Differential Virulence of *Xanthomonas campestris* pv. *pruni* to Peach, Plum, and Apricot Cultivars

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ABSTRACT

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Virulence of five strains of *Xanthomonas campestris* pv. *pruni* to 10 peach, plum, and apricot cultivars was determined after inoculation of detached leaves and leaves on trees in a greenhouse. Virulence of these five strains and 10 additional strains of *X. c. pruni* to four peach cultivars was also measured on detached leaves. A highly significant interaction occurred between strains and cultivars. Differential virulence exhibited by *X. c. pruni* strain Xcp2 on detached leaves of plum cultivars Golden King, Reubennel, and Songold corresponded to results obtained in the greenhouse. However, the differential virulence of strain Xcp2 to Royal apricot in the greenhouse was not apparent on detached leaves. Some strains of *X. c. pruni* were differentially virulent to detached leaves of peach cultivars Olympia, Bokkeveld, and Hantam.

Additional keywords: bacterial spot, *Prunus armeniaca*, *Prunus persica*, *Prunus salicina*.

Bacterial spot disease of stone fruits caused by *Xanthomonas campestris* pv. *pruni* (Smith) Dye, occurs on most cultivars of plum (*Prunus salicina* Lindley), peach (*Prunus persica* (L). Batsch), and apricot (*Prunus armeniaca* L.) trees grown in South Africa (7). Attempts have been made to differentiate strains of *X. c. pruni* in order to understand the epidemiology of the disease. Strains show only minor differences in antigenicity (9), phage sensitivity (8), and growth on a selective medium (2). All strains tested had plasmids with 10 size classes identified, and the number of plasmids in a strain ranged from 1 to 4 (22). However, there was no relationship between presence of plasmids and pathogenicity on detached peach leaves.

Virulence of strains of *X. c. pruni* is known to differ on peach (1). However, in these tests virulence was evaluated on a single cultivar. My attempts (*unpublished*) to reproduce disease symptoms by inoculating greenhouse-grown plum seedlings with a single strain of *X. c. pruni* were frequently unsuccessful. In contrast, inoculation of Golden King plum with a strain recovered from this cultivar, invariably results in disease symptoms (5,6).

A greenhouse leaf inoculation technique (1) and a detached leaf bioassay (21) have been developed to measure virulence of strains of *X. c. pruni*. The purpose of this study was to determine if differential virulence could be detected for strains of *X. c. pruni* to different peach, plum, and apricot cultivars by inoculating detached leaves and leaves on trees in a greenhouse.

MATERIALS AND METHODS

Bacterial strains and inoculum. Fifteen strains of *X. c. pruni* were used (Table 1). Lyophilized cultures were revived on Difco nutrient agar (NA) at 27 C and subcultured monthly on NA slants. Inoculum suspensions were prepared from 48-hr-old cultures on NA. A loopful of bacterial growth was suspended in sterile distilled water (SDW) and adjusted to $A_{620nm} = 0.027$ corresponding to approximately 5.6×10^7 colony-forming units (cfu) per milliliter as determined by dilution plating on NA. Suspensions were diluted further in SDW to contain about 1×10^6 or 5×10^4 cfu/ml and used within 1 hr.

Plant material. Cultivars of apricot (Royal), peach (Bokkeveld, Hantam, Olympia, San Pedro, Selection 1/7/19, Waveren), and plum (Golden King, Reubennel, Songold) were budded in a nursery on Marianna plum rootstocks. Two-year-old trees were pruned, removed from the soil, and planted in 20-cm-diameter pots containing a mixture of equal volumes of loam, peat, and sand. The potted trees were grown in a lathhouse and received weekly applications of balanced nutrient solution (12).

