Pathogenicity and Numerical Analysis of Phenotypic Features of *Pseudomonas syringae* Strains Isolated from Deciduous Fruit Trees

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ABSTRACT

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Eighty South African strains of *Pseudomonas syringae* and 33 reference strains were subjected to numerical phenetic analysis using 215 unit characters. Data were analyzed using unweighted pair-grouping clustering on the simple matching (S_{sm}) coefficient. Three phenons were distinguished at 75.8% S_{sm}. Most South African strains were assigned to one of two major phenons: *P. s.* pv. *syringae* (phenon 1) and race 1 of *P. s.* pv. *morsprunorum* (phenon 2). Four *P. s.* pv. *savastanoi* strains formed a tight subgroup within phenon 2. Phenon 3 comprised the reference strain of *P. s.* pv. *morsprunorum* race 2, two local race 2 strains, and a *P. s.* pv. *papulans* strain. Pathogenicity tests with five selected strains on apple, pear, plum, and cherry trees confirmed the clear distinction between the two pathovars and between races 1 and 2 of *P. s.* pv. *morsprunorum*. No significant differences in pathogenicity were detected when comparing *P. s.* pv. *syringae* strains originally isolated from a weed or from pear or nectarine trees.

Additional key words: bacterial canker, epidemiology, host specificity.

Bacterial canker of stone fruit trees in South Africa is caused by *Pseudomonas syringae* pv. *syringae* van Hall and *P. s.* pv. *morsprunorum* (Wormald) Young et al (25,27). Significant differences were found between groups of strains within each pathovar, and intermediate forms were also recognized. It was also recently established that *P. s.* pv. *syringae* causes blister bark of apple (21) and blossom blast of pear (22) in South Africa. These findings suggest the existence of a heterogeneous population of *P. syringae* clustered around *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum*. However, the relationship between these pathogens and their stone and pome fruit hosts is uncertain. Orchards of stone and pome fruit crops are often situated close together in the southwestern Cape Province, the major deciduous fruit growing area of South Africa, and this could have epidemiologic implications if pathogens survive on or infect both of these fruit crops.

This study examines the phenotypic grouping of strains of *P. syringae* isolated from stone and pome fruit hosts from different localities in South Africa. Isolates from other hosts and reference strains were included. The pathological behavior of selected virulent strains from stone and pome fruit orchards was compared on apple, pear, plum, and cherry trees.

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MATERIALS AND METHODS

Bacterial strains. The source and original host plants of the 113 strains used in this investigation are listed in Table 1. Eighty (selected at random from a larger collection) strains were isolated in South Africa. Isolation of most of these has been reported (21,22,25,27). All local strains were assigned to either *P. s.* pv. *syringae*, *P. s.* pv. *morsprunorum*, or to intermediate forms of these pathovars. Pathotype strains of *P. s.* pv. *syringae* (lilac strain PDDCC 3023) and *P. s.* pv. *morsprunorum* (PDDCC 5795) and 31 other reference strains were included in the investigation.

Single colonies from freshly revived lyophilized cultures were transferred to Difco nutrient agar slants supplemented with 2% glycerol and 4% $CaCO_3$. These cultures were kept at room temperature and transferred monthly during the duration of the investigation.

Characterization of strains. Unless stated otherwise, inoculum used for tests was prepared from overnight slant cultures on nutrient-yeast extract-glycerol agar (NYGA) (0.05% Difco peptone, 0.3% Difco yeast extract, 2% glycerol, and 2% agar). Cultures were suspended in sterile distilled water and standardized to a turbidity of 1.5×10^8 cells per milliliter according to the McFarland scale (16), corresponding to 3×10^7 colony-forming units (cfu) per milliliter. Uninoculated control media were included in all tests, which were repeated at least twice on separate days. An incubation temperature of 26 C was used throughout. Most key tests used in previous studies to characterize South African isolates (21,22,25,27) had to be repeated to accommodate the reference

strains.

The Gram stain was performed according to Harrigan and McCance (9). Cytochrome oxidase activity was tested with Difco oxidase-differentiation disks and catalase activity by suspending bacterial cells in 10% H_2O_2 (9). Colony characteristics of strains were determined on King's medium B (MB) (13) and on Difco nutrient agar supplemented with 5% sucrose (NSA) (18). In addition, pigmentation and levan production were checked on MB and NSA, respectively. Pigmentation was also observed in the corresponding nutrient-sucrose broth (NSB). Action on Difco litmus milk was followed regularly for 21 days. Casein hydrolysis was determined on Yeastrel agar supplemented with 30% Difco skim milk powder (1). Pigmentation was also recorded on this medium. The GATTa determinative tests for gelatin liquefaction (G), esculin hydrolysis (A), tyrosinase activity (T), and tartrate utilization (Ta) were according to Latorre and Jones (17). Salt tolerance was monitored after 7 days in Difco nutrient broth containing 5, 6, and 8% NaCl.

The API 50CH, API 50AO, and API 50AA (API System S.A., Montalieu Vecieu, France) galleries were used to assay for utilization of carbohydrates, organic acids, and amino acids and amines (49 of each), respectively. These microtests were performed under aerobic conditions. Tubes and cupules were filled with a basal (inoculated) medium as described by Kersters et al (12). Oxoid agar 4 (0.5%) was used instead of Oxoid agar 1. Results were recorded after 7 days of incubation.

Nineteen enzyme activities were investigated using the API-ZYM system. Two drops of standardized inoculum were added to appropriate cupules with a Pasteur pipette. After 18 hr, appropriate reagents were added according to the manufacturer's instruction. Plates were left for 10 min under intense light before scoring results according to the API standard color chart.

Susceptibility of strains to 20 antibiotics (concentrations listed in Table 2) incorporated in Oxoid sensitivity disks was recorded after 24 hr on NYGA plates seeded with a layer of soft nutrient agar (0.7% agar) containing 0.1 ml of standardized inoculum. Strains showing inhibition zones >1 mm were scored as susceptible.

Strains were tested for production of syringomycin (8), ice nucleation activity (19), and ability to elicit a hypersensitive reaction (HR) in tobacco leaves (14).

