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Selecting for Common Bacterial Blight Resistance in Common Bean: Effects of Multiple Leaf Inoculation and Detached Pod Inoculation Test

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Abstract. Breeding for resistance is an important strategy to manage common bacterial blight disease caused by *Xanthomonas campestris* pv. *phaseoli* (E. Smith) Dye (*Xcp*) in common bean (*Phaseolus vulgaris* L.). It is necessary to determine if prior inoculation of the first trifoliolate leaf with *Xcp* influences subsequent reactions in other plant organs by increasing or decreasing resistance to *Xcp*. It is difficult to get an accurate estimate of heritability of disease reaction in pods since environment greatly affects the heritability estimate if flowering occurs over extended time periods. Thus, the disease reaction in attached pods versus detached pods was compared. A split-split plot design with two replications (growth chambers as blocks) was used, with bean lines as the whole-plot factors, *Xcp* strains as subplot factors, and bacterial inoculation treatments for leaf reactions or pod treatments as split-split plot factors. The first trifoliolate leaves, later developed leaves, and attached and detached pods were inoculated. No effects of prior inoculation on the disease reactions of subsequently inoculated leaves and pods were observed, indicating that the different plant organs can be inoculated at different times. The fact that detached and attached pods showed similar disease symptoms would suggest use of the former to reduce environment variance and improve heritability estimates of resistance.

Common bean (*Phaseolus vulgaris*) is an important food legume, especially in tropical and subtropical Latin America and subsaharan Africa. One of the major problems affecting production of beans in many parts of the world is common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (E. Smith) Dye (*Xcp*) (Kyle, 1972; Saettler, 1989; Yoshii et al., 1978; Zaumeyer and Thomas, 1957). There is no satisfactory chemical control for CBB. Considerable breeding has been conducted to develop cultivars and lines of dry beans resistant to *Xcp* (Coyne and Schuster, 1983; Michaels, 1992; Park and Dhanvantari, 1987; Singh, 1996). Growing resistant cultivars is now being adopted as a management method.

Most reports indicate that the reaction to *Xcp* in *P. vulgaris* is quantitatively inherited (Coyne and Schuster, 1983; Saettler, 1989), but a few reports indicate that the reaction to *Xcp* is simply inherited (Adams et al., 1988; Silva et al., 1989). Resistance in *P. vulgaris* to *Xcp* is expressed as a low compatibility reaction (Saettler, 1989; Singh et al., 1996). Low heritability estimates of the leaf and pod reactions to *Xcp* in dry beans were reported (Aggour and Coyne, 1989; Arnaud-Santana et al., 1994; Arnaud-Santana and Coyne, 1996; Coyne et al., 1965; Silva et al., 1989;). However, Pompeu and Crowder (1972) reported high heritabilities for the reactions of leaves to *Xcp*. Segregating bean progenies often differ in the number of days for flowering and pod development. Varying temperatures occurring at different times during pod development affect the reaction of the pods to *Xcp*, thus increasing the environmental variance leading to lower heritability estimates for the disease reactions. Some workers investigated the disease reaction to *Xcp* in different plant organs, such as leaves and pods (Arnaud-Santana et al., 1994; Schuster et al., 1983; Silva et al., 1989), but did not determine if prior inoculation of a plant organ affected the reaction of later inoculated plant organs (leaves and pods).

There are some reports about effects of prior inoculation with pathogens on disease reactions developing from subsequent inoculations. Induced systemic resistance to pathogens was observed in tobacco (*Nicotania tabacum* L.) inoculated with heat-killed bacteria (Lozano and Sequeira, 1970) and cucumber (*Cucumis sativus* L.) with living bacteria (Caruso and Kuc, 1979). Hypersensitive symptoms were confined only to the inoculated areas, indicating no evidence for induced systemic resistance to bacteria causing halo blight disease in bean leaves (Lyon and Wood, 1977; Meier et al., 1993). However, in screening for resistance we need to determine if prior inoculation of leaves with virulent or low-virulent strains of *Xcp* would affect subsequent reactions of the leaves and pods. If there is no effect, this would allow inoculation of different plant organs to evaluate the disease reaction.

Detached pods (Klement and Lovrekovich, 1961), detached leaves (Arunakumari and Vidaver, 1986), and detached rooted leaves (Mohamed et al., 1993) have been used to detect *Xcp* reaction in common bean. Detached stems have been used to evaluate the reaction to *Xcp* and two other bacterial pathogens in beans (Lienert and Schwartz, 1994). If similar reactions to *Xcp* occurred on inoculated detached pods and attached pods, then better control of environmental conditions could be used to obtain more consistent disease reactions on detached pods.

