Variation in Virulence, Plasmid Content, and Genes for Coronatine Synthesis Between *Pseudomonas syringae* pv. *morsprunorum* and *P. s. syringae* from *Prunus*

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ABSTRACT

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Strains of *Pseudomonas syringae* pv. *morsprunorum* and *P. s. syringae* from *Prunus* were examined for virulence to cherry, plasmid DNA content, and coronatine synthesis genes. Michigan strains of *P. s. syringae* spread faster through cherry rootstock plantlets than did strains of *P. s. morsprunorum*. Twenty-six strains of *P. s. morsprunorum*, regardless of geographic origin, contained three to seven plasmids of variable size. Twenty-two strains of *P. s. syringae* contained zero to two plasmids. Among 319 field strains of *P. s. morsprunorum* from cherry (*Prunus cerasus* and *Prunus avium*) orchards in Michigan, 98% contained genomic DNA that hybridized with two DNA probes for coronatine synthesis genes cloned from *P. s. tomato*. Conversely, genomic DNA from strains of *P. s. syringae* from cherry, except one strain, did not hybridize with either probe for coronatine synthesis genes. Southern analysis of plasmid DNA indicated that the genes for coronatine synthesis were located on the same 105-kb plasmid in Michigan strains of *P. s. morsprunorum* as in English strain PM567, which was used as a standard. In a bioassay for coronatine, strains of *P. s. morsprunorum* with homologous DNA for coronatine synthesis, but not strains lacking homologous DNA, induced hypertrophy on potato tuber slices. These differences in virulence, plasmid profile, and coronatine production provide further evidence that *P. s. morsprunorum* is distinct from stone fruit strains of *P. s. syringae*.

Bacterial canker is a sporadic but economically important disease of several *Prunus* spp. In Michigan in the United States (12,16,26,32) and in Ontario, Canada (1,9), *Pseudomonas syringae* pv. *morsprunorum* (Wormald) Young et al and *P. s. syringae* van Hall can elicit bacterial canker lesions on sweet (*Prunus avium* (L.) L.) and sour (*Prunus cerasus* L.) cherry leaves and fruits. Outbreaks of bacterial canker usually occur in years when prolonged wet periods are accompanied by freezing temperatures during or shortly after bloom. Frost-induced water soaking has been shown to increase the ingress of *P. s. syringae* and *P. s. morsprunorum* into sour cherry leaves (30).

Sour cherry leaves infected with *P. s. morsprunorum* exhibit necrotic lesions that may be surrounded by systemic chlorosis or yellow halos. The origin of the chlorosis associated with lesions on cherry leaves likely involves the biosynthesis of coronatine by the pathogen (3,20). Coronatine is produced by *P. s. morsprunorum* and a few other pathovars of *P. syringae*, but not by *P. s. syringae* (11). In *P. s. morsprunorum* strains PM567 and PM3714, the genes for coronatine synthesis are located on 105- and 95-kb plasmids, respectively (3). Although strains PM567 and PM3714 are confirmed coronatine producers (3, 20), synthesis of coronatine by other strains of *P. s. morsprunorum* could not be confirmed by bioassay (20,23) or by hybridization with a fragment from the coronatine-synthesis region of *P. s. tomato* DC3000 chromosomal DNA (8,17,21).

The objective of this study was to determine the variability in virulence and in plasmid DNA content between *P. s. morsprunorum* and *P. s. syringae*. We also evaluated whether coronatine synthesis genes were present in the pathovars of *P. syringae* isolated from cherry in Michigan and whether the genes were of chromosomal or plasmid origin. A brief portion of this work was reported previously (25).

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MATERIALS AND METHODS

Bacterial strains and plasmids. Strains Psm212, Psm210, and Psm219 of *P. s. morsprunorum* were isolated from plum, sweet cherry, and prune blossoms, respectively, as described by Sundin et al (32). *P. s. syringae* strain Pss315 was isolated from sweet cherry, and strains Pss224 and Pss219 were isolated from sour cherry. Strains from other geographical regions were supplied by colleagues in the respective countries listed in Table 1. The strains examined for coronatine synthesis genes were isolated from apple and cherry orchards in Michigan in 1990 and 1991 (19,26,29). Strain PM567 of *P. s. morsprunorum* was supplied by C. L. Bender, Oklahoma State University, Stillwater, and was originally from England (3). The identity of the strains from stone fruit trees was confirmed based on the production of fluorescence when grown on King's medium B (KB) (14), a negative cytochrome oxidase reaction (15), and GATTa tests for separation of *P. s. syringae* from *P. s. morsprunorum* (16). The strains from apple were identified previously (29).