Numerical analysis of phenotypic features. Phenotypic features were scored 2 (positive), 1 (negative), or 0 (no comparison for a missing or doubtful result). The average of features per strain recorded as 0 was only 0.03%. Multistate features like colony appearance and pigmentation were divided into several mutually exclusive states. The API 50 test scores on the manufacturer's scale (0–5) were converted to the following for numerical analysis: 0 = 1, 1–2 = 0, and 3–5 = 2. In the API-ZYM tests, 0 was scored as negative and 1–5 as positive.

The similarity coefficient S_{sm} (37) was determined on a Sperry Univac 1100 computer with the Bonham-Carter (2) program modified by K. Kersters (Laboratorium voor Microbiologie en Microbiële Genetica, Rijksuniversiteit, Ghent, Belgium). Strains were clustered by the unweighted average pair-group method (34). The S_{sm} included both positive and negative matches in the calculation. After similarity coefficients had been calculated

TABLE 1. Origin of *Pseudomonas syringae* strains used in numerical analysis of phenotypic features

Bacteria and original host	Strain number[a]	Source and year isolated
P. s. pv. *cannabina*		
Cannabis sativa	CN62	Italy
P. s. pv. *coronafaciens*		
Avena sativa	CR4	Italy
P. s. pv. *morsprunorum*		
Prunus avium	123, 133, 146, 428	South Africa, 1981
	454, 627, 712, 753	South Africa, 1982
	PDDCC 3715 (= NCPPB 1782 = CF8)	Italy, 1965
	PDDCC 568	United Kingdom
	C304 (race 2)	United Kingdom, 1971
	III.11 (race 1)	United Kingdom, 1977
	CF7, CF8, CF/F1	Italy
P. domestica	PDDCC 5795 (= ATCC 19322 = NCPPB 48) (pathotype strain)	United Kingdom
P. salicina	182, 239	South Africa, 1981
	632, 634, 783	South Africa, 1982
P. s. pv. *papulans*		
Malus pumila	PDDCC 4046	Canada, 1974
P. s. pv. *phaseolicola*		
Phaseolus vulgaris	IL3	Italy
P. s. pv. *savastanoi*		
Olea europaea	PS202, PS204	Italy
Nerium oleander	PS205, PS214	Italy
P. s. pv. *syringae*		
Citrus sinensis	PS121, PS122	Italy
Malus pumila	L195, L314, L532, L583, L605, L677, L810, L840	South Africa, 1983
	L796, L985, L1114, L1146, L1166, L1181, L1397, L2222	South Africa, 1984
	PDDCC 457	New Zealand, 1954
Phaseolus vulgaris	RA3	Unknown
Prunus armeniaca	15, 139, 294, 312, 703, R1, R4	South Africa, 1981
	670	South Africa, 1982
P. avium	51, 53	South Africa, 1981
	533, 715, 578, 584	South Africa, 1982
	S157	United Kingdom, 1979
	PDDCC 504	Unknown
P. persica	5(2), 5h, S8, SA9	South Africa, 1981
	NN, NV	South Africa, 1983
	PDDCC 509	New Zealand
P. salicina	164, 244, 724, 786, D10	South Africa, 1981
	4(4), 185, 777, 793, 832	South Africa, 1982
	PDDCC 516	New Zealand, 1962
Pyrus communis	L280, L569, L781, L843, L849, L850, L683	South Africa, 1983
	L783, L795, L870, L1069, L1592	South Africa, 1984
	PDDCC 397	New Zealand, 1962
	SP1	Italy
	S9	United Kingdom
Rubus strigosus	UBC P429	Canada, 1976
Syringa vulgaris	PDDCC 3023 (= NCPPB 281) (pathotype strain)	United Kingdom, 1950
Vicia villosa	HVA27	Unknown
Weed in plum orchard	732	South Africa, 1982
Intermediate forms[b]		
Aspalathus linearis	618	South Africa, 1982
Malus pumila	L815, L908	South Africa, 1983
	L1323	South Africa, 1984
Prunus salicina	D6	South Africa, 1981
	635	South Africa, 1982
Pyrus communis	L681, L847	South Africa, 1983
Rubus strigosus	UBC P45,[c] UBC P202[c]	Canada, 1975
Syringa vulgaris	UBC P231[c]	Canada, 1972

[a] ATCC = American Type Culture Collection, Rockville, MD; NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, Hertfordshire, UK; PDDCC = Plant Diseases Division Culture Collection, Auckland, NZ; UBC = University of British Columbia, Vancouver, Canada.

[b] As defined by Latorre and Jones (17).

[c] Strains received as *P. s.* pv. *syringae*.

TABLE 2. Characteristics of major phenons,[a] groups,[b] and subgroups[c] defined by unweighted pair-group analysis on the simple matching coefficient