Greenhouse tests. Virulence of *X. c. pruni* strains Xcp1, Xcp2, Xcp3, Xcp4, and Xcp5 was compared on leaves of the 10 cultivars in a greenhouse at 27 C. The method of inoculation was based on techniques described previously (1,5). Actively growing trees were transferred to a controlled environment (27 C, relative humidity 95–100%) 4–8 hr before inoculation. A split-plot experimental design was used for main plot (cultivars) and subplot (bacterial strains) treatments replicated 10 times at random. The first four young but fully expanded leaves from the tip of a shoot on each of 10 trees per cultivar were used per inoculum suspension. Two circular areas on the abaxial side of each leaf were exposed by positioning a polyvinyl carbon shield with a 13-mm-diameter hole against the lamina. Inoculum suspensions were applied onto these areas with a spray gun connected to a compressed air supply (1.7 kg/cm^2) until the underlying tissue became uniformly water soaked. Trees were returned to the greenhouse 1–4 hr after inoculation. The number of bacterial spots per inoculation area was recorded 4–5 wk after inoculation, and the mean number per shoot was used to calculate the disease severity value per treatment. The logarithmic transformation [$\log(X+1)$] (23) was applied to all mean numbers to achieve normality and homogeneity of variances.

TABLE 1. Origin of strains of *Xanthomonas campestris* pv. *pruni* used for testing differential virulence

Strain	Host	Location	Year of isolation
Xcp1	Peach (Elberta)	Ceres, South Africa	1984
Xcp2	Plum (Golden King)	Stellenbosch, South Africa	1984
Xcp3	Peach (San Pedro)	Elsenburg, South Africa	1985
Xcp4	Plum (Santa Rosa)	Ceres, South Africa	1984
Xcp5	Apricot (Supergold)	Malmesbury, South Africa	1985
Xcp9	Plum	New Zealand	1953
Xcp58	Peach	Zimbabwe	1979
Xcp62	Apricot (Blenril)	North America	...
Xcp68	Cherry	Indooroopilly, Australia	1969
Xcp71	Peach (Elberta)	Indooroopilly, Australia	1969
Xcp83	Unknown	Canada	1978
Xcp89	Almond (Thiobelle)	Ceres, South Africa	1986
Xcp93	Plum (Laetitia)	Elsenburg, South Africa	1986
Xcp94	Peach	Paarl, South Africa	1986
Xcp96	Plum (Eldorado)	Stellenbosch, South Africa	1986

A split-plot analysis of variance (ANOVA) of transformed data was performed to analyze differences in virulence of strains, susceptibility of cultivars, and interactions between strains and cultivars. Statistical comparisons between treatment means were made with Student's LSD (23).

Detached leaf tests. Virulence of strains of *X. c. pruni* was compared as described by Randhawa and Civerolo (21) on detached leaves from trees grown in the lathhouse. However, each leaf was inoculated at 10 sites, and 30 leaves were used per treatment. Leaves of each of the 10 cultivars were inoculated with strains Xcp2 and Xcp3 (5×10^4 cfu/ml). In another experiment, all 15 strains (1×10^6 cfu/ml) were inoculated on leaves of peach cultivars Bokkeveld, Hantam, Olympia, and San Pedro. Percentages of inoculated areas with water-soaked spots were recorded 14 days after inoculation. The angular transformation for binomial proportions (23) was applied to all values to achieve normality and homogeneity of variances. A two-way analysis of variance (ANOVA) of transformed values was used to analyze differences in virulence of strains, susceptibility of cultivars, and interactions between strains and cultivars. Statistical comparisons between treatments were made with Student's LSD (23).

RESULTS

Greenhouse tests. Water-soaked spots developed 7–14 days after inoculation on surfaces of leaves. Typical spots of the disease (0.5–1.5 mm diameter) were apparent after 4 wk and were well-separated on inoculated leaf areas of all cultivars.