Two selected regions from cosmid pSAY1 containing coronatine-synthesis genes from strain PT23.2 of *P. s. tomato* (2,3) were supplied by C. L. Bender. Plasmid pCLB4 contained a 2.2-kb *Hin*dIII fragment, and pSAY1.11 contained a 2.5-kb *Sac*I fragment from pSAY1. The plasmids were received and amplified in *Escherichia coli* strain HB101. Strain HB101 was maintained on Luria-Bertani (LB) medium (18).

Plantlet assay for virulence. Shoot-tip cultures of Mazzard (*Prunus avium*) and GM-79 (*Prunus canescens*) cherry rootstocks were supplied by Marilyn Canfield, Oregon State University, Corvallis; and Hybrid II, from a cross of English Morello × Sumadinka, was supplied by A. F. Iezzoni, Michigan State University, East Lansing. Bacteria grown overnight on KB agar at 25 C were suspended in sterile distilled water and adjusted with sterile water to an optical density of 0.4–0.45 at 620 nm in a Bausch & Lomb Spectrophotometer (approximately 10^8 cfu/ml). Inoculations were performed on shoots 3–4 cm high after 4–5 wk of growth in 125-ml flasks on Murashigi and Skoog medium without glycine (MS medium) (22). The tip of each shoot was cut with a small scissors previously dipped into the suspension of bacteria (24). After inoculation, the flasks were kept at room temperature under fluorescent light with a 16-hr photoperiod. Plantlets were rated for symptom development up to 29 days after inoculation. The severity of necrosis was scored using the following scale: 0 = no necrosis, 1 = trace of brown discoloration at the point of inoculation, 2 = up to 2 mm of necrosis, 3 = 2–5 mm of necrosis, 4 = 5–10 mm of necrosis, and 5 = more than 10 mm of necrosis. Each set of inoculations was done on 18 to 20 shoots, and the experiment was repeated.

Preparation of DNA probe. Each

plasmid was isolated from *E. coli* by alkaline lysis extraction, purified by cesium chloride centrifugation, and digested with restriction enzyme *Hin*dIII or *Sac*I. The fragments were separated by electrophoresis on 0.7% agarose gels (18). A 2.2-kb *Hin*dIII restriction fragment from pCLB4 (probe CLB4) and a 2.5-kb *Sac*I restriction fragment from pSAY1.11 (probe SAY1.11) were electroeluted onto TEAE membranes (Schleicher & Schuell, Inc., Keen, NH), then released from the membranes by incubation in a high salt solution (20 mM Tris, pH 8.0, 0.1 mM EDTA, and 1.0 M NaCl) for 30 min at 65 C with intermediate shaking. The eluted DNA was precipitated with ethanol, dissolved in TE buffer (10 mM Tris.Cl, pH 7.4, and 1 mM EDTA, pH 8.0), and labeled with α-^{32}P-dCTP using a Random Primed DNA Labeling Kit (United States Biochemical Corp., Cleveland, OH).

Colony hybridizations. Two replicates of each isolate were transferred with sterile toothpicks to Colony/Plaque Screen Hybridization Transfer Membranes (NEN Research Products, Boston, MA) that had been placed on the surface of KB agar. Strain PM567 of *P. s. morsprunorum* was included on each membrane as a positive control. The bacteria were allowed to grow for 24 hr at 20 C before the cells were lysed and the DNA was denatured and fixed to the membranes according to the manufacturer's instructions. Hybridizations with each of the two radiolabeled restriction fragments were performed overnight, and the membranes were washed according to manufacturer's recommended procedures. Autoradiographs of membranes were carried out with X-ray film at −70 C.

