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Bacterial blight caused by *Pseudomonas syringae* pv. *syringae* shown to be an important disease of field pea in south eastern Australia

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Abstract *P. syringae* pv. *syringae* was shown to be the cause of bacterial blight in 40% of field pea crops showing symptoms of bacterial blight during 2005 compared to 47.5% caused by *P. syringae* pv. *pisi* and the 12.5% of crops infected by both pathovars. A replicated field experiment conducted in the presence of stubble infected with *P. syringae* pv. *syringae* quantified yield losses in commercial cultivars due to this pathovar. Within this study field pea cultivars could be divided into two groups based on resistance or susceptibility to bacterial blight caused by *P. syringae* pv. *syringae.* The average yield loss in the resistant cultivars in the presence of infected field pea stubble was 23%, whereas in the susceptible cultivars the yield loss was 75%. In one cultivar a yield loss of 94% was measured. Variability between cultivars and breeding lines in their responses indicates potential for breeders to develop *P. syringae* pv. *syringae* resistant cultivars. Studies into the survival of *P. syringae* pv. *syringae* on infected field pea stubble showed that the pathogen could not be recovered after 34 weeks.

Keywords *Pseudomonas syringae* pv. *pisi*

Introduction

There were many reports of bacterial blight in crops of field pea (*Pisum sativum*) in south-eastern Australia during the late 1990s and early 2000s. *Pseudomonas syringae* pv. *pisi* has been regarded as the most important cause of bacterial blight in Australia and internationally (Hollaway et al. 2007).

However, *P. syringae* pv. *syringae* has been reported to occur in pea crops in Australia (Wimalajeewa and Nancarrow 1984; Clarke 1990) and overseas (Taylor and Dye 1972; Jindal and Bhardwaj 1989; Lawyer and Chun 2001), but has been regarded as less important than *P. syringae* pv. *pisi,* which is reported to cause disease over a wider range of environmental conditions (Taylor and Dye 1972; Lawyer and Chun 2001). The symptoms of bacterial blight (also known as brown spot) in field peas have been described previously (Taylor and Dye 1972; Lawyer and Chun 2001). All aerial plant parts are susceptible to attack throughout the growing season and affected stipules develop characteristic water-soaked, fan-shaped lesions which turn brown and papery. Disease caused by *P. syringae* pv. *syringae* or *P. syringae* pv. *pisi* cannot be distinguished between by field symptoms.

There is little reported information regarding yield loss caused by *P. syringae* pv. *syringae* in field pea. Jindal and Bhardwaj (1989) reported a severe outbreak in northern India attributed to *P. syringae* pv. *syringae*, but the extent of yield loss was not quantified. Likewise, there have been limited studies into the epidemiology of *P. syringae* pv. *syringae* in field peas. It is thought that *P. syringae* pv. *syringae* survives from one season to the next on seed and/or infected crop residues (Lawyer and Chun 2001) in a similar way to *P. syringae* pv. *pisi* (Hollaway et al. 2007). Studies of brown spot in common bean (*Phaseolus vulgaris)*, caused by *P. syringae* pv. *syringae* indicate that disease development is favoured by cool, wet conditions with ideal temperatures between 12 and 25°C (Hirano and Upper 1990). Disease development is often favoured by frosts due to the ice nucleating properties of the pathogen (Maki et al. 1974).

Pathogenic variation of *P. syringae* pv. *pisi* is well documented with a race structure having been determined

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and resistance identified in cultivars (Taylor et al. 1989; Bevan et al. 1995; Hollaway and Bretag 1995b). There have been limited studies internationally into the presence or absence of host plant resistance or pathogenic variability toward field pea with regard to *P. syringae* pv. *syringae*. Butler and Fenwick (1970) reported the presence of pathogenic variability within limited isolates of *P. syringae* pv. *syringae* toward field peas.

There appears to have been an increased incidence of bacterial blight in field peas caused by *P. syringae* pv. *syringae* since the introduction of new semi-leafless cultivars in south-eastern Australia. New cultivars released during the late 1990s and early 2000s possessed many desirable agronomic traits which included more upright growth habitat and higher grain yields than the older cultivars. This has resulted in a significant increase in the area sown to new semi-leafless cultivars, such as cv. Kaspa.

These studies were undertaken to 1) determine the importance of *P. syringae* pv. *syringae* as a cause of bacterial blight of field peas in south east Australia; 2) quantify disease susceptibility and associated yield loss in current commercial cultivars; 3) evaluate a field screening method that could be adopted by field pea breeders, and 4) determine the survival period of *P. syringae* pv. *syringae* in infected stubble.

Materials and methods

Cause of bacterial blight in symptomatic field pea crops

Collection of symptomatic field pea plants

During 2005, 40 field pea crops exhibiting symptoms of bacterial blight were sampled from South Australia, Victoria and New South Wales at the 8th to 12th node growth stage (Knott 1987).

From each crop, two or three symptomatic plants were collected and placed in a plastic zip-lock bag and transported back to Horsham for testing. Plants were stored at 4°C until processed for testing.