Mean numbers of spots recorded for each strain on the 10 different cultivars are shown in Figure 1. A highly significant interaction ($P < 0.001$) occurred between strains and cultivars (Table 2). Strain Xcp2 was the most virulent strain on all plum and apricot cultivars, but caused little disease on the six peach cultivars. Strains Xcp1, Xcp4, and Xcp5 also had differential virulence. Disease severity values were significantly higher ($P < 0.05$) for strain Xcp1 than strain Xcp5 on cultivars Golden King, Songold, San Pedro, and Selection 1/7/19, but significantly lower for Xcp1 than Xcp5 on Bokkeveld. Similarly, disease values were significantly higher for Xcp5 than Xcp4 on Bokkeveld, but significantly lower for Xcp5 than Xcp4 on Waveren and Royal. Strain Xcp3 showed no differential virulence but was more aggressive ($P < 0.05$) than any other strain based on transformed data; mean disease severity values for strains Xcp1, Xcp2, Xcp3, Xcp4, and Xcp5 were 8.2, 10.1, 12.4, 6.5, and 5.0, respectively. Songold plum and Royal apricot were the most susceptible cultivars with mean disease severity values of 23.8 and 25.1, respectively. Mean values for other cultivars were much lower and ranged from 1.7 to 7.9.

Detached leaf tests. Water-soaked spots developed 9–14 days after inoculation on the 2–4-mm-diameter areas infiltrated with inoculum suspensions. Counts of spots that were often confluent with both inoculum concentrations were not attempted. Confluent spots were not correlated to any specific strains.

Mean percentages of inoculated areas with water-soaked spots for strains Xcp2 and Xcp3 on leaves of the 10 different cultivars, and for the 15 strains on leaves of peach cultivars Bokkeveld, Olympia, Hantam, and San Pedro are shown in Tables 3 and 4,

TABLE 2. Summary of analysis of variance for severity of bacterial spot disease on plum, peach, and apricot cultivars inoculated with five strains of *Xanthomonas campestris* pv. *pruni* in the greenhouse

Source of variation	df	MS[a]	*P*
Cultivars	9	11.66	<0.001
Strains	4	5.50	<0.001
Strains × cultivars	36	1.36	<0.001
Error	359	0.14	

[a]Calculated from transformed [Log(X + 1)] data.