There are no reports on the effects of prior inoculation of leaves of common bean with strains of *Xcp* on the reactions of later- inoculated leaves or pods with *Xcp* strains. The objectives of this study were as follows: 1) to study the effect of initial inoculation of bean leaves with *Xcp* on the disease reactions of subsequently inoculated leaves and pods and 2) to compare the *Xcp* reaction in attached bean pods with detached pods.

Materials and Methods

Two experiments were conducted in two growth chambers. A split-split plot design with two replications (growth chambers as

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Table 1. Disease reactions [% of inoculated leaf area with common bacterial blight (CBB) symptoms] of first trifoliolate leaves of four bean lines 14 d after inoculation with two strains of *Xanthomonas campestris* pv. *phaseoli* (Xcp) in Expt. 1.[z]

	Susceptible		Resistant	
Xcp strain	PC 50	HT 7719	XAN 159	BAC 6
	(% of inoculated area with CBB symptoms)			
V_3S_8	100	88	65	35
Xps	53	38	23	33

[z]$LSD_{0.05}$ = 13, to compare between means of same line for two strains. $LSD_{0.05}$ = 17, to compare between means of same strain for any two lines.

Table 2. Disease reactions [% of inoculated leaf area with common bacterial blight (CBB) symptoms] of first trifoliolate leaves of four bean lines 14 d after inoculation with two strains of *Xanthomonas campestris* pv. *phaseoli* (Xcp) in Expt. 2.[z]

	Susceptible		Resistant	
Xcp strain	PC 50	HT 7719	XAN 159	BAC 6
	(% of inoculated area with CBB symptoms)			
V_3S_8	70	100	16	25
Xps	18	43	3	37

[z]$LSD_{0.05}$ = 34, to compare between means of same line for two strains. $LSD_{0.05}$ = 30, to compare between means of same strain for any two lines.

blocks) was used, with bean lines as the whole-plot factors, strains as subplot factors, and bacterial inoculation treatments for leaf reactions or pod treatments as split-split plot factors. The experimental unit consisted of two plants per 15-cm clay pot containing a potting mixture of equal parts of sand, sphagnum peat, vermiculite, and soil (sharpsburg silty clay loam). The plants were fertilized once a week with 20N–10P–20K fertilizer. 'PC 50' (CBB susceptible, Arnaud-Santana et al., 1994), HT 7719 (CBB susceptible, Arnaud-Santana et al., 1994), XAN 159 (CBB resistant, McElroy, 1985), and BAC 6 (CBB resistant, Mohan, 1981) were tested. The plants were first grown in the greenhouse until first trifoliolate leaves were inoculated. The plants were placed immediately in the growth chambers after inoculation under a temperature regime of 24 ± 2/21 ± 2 °C day/night, respectively, and a photoperiod of 12 h. The average light intensity in the center of the growth chambers was 118 ± 21 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

The two Nebraska *Xcp* strains, V_3S_8 and Xps (of high and low virulence, respectively), were used for inoculations. The *Xcp* strains were grown on MXP medium (Claflin et al., 1987) for 48 to 72 h at 27 °C. The cultures were gradually transferred to 5 mL of 12.5 mM potassium phosphate buffer (PB) (pH 7.1) until diluted to read 0.1 O.D. on a spectrophotometer (Spectronic 20; Bausch and Lomb, Rochester, N.Y.) scale at 640 nm. The final concentrations of 10^7 colony-forming units/mL were prepared and used for inoculations.

The first and second experiments were planted 31 May 1993 and 26 Sept. 1993, respectively. The first fully expanded trifoliolate leaves were inoculated 21 d after planting using the multiple needle method (MNM) (Andrus, 1948). Subsequently, the pods were inoculated at pod filling stage, and then fully expanded leaves behind the growing point were inoculated with MNM at the same time as the pods. The time of pod inoculation was different among lines due to different dates of flowering.

The following inoculation treatments were used: 1) inoculation of the first trifoliolate, then separate subsequent inoculations of another trifoliolate (fully expanded behind growing point) and green pods at pod filling stage with a virulent *Xcp* strain (V_3S_8) and low-virulent strain (*Xps*), 2) same as above except no inoculation of first trifoliate, and 3) inoculation with buffer only for control.