Plasmid isolation and preparation of Southern blots. In 1988 and 1989, plasmid DNA was isolated by alkaline lysis (4) from 3-ml cultures shaken overnight in LB broth. The plasmids were separated by electrophoresis in 0.5% agarose gels immersed in Tris-borate buffer (18). The gels were stained with ethidium bromide, visualized by UV light, and photographed (31). Plasmids in strain SW2 of *Erwinia stewartii* were included for estimating the size of detected plasmids (5). In 1990 and 1991, plasmid DNA of *P. s. morsprunorum* was isolated by alkaline lysis extraction (18). The extracted plasmid DNA was digested with *Hin*dIII, then subjected to gel electrophoresis and Southern transfer to GeenScreen Plus Hybridization Transfer Membrane (NEN Research Products). Plasmid DNA that was not to be digested was isolated by the method of Kado and Liu (13). The gels were incubated in 0.25 N HCl for 15 min at room temperature prior to alkaline treatment. Transfer of DNA proceeded for 24 hr. Hybridizations with probe CLB4 were carried out as described for the colony hybridizations.

Bioassay for coronatine. Isolates of *P. s. morsprunorum* were assayed for their ability to elicit hypertrophic outgrowths on potato slices (10,28). Bacteria from single colonies were transferred to 500 ml of Woolley's medium (34) in 2-L flasks. After the cultures were incubated for 6 days on a shaker at 20 C, the bacteria were removed by centrifugation at 2,987 *g* for 5 min. Culture supernatants were concentrated by rotary evaporation (bath temperature less than 50 C) and then centrifuged at 26,890 *g* for 5 min at 4 C. The resulting supernatant was acidified (pH 2.5) with 12 N HCl and extracted three times with ethyl acetate. The extracts were concentrated, dried, and evaporated. The residue was dissolved in 1 ml of methanol and stored at −20 C. Aliquots (10 μl) of the residue were applied to 13-mm-diameter filter-paper disks. After air-drying, the disks were placed on the upper surface of freshly cut 6 × 13 mm potato tuber slices in petri dishes on moistened filter paper at 20 C. Each residue was tested on four potato slices. Slices treated with extracts from noninoculated medium served as controls.

Table 1. Plasmid content of strains of *Pseudomonas syringae* pv. *morsprunorum* and *P. s. syringae* from stone fruit trees in Michigan and in other parts of the world

Pathovar Strain	Host	Origin[a]	Plasmids (no.)	Size of plasmids (kb)
P. s. morsprunorum[b]				
212-02	Sweet cherry	MI	3	120, -[c], 37
C-17	Cherry	Eng.	3	120, 78, 47
P-243	Sour cherry	Pol.	3	120, 100, 78
634	Plum	S.A.	3	98, 74, 47
102-A3	Prune	MI	4	82, 46, 35, 5
102-A7	Prune	MI	4	120, 46, 35, 5
106-A2	Sour cherry	MI	4	100, 50, 46, 35
115-A4	Prune	MI	4	120, 66, 50, 35
C-185	Cherry	Eng.	4	120, 98, 54, 46
P-204	Sour cherry	Pol.	4	98, 74, 64, 2.4
101-A7	Prune	MI	5	120, 90, 66, 50, 7
102-A5	Prune	MI	5	84, 78, 66, 50, 35
110-B2	Prune	MI	5	100, 66, 50, 29, 5
111-B4	Prune	MI	5	100, 60, 46, 40, 10
115-A1	Prune	MI	5	90, 60, 50, 29, 5
210-02	Plum	MI	5	90, 64, 46, 35, 10
211-01	Plum	MI	5	90, 64, 46, 35, 10
218-01	Prune	MI	5	100, 60, 50, -, 10
218-03	Prune	MI	5	90, 60, 50, 35, 10
101-A1	Prune	MI	6	120, 106, 60, 52, 46, 35
103-A2	Prune	MI	6	74, 58, 52, 50, 42, 35
103-A6	Prune	MI	6	84, 68, 50, 46, 28, 5
103-A8	Prune	MI	6	110, 78, 68, 46, 35, 5
627	Sweet cherry	S.A.	6	88, 78, 64, 56, 35, 18
103-B1	Prune	MI	7	106, 74, 60, 58, 52, 46, 35
211-10	Plum	MI	7	90, 60, 50, -, 29, 10, 5
P. s. syringae[b]				
105-A1	Prune	MI	0	
112-A1	Sour cherry	MI	0	
114-A1	Prune	MI	0	
201-02	Sour cherry	MI	0	
203-02	Sour cherry	MI	0	
220-02	Sweet cherry	MI	0	
223-01	Plum	MI	0	
224-01	Sour cherry	MI	0	
W4N9	Sweet cherry	WA	0	
W4N108	Sweet cherry	WA	0	
JP 442	Plum	Eng.	0	
PSS 10	Sour cherry	Ger.	0	
No. 1835	Sour cherry	Pol.	0	
No. 2905	Sour cherry	Pol.	0	
110-A1	Prune	MI	1	28
203-06	Sour cherry	MI	1	37
203-13	Sour cherry	MI	1	40
219-05	Sour cherry	MI	1	42
222-04	Plum	MI	1	60
223-04	Plum	MI	1	25
S-150	Cherry	Eng.	2	68, 50
PS2	Sour cherry	MI	2	-, -

