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Evaluation of Virulence of Strains of *Xanthomonas campestris* pv. *pruni* on Peach and Plum Cultivars

OLINDA M. MARTINS[1]

Abstract

Virulence of ten strains of *Xanthomonas campestris* pv. *pruni* obtained from nectarine, peach and plum orchards of different regions in Brazil was determined by means of a pressure infiltration inoculation procedure on peach and plum cultivars in the greenhouse. Analysis of variance revealed significant differences among cultivars, bacterial strains, and the interaction between cultivars and strains.

Introduction

Brazil has 22,800 hectares (ha) of peach (*Prunus persica* (L.) Batsch) orchards with about 10,000 ha in the state of Rio Grande do Sul (RS) (17), where canning varieties predominate. Bacterial spot caused by *Xanthomonas campestris* pv. *pruni* (Smith) Dye, causes serious damages to nectarine (*P. persica* var. *nucipersica* (L.) Batsch), peach and plum (*P. salicina* Lindl.) orchards in Southern Brazil. Disease development is favored by environmental conditions in addition to the fact that many high quality commercial cultivars are susceptible to this pathogen.

Chemical control of bacterial spot has been ineffective (4, 20). An effective degree of control with antibiotics has been searched (4, 14). The search for sources of resistance of *Prunus* spp. to bacterial spot has been a consideration of many researchers (3, 4, 9, 10, 13, 15, 18, 30). In a breeding program for resistance it is necessary to consider the interaction between host and the pathogen. In this regard, virulence of strains of *X. campestris* pv. *pruni* differs on *Prunus* spp. (1, 16), as well as minor differences in antigenicity (7) and phage sensitivity (8).

The purpose of this investigation was to study levels of virulence in different strains of *X. campestris* pv. *pruni* on peach and plum cultivars of commercial interest in Brazil, and the reaction of these hosts through an artificial inoculation procedure in the greenhouse.

Materials and Methods

Plant material. Three peach cultivars 'Cristal Taquari,' (susceptible; old Southern Brazil selection) 'Gaucho' (Brazilian selection, a progeny of 'Delicioso') and the resistant 'Norman' ('Sunhigh' x 'Redskin') along with two plum cultivars 'Pluma 7' (susceptible: 'The First' x 'Santa Rosa,' origin Brazil) and 'The First' (resistant; origin USA) were tested. These plants were budded on cv. 'Capdebosq ('Lake City' x 'Intermediario,' origin Brazil), op and grown in 30 x 30 x 20 cm plastic bags containing 3:1 (V/V) mixture of sterilized soil and vermiculite, and received weekly applications of a balanced nutrient solution. Plants with 10-week-old shoots were inoculated.

Bacterial isolates. Ten representative strains were chosen from commercial orchards of peach, plum and nectarine in different Brazilian States (Table 1). They were identified (11) and maintained in the bacterial collection of the Agriculture and Animal Research Center for Temperate Climate Regions (CPACT/EMBRAPA). All cultures were stored under desiccation (19).

Inoculum preparation and inoculation procedure. Inoculum was obtained from 48-hour-old cultures streaked on

[1]EMBRAPA/CPACT Agriculture and Animal Research Center for Temperate Climate Regions. c.p. 403. 96001-970. Pelotas. RS. Brazil.

523 media (12). A colony was cultured in 50 ml of 523 broth media in a shaker for 14 hr under 150 rpm, at 28C. Bacteria were pelleted by centrifugation at 1309g for 15 min. and suspended in sterile distilled water. The turbidity of the suspension containing 10^8 cfu/ml was adjusted in the spectrophotometer (Varian 634), according to the following equation line based on serial dilutions at 550nm wavelength: y = 507.36 - 56.16X, where, y = transmittance (%), X = log of the concentration. Plants were kept at 27C and relative humidity of 90 to 100% 48 hr before inoculation. Inoculum suspension was applied to the abaxial surface of the leaves with a spray gun connected to a compressed air supply (1.7 kg/cm^2) (5, 6) until the tissue became uniformly water-soaked. Control plants were sprayed with sterile distilled water. After inoculation plants were held in a controlled environment at 27C and 90 to 100% relative humidity for 4 days (2) before being returned to greenhouse conditions with temperature ranging from 16C to 39C.