For each treatment two attached and two detached pods were punctured with a dissecting needle at the pedicel ends, and then a Pipetteman was used for pod inoculations as described by Arnaud-

Table 3. Disease reactions [% of inoculated leaf area with common bacterial blight (CBB) symptoms] of fully expanded later leaves of four bean lines 14 d after inoculation with two strains of *Xanthomonas campestris* pv. *phaseoli* (Xcp) in Expt. 1.[z]

		Susceptible		Resistant	
Xcp strain	Inoculation[y]	PC 50	HT 7719	XAN 159	BAC 6
		(% of inoculated area with CBB symptoms)			
V_3S_8	1	95	75	33	30
	2	90	70	28	25
Xps	1	---	---	---	---
	2	48	38	20	30

[z]$LSD_{0.05}$ = 15, to compare between means of same line for any two inoculation treatments. $LSD_{0.05}$ = 35, to compare between means for different lines at same or any inoculation treatments.
[y]1= Inoculation of first and a subsequent trifoliolate leaf at pod filling. 2 = No inoculation of first trifoliolate and inoculation of subsequent trifoliolate leaves at pod filling.

Table 4. Disease reactions [% of inoculated leaf area with common bacterial blight (CBB) symptoms] of fully expanded later leaves of four bean lines 14 d after inoculation with two strains of *Xanthomonas campestris* pv. *phaseoli* (Xcp) in Expt. 2.[z]

		Susceptible		Resistant	
Xcp strain	Inoculation[y]	PC 50	HT 7719	XAN 159	BAC 6
		(% of inoculated area with CBB symptoms)			
V_3S_8	1	85	100	0	0
	2	83	88	10	10
Xps	1	50	37	5	20
	2	38	42	0	23

[z]$LSD_{0.05}$ = 19, to compare between means of same line for any two inoculation treatments. $LSD_{0.05}$ = 40, to compare between means for different lines at same or any inoculation treatments.
[z]1 = Inoculation of first and a subsequent trifoliolate leaf at pod filling. 2 = No inoculation of first trifoliolate and inoculation of subsequent trifoliolate leaves at pod filling.

Santana et al. (1994). Ten microliters of *Xcp* suspensions was deposited inside the pod through the puncture. Inoculated detached pods were placed in sealed 5 × 7-inch plastic bags containing several drops of water, punched with two small holes, and suspended from a bamboo stake supporting the same plant. Necrotic, water-soaking, and chlorotic symptoms developed on the inoculated leaves. Water-soaked areas were visible along the pod suture or along both sides of pod walls 5 to 6 d after inoculation. Leaf and pod disease reactions were recorded 14 d after inoculation. The ratings for the disease reactions are shown in Table 1.

Correlations between disease reactions of different plant parts were estimated separately for each experiment using original values. Analyses of variance were conducted using the Statistical Analysis System (SAS Institute, Cary, N.C.). Analyses were done separately for the two experiments because the Xps strain was not used for prior inoculation in Expt. 1.

Results and Discussion

The disease reactions of the first trifoliolate bean leaves showed significant differences between lines in both experiments. Differences in leaf reactions to the two *Xcp* strains also were detected. A significant line × strain interaction was found because both resistant lines were resistant to two *Xcp* strains. Strain V_3S_8 was more virulent on the first trifoliolate leaves of both susceptible lines than strain Xps (Tables 1 and 2). The resistant lines XAN 159 and BAC6 developed low compatibility reactions. No hypersensitive reactions to *Xcp* were observed on these resistant lines. Even with no visual leaf symptoms, a low bacterial concentration may be present in the leaves of the resistant tepary bean (*P. acutifolius*) plants inoculated with low concentrations of *Xcp* (Scharen, 1959), indicating lack of hypersensitivty.

The reactions of fully expanded leaves at the pod filling stage indicated significant interactions between lines and strains. The interaction occurred because the lowest disease reactions of the two resistant lines were nearly uniform with both strains, while the two susceptible lines varied in response to these strains (Tables 3 and 4). Strain V_3S_8 was more virulent than strain Xps on both susceptible lines (Tables 3 and 4). This agrees with Fujimoto's (1985) observations on the virulence of these two strains on leaves of 'Dark Red Kidney' and 'Arroyo Loro No. 1'. No significant differences were observed among reactions to either *Xcp* strain used in inoculations 1 and 2 in all lines in Expt. 2, and V_3S_8 used in Expt. 1 (Tables 3 and 4). This indicates that the prior leaf inoculations with *Xcp* had no effect on disease reactions resulting from subsequent inoculations of later developed leaves.