[a] Countries and their abbreviations are as follows: England (Eng.), Germany (Ger.), Poland (Pol.), and South Africa (S.A.). States in the United States are abbreviated as Michigan (MI) and Washington (WA).
[b] Pathovar identification based on results from the GATTa tests (16).
[c] Undetermined.

RESULTS

Evaluation of virulence in plantlet assays. The development of necrosis on

plantlets of cherry inoculated with strains of *P. s. syringae* was more severe than on plantlets inoculated with strains of *P. s. morsprunorum* (Fig. 1). Five days after inoculation, disease ratings for plantlets inoculated with strains of *P. s. syringae* were 2–3 (except for strain Pss219 on GM-79) compared to ratings of 0–1 for plantlets inoculated with strains of *P. s. morsprunorum*. More necrosis was observed 29 days after inoculation on plantlets inoculated with *P. s. syringae* than on plantlets inoculated with *P. s. morsprunorum*. Populations of bacteria recovered from shoots of Hybrid II 1, 4, and 7 days after inoculation were $\log_{10}$ 5.97, 7.14, and 7.45 cfu/cm of shoot tissue for strain Psm212; 6.90, 7.98, and 7.80 cfu/cm for strain Pss224; and 7.15, 8.07, and 7.80 cfu/cm for strain Pss315, respectively.

Plasmid profiles. The plasmid content of *P. s. morsprunorum* from the United States, England, Poland, and South Africa was highly variable (Table 1). All 26 strains examined by electrophoresis contained plasmids ranging from about 5 to 120 kb. The number of plasmids per strain varied from three to seven.

The plasmid content for strains of *P. s. syringae* differed considerably from the plasmid DNA content for strains of *P. s. morsprunorum*. Only eight of 22 strains of *P. s. syringae* contained detectable plasmids (Table 1). The number of plasmids in the eight strains varied from one for each of six strains from Michigan to two for strains from England and Michigan. The size of the plasmids ranged from 25 to 68 kb.

Colony hybridization and Southern analysis. Probes CLB4 and SAY1.11 hybridized to DNA from 310 of 319 strains of *P. s. morsprunorum*, but not to DNA from 5 strains of *P. s. papulans*, 7 of *P. fluorescens*, 2 of *P. aeruginosa*, 2 of *P. putida*, 14 of *Erwinia amylovora*, 3 of *E. herbicola*, 2 of *Acinetobacter*, 2 of *Aeromonas*, 2 of *Flavobacterium*, and 43 of *Pseudomonas* spp., used as controls. Probe CLB4 but not probe SAY1.11 hybridized to DNA from one of 76 strains of *P. s. syringae*. Strains with DNA that hybridized with both probes were considered to have a *cor*$^+$ genotype, others a *cor*$^-$ genotype.

When the plasmids were examined by Southern analysis, probe CLB4 hybridized to the largest plasmid (estimated to be 105 kb by Bender et al [3]) in PM567 and to a similar-sized plasmid from each of the 14 *cor*$^+$ strains of *P. s. morsprunorum* from Michigan. Probe CLB4 did not hybridize with any of the plasmids in the nine *cor*$^-$ strains of *P. s. morsprunorum*. Plasmids were not detected in the isolate of *P. s. syringae* with DNA that hybridized with probe CLB4 on dot blots.

Probe CLB4 hybridized to a 2.2-kb fragment in *Hin*dIII digests of plasmid DNA from seven *cor*$^+$ strains of *P. s. morsprunorum* but not to digested plasmid DNA from a *cor*$^-$ strain (Fig. 2). There was no polymorphism for the 2.2-kb *Hin*dIII fragment among strains of *P. s. morsprunorum*.