Diagnostic character[d]	Percentage of isolates positive										
	1	1A	Pss 1	1B	Pss 2	2	2A	Psm	2B	Psv	3
Colony characters on NSA											
Cream	44	54	62	37	36	8	10	0	0	0	25
White	24	17	0	27	12	73	85	100	33	0	75
Greenish yellow	24	8	0	33	48	15	0	0	67	100	0
Pale yellow	6	17	15	2	4	0	0	0	0	0	0
Dome	46	50	54	39	32	54	70	82	0	0	25
Umbonate	32	29	15	35	44	42	30	18	83	75	75
Umbilicate	5	4	0	6	12	0	0	0	0	0	0
Convex	15	17	31	16	16	4	0	0	17	25	0
Entire	92	96	100	90	88	81	100	100	17	25	75
Undulate	8	4	0	10	12	19	0	0	83	75	25
Levan production	76	92	92	67	52	81	95	100	33	0	100
Colony characters on MB											
Fluorescent	100	100	100	100	100	81	80	100	83	75	75
Green	91	96	100	88	96	23	30	0	67	75	0
Brown	8	4	0	10	4	62	55	100	17	0	75
Translucent	95	100	100	92	92	100	100	100	100	100	75
Opaque	16	29	15	12	12	0	0	0	0	0	25
Rough	5	4	0	6	4	4	5	9	0	0	25
Smooth	94	96	100	92	96	96	95	91	100	100	75
Umbonate	10	4	0	12	12	4	5	9	0	0	0
Umbilicate	73	79	85	73	72	89	90	82	83	100	75
Convex	4	8	15	2	4	4	0	0	17	0	0
Biumbilicate	14	13	0	14	8	8	10	18	0	0	25
Entire	94	92	100	96	100	89	90	91	83	100	75
Undulate	14	0	0	24	8	12	10	9	17	0	25
Growth in NSB											
White	4	0	0	2	0	69	85	100	17	25	50
Yellow	99	100	100	98	100	35	15	0	83	75	50
Litmus milk reaction											
Alkalinization	49	50	54	53	52	81	90	91	50	25	75
Peptonization	42	42	46	37	44	15	5	0	50	75	0
Reduction	4	4	0	4	4	0	0	0	0	0	0
Casein hydrolysis	51	75	85	37	28	19	15	27	33	50	25
Green pigment on casein medium	5	8	0	4	8	4	5	9	0	0	0
GATTa tests[e]											
Gelatin liquefaction (G)	96	100	100	94	80	12	10	0	17	25	50
Esculin hydrolysis (A)	94	96	100	94	100	12	15	0	0	0	25
Tyrosinase activity (T)	1	0	0	2	4	77	95	100	17	25	100
Na-tartrate utilization (Ta)	0	0	0	0	0	88	95	100	67	100	75
Tolerance to NaCl											
5%	94	92	92	94	96	77	75	73	83	100	75
6%	86	79	77	88	84	54	45	18	83	100	25
8%	38	38	46	37	24	27	25	0	33	50	0
Carbohydrate utilization											
Glycerol	94	100	100	94	100	85	85	100	83	100	100
Erythritol	90	88	100	94	100	73	95	100	0	0	100
D-Arabinose	46	71	100	37	32	62	80	91	0	0	0
L-Arabinose	95	100	100	96	100	92	95	100	83	100	100
Ribose	99	100	100	100	100	85	95	100	67	75	100
D-Xylose	95	100	100	94	100	73	90	100	17	25	50
L-Xylose	20	17	15	22	8	23	30	18	0	0	0
Adonitol	6	4	0	8	0	8	10	9	0	0	25
D-Galactose	96	100	100	96	100	85	90	100	83	100	100
D-Glucose	96	100	100	96	100	81	80	82	83	100	100
D-Fructose	96	100	100	96	100	96	100	100	83	100	100
D-Mannose	99	100	100	100	100	96	100	100	83	100	100
L-Sorbose	3	0	0	4	0	4	5	0	0	0	0
Rhamnose	67	75	62	67	72	23	25	18	17	25	25
Dulcitol	3	0	0	4	0	0	0	0	0	0	0
Inositol	99	100	100	100	100	65	65	82	67	100	100
Mannitol	98	100	100	98	100	62	60	64	67	100	50
Sorbitol	95	96	100	96	100	96	100	100	17	25	100
Methyl-D-mannoside	3	0	0	4	0	4	5	0	0	0	0
Methyl-D-glucoside	4	0	0	6	4	0	0	0	0	0	0
N-Acetylglucosamine	14	21	0	12	0	0	0	0	0	0	0
Amygdalin	3	0	0	4	0	0	0	0	0	0	0
Arbutin[f]	87	96	100	84	100	8	10	0	0	0	50
Esculin[f]	91	100	100	86	100	12	15	0	0	0	50
Salicin	6	8	15	4	4	0	0	0	0	0	25
D-Cellobiose	14	17	15	12	4	8	10	0	0	0	0
Maltose	9	13	15	6	0	4	5	9	0	0	25

(CONTINUED)

TABLE 2 cont.