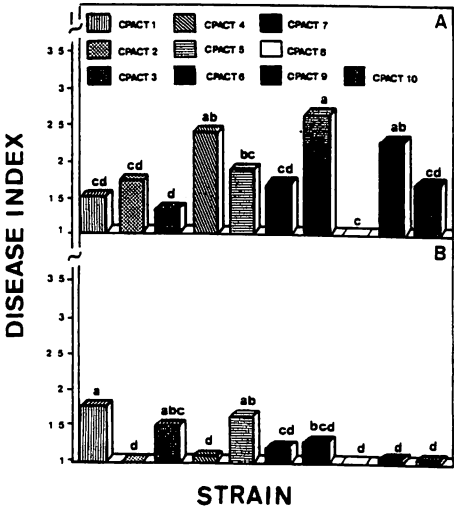

Figure 1. Disease index caused by ten strains of *Xanthomonas capestris* pv. *pruni* on plum cultivars 'Pluma 7' (A) and 'The First' (B). Bars topped by the same letter mean that transformed data log (x + 1) are not significantly different ($P < 0.01$) according to Duncan's Multiple Range Test.

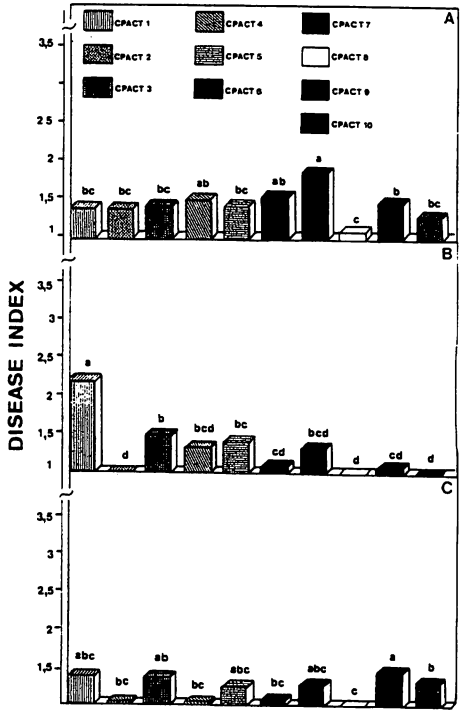

Figure 2. Disease index caused by ten strains of *Xanthomonas campestris* pv. *pruni* on peach cultivars 'Cristal Taquari' (A) 'Gaucho' (B) and 'Norman' (C). Bars topped by the same letter mean that transformed data log (x + 1) are not significantly different ($P < 0.01$) according to Duncan's Multiple Range Test.

Experimental design and disease assessment. The experimental design was a randomized complete factorial replicated three times. Disease index of eight leaves per plot was rated on a 1 to 5 scale. where, 1 = 0% to 2% of the leaf surface affected, 2 = 2% to 6% of the leaf surface affected, 3 = 6% to 12% of the leaf surface affected. 4 = 12% to 15% of the leaf surface affected, and 5 = over 15% of the leaf surface affected. Data were analyzed after transformation to log (x + 1).

Table 1. Origin of strains of *Xanthomonas campestris* pv. *pruni*, Brazil.

Bacterial Strains	Date of Isolation	Location	Organ of Isolation	Host
CPACT 1	1990	Irati, PR[2]	leaf	nectarine
CPACT 2	1990	Pelotas, RS	leaf	peach
CPACT 3	1990	Vacaria, RS	leaf	nectarine
CPACT 4[1]	1991	Paranapanema, SP	leaf	plum
CPACT 5	1991	Videira, SC	leaf	plum
CPACT 6	1991	Pelotas, RS	fruit	plum
CPACT 7	1991	Ijui, RS	leaf	peach
CPACT 8	1992	Erechim, RS	fruit	plum
CPACT 9	1993	Piratini, RS	leaf	peach
CPACT 10	1993	São Joaquim, SC	leaf	plum

[1]Strain obtained from collection of the Instituto Biologico de São Paulo (Biological Institute of São Paulo).
[2]PR = Paraná; RS = Rio Grande do Sul; SC = Santa Catarina; SP = São Paulo.

Results

Virulence of strains of *X. campestris* pv. *pruni*. Ten bacterial strains isolated from nectarine, peach and plum from representative regions (Table 1) showed wide differences in virulence on peach and plum cultivars (Figs. 1 and 2). Strain CPACT 8 was avirulent on 'Pluma 7,' 'The First,' 'Gaucho' and 'Norman,' and caused less than 1% of the leaf spot on 'Cristal Taquari' (Figs. 1 and 2). The values for CPACT 4 were high on the susceptible plum cv. 'Pluma 7' and on the peach cv. 'Cristal Taquari,' although this strain was among the less virulent strains on 'The First' and 'Norman.' Strains CPACT 9 and CPACT 10 were among the lowest in virulence on 'Gaucho' and 'The First,' although a highly significant interaction occurred between these strains and 'Norman.' Strain CPACT 2, avirulent

Table 2. Bacterial spot index[a] on peach and plum cultivars inoculated with ten strains of *Xanthomonas campestris* pv. *pruni*.