Bioassay for coronatine production. Potato tuber slices treated with extracts from 6-day-old cultures in Woolley's medium of 10 *cor*$^+$ strains of *P. s. morsprunorum* developed hypertrophic outgrowths in 3–4 days. None of the potato tuber slices treated with extracts from cultures of the nine *cor*$^-$ strains of *P. s. morsprunorum* and none treated with extracts from Woolley's medium alone exhibited outgrowths 4 days after treatment.

DISCUSSION

Our results showing that Michigan strains of *P. s. syringae* spread faster through cherry tissue than strains of *P. s. morsprunorum* verify a similar study conducted in England with a North American strain of *P. s. syringae* (6). Our results also indicate that tissue culture techniques can be used to test the pathogenicity of isolates when cherry fruitlets or actively growing shoot tissues are not available for inoculation.

The detection of genes for synthesis of the phytotoxin coronatine in recently isolated field strains of *P. s. morsprunorum* from Michigan suggests that the production of coronatine by this pathogen may be widespread in nature. Only 2% of the strains lacked genes for coronatine synthesis. Previously, a small number of strains, including *P. s. morsprunorum* PM567 from England and PM3714 from Italy, were shown to produce coronatine in liquid culture (20) and to contain DNA for coronatine synthesis homologous with that from strain PT23.3 of *P. s. tomato* (3). Our conclusion that the *cor*$^+$ genes in *P. s. morsprunorum* are located on plasmid DNA supports a similar conclusion for

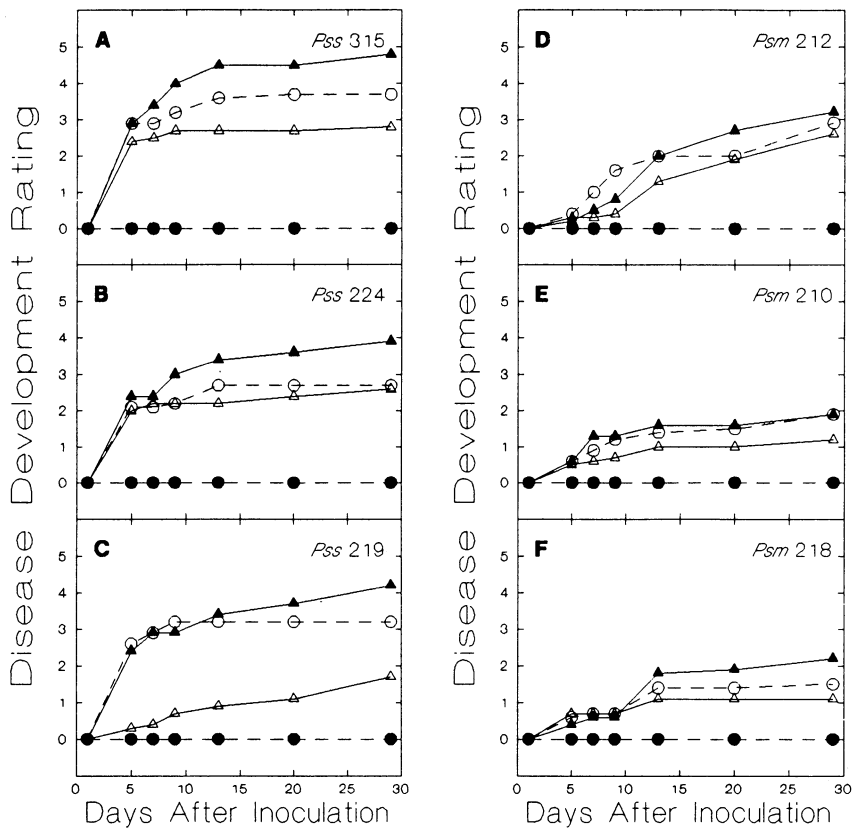

Fig. 1. Development of necrosis on plantlets of GM-79 (△), Hybrid II (○), and Mazzard (▲) cherry rootstocks grown in vitro for 29 days. Plantlets were inoculated with three strains of *Pseudomonas syringae* pv. *syringae* **(A–C)** or of *P. s. morsprunorum* **(D–F)** by cutting the tips with scissors dipped into suspensions containing 10^8 cfu/ml of the respective strains. Control plantlets (●) were cut with scissors dipped into sterile water.