Diagnostic character[d]	Percentage of isolates positive										
	1	1A	Pss 1	1B	Pss 2	2	2A	Psm	2B	Psv	3
Lactose	1	0	0	0	0	0	0	0	0	0	0
D-Melibiose	6	0	0	10	4	0	0	0	0	0	25
Sucrose	96	100	100	94	100	92	90	100	100	100	100
Trehalose	67	96	92	53	100	42	50	46	17	25	100
Inulin	8	0	0	12	0	0	0	0	0	0	0
D-Melezitose	8	0	0	12	0	0	0	0	0	0	0
D-Raffinose	77	92	92	69	52	73	90	100	17	25	75
Starch	6	0	0	10	0	4	5	0	0	0	25
Glycogen	11	0	0	18	8	8	5	0	17	25	25
Xylitol	15	8	0	22	4	0	0	0	0	0	25
Gentiobiose	10	4	8	14	0	0	0	0	0	0	0
D-Lyxose	86	100	100	80	100	62	80	91	0	0	75
D-Tagatose	1	0	0	2	4	4	5	0	0	0	0
D-Fucose	10	0	0	16	4	8	10	0	0	0	25
L-Fucose	10	0	0	16	4	0	0	0	0	0	0
D-Arabitol	99	100	100	100	100	62	70	91	33	50	50
L-Arabitol	14	0	0	22	0	4	5	0	0	0	25
Gluconate	94	100	100	90	100	85	85	91	100	100	100
2-Ketogluconate	33	33	15	37	28	31	40	55	0	0	25
5-Ketogluconate	1	0	0	2	0	0	0	0	0	0	25
Organic acid utilization											
Acetate	73	50	46	82	80	23	30	46	0	0	0
Propionate	42	38	31	45	4	12	15	9	0	0	0
Butyrate	53	29	31	67	44	8	0	18	0	0	0
Isobutyrate	20	13	0	24	8	4	5	9	0	0	0
N-Valerate	66	46	62	73	80	19	25	36	0	0	25
Isovalerate	9	4	0	12	8	0	0	0	0	0	50
N-Caproate	81	79	85	80	100	62	80	73	33	50	100
Heptanoate	93	88	100	94	100	73	75	55	67	75	100
Caprylate	90	88	100	90	100	73	75	73	67	75	100
Pelargonate	92	92	100	94	100	81	80	64	83	100	100
Caprate	96	96	100	96	100	85	85	73	83	100	100
Oxalate	10	4	0	8	0	73	10	18	17	25	0
Malonate	37	25	8	41	40	81	20	73	17	0	25
Succinate	99	96	92	100	100	100	100	100	100	100	100
Maleate	23	38	38	16	12	39	50	46	0	0	75
Fumarate	98	100	100	96	96	96	100	100	83	100	100
Glutarate	91	92	100	92	100	42	55	46	0	0	75
Adipate	8	4	0	8	0	0	0	0	0	0	100
Pimelate	5	0	0	6	0	4	5	0	0	0	100
Suberate	4	4	0	4	0	4	5	9	0	0	100
Azelate	5	8	0	4	0	8	10	0	0	0	75
Sebacate	8	13	0	6	0	4	5	0	0	0	100
Glycolate	9	0	0	14	8	4	5	0	0	0	75
DL-Lactate	98	96	92	98	100	15	20	36	0	0	100
DL-Glycerate	100	100	100	100	100	100	85	91	100	100	100
DL-3-Hydroxybutyrate	92	96	92	92	92	42	85	55	0	0	100
D-Malate	99	100	100	100	100	92	85	91	83	100	100
L-Malate	100	100	100	100	100	92	100	100	67	75	100
D-Tartrate	27	25	0	27	32	15	20	18	0	0	0
L-Tartrate	15	29	23	10	0	81	85	73	67	100	75
Mesotartrate	86	88	100	88	100	81	85	82	67	100	100
Pyruvate	99	100	100	98	100	96	95	100	100	100	100
Levulate	5	8	0	4	0	4	5	9	0	0	100
2-Ketoglutarate	100	100	100	100	100	81	100	100	17	25	100
Citraconate	9	13	0	78	0	4	5	9	0	0	75
Itaconate	10	4	8	14	0	0	0	0	0	0	100
Mesoconate	10	0	0	14	0	0	0	0	0	0	75
Aconitate	99	100	100	98	100	94	100	91	83	100	100
Citrate	98	100	100	96	96	100	100	100	100	100	100
Phenylacetate	13	17	0	10	0	0	0	0	0	0	25
Benzoate	10	17	0	8	0	0	0	0	0	0	50
m-Hydroxybenzoate	9	17	0	4	0	0	0	0	0	0	0
p-Hydroxybenzoate	90	100	100	86	100	77	80	73	67	100	25
L-Mandelate	5	0	0	4	0	0	0	0	0	0	0
Phthalate	3	0	0	0	0	0	0	0	0	0	25
Isophthalate	3	0	0	0	0	0	0	0	0	0	0
Terephthalate	1	4	0	0	0	0	0	0	0	0	0
Amino acid and amine utilization											
Glycine	15	45	85	0	0	19	10	0	50	50	0
D-Alanine	23	71	100	0	0	42	35	9	67	75	0
L-Alanine	23	71	100	0	0	46	40	9	67	75	0
L-Leucine	29	92	77	0	0	23	25	9	17	25	25

(CONTINUED)

TABLE 2 cont.

Diagnostic character[d]	Percentage of isolates positive										
	1	1A	Pss 1	1B	Pss 2	2	2A	Psm	2B	Psv	3
L-Isoleucine	6	17	15	0	0	12	10	0	17	0	25
L-Norleucine	3	4	8	0	0	0	0	0	0	0	25
L-Valine	10	29	31	0	0	8	5	0	17	25	0
DL-Norvaline	18	46	62	2	0	0	0	0	0	0	25
DL-2-Aminobutyrate	5	4	0	4	4	4	5	0	0	0	0
L-Serine	30	92	100	0	0	46	40	18	67	75	0
L-Threonine	24	71	69	0	0	12	10	0	17	25	25
L-Cysteine	18	54	77	0	0	15	15	0	17	25	0
L-Methionine	1	0	0	0	0	0	0	0	0	0	25
L-Phenylalanine	19	50	31	0	0	12	15	0	0	0	100
L-Tyrosine	20	50	31	2	0	15	25	9	0	0	100
L-Histidine	68	100	100	51	56	62	65	46	67	75	75
D-Tryptophan	3	0	0	0	0	4	5	0	0	0	25
L-Tryptophan	8	17	0	0	0	8	10	0	0	0	75
Trigonelline	32	83	100	4	0	42	45	18	33	0	0
L-Aspartate	30	92	100	0	0	39	30	9	67	75	0
L-Glutamate	35	100	100	2	0	54	45	18	83	75	100
L-Ornithine	76	33	31	94	92	35	45	64	0	0	0
L-Lysine	65	17	15	86	88	35	45	64	0	0	0
L-Citrulline	6	8	0	6	0	4	5	0	0	0	0
L-Arginine	96	96	100	96	100	89	100	82	83	75	75
DL-Kynurenine	8	13	0	0	0	4	0	0	17	25	25
L-Proline	35	100	100	2	0	62	55	27	83	75	100
Betaine	34	100	100	2	0	58	55	27	67	50	100
Creatine[g]	0	0	0	0	0	0	0	0	0	0	0
Alanine	13	29	23	4	4	8	10	0	0	0	0
DL-3-Aminobutyrate	57	4	0	78	72	12	15	27	0	0	50
DL-4-Aminobutyrate	96	100	100	94	100	77	95	82	33	25	100
DL-5-Aminobutyrate	72	96	100	59	48	31	40	27	0	0	0
2-Aminobutyrate	6	13	0	4	0	0	0	0	0	0	25
Urea	3	8	15	0	0	0	0	0	0	0	0
Acetamate	37	8	0	45	56	4	5	9	0	0	0
Sarcosine	22	67	85	0	0	15	15	0	33	50	0
Ethylamine	20	0	0	26	16	4	5	9	0	0	0
Butylamine	10	0	0	10	4	0	0	0	0	0	0
Amylamine	4	4	0	2	4	4	5	9	0	0	0
Ethanolamine	72	88	85	63	52	35	45	27	0	0	0
Benzylamine	3	0	0	0	0	0	0	0	0	0	0
Diaminobutane	86	79	77	88	88	35	45	36	0	0	25
Spermine	47	38	38	47	36	31	40	27	0	0	25
Histamine	22	8	0	26	16	15	20	9	0	0	0
Tryptamine	11	13	0	10	0	0	0	0	0	0	0
Glucosamine	52	45	23	53	32	19	25	27	0	0	50
Enzyme activity											
Phosphatase, alkaline	34	33	31	35	24	12	10	0	17	25	0
Esterase (C 4)	81	83	92	82	84	77	80	100	67	75	75
Esterase lipase (C 8)	94	100	100	92	100	100	100	100	100	100	100
Lipase (C 14)	22	21	23	22	0	12	10	18	17	25	0
Valine arylamidase	47	54	62	39	68	77	70	91	100	100	100
Chymotrypsin	6	0	0	10	0	0	0	0	0	0	0
Phosphatase, acid	84	88	92	80	100	100	100	100	100	100	100
Phosphoamidase	77	88	92	71	80	96	95	91	100	100	75
α-Galactosidase	3	0	0	0	0	0	0	0	0	0	0
β-Galactosidase	0	0	0	0	0	0	0	0	0	0	25
α-Glucosidase	3	0	0	4	0	0	0	0	0	0	0
β-Glucosidase	66	75	77	61	80	8	5	0	17	25	25
N-Acetyl-β-Glucosaminidase	4	0	0	6	0	0	0	0	0	0	0
α-Mannosidase	4	0	0	6	0	0	0	0	0	0	0
Antibiotic sensitivity											
Lincomycin, 2 μg	0	0	0	0	0	4	0	0	17	25	0
Penicillin G, 10 U	4	4	0	4	80	0	0	0	0	0	25
Novobiocin, 30 μg	44	38	54	45	56	39	25	18	83	75	25
Neomycin, 30 μg	94	96	100	92	96	100	100	100	100	100	50
Methicillin, 10 μg	1	4	0	0	0	19	10	9	50	50	0
Gentamycin, 10 μg	98	96	100	98	100	94	90	91	100	100	75
Erythromycin, 10 μg	80	79	77	84	100	62	80	100	0	0	100
Colistin sulphate, 10 μg	81	79	92	80	92	96	95	91	100	100	75
Ampicillin, 10 μg	52	75	92	78	96	96	95	100	100	100	75
Fusidic acid, 10 μg	17	17	31	12	12	31	20	18	67	50	50
Nitrofurantoin, 200 μg	11	4	8	12	4	62	70	72	33	25	25
Bacitracin, 10 U	15	25	31	10	4	39	30	36	67	75	25