The pod reactions to *Xcp* indicated that there was no significant interaction between lines and pod treatments, inoculations and pod treatments, or lines with inoculations and pod treatments in either experiment, but the line × strain interaction was significant. Detached pods and attached pods showed similar disease reactions. No, or slight, symptoms on pods were observed in the two resistant lines

Table 5. Length (mm) of disease lesion from site of inoculation of attached versus detached pods of four bean lines 14 d after inoculation with two strains of *Xanthomonas campestris* pv. *phaseoli* (Xcp) in Expt. 1.[z]

Xcp strain	Inoculation treatment[y]	Pod treatment	Lesion length (mm)			
			Susceptible		Resistant	
			PC 50	HT7719	XAN 159	BAC 6
V_3S_8	1	Attached	4.9	4.9	1.0	1.0
		Detached	5.0	5.0	1.0	1.0
	2	Attached	4.7	4.8	1.0	0.8
		Detached	4.7	5.0	1.0	1.0
Xps	1	Attached	---	---	---	---
		Detached	---	---	---	---
	2	Attached	2.0	3.3	1.0	0.8
		Detached	1.9	3.3	1.0	0.8

[z]$LSD_{0.05}$ = 0.18, to compare between means of same line for any pod treatments. $LSD_{0.05}$ = 0.58, to compare between means of same line at any different inoculations. $LSD_{0.05}$ = 0.82, to compare between means of different lines at any combination of inoculations and pod treatments.
[y]1 = Inoculation of first trifoliolate leaf and subsequent inoculation of pods. 2 = Inoculation of pods without prior inoculation of first trifoliolate leaf.

Table 6. Length (mm) of disease lesion from site of inoculation of attached versus detached pods of four bean lines 14 d after inoculation with two strains of *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) in Expt. 2.[z]

Xcp strain	Inoculation treatment[y]	Pod treatment	Lesion length (mm)			
			Susceptible		Resistant	
			PC 50	HT7719	XAN 159	BAC 6
V_3S_8	1	Attached	4.2	3.8	0.8	1.0
		Detached	4.3	3.6	1.0	1.0
	2	Attached	4.3	4.0	1.0	1.0
		Detached	4.4	4.1	1.0	0.8
Xps	1	Attached	2.5	2.6	1.0	1.0
		Detached	2.8	2.6	1.0	1.0
	2	Attached	2.7	2.7	0.8	1.0
		Detached	2.5	2.7	1.0	1.0

[z]$LSD_{0.05}$ = 0.24, to compare between means of same line for any pod treatments. $LSD_{0.05}$ = 0.40, to compare between means of same line at any different inoculations. $LSD_{0.05}$ = 0.64, to compare between means of different lines at any combination of inoculations and pod treatments.
[y]1 = Inoculation of first trifoliolate leaf and subsequent inoculation of pods. 2 = Inoculation of pods without prior inoculation of first trifoliolate leaf.

(Tables 5 and 6). A significant interaction occurred between lines and inoculation treatments. Strain V_3S_8 was more virulent than Xps on the two susceptible lines. The comparison of inoculations with either the virulent or low virulent strains in all lines in Expt. 1 and low virulent strain V_3S_8 (only this strain was available for comparison) in Expt. 2 showed that there were no significant differences between pod reactions of prior leaf inoculated plants and those not receiving a prior leaf inoculation (Tables 5 and 6).

High positive Pearson correlation coefficients were observed between disease reactions to *Xcp* in attached pods and detached pods (r = 0.98 for both experiments) and first trifoliolate and fully expanded leaves (r = 0.90 for Expt. 1 and r = 0.88 for Expt. 2). Some workers have reported low Pearson correlations for disease reactions to *Xcp* among leaves, pods (Aggour and Coyne, 1989), and seeds (Arnaud-Santana et al., 1994) in segregating populations. Similar reactions to *Xcp* between leaves and pods in inbred lines 'PC 50', BAC 6, and XAN 159 were reported (Arnaud-Santana et al., 1993), which agrees with our findings.

Separate prior inoculations with virulent or low virulent *Xcp* strains did not influence the reactions from subsequent inoculation of different plant parts with *Xcp*. The effects of avirulent strains or dead cells of *Xcp* on the induction of resistance in common bean was not determined in this investigation because these are not the types of cells used by breeders in screening for resistance to *Xcp*.

In conclusion, inoculation of detached pods of common bean with *Xcp* under controlled temperature conditions showed similar disease reactions as attached pods. Inoculation of detached pods under controlled temperature conditions can be used to reduce environment variation affecting disease development on pods and so could be used to improve heritability estimates for more effective selection and for better detection of quantitative trait loci with molecular markers in segregating populations if desired.

There was no effect of prior inoculation of *Xcp* on the disease reactions of subsequently inoculated leaves and pods of the common bean lines tested. Thus, different plant organs of the same common bean plant can be used by breeders to assess disease reaction to *Xcp* at different or simultaneous times of inoculation without affecting the disease reaction in each plant organs.

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