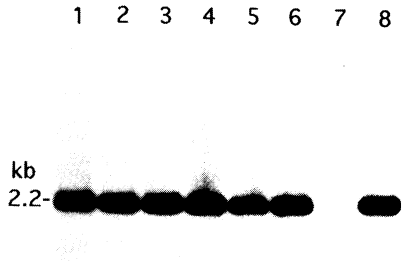

Fig. 2. Hybridization of a ^{32}P-labeled 2.2-kb *Hin*dIII fragment (probe CLB4) from the region of *Pseudomonas syringae* pv. *tomato* plasmid pPT23A involved in coronatine synthesis to a nylon membrane containing *Hin*dIII digested plasmid DNA from eight strains of *P. s. morsprunorum*.

strains PM567 and PM3714. Among five coronatine-producing pathovars of *P. syringae,* pathovars *atropurpurea, glycinea, morsprunorum,* and *tomato* contain plasmid-borne genes for coronatine synthesis (3); while in pathovar *maculicola,* the location of coronatine-synthesis genes has not been precisely defined (33).

Although *P. s. morsprunorum* and *P. s. syringae* occur together as epiphytes and as pathogens of stone fruit crops in Michigan (12,16,19,26,32) and in other parts of the world (1,7,9,27), there appears to be a high level of genetic isolation of the two pathovars. Strains of *P. s. morsprunorum* but not *P. s. syringae* contained plasmid-mediated genes for coronatine synthesis as part of their gene pool. Previously, strains of *P. s. syringae* but not *P. s. morsprunorum* from the same orchards were shown to have plasmid-mediated genes for copper resistance (31). In this study, strains of *P. s. morsprunorum* had a higher number of plasmids and exhibited a higher diversity in plasmid profiles than did strains of *P. s. syringae.* These examples are evidence that the pathovars have mingled very little at the genetic level.