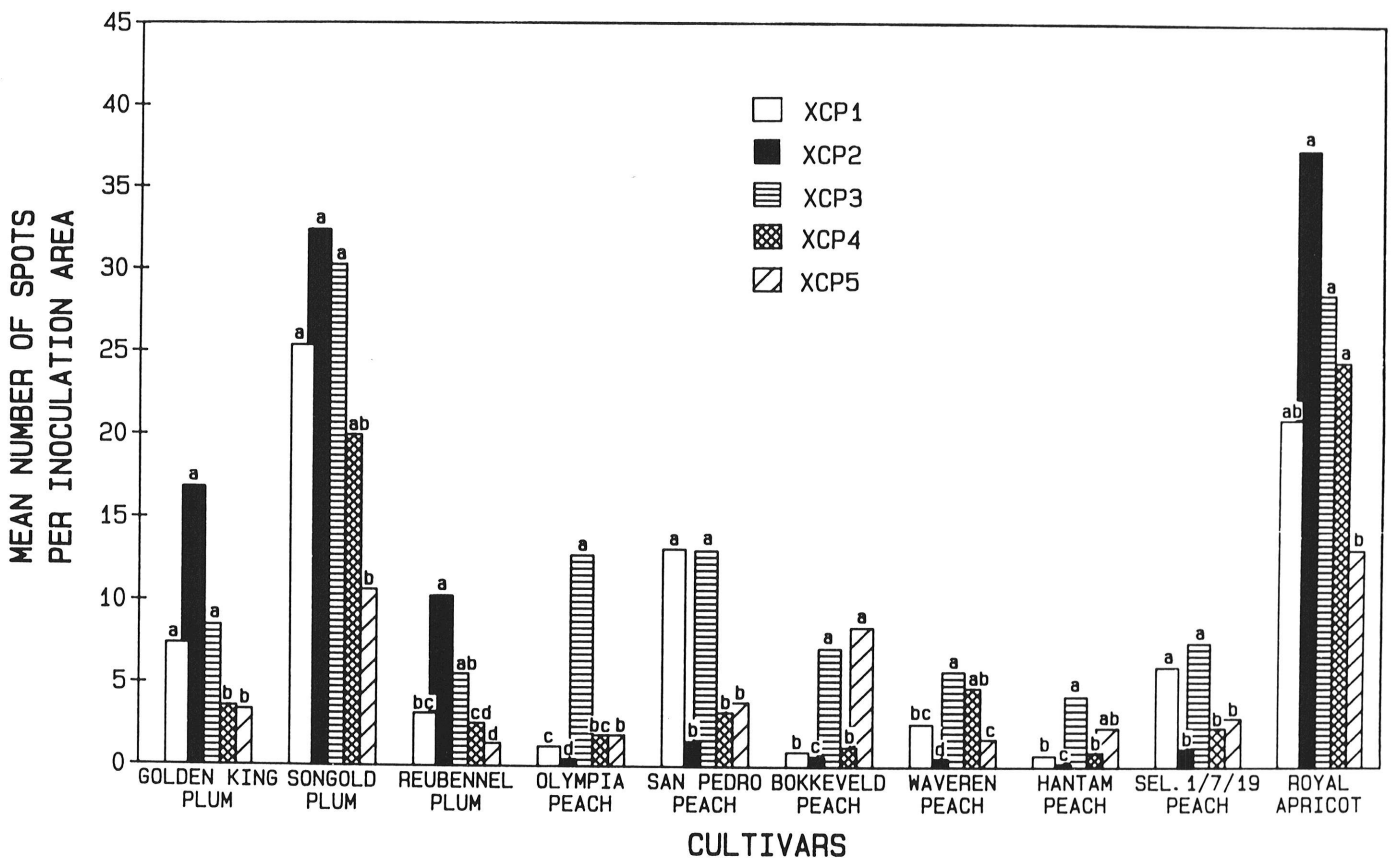

Fig. 1. Mean number of spots on leaves of 10 plum, peach, and apricot cultivars inoculated with five *Xanthomonas campestris* pv. *pruni* strains (Xcp1, Xcp2, Xcp3, Xcp4, and Xcp5) in a greenhouse. The first four young but fully expanded leaves from the tip of a shoot on 10 trees per treatment were each spray-inoculated onto two circular areas on the abaxial side. Bars topped by the same letter indicate that transformed means log(X + 1) are not significantly different ($P < 0.05$) according to Student's LSD (23).

respectively. A highly significant interaction ($P < 0.001$) occurred between strains and cultivars. As in the greenhouse, strain Xcp2 exhibited differential virulence to plum cultivars. Disease values of strains Xcp2 and Xcp3 were high (41–83%) on Golden King, Reubennel, and Songold plums, but were much lower for Xcp2 (2–11%) than for Xcp3 (24–71%) on the six peach cultivars (Table 3). Disease values for both strains were low (4%) on Royal apricot.

Strain Xcp2 caused more disease on Bokkeveld, Olympia, Hantam, and San Pedro (16–32%) at the high inoculum concentration (Table 4) compared with the low concentration, but the values were still much lower than those for 13 other strains. Strain Xcp9 had no effect on San Pedro, Hantam, or Olympia, but a low disease value (26%) was recorded on Bokkeveld. Disease values for strains Xcp1, Xcp3, Xcp4, Xcp5, Xcp58, Xcp62, Xcp68, Xcp71, Xcp83, Xcp89, Xcp93, Xcp94, and Xcp96 were high (86–100%) on San Pedro and did not differ significantly ($P < 0.05$) from each other. However, except for strain Xcp93, values for all these strains were significantly lower on Olympia than on San Pedro, Hantam, or Bokkeveld. The values for strain Xcp93 (97–100%) were high on all four cultivars. Strain Xcp93 therefore exhibited differential virulence to Olympia, Bokkeveld, and Hantam. Similarly, the high disease values of strains Xcp5, Xcp62, Xcp89, Xcp94, and Xcp96 on Hantam (93–99%), Xcp4, Xcp62, Xcp83, Xcp94, and Xcp96 on Bokkeveld (80–87%), and Xcp94 and Xcp96 on Olympia (81–82%) also indicate differential interactions.