(CONTINUED)

TABLE 2 cont.

Diagnostic character[d]	Percentage of isolates positive										
	1	1A	Pss 1	1B	Pss 2	2	2A	Psm	2B	Psv	3
Kanamycin, 30 μg	96	96	100	96	100	96	95	91	100	100	100
Streptomycin, 10 μg	98	96	100	98	100	96	95	91	100	100	75
Sulfafurazole, 100 μg	20	17	15	20	16	31	40	18	0	0	100
Cephaloridine, 25 μg	56	54	62	57	72	85	80	91	100	100	75
Oxytetracycline, 30 μg	98	96	100	98	100	96	95	91	100	100	100
Polymyxin, 300 U	99	96	100	100	100	100	100	100	100	100	100
Chloramphenicol, 30 μg	96	96	100	98	100	100	100	100	100	100	100
Syringomycin production	81	83	85	82	64	12	10	0	17	25	0
Ice nucleation activity	78	83	85	75	88	23	20	18	33	25	25
Hypersensitivity in tobacco	90	96	100	86	96	85	90	82	67	50	100

[a] Phenons 1 (79 strains), 2 (26 strains), and 3 (4 strains).
[b] Groups 1A (24 strains) and 1B (51 strains) of phenon 1 and groups 2A (20 strains) and 2B (6 strains) of phenon 2.
[c] Subgroups Pss 1 (*Pseudomonas syringae* pv. *syringae*) (13 strains of group 1A), Pss 2 (25 strains of group 1B), Psm (*P. s.* pv. *morsprunorum*; 11 strains of group 2A), and Psv (*P. s.* pv. *savastanoi*; 4 strains of group 2B).
[d] NSA = Difco nutrient agar + 5% sucrose; MB = King's medium B (13); NSB = Difco nutrient broth + 5% sucrose.
[e] According to Latorre and Jones (17).
[f] Hydrolysis instead of growth taken as positive.
[g] Strain PDDCC 397 (not in phenons 1–3) utilized creatine.

pairwise for all strains under study, data were transposed into a dendrogram. Similarity levels at 75.8 and 80% S_{sm} defined distinct phenons and groups, respectively. The subgroups were not distinguished at a specific S_{sm} level but refer to the inner core of each group. Redundant characters were not used for calculating coefficients of associations.

Pathogenicity. Five virulent strains of *P. syringae* isolated in South Africa were selected from the collection used in the present investigation. They were chosen after screening all local strains for pathogenicity by a hypocotyl bioassay (4) on pear and apple seedlings.

Two-year-old apple (Topred), pear (Clapp's Favorite), cherry (Black Tartarian), and plum (Eldorado) trees grown under field conditions were used. Vegetative shoots at least 15 cm long were tagged for inoculation. A hypodermic syringe fitted with a 26-gauge needle was used to inject 0.01 ml of standardized bacterial suspension (3×10^7 cfu/ml) into each of the three upper internodes of two separate shoots on a test tree. Each bacterial strain was injected into four trees (i.e., 24 infiltration sites) of each cultivar. This procedure introduced inoculum doses of $1.4–2.8\times 10^4$ cfu per site. Control shoots were injected with sterile distilled water.

Canker length was measured after 4 wk in terms of round numbers on a 0–4 scale where 0 = no necrosis, 1 = >0–5 mm, 2 = >5–10 mm, 3 = >10–20 mm, and 4 = >20 mm. At the same time, the mean number of bacteria per inoculated internode was determined for each strain on one shoot of a single tree. Each of the three internodes was surface-disinfested with 70% ethanol, cut into small pieces, and shaken vigorously for 1 min in a test tube containing 10 ml of sterile distilled water. After 1–2 hr, 10-fold dilutions of the suspension were plated onto MB. Colony counts were made after 3 days of incubation at 26 C. The identity of bacterial isolates with typical colonies was verified by the oxidase, GATTa, and HR tests mentioned previously.