	Bacterial Strain									
Cultivar	CPACT 1	CPACT 2	CPACT 3	CPACT 4	CPACT 5	CPACT 6	CPACT 7	CPACT 8	CPACT 9	CPACT 10
Peach										
Cristal Taquari	1.31 bc[b]	1.30 ab	1.36 a	1.44 b	1.36 b	1.46 a	1.79 b	1.06 a	1.40 b	1.25 ab
Gaucho	2.12 a	1.00 c	1.42 a	1.27 bc	1.36 b	1.06 b	1.28 c	1.00 a	1.06 c	1.00 b
Norman	1.28 c	1.03 bc	1.31 a	1.03 c	1.21 b	1.06 b	1.24 c	1.00 a	1.38 b	1.27 ab
Plum										
Pluma 7	1.43 bc	1.58 a	1.28 a	2.16 a	1.75 a	1.51 a	2.46 a	1.00 a	2.07 a	1.56 a
The First	1.64 b	1.00 c	1.42 a	1.06 c	1.51 ab	1.15 b	1.21 c	1.00 a	1.03 c	1.03 b

Analysis of variance

Source of variation	df	MS[c]	P
Strains	9	0.6534137	< 0.001
Cultivars	4	1.5744076	< 0.001
Strains x cultivars	36	0.2356494	< 0.001
Error	1148	0.0435355	

[a]Rated on a 1 to 5 scale, according to the percentage of the leaf surface affected: 1 = 0% to 2%, 2 = 2% to 6%, 3 = 6% to 12%, 4 = 12% to 15%, and 5 = over 15%.
[b]Means followed by the same letter within columns are not significantly different ($P < 0.01$) according to Duncan's Multiple Range Test.
[c]Calculated from transformed data log (x + 1).

on 'Gaucho,' 'Norman' and 'The First' showed only moderate virulence on 'Pluma 7' and 'Cristal Taquari.' Strain CPACT 7 was highly virulent on 'Cristal Taquari,' 'Pluma 7, and 'Norman,' however, only moderately virulent on 'Gaucho' and 'The First.' Strains CPACT 3 and CPACT 5 showed a highly significant interaction between cultivars 'Norman' and 'The First.' These strains showed moderate virulence level to all other cultivars, except strain CPACT 3, whose disease index on 'Pluma 7' was low. Strain CPACT 1 was highly virulent on 'Gaucho,' 'Norman' and 'The First,' and caused different virulence levels on other cultivars.

Reaction of peach and plum cultivars Analysis of variance data revealed highly significant effects ($P < 0.001$) for isolates, peach and plum reactions, and strains x cultivars interaction (Table 2). The inoculation procedure of pressure infiltration distinguished different reactions between cultivars. A wide differentiation among peach and plum cultivars was shown for most isolates, except for strains CPACT 3 and CPACT 8, where no significant differences were observed for all cultivars (Table 2). The highest susceptibility was detected in 'Pluma 7' for almost all strains, although, 'Gaucho' had the highest disease index when inoculated with strain CPACT 1. 'Gaucho,' 'Norman' and 'The First' showed a high level of resistance (Table 2).

Discussion

Large differences in virulence between strains were revealed on peach and plum cultivars 40 days after inoculation. Highly significant differences among cultivars, isolates, and cultivar x isolate effects suggested that variation of the pathogen must be considered in a selection for disease resistance. Based on the variation of the bacterial strains it would be important to choose a highly virulent strain in the selection of resistant germplasm. Variations in virulence among strains of *X. campestris* pv. *pruni* were detected by means of a detached-leaf bioassay (1, 6). Dose-response relationships between strains of the bacteria and peach seedling leaves were well established (1). A high concentration (10^8 cfu/ml) revealed differences in virulence (Figs. 1 and 2). The interaction between strains CPACT 9 and CPACT 10 and the peach cv. 'Norman' confirms that highly virulent strains can overcome host resistance, considering the high resistance level of 'Norman' to other strains. The data showed no correlation between source of the strain and virulence on plum or peach. This is in agreement with previous data (6).

Disease index rated for cultivars varied, showing different resistance levels (Table 2). Generally, the susceptible plum cv. 'Pluma 7' showed high disease values, whereas, the peach cv. 'Norman' had the lowest disease index (Table 2), confirming its high resistance level (3). It was found a desirable resistance level on 'Gaucho,' in agreement with field observations.

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