TABLE 3. Mean percentages of inoculated areas with water-soaked spots[y] on detached leaves of 10 peach, plum, and apricot cultivars inoculated with *Xanthomonas campestris* pv. *pruni* strains Xcp2 and Xcp3 (5×10^4 colony-forming units per milliliter) and summary of analysis of variance

Cultivar	Xcp2	Xcp3
Reubennel plum	69 a	65 bc
Golden King plum	55 a	56 c
Songold plum	41 b	83 a
San Pedro peach	11 c	71 ab
Waveren peach	11 c	52 c
Bokkeveld peach	7 c	47 cd
Selection 1/7/19 peach	5 c	44 d
Hantam peach	4 c	39 de
Olympia peach	2 c	24 f
Royal apricot	4 c	4 g
Strain mean	20.9	48.5

Analysis of variance: Source of variation	df	MS[z]	P
Cultivars	9	12,973.96	<0.001
Strains	1	55,850.86	<0.001
Strains × cultivars	9	3,923.51	<0.001
Error	580	374.70	

[y] Values are means of 30 replications of 10 inoculated areas per leaf. Means followed by the same letter within columns are not significantly different ($P < 0.05$) according to Student's LSD (23) performed on the transformed (arcsin $\sqrt{x}$) data.
[z] Calculated from transformed data.

DISCUSSION

Tests in the greenhouse and on detached leaves kept under controlled environmental conditions demonstrated differential virulence of strains of *X. c. pruni* to peach, plum, and apricot cultivars. The highly significant interactions ($P < 0.001$) between strains and cultivars (Tables 2–4), and the significant changes ($P < 0.05$) in virulence rankings of strains on different cultivars, are both indicators of differential virulence (24).

The differential virulence of strain Xcp2 to plum cultivars in the greenhouse corresponded to results obtained on detached leaves. However, differential virulence of strain Xcp2 to Royal apricot in the greenhouse was not confirmed on detached leaves. This might have been due to ineffective inoculation. Unlike peach and plum leaves, detached Royal apricot leaves are not easily infiltrated by bacterial suspensions. The results shown in Figure 1 were confirmed 1 yr later when virulence of strains Xcp2 and Xcp3 were measured on Golden King plum and Olympia peach. Disease severity values for strain Xcp2 and an isolate of strain Xcp2 recovered from a spot on an inoculated Olympia leaf were both low (0.1 and 0.2, respectively) when remeasured on Olympia. In contrast to results obtained in the greenhouse, disease severity values were much lower for strain Xcp2 (41%) than for Xcp3 (83%) on detached leaves of Songold plum (Table 3). Furthermore, the differential virulence of strains Xcp1 and Xcp5 on Bokkeveld and San Pedro peaches in the greenhouse (Fig. 1) was not apparent on detached leaves (Table 4).

Differences in symptom expression by strains of *X. c. pruni* on attached and detached leaves probably influenced the results obtained in my investigation. Water-soaked spots on detached leaves were not conspicuously different in size and appearance than on attached leaves. However, inoculated detached peach leaves often develop water-soaked spots, whereas nontypical greyish white spots appear on attached leaves (21). Nontypical greyish white spots (0.1–0.5 mm diameter), which frequently occurred with well-defined spots on leaves in the greenhouse, were not used to assess disease severity. However, the detached leaf bioassay provides a simple method to detect differential virulence of strains of *X. c. pruni* to peach and plum cultivars and to evaluate host susceptibility. For example, the detached leaf response to a strain of *X. c. pruni* (1×10^6 cfu/ml) distinguished differential field resistance (17,25) of 21 out of 22 peach cultivars (21).