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LITERATURE CITED

1. Allen, W. R., and Dirks, V. A. 1978. Bacterial canker of sweet cherry in the Niagara Peninsula of Ontario: *Pseudomonas* species involved and cultivar susceptibilities. Can. J. Plant Sci. 58:363-369.
2. Bender, C. L., Malvick, D. K., and Mitchell, R. E. 1989. Plasmid-mediated production of the phytotoxin coronatine in *Pseudomonas syringae* pv. *tomato.* J. Bacteriol. 171:807-812.
3. Bender, C. L., Young, S. A., and Mitchell, R. E. 1991. Conservation of plasmid DNA sequences in coronatine-producing pathovars of *Pseudomonas syringae.* Appl. Environ. Microbiol. 57:993-999.
4. Birnboim, H. C. 1983. A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol. 100:243-255.
5. Coplin, D. L., Rowan, R. G., Chisholm, D. A., and Whitmoyer, R. E. 1981. Characterization of plasmids in *Erwinia stewartii.* Appl. Environ. Microbiol. 42:599-604.
6. Crosse, J. E., and Garrett, C. M. E. 1966. Bacterial canker of stone-fruits. VII. Infection experiments with *Pseudomonas mors-prunorum* and *Ps. syringae.* Ann. Appl. Biol. 58:31-41.
7. Crosse, J. E., and Garrett, C. M. E. 1970. Pathogenicity of *Pseudomonas morsprunorum* in relation to host specificity. J. Gen. Microbiol. 62:27-34.
8. Cuppels, D. A., Moore, R. A., and Morris, V. L. 1990. Construction and use of a nonradioactive DNA hybridization probe for detection of *Pseudomonas syringae* pv. *tomato* on tomato plants. Appl. Environ. Microbiol. 56:1743-1749.
9. Dhanvantari, B. N. 1969. Occurrence of bacterial canker of sweet cherry and plum in Ontario. Can. Plant Dis. Surv. 49:5-7.
10. Gnanamanickam, S. S., Starratt, A. N., and Ward, E. W. B. 1982. Coronatine production *in vitro* and *in vivo* and its relation to symptom development in bacterial blight of soybean. Can. J. Bot. 60:645-650.
11. Gross, D. C. 1991. Molecular and genetic analysis of toxin production by pathovars of *Pseudomonas syringae.* Annu. Rev. Phytopathol. 29:247-278.
12. Jones, A. L. 1971. Bacterial canker of sweet cherry in Michigan. Plant Dis. Rep. 55:961-965.
13. Kado, C. I., and Liu, S. T. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145:1365-1373.
14. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-313.
15. Kovacs, N. 1956. Identification of *Pseudomonas pyrocynanea* by the oxidase reaction. Nature 178:703.
16. Latorre, B. A., and Jones, A. L. 1979. *Pseudomonas morsprunorum,* the cause of bacterial canker of sour cherry in Michigan, and its epiphytic association with *P. syringae.* Phytopathology 69:335-339.
17. Ma, S.-W., Morris, V. L., and Cuppels, D. A. 1991. Characterization of a DNA region required for production of the phytotoxin coronatine by *Pseudomonas syringae* pv. *tomato.* Mol. Plant-Microbe Interact. 4:69-74.
18. Maniatis, R., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
19. Melakeberhan, H., Jones, A. L., Sobiczewski, P., and Bird, G. W. 1993. Factors associated with the decline of sweet cherry trees in Michigan: Nematodes, bacterial canker, nutrition, soil pH, and winter injury. Plant Dis. 77:266-271.
20. Mitchell, R. E. 1982. Coronatine production by some phytopathogenic pseudomonads. Physiol. Plant Pathol. 20:83-89.
21. Moore, R. A., Starratt, A. N., Ma, S.-W., Morris, V. L., and Cuppels, D. A. 1989. Identification of a chromosomal region required for biosynthesis of the phytotoxin coronatine by *Pseudomonas syringae* pv. *tomato.* Can. J. Microbiol. 35:910-917.
22. Murashigi, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473-479.
23. Nishiyama, K., and Ezuka, A. 1978. Species of bacteria producing coronatine, a new physiologically active substance. Ann. Phytopathol. Soc. Jpn. 44:179-183.
24. Norelli, J. L., Aldwinckle, H. S., and Beer, S. V. 1988. Virulence of *Erwinia amylovora* strains to *Malus* sp. Novole plants grown in vitro and in the greenhouse. Phytopathology 78:1292-1297.
25. Paterson, J. M., and Jones, A. L. 1989. Characterization of indigenous plasmids in *Pseudomonas syringae* pv. *morsprunorum* and *P. s.* pv. *syringae* from cherry. (Abstr.) Phytopathology 79:1179.
26. Paterson, J. M., and Jones, A. L. 1991. Detection of *Pseudomonas syringae* pv. *morsprunorum* on cherries in Michigan with a DNA hybridization probe. Plant Dis. 75:893-896.
27. Roos, I. M. M., and Hattingh, M. J. 1987. Pathogenicity and numerical analysis of phenotypic features of *Pseudomonas syringae* strains isolated from deciduous fruit trees. Phytopathology 77:900-908.
28. Sakai, R., Nishiyama, K., Ichihara, A., Shiraishi, K., and Sakamura, S. 1979. The relation between bacterial toxic action and plant growth regulation. Pages 165-179 in: Recognition and Specificity in Plant Host-Parasite interaction. J. M. Daly and I. Uritani, eds. University Park Press, Baltimore.
29. Sobiczewski, P., Chiou, C.-S., and Jones, A. L. 1991. Streptomycin-resistant epiphytic bacteria with homologous DNA for streptomycin resistance in Michigan apple orchards. Plant Dis. 75:1110-1113.
30. Süle, S., and Seemüller, E. 1987. The role of ice formation in the infection of sour cherry leaves by *Pseudomonas syringae* pv. *syringae.* Phytopathology 77:173-177.
31. Sundin, G. W., Jones, A. L., and Fulbright, D. W. 1989. Copper resistance in *Pseudomonas syringae* pv. *syringae* from cherry orchards and its associated transfer in vitro and in planta with a plasmid. Phytopathology 79:861-865.
32. Sundin, G. W., Jones, A. L., and Olson, B. D. 1988. Overwintering and population dynamics of *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *morsprunorum* on sweet and sour cherry trees. Can. J. Plant Pathol. 10:281-288.
33. Wiebe, W. L., and Campbell, R. N. 1993. Characterization of *Pseudomonas syringae* pv. *maculicola* and comparison with *P. s. tomato.* Plant Dis. 77:414-419.
34. Woolley, D. W., Pringle, R. B., and Braun, A. C. 1952. Isolation of the phytopathogenic toxin of *Pseudomonas tabaci,* an antagonist of methionine. J. Biol. Chem. 197:409-417.