The results obtained were subjected to the usual analysis of variance for randomized block designs (35).

RESULTS

Clustering of strains. A dendrogram illustrates the phenotypic similarities among the 113 strains (Fig. 1). All strains were associated at 71.4% S_{sm}. Three phenons and four solitary strains were distinguished at 75.8% S_{sm}. Seventy-two of the 75 (96%) strains assumed to be *P. s.* pv. *syringae* were allocated to phenon 1. Pathotype strain PDDCC 3023 of *P. s.* pv. *syringae* joined this phenon at 76.8% S_{sm}. Most (15/18) *P. s.* pv. *morsprunorum* strains, including pathotype strain PDDCC 5795 and race 1 strain III.11, clustered together in phenon 2. Reference strain C304 of race 2 of *P. s.* pv. *morsprunorum* associated with two local *P. s.* pv. *morsprunorum* strains (123 and 146), previously shown to belong to race 2 (27), and *P. s.* pv. *papulans* in phenon 3. The four *P. s.* pv. *savastanoi* strains clustered tightly to form the inner core of group 2B.

Characterization of clusters. All strains were Gram-negative, oxidase-negative, and catalase-positive. None utilized the carbohydrates methylxyloside or D-turanose, organic acids *o*-hydroxybenzoate or D-mandelate, amino acids 3- and 4-aminobutyrate, or formed the enzymes cystine arylamidase, trypsin, β-glucuronidase, or α-fucosidase. All strains produced leucine arylamidase. Nalidixic acid was the only antibiotic that inhibited all strains. These results were therefore not used to characterize clusters.

The distribution of test responses with different reactions within or between phenons, groups, and subgroups is listed in Table 2. Characterization of phenons was based on a combination of all 215 valid features. Some tests (growth in NSB, gelatin liquefaction, hydrolysis of esculin and arbutin, tyrosinase activity, and utilization of Na tartrate) were specific and could discriminate one phenon, group, or subgroup from another.

Phenon 1 contained most of the green fluorescent GATTa$^+$ (*P. s.* pv. *syringae*) strains. Most of these strains hydrolyzed arbutin, produced syringomycin, showed ice nucleation activity, and exhibited yellow growth in NSB. At 80% S_{sm}, two groups (1A and 1B) were distinguished. Group 1A comprised almost entirely typical levan-forming GATTa$^+$ strains. Within this group, 13 *P. s.* pv. *syringae* strains (including 6 reference strains from abroad) formed a tight subgroup (*P. s.* pv. *syringae* 1) at 87% S_{sm}. Group 1B contained most of the *P. s.* pv. *syringae* strains isolated in South Africa. At 88% S_{sm}, a subgroup (*P. s.* pv. *syringae* 2) of this group was distinguished. It contained most of the non-levan-forming strains isolated from fruit trees in the southwestern Cape Province of South Africa.

All strains of subgroup *P. s.* pv. *syringae* 1 utilized D- and L-alanine, L-serine, trigonelline, L-aspartate, L-glutamate, L-proline, and betaine. None of the strains of subgroup *P. s.* pv. *syringae* 2 metabolized these compounds.

Phenon 2 included GATTa (*P. s.* pv. *morsprunorum*) strains from stone fruit, an intermediate form from lilac, four strains of *P. s.* pv. *savastanoi*, and single strains of *P. s.* pv. *cannabina*, *P. s.* pv. *coronafaciens*, and *P. s.* pv. *phaseolicola*. Strains of *P. s.* pv. *syringae* were absent. At 80% S_{sm}, the *P. s.* pv. *morsprunorum* and *P. s.* pv. *savastanoi* strains separated to form groups 2A and 2B, respectively. At 84% S_{sm}, seven South African and four reference strains (including *P. s.* pv. *morsprunorum* pathotype strain PDDCC 5795 and *P. s.* pv. *morsprunorum* race 1 strain III.11) clustered to form a *P. s.* pv. *morsprunorum* subgroup within group 2A. These 11 strains were GATTa$^-$, produced levan, failed to hydrolyze esculin or arbutin, did not produce syringomycin, and showed white growth in NSB. Four strains formed the tight

