 and PI 19 were the least virulent. On excised shoots and twigs of peach and cherry, strains BPI 176, BPI 203, PI 2, PI 14, PI 18 and PI 19 were the most virulent and strains BPI 4 and BPI 20 were the least virulent. Moderate virulence was evinced by strains BPI 689 and BPI 992. These pathogenicity assays are proposed as rapid and reproducible screening systems to evaluate the susceptibility of apple, pear, cherry and peach cultivars to this bacterial pathogen.

**KEY WORDS:** Excised twig; *Pseudomonas syringae* pv. *syringae*; pathogenicity; virulence.

Infection of trees by *Pseudomonas syringae* pv. *syringae* (*Pss*) is a potentially serious problem that may limit the establishment and sustained productivity of pome and stone fruit orchards (7). Variability in pathogenicity among isolates of *Pss* has been recognized for a long time (9). Scheck *et al.* (5) evaluated the pathogenicity of 522 *P. syringae* pv. *syringae* strains with a lilac tissue culture bioassay and syringomycin DNA probes and found that 59% were pathogenic and hybridized with the syringomycin probes, while 19% were non-pathogenic and did not hybridize with syringomycin probes. Little *et al.* (3) found host specialization within the diverse pathovars of *P. syringae* pv. *syringae*. The availability of a rapid and reliable laboratory method to evaluate the pathogenicity and virulence of a large number of strains of *Pss* on fruit trees could be very

helpful, because the symptoms develop quickly after inoculation (4,8). The goal of this study was to test the pathogenicity and virulence of ten *Pss* strains originating from different plants to apple, pear, cherry and peach trees under laboratory conditions. Also, a new method to evaluate the pathogenicity and virulence of *Pss* on trees was developed and compared with the excised twig and excised shoot methods.

Four strains of *Pss* from citrus (BP 14, BPI 176, BPI 203 and PI 18, all isolated from infected stems); four from pear (BPI 20, BPI 992 and PI 19, isolated from infected stems, and BPI 689, isolated from an infected flower); and two from stone trees (PI 14 from peach and PI 2 from cherry, both isolated from infected shoots) were used in this study. Bacteria were grown on King's medium at 25°C.

Received June 2, 2004; accepted Oct. 31, 2004; <http://www.phytoparasitica.org> posting March 4, 2005.

<sup>1</sup>National Agriculture Research Foundation (NAGREF), Pomology Institute, P.C. 59200, Naoussa, Greece.

\*Corresponding author [e-mail: thomi-1@otenet.gr].

In the first experiment, King's medium, amended with 1.5 g boric acid, 8 mg cephalixin and 20 mg cycloheximide per liter, was dispensed in sterile Pyrex jars to obtain a layer of ~10 mm. These were inoculated with the *Pss* strains and incubated at 25°C in the dark for 7 days. One-year-old dormant shoots, ~50 cm in length and 10–15 mm in diameter, were collected from 4-year-old apple (cv. 'Starking'), pear (cv. 'Kontoula 1/6'), cherry (cv. 'Burlat') and peach (cv. 'Andross') trees. Preparation of twigs, inoculation and data collection were as described by Krzesinska and Azarenko (2). Eight replicate jars were used for each *Pss* strain, two for each cultivar. Two non-inoculated jars were used as the control.

In the second experiment, 2-year-old dormant shoots, ~80 cm in length and 2 cm in diameter, were collected from apple, pear, cherry and peach trees. The same trees as above were used. Segments, ~10 cm in length, were cut from the center of each shoot. Wounding of segments, inoculation and data collection were as described previously by Thomidis (6). There were 80 segments, 20 for each test plant, used for each *Pss* strain. Twenty non-inoculated segments from each plant were used as the control.

In the third experiment, fine vermiculite was placed in Pyrex jars to form a layer of ~10 mm. Jars were closed and sterilized in an autoclave at 121°C for 15 min. Bacteria were grown in King's medium, mixed with sterile-distilled water to produce a concentration of  $\sim 1 \times 10^9$  CFU ml<sup>-1</sup>, after which the vermiculite was saturated with test strains under aseptic conditions. Preparation of twigs, inoculation and data collection were as described by Krzesinska and Azarenko (2). Again, eight replicate jars were used for each *Pss* strain, two for each plant. Two non-inoculated jars were used as control.

