

## Resistance to common bacterial blight in selected accessions of *Phaseolus* species

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### Abstract

Nine *Phaseolus* accessions showed consistent reactions to common bacterial blight in two field trials and one glasshouse test. An accession of *P. lunatus*, Bridgeton, showed a high level of resistance followed by *P. vulgaris* accessions, PI 207262 and CPI 9584, with intermediate levels of resistance, and BAC 135 with an inconsistent lower level of resistance. The *P. vulgaris* accessions Antioquia, BAC 51 and BAC 125, had inconsistent reactions between tests with susceptibility levels approaching that of the susceptible check. BAC 57 and BAC 134 had consistent reactions with expressions of moderate susceptibility.

Severity of disease in the leaf canopy in field trials increased with time except for the accessions Antioquia and Bridgeton. In the glasshouse test the size of the lesion increased with inoculum concentration although to a lesser degree on those accessions which were resistant in the field trials.

### INTRODUCTION

Common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* (Smith 1897; Dye 1978) is an important disease on navy beans (*Phaseolus vulgaris* L.) in Queensland (Moffett and Middleton 1979, Redden *et al.* 1985a). A germplasm collection of 1462 accessions of mainly *P. vulgaris* was screened for reaction to common bacterial blight as part of the navy bean breeding programme of the Queensland Department of Primary Industries (DPI) (Redden *et al.* 1985b). Through primary and secondary field screening, 16 accessions were identified as showing both a lower level of disease and a slower rate of disease spread from the lower to the upper leaf canopy than in the susceptible check plants. This study was planned to examine nine of these accessions chosen for their resistance to common bacterial blight, and to compare them for rust reaction and yield.

Available resistance to common bacterial blight (*X. campestris*) is always incomplete, and is quantitatively inherited (Webster *et al.* 1980; Redden *et al.* 1985b). It may be expressed differentially on foliage and on pods (Coyne and Schuster 1974). In this breeding programme the emphasis has been upon foliar resistance (Redden *et al.* 1985a).

### MATERIALS AND METHODS

#### Field trials

Two trials with nine accessions of *Phaseolus*, previously identified as resistant to common bacterial blight (Redden *et al.* 1985b) and a susceptible check, Gallaroy, (Table 1) were sown at Kingsthorpe, 22 km west of Toowoomba, on 29 January 1985, and on 24 January 1986. The plot size was 4 rows 0.7 m apart and 5 m long with 1 m wide alleyways. The alleyways were sown with Gallaroy to act as a spreader for common bacterial blight. A randomised complete block design with three replicates was used.

The nine accessions sown included Bridgeton, a lima bean (*P. lunatus*); five accessions from CIAT (BAC series 51, 57, 125, 134, 135); PI 207262 reported as resistant in the USA (Coyne and Schuster 1973a); Antioquia a large yellow seeded bean; and CPI 95894 originally accessed by CIAT as G 121817. These accessions varied in growth habit from a small bush with small leaves (PI 207262) to a relatively tall bush (0.70 m) with large leaves (Antioquia), and varied in maturity from early (Gallaroy) to late (Bridgeton). All accessions were of semi-determinate to indeterminate bush habit, (types II and III, Singh 1982).

Inoculum of common bacterial blight was obtained from diseased green plants of the variety Gallaroy, sown in a special nursery a month before each trial and inoculated with laboratory cultivars of *X. campestris* pv. *phaseoli*. The inoculation method described by Innes and Last (1961) was used. The plants were macerated, placed in hessian bags and soaked in 100 L drums of water for about four hours. The exudate was decanted into a high pressure sprayer and applied to the foliage of all entries using compressed air. The inoculations were made at 39 and 49 days after sowing in 1985, and at 26 days after sowing in 1986. A light spray irrigation of 10 to 20 mm was given in the evening after each inoculation. Otherwise the trials were non-irrigated.

The disease was visually rated for the entire leaf canopy on a scale of 1 (no disease) to 9 (high infection) as in a previous study (Redden *et al.* 1985b), at 64, 71 and 78 days after sowing in 1985, and at 49, 61 and 68 days after sowing in 1986. Beyond these dates leaf senescence of the earlier varieties precluded comparisons, while prior to these initial dates disease development was not obvious in a majority of plots.