Detached leaves of Bokkeveld, Hantam, Olympia, and San Pedro peaches were equally susceptible or resistant ($P < 0.05$) to strain Xcp93 and strains Xcp2 and Xcp9, respectively. However, the level of general susceptibility to *X. c. pruni* is probably higher in San Pedro than in the other three cultivars. This was indicated by the high disease severity values (86–100%) of 12 strains (Table 4) that were not significantly different from each other on San Pedro, but significantly lower on Hantam, Bokkeveld, or Olympia than on San Pedro.

In general, differential virulence and cultivar from which the bacterial strain had originally been isolated were not correlated.

TABLE 4. Mean percentages of inoculated areas with water-soaked spots[y] on detached leaves of four peach cultivars inoculated with 15 strains of *Xanthomonas campestris* pv. *pruni* (1×10^6 colony-forming units per milliliter) and summary of analysis of variance

	Strain															Cultivar
Cultivar	Xcp93	Xcp94	Xcp96	Xcp62	Xcp5	Xcp89	Xcp4	Xcp83	Xcp71	Xcp58	Xcp3	Xcp1	Xcp68	Xcp2	Xcp9	mean
San Pedro	100 a	100 a	99 a	96 a	99 a	94 a	99 a	100 a	88 a	87 a	86 a	95 a	86 a	32 a	0 a	84.1
Hantam	97 a	99 a	94 ab	98 a	93 b	94 a	69 ab	79 b	60 b	77 a	78 a	69 b	45 b	20 a	0 a	71.5
Bokkeveld	97 a	87 b	83 b	87 b	75 bc	76 b	80 b	82 b	75 a	61 a	55 b	49 b	43 c	21 b	26 a	66.5
Olympia	98 a	81 b	82 b	70 b	64 c	65 b	65 c	51 c	54 b	46 c	52 b	41 c	27 c	16 a	0 a	54.1

Analysis of variance: Sources of variation	df	MS[z]	P
Cultivars	3	35,962.97	<0.001
Strains	14	52,124.00	<0.001
Strains × cultivars	42	1,278.69	<0.001
Error	1,740	339.25	

[y] Values are means of 30 replications of 10 inoculated areas per leaf. Means followed by the same letter within columns are not significantly different ($P < 0.05$) according to Student's LSD (23) performed on the transformed (arcsin $\sqrt{x}$) data.
[z] Calculated from transformed data.

Strain Xcp2 was first isolated from Golden King plum. However, strain Xcp93 with differential virulence to peach cultivars Olympia, Bokkeveld, and Hantam, was also originally found on plums. Strain Xcp93 was recovered from a single canker detected in an orchard of plum cultivar Laetitia, which is apparently resistant to *X. c. pruni* (7). Differential virulence of strains of *E. amylovora* to apple cultivars was also not associated with the original host cultivar (19).

The inconsistent pattern of epidemic outbreaks of bacterial spot in the stone fruit-growing areas of South Africa may be due to differential interactions between pathogenic variants of *X. c. pruni* and different stone fruit cultivars. Severe symptoms of the disease frequently appear on leaves of different cultivars in nurseries without being apparent on other susceptible cultivars in the same nursery block. Furthermore, certain apricot, peach, and plum cultivars appear to be resistant compared with other cultivars in some geographic regions but highly susceptible when evaluated in different regions (H. J. du Plessis, *unpublished*). Different virulence groups of *X. c. oryzae* (10,11,15,18) are also found only in certain geographic regions (13,14).

My results show that the selection and evaluation of bacterial spot resistance of apricot, peach, and plum cultivars might be influenced substantially by the strains of *X. c. pruni* used in screening. Like other bacterial diseases (3,4,15,16,20), screening for resistance should be performed with strains representative of the complete range of differential virulence known to occur in *X. c. pruni*.

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