The experimental design used throughout the experiments was completely randomized. Data were analyzed by one-way analysis of variance. To combine experiments, Bartlett's test of homogeneity of variance was used and treatment means were separated by Duncan's Multiple Range Test ( $P=0.05$ ).

Overall, results from the three pathogenicity tests used in this study were in good agreement. These methods can be useful techniques to assess the susceptibility to *Pss*; they are reliable and quick, allow ample replication, and can be

adapted to accommodate almost any type of woody plant host. In modified excised twig assay, inocula are uniformly arranged throughout the substrate (necrosis begins uniformly from the base of twigs), in contrast to the excised twig assay, where the inocula are only on the surface of the substrate (necrosis begins from the level of the substrate surface). Because of the uniform inoculation of the bases of the twig segments in the modified excised twig assay, lesions developed only upward rather than in several directions, as occurred with the excised shoot method and the excised twig assay.

All the *Pss* strains tested were pathogenic on apple, pear, cherry and peach trees (Table 1). In contrast, Yessadcarreau *et al.* (9) found that some strains of *Pss* showed host-specificity. The extent of tissue colonized varied considerably among strains and cultivars. On excised shoots and twigs of apple and pear, the strains BPI 176, BPI 203, PI 14 and PI 2 were the most virulent and strains BPI 689, BPI 992, BPI 4, BPI 20, PI 18 and PI 19 were the least virulent. On excised shoots and twigs of peach and cherry, strains BPI 176, BPI 203, PI 14, PI 2, PI 18 and PI 19 were the most virulent and strains BPI 4 and BPI 20 were the least virulent. Moderate virulence was evinced by strains BPI 689 and BPI 992 (Table 1). It is well-known that *Pss* produces two groups of cyclic lipodepsipeptides (syringomyins and syringopeptins), which significantly contribute to bacterial pathogenesis (1). The different level of production of these lipodepsipeptides by the strains and the possibly different susceptibility of tree species may be a good explanation for the different virulence of the *Pss* strains tested. It is surprising that strains BPI 20 and PI 19 originating from pear were less virulent on pear than were the strains originating from citrus, peach and cherry trees. These findings show that the virulence of the *Pss* strains tested probably is not controlled by mechanisms related to the host specificity, but no reasonable explanation can be given. Vicente *et al.* (7) conducted pathogenicity tests with *Pss* strains and found a range of virulence among isolates. Little *et al.* (3) found that strains of *Pss* were moderately to highly pathogenic on 'Lovell' peach seedlings regardless of the host origin, whereas strains of other pathovars exhibited low or no pathogenicity.

TABLE 1. Pathogenicity and virulence of ten *Pseudomonas syringae* pv. *syringae* strains on apple, pear, peach and cherry excised shoots and twigs<sup>z</sup>