Grain yields were recorded from the central two rows of each plot using an experimental harvester. The occurrence of other diseases, especially rust (*Uromyces appendiculatus*), in each of the trials was noted.

### Glasshouse trial

The reactions of the nine accessions and Gallaroy to three different inoculum concentrations of *X. campestris* pv. *phaseoli* were compared in a glasshouse trial.

Inoculum was prepared from a 48 h culture of the bacterium on sucrose peptone agar (Hayward 1960) by standardising a sterile distilled water suspension to  $10^7$ ,  $10^5$  or  $10^3$  cells/mL.

Plants at the four leaf stage were spray inoculated at 34.5 kPa using a chromatographic atomiser. Both leaf surfaces were inoculated to run-off without producing the water soaked areas that indicate infiltration of the leaf tissue with inoculum. Inoculated plants were kept at 100% relative humidity for 48 h and then placed in a random design on a glasshouse bench. Eighteen plants of each accession (two plants per pot) were inoculated per inoculum treatment.

Leaves were rated three weeks after inoculation using a rating system based on per cent leaf area damaged (James 1971). The reaction of each accession was assessed by a disease severity index.

$$\text{Disease severity} = \frac{\text{leaf no.} \times \% \text{ leaf damage}}{\text{total no. of diseased leaves}}$$

## RESULTS

### Field evaluation

During the observation period, 25 to 39 days after inoculation in 1985 and 32 to 51 days after inoculation in 1986 (Table 1), the severity of common bacterial blight increased with time on all accessions except Antioquia and Bridgeton. Bridgeton was disease free on all observation dates except the final one in 1985. Gallaroy showed the highest disease levels on the final observations in 1985 and on all observation dates in 1986. The *P. vulgaris* accessions with consistently the lowest disease levels were PI 207262 and CPI 19584, with the latter tending to be the least infected on the final observation date in each year. The final disease readings were slightly closer to those of Bridgeton than Gallaroy, and can be classed as moderately resistant. BAC 135, the fourth most resistant accession had promising initial reactions each year, and was classed as moderately resistant in 1985, but moderately susceptible in 1986.

Table 1. Common bacterial blight ratings\* for 10 *Phaseolus* accessions over 2 years and 3 observation dates in field trials

Accession	Means over dates and years†	Means over dates by years		Difference between years	Means over years, by dates			Difference date 3 - date 1
		1985	1986		Date 1	Date 2	Date 3	
Bridgeton	1.1	1.2	1.0	0.2	1.0	1.0	1.3	0.3
CPI 95894	2.9	2.7	3.2	-0.5	2.7	2.5	3.6	0.9
PI 207262	3.1	3.2	3.0	0.2	2.5	3.0	3.8	1.3
BAC 135	3.4	2.8	4.0	-1.2	2.5	3.5	4.2	1.7
BAC 57	4.1	3.9	4.3	-0.4	3.1	4.1	5.1	2.0
BAC 51	4.3	3.7	4.9	-1.3	3.7	4.4	4.8	1.1
BAC 125	4.6	4.1	5.0	-0.9	3.9	4.6	5.2	1.3
BAC 134	4.7	4.8	4.6	-0.2	4.2	4.8	5.0	0.8
Antioquia	4.8	5.3	4.3	1.0	4.8	4.7	5.0	0.2
Gallaroy	5.4	4.9	5.9	-1.0	4.2	5.5	6.6	2.4
Mean		3.7	4.0		3.3	3.8	4.6	
LSD ( $P=0.05$ )	0.46		0.21	0.65		0.25		0.8

\* Scale 1 = no disease to 9 = fully susceptible.

† Year × date × accession interaction, and year × date interaction both non significant.