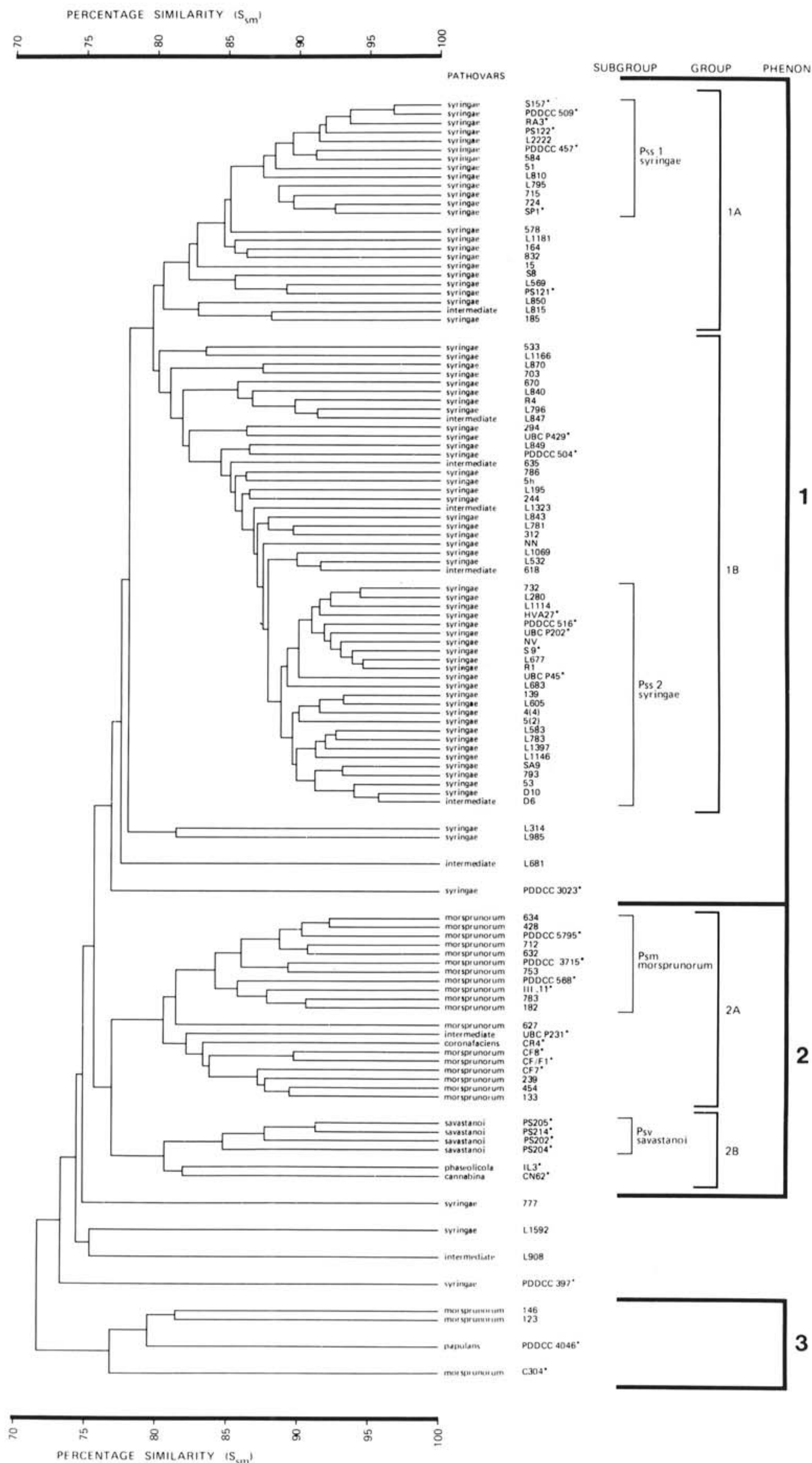

Fig. 1. Dendrogram of S_{sm} similarity coefficients grouped by unweighted average linkage cluster analysis and showing phenotypic similarities among 80 South African and 33 reference strains (*) of *Pseudomonas syringae*.

subgroup *P. s.* pv. *savastanoi.*

Pathogenicity. Response to infection depended upon the host-pathogen combination (Fig. 2). Canker length caused by different strains on cherries differed considerably, with *P. s.* pv. *morsprunorum* strain 123 being the least virulent. The two *P. s.* pv. *morsprunorum* test strains were not detected in inoculated apple, pear, or plum and did not cause cankers on these hosts. These two strains colonized and caused cankers exclusively on cherry trees. Obviously the absence of cankers or bacteria was statistically significant.

The three *P. s.* pv. *syringae* test strains effectively colonized apple, pear, plum, and cherry tissue. In addition, these strains caused brown sunken cankers that spread from the point of introduction on apple, pear, and cherry.

None of the five test strains caused symptoms on plum shoots. However, the three *P. s.* pv. *syringae* strains were present in high numbers in plum tissue. Internal distribution of bacteria was generally confined to each inoculated internode. In a few samples, bacteria were evident in adjacent internodes, petioles, and leaves. Cankers or bacteria were not found on control trees.

DISCUSSION

The phenotypic dendrogram (Fig. 1) based on 215 cultural, biochemical, nutritional, and physiological properties of a collection of pathogenic pseudomonads from deciduous fruit trees in South Africa indicated that most strains could be assigned to one of two major, distinct phenons: *P. s.* pv. *syringae* and race 1 of *P. s.* pv. *morsprunorum*. Comparative results obtained with reference strains substantiated this conclusion. Furthermore, the pathotype strain of *P. s.* pv. *morsprunorum* fell into the inner core of the *P. s.* pv. *morsprunorum* subgroup. However, the pathotype strain of *P. s.* pv. *syringae* associated weakly as a satellite strain with the main body of *P. s.* pv. *syringae* strains. It was not allocated to either of the phenotypic groups at 80% S_{sm} in phenon 1. A possible explanation is that lilac, the host from which the phenotypic strain was isolated, does not occur commonly in South Africa and is not closely related to the Rosaceae.

No distinction could be made between strains of *P. s.* pv. *morsprunorum* based on cultural properties. Overall cultural differences between *P. s.* pv. *syringae, P. s.* pv. *morsprunorum, P. s.* pv. *savastanoi*, and other pathogenic strains of *P. syringae* were minor. On MB, strains of *P. s.* pv. *syringae* produced bright yellow-green to blue-green, diffusible pigments, whereas those of *P. s.* pv. *morsprunorum* were pale brown or blue green. Some of the *P. s.* pv. *syringae* strains did not produce levan and thus failed to form typical dome-shaped colonies on NSA. These strains grouped tightly together at 89.5% S_{sm} in subcluster 1B. They were all isolated from deciduous fruit trees growing in the southwestern Cape Province.

The biochemical and physiological reactions of the *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum* subgroups supported the reliability of the GATTa determinative tests (17) proposed to separate these two pathovars. However, the phenotypic heterogeneity of *P. s.* pv. *syringae*, and to a lesser extent of *P. s.* pv. *morsprunorum*, appears to be more extensive than indicated by some other diagnostic schemes (1,6,18,23,30). In these schemes, tests for levan production, casein hydrolysis, β-glucosidase, and utilization of L-leucine and L-tartrate as sole carbon sources were used to distinguish between *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum*. In our investigation, strains of both pathovars utilized L-tartrate, whereas Na-tartrate, which is included in the GATTa determinative tests (17), gave unambiguous results (Table 2). Other authors have also commented on the phenotypic heterogeneity of *P. s.* pv. *syringae, P. s.* pv. *morsprunorum* (31,36), and other pathovars of *P. syringae* (5,24).

The reaction of the *P. s.* pv. *morsprunorum* strains in biochemical tests showed a high level of homogeneity and corresponded well with the scheme proposed by Garrett et al (6). This pathovar is virtually restricted to cherry trees in South Africa. Only 3 of 21 strains deviated markedly from the group pattern: reference strain C304 of *P. s.* pv. *morsprunorum* race 2 and two local strains of this race. This contrasts with the *P. s.* pv. *syringae* strains from fruit trees in South Africa slotted into two distinct phenotypic groups within phenon 1. Three solitary strains of *P. s.* pv. *syringae* were excluded from this grouping. Strains in group 1A were nutritionally more versatile, utilizing a wider range of amino acids than those in group 1B. Most of the non-levan-producing strains occurred in a subgroup of group 1B.