Strains	Excised twig assay (cm)				Excised shoot method (cm)				Modified excised twig assay (cm)			
	Apple	Pear	Cherry	Peach	Apple	Pear	Cherry	Peach	Apple	Pear	Cherry	Peach
BPI 4	0.3 a <sup>y</sup>	0.6 a	2.3 a	2.0 a	0.3 a	0.3 a	1.3 a	1.3 a	0.2 a	0.8 ab	1.2 a	1.0 a
BPI 20	0.5 a	0.6 a	2.4 a	2.8 b	0.7 ab	0.5 a	1.1 a	1.2 a	0.3 a	1.1 bc	1.1 a	1.1 a
BPI 176	1.9 b	2.0 b	4.0 c	3.5 c	1.8 c	1.4 b	2.5 c	1.5 a	0.7 b	1.1 bc	2.7 b	1.6 b
BPI 203	1.7 b	1.8 b	4.0 c	3.2 bc	1.7 c	1.5 b	3.1 d	1.4 a	1.6 c	1.5 c	2.4 b	1.5 b
PI 2	2.0 b	1.8 b	4.1 c	3.2 bc	1.9 c	1.5 b	3.0 d	1.4 a	1.4 c	1.4 c	2.5 b	1.5 b
PI 14	1.9 b	1.9 b	4.0 c	3.1 bc	1.8 c	1.5 b	3.0 d	1.5 a	1.5 c	1.5 c	2.5 b	1.6 b
BPI 689	0.5 a	0.5 a	3.3 b	2.5 ab	0.4 ab	0.5 a	2.1 bc	1.6 a	0.4 ab	0.5 ab	1.3 a	1.2 ab
BPI 992	0.6 a	0.6 a	2.9 ab	2.4 ab	0.8 b	0.7 a	1.9 b	1.4 a	0.3 a	0.4 a	1.1 a	1.3 ab
PI 18	0.3 a	0.4 a	4.2 c	3.4 c	0.3 a	0.6 a	3.1 d	1.4 a	0.7 b	0.3 a	2.9 b	1.4 b
PI 19	0.4 a	0.4 a	4.2 c	3.1 bc	0.6 ab	0.5 a	3.2 d	1.5 a	0.4 ab	0.2 a	2.6 b	1.5 b

<sup>z</sup>Values are the means of three experiments, each with 20 replicates; results were similar according to Bartlett's test of homogeneity of variance and the data were therefore combined.

<sup>y</sup>Within columns, values followed by a common letter do not differ according to Duncan's Multiple Range Test ( $P=0.05$ ).

#### ACKNOWLEDGMENT

We would like to express our thanks to Dr. A.S. Alivisatos, Director of the Benaki Phytopathological Institute in Athens for providing the cultures of *Pseudomonas syringae* pv. *syringae*.

#### REFERENCES

1. Dalla Serra, M., Fagioli, G., Nordera, P., Bernhart, I., Della Volpe, C., DiGiorgio, D. *et al.* (1999) The interaction of lipodepsipeptide toxins from *Pseudomonas syringae* pv. *syringae* with biological and model membranes: A comparison of syringotoxin, syringomycin, and two syringopeptins. *Mol. Plant-Microbe Interact.* 12:391-400.
2. Krzesinska, E.Z. and Azarenko, A.N. (1992) Excised twig assay to evaluate cherry rootstocks for tolerance to *Pseudomonas syringae* pv. *syringae*. *HortScience* 27:153-155.
3. Little, E.L., Bostock, R.M. and Kirkpatrick, B.C. (1998) Genetic characterization of *Pseudomonas syringae* pv. *syringae* strains from stone fruits in California. *Appl. Environ. Microbiol.* 64:3818-3823.
4. Moragrega, C., Llorente, I., Manceau, C. and Montesinos, E. (2003) Susceptibility of European pear cultivars to *Pseudomonas syringae* pv. *syringae* using immature fruit and detached leaf assays. *Eur. J. Plant Pathol.* 109:319-326.
5. Scheck, H.J., Canfield, M.L., Pscheidt, J.W. and Moore, L.W. (1997) Rapid evaluation of pathogenicity in *Pseudomonas syringae* pv. *syringae* with a lilac tissue culture bioassay and syringomycin DNA probes. *Plant Dis.* 81:905-910.
6. Thomidis, T. (2000) Field susceptibility of four peach rootstocks to *Phytophthora citrophthora* and *P. syringae*. *Phytopathol. Mediterr.* 39:404-409.
7. Vicente, J.G., Alves, J.P., Russell, K. and Roberts, S.J. (2004) Identification and discrimination of *Pseudomonas syringae* isolates from wild cherry in England. *Eur. J. Plant Pathol.* 110:337-351 .
8. Yessad, S., Manceau, C. and Luisetti, J. (1992) A detached leaf assay to evaluate virulence and pathogenicity of strains of *Pseudomonas syringae* pv. *syringae* on pear. *Plant Dis.* 76:370-373.
9. Yessad-Carreau, S., Manceau, C. and Luisetti, J. (1994) Occurrence of specific reactions induced by *Pseudomonas syringae* pv. *syringae* on bean pods, lilac and pear plants. *Plant Pathol.* 43:528-536.