The common blight reactions of other accessions tended to be moderately susceptible but varied from year to year. Antioquia showed uniform levels of infection over time, but disease was more severe in 1985 than in 1986. Each year disease severity for BAC 134 was initially high with a tendency for final levels to plateau at a lower level of susceptibility than Gallaroy. The accessions BAC 125 and BAC 51 were both moderately susceptible each year with a slower rate of disease increase over time compared with Gallaroy. BAC57 was also moderately susceptible and exhibited a rapid increase in disease severity over time which was exceeded only by Gallaroy. The correlation for mean disease ratings of accessions between years ( $R^2=0.84$ ) was significant ( $P<0.01$ ).

### Yield and rust data

In both 1985 and 1986 droughts occurred in the growing season. This was during early growth in 1985, and from flowering onwards in 1986. Yields were lower in 1985 (Table 2) than in 1986.

**Table 2. Grain yield and rust reactions for 1985 and 1986 field evaluations of 10 *Phaseolus* accessions**

Accession	Grain yield kg/ha		Rust 1986*
	1985	1986	
Bridgeton	540	896	1
CPI95894	264	622	1
PI 207262	286	831	1
BAC 135	361	862	1
BAC 57	378	816	1
BAC 51	425	1006	4.7
BAC 125	414	899	1
BAC 134	385	982	1
Antioquia	324	529	1
Gallaroy	438	973	6.0
LSD ( $P=0.05$ )	141	334	0.6

\* 1 = resistant to 9 = susceptible.

### Glasshouse trial

Glasshouse observations (Table 3) recorded reactions at the inoculation site only, whereas the field observations recorded disease spread in the canopy from infected leaves to new growth.

Gallaroy was the most susceptible at the highest inoculum concentration ( $10^7$  cells/mL). Similarly, this concentration produced reactions in most accessions that were the most closely related to field observations. Both CPI 95894 and PI 207262 tended to be more resistant than other *P. vulgaris* accessions but with PI 207262 displaying more resistance. At the lower inoculum concentrations both Antioquia and BAC 57 were relatively resistant. The remaining BAC accessions were similar to, or more susceptible (BAC 51 and BAC 134) than Gallaroy. These reactions to lower inoculum levels were less consistent with field observations. The correlation ( $R^2=0.82$ ), between the mean field scores (over years) and the glasshouse trial using  $10^7$  cells/mL inoculum for disease ratings of all entries was significant ( $P<0.01$ ).

**Table 3. Reaction of bean lines to three inoculum concentrations ( $10^3$ ,  $10^5$ ,  $10^7$  cells/mL) of *Xanthomonas campestris* pv. *phaseoli* in the glasshouse expressed as a disease severity\* index**

Line	Disease severity index		
	$10^7$	$10^5$	$10^3$
Bridgeton	13.97	6.78	0
Antioquia	22.72	15.35	1
PI 207262	14.27	16.1	9.62
CPI 95894	18.52	19.41	10
BAC 51	26.35	19.91	14.4
BAC 57	23.09	14.15	4
BAC 125	20	21.54	5.5
BAC 134	23.24	24.89	3.25
BAC 135	20.81	18.8	10
Gallaroy check	32.02	19.06	8.87

\* Disease severity based on % leaf area affected (James, C. (1971) *Canadian Plant Disease Survey* 51 (2), 62).

### DISCUSSION

In this study the accessions Bridgeton, PI 207262 and CPI 95894, displayed a higher level of resistance than the accessions (prefixed BAC and BAT) from CIAT. This is in contrast with the previous screening (Redden *et al.* 1985b) in which the latter tended to be more resistant. This discrepancy may relate to environmental effects, to the smaller single row plots of the initial studies, or less uniform inoculum application. Plant reaction was shown

to be very sensitive to inoculum concentration in this study. The results of the two studies agree in classifying all nine accessions as showing varying degrees of resistance in comparison with cv. Gallaroy, the susceptible check. With field ratings, the low scores (1 to 4) indicated disease spread from the lower to the upper canopies, and higher scores referred to degree of disease spread in the upper canopy layers.