Despite their diversity, no obvious phenotypic differences were detected between *P. s.* pv. *syringae* strains isolated from pome and stone fruit trees in South Africa. Gross et al (7) were also unable to distinguish between *P. s.* pv. *syringae* strains from stone and pome fruit trees by bacteriocin and phage typing. Furthermore, single strains of *P. s.* pv. *syringae* isolated from trees and weeds in pome and stone fruit orchards were pathogenic on apple, pear, and cherry, with each individual strain showing a corresponding level of virulence on these hosts. Similar findings were recorded by others (4,7,31). Our findings, together with these and earlier reports (29,32,33,38), indicate that a diverse group of *P. s.* pv. *syringae* strains, attacking both pome and stone fruit trees, occurs in many fruit-growing areas of the world.

The ability of three *P. s.* pv. *syringae* test strains to colonize plum tissue without causing visible symptoms was exceptional since cankers developed on apple, pear, and cherry trees. We propose that each host fruit variety supports a heterogeneous population of *P. s.* pv. *syringae* and that some of these strains are more virulent on other host trees. Symptoms are possibly expressed in some

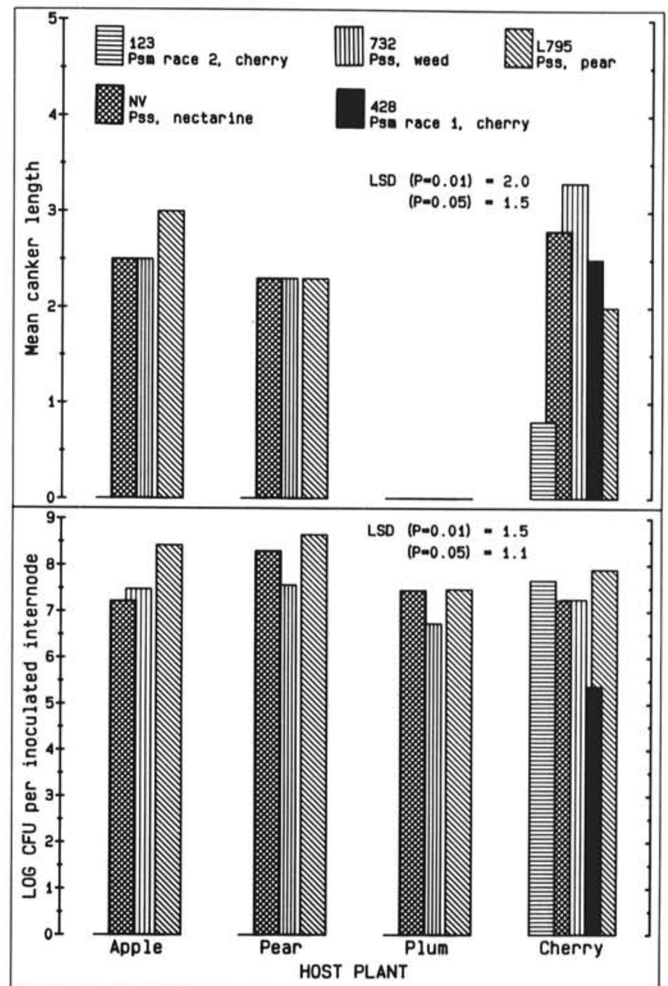

Fig. 2. Pathogenic behavior of five *Pseudomonas syringae* strains on different pome and stone fruit hosts in field test. Canker length is expressed on 0–4 scale where 0 = no necrosis, 1 = >0–5 mm, 2 = >5–10 mm, 3 = >10–20 mm, and 4 = >20 mm. Each bar represents mean of 12 replicates. LSD values correspond with ordinary *t* test of significance.

host-pathogen combinations only if conditions promote the particular strain or if the tree is severely stressed. Bacterial canker of plum in South Africa is favored by hot, dry, windy conditions prevailing in midsummer (26). Although ice nucleation activity of *P. s.* pv. *syringae* predisposes some hosts to the pathogen (7,11,15,20), it is not yet known whether this is important in the development of canker of plum and other fruit trees in South Africa.

Clear distinctions were evident between *P. s.* pv. *syringae, P. s.* pv. *morsprunorum*, and *P. s.* pv. *savastanoi* (Fig. 1). *P. s.* pv. *morsprunorum* seemed to be more closely related to *P. s.* pv. *savastanoi* than to *P. s.* pv. *syringae*. In this regard, our results correlated well with two- and three-dimensional models based upon phenotypic characterization and DNA homologies of nomenspecies of the *P. syringae* group (10). We also support the existence of races 1 and 2 of *P. s.* pv. *morsprunorum*. Additional confirmation for this came from the pathogenicity tests where it was shown that the two races differed significantly from each other. Furthermore, the distinction between *P. s.* pv. *morsprunorum* and *P. s.* pv. *syringae* was also evident from the pathogenicity tests. Both *P. s.* pv. *morsprunorum* test strains, originally isolated from sweet cherry, produced cankers on this host only.

The apparent phenotypic homogeneity and host specificity of *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum* strains isolated in the United Kingdom (3) possibly reflect the high degree of selection pressure exerted by the host or the environment. In contrast, the overall diversity of South African strains of *P. syringae* isolated from deciduous fruit trees is striking. Each climatic region, in combination with the host and cultural practices, appears to allow the development of certain groups of bacteria. For example, *P. s.* pv. *morsprunorum* predominates on cherries in the summer rainfall region, whereas *P. s.* pv. *syringae* is favored on both stone and pome fruit trees in the southwestern Cape Province, a winter rainfall region (26,27). *P. s.* pv. *syringae*, but not *P. s.* pv. *morsprunorum*, also survives on the surface of weeds during the mild, wet winter months (28). The nutritional diversity of *P. s.* pv. *syringae* conceivably enables it to multiply on stone and pome fruit trees, weeds, and possibly other hosts in the southwestern Cape Province. This apparent lack of host specificity complicates epidemiologic studies and development of disease control strategies.

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