The lower overall yields in 1985 reflected less growth of branches and foliage than in 1986, although in both years rainfall was below average (82 to 210 mm during growth), and temperatures above average (February maximum 31°C, minimum 17.5°C each year). The visually rated infection scale represented a disease spread within a larger leaf canopy in 1986. The superior yield achieved by BAC 51, BAC 134, BAC 125, Gallaroy and Bridgeton was not a reflection of growth habit. In general, grain yield and common bacterial blight reactions appeared to be unrelated.

The most promising resistance was expressed by the lima bean, Bridgeton. It may be possible with special embryo culture techniques to achieve an interspecific cross between *P. lunatus* and *P. vulgaris* (Honma and Haecht 1959), but the hybrid may not be fertile, and as yet no gene transfer between the two species has been achieved. For navy bean breeding the best sources of resistance are the accessions PI 207262 and CPI 95894. In earlier screening with continuous irrigation (Redden *et al.* 1985*b*) the poor expression of common blight resistance by Great Northern No. 1 selection 27, which is resistant in the USA (Coyne and Schuster 1973*a*), may be evidence for the existence of different strains of *X. campestris* pv. *phaseoli* in the USA and Queensland. The occurrence of such strain differences has been documented, with PI 207262 noted as relatively resistant to three diverse sources of the pathogen (Coyne and Schuster 1973*b*).

The common blight reactions of the BAC accessions and Antioquia tended to be more variable than those of Bridgeton, PI 207262, and CPI 95894. The latter accessions had a stable consistent expression of resistance between seasons. This was unrelated to yield levels or to any other obvious agronomic characteristic. However, this level of resistance in the two *P. vulgaris* accessions may be inadequate to prevent yield losses due to common bacterial blight and to prevent seed transmission of the disease (Weller and Saettler 1980); a potential hazard for production of seed crops. Webster *et al.* (1980) have suggested that expression of resistance by PI 207262 may be affected by daylength. Daylength was below 12 hours during the reproductive phase each year in the current study, and may have lowered the resistance of PI 207262 compared with field tests under the longer daylengths typical of North America (Webster *et al.* 1980). Temperature and moisture stress factors during pod fill (April) may possibly be implicated in the variable reactions shown by the BAC lines. The development of common bacterial blight symptoms is positively correlated with temperature, which for *P. vulgaris* is likely to be increased by moisture stress (Goss 1940).

Disease levels in the field increased with age on all entries except Bridgeton in 1986. Coyne *et al.* (1973) report that plants are more susceptible in the reproductive stage than the seedling stage, and emphasise the importance of both the developmental stage and of the interval after inoculation in resistance studies. In this study, field inoculation was at the beginning of flowering of the earliest entry, Gallaroy in 1985, but pre-flowering in 1986. The disease recording dates over a 2 to 3 week period overlapped in respect to growth stages between years and may possibly have influenced differences in results between seasons.

Rust was important only in 1986, with only two accessions, Gallaroy and BAC 51 being susceptible. The data did not indicate a synergistic effect where joint bacterial blight and rust infections occurred.

The concentrations of inoculum used in the field were not standardised, although similar procedures were used in each case, with similar lesions each year and only two diseases observable, rust and common bacterial blight. Although the source plots for inoculum were infected with cultures of common bacterial blight, and only this disease and rust appeared to be present on plants at the flowering stage when macerated for preparing field inoculum, the presence of other pathogens or other strains of common bacterial blight in the field trials cannot be ruled out. For this reason the glasshouse tests are important, involving a single strain of pure inoculum under controlled conditions. Differences in the initial inoculum concentration affects disease severity at the initial infection site (Coyne *et al.* 1973) and could influence secondary spread in the leaf canopy. Although the inoculum concentrations used in the glasshouse test were lower the results were similar to those of Coyne *et al.* (1973), with increased disease severity and lowered resistance as inoculum concentration increased.

Despite the various factors affecting the expression of symptoms to common bacterial blight, it is noteworthy that the results of the two field and one glasshouse tests reported are comparable. This adds confidence to the relative rankings of disease resistance among the accessions studied. Higher levels of resistance than expressed by PI 207262 and CPI 95894 are required. If these were crossed with BAC 125 and BAC 134 it may be possible to obtain transgressive segregation for greater resistance in combination with acceptable yielding ability.

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