Isolation of Pseudomonas *species*

Tests to determine the causal organism(s) were undertaken within two days of sampling the plant material. Leaf and stem tissue with symptoms of bacterial blight was finely chopped using sterile scissors. Ten gram of tissue was added to a 250 ml Schott bottle containing 100 ml of sterile distilled water and soaked overnight at 21°C±4°C . The resulting suspension was serially diluted (1:1, 1:10, 1:100 and 1:1000) with sterile distilled water. Using a glass rod, 100 μl of each of the dilutions was spread onto sucrose-nutrient agar supplemented with boric acid, cefuroxime, cycloheximide and cephalexin (SNAC) (Hollaway and Bretag 1995a). Colonies similar in appearance to *P. syringae* were subcultured after 48 h incubation at 21°C± 2°C and maintained on King's medium B (King et al. 1954) until identified. A set of four *Pseudomonas* spp. reference cultures obtained from the Biological and Chemical Research Institute, Rydalmere (DAR 69866 *P. syringae* pv. *pisi*, DAR 58721 *P. viridiflava*, DAR 35680 *P. syringae* pv. *syringae* and DAR 55534 *P. cichorii*) were also maintained on King's medium B and used in all subsequent pathogenicity and biochemical tests for reference.

Bacterial identification

Bacterial isolates were identified using pathogenicity and biochemical tests. Isolates were first tested for pathogenicity on field pea seedlings at the 3 to 4 leaf stage using the methods described by Hollaway and Bretag (1995a). Bacteria were scraped from the surface of a 48 h King's B culture plate using a toothpick. For each isolate, a field pea seedling (cv. Kaspa) was then stabbed with the toothpick in two distinct stem locations. Once inoculated the plants were placed in dew chambers at 25°C±3°C and 100% relative humidity for 48 h. Plants were transferred to a controlled environment with a 24 h photoperiod at 15°C±3°C for a further 48 h. They were then returned to the glasshouse for the disease to develop and were assessed three days later. A positive result was recorded if a water soaked lesion had developed around the inoculation site. Whether or not the lesion was localised or extensive was also recorded. *P. syringae* pv. *syringae* usually causes an extensive lesion that causes stem collapse, whilst *P. syringae* pv. *pisi* tends to be more localised (Mazarei and Kerr 1990). Isolates that were positive on pea stem were tested for oxidase reaction (Kovacs 1956), levan production (Lelliott et al. 1966), fluorescence (Hildebrand et al. 1988), pectolytic activity (Hildebrand et al. 1988) and pathogenicity of common bean (*Phaseolus vulgaris*) and lemon (*Citrus limonium*) fruits (Wimalajeewa and Nancarrow 1984). Isolates that were oxidase negative, levan positive and potato rot negative belong to the *P. syringae* group of bacteria (Fahy and Lloyd 1983; Hildebrand et al. 1988). The two pathovars are differentiated by their reaction on lemon and bean fruit as *P. syringae* pv. *pisi* does not cause a reaction on lemon and bean fruit whereas *P. syringae* pv. *syringae* does (Wimalajeewa and Nancarrow 1984).

Disease scores and yield loss in pea cultivars infected with *P. syringae* pv. *syringae*

A field experiment was conducted during 2006 near Horsham at the Department of Primary Industries' Plant Breeding Centre in the Wimmera region of Victoria. The field site has

an average annual rainfall of 450 mm and a friable grey soil type. Monthly rainfall data and the number of frost days observed for 2005–2007 at Horsham are shown in Table 1. The site was cropped to lupins (*Lupinus* spp.) the previous year and had not been cropped to field peas for at least 5 years. The site was flood irrigated with approximately 50 mm of water 4 weeks prior to sowing to allow for early sowing to encourage disease development.

Eleven pea cultivars (Table 2) were sown on 17 May 2006. Each cultivar was sown at 100 kg of seed per ha in 6-row plots, 6 m long, with a row spacing of 15 cm. Seed was obtained from Tony Leonforte (Department of Primary Industries, Horsham, Victoria). Double superphosphate (0% N, 9% P, 0% K) was applied in furrow at seeding at a rate of 75 kg/ha. Galant West® (Haloxyfop 130 g/L) was applied (19 May) at a rate of 200 ml/ha to control grass weeds and volunteer cereals and Select® (Clethodim 240 g/L) was applied (11 July) at a rate of 150 ml/ha to control broad-leaved weeds.

The experimental design was a randomised split block with three replicates. The main plot comprised the 11 pea cultivars which were split into three sub-plots. The first sub-plot had field pea stubble naturally infected with *P. syringae* pv. *syringae* spread at a rate of 2,500 kg/ha on 28 June 2006 following the sowing of uninoculated seed. The second sub-plot was sown with seed artificially infected with *P. syringae* pv. *syringae* prior to sowing in addition to receiving the stubble treatment as described above. Seed was artificially infected by soaking seed in a bacterial suspension (1×10^6 colony forming units/ml) for 20 min while under vacuum and then air dried. The third sub-plot was the nil treatment and was sown with uninoculated seed.

Table 1 Monthly rainfall (mm) and the number of frost days observed at Horsham and Wagga Wagga during 2005–2007 (Bureau of Meteorology)

Month	2005		2006		2007		Long term average (1957–2008)	
	Rain (mm)	Frost days	Rain (mm)	Frost days	Rain (mm)	Frost days	Rain (mm)	Frost days[A]
Horsham, Victoria								
January	39.2	0	25.8	0	64.3	0	23.3	0
February	31.2	0	5.4	0	12.6	0	24.7	0
March	4.4	0	6.4	0	9.6	0	23.3	0
April	11	0	38.8	1	67.4	0	31.7	1
May	13	9	26.6	7	70.4	0	46.8	3
June	58.4	9	5.8	22	6.8	10	49.7	8
July	28.6	8	31.6	15	47.8	10	46.8	9
August	36.8	9	19.6	13	20.2	10	48.5	7
September	30.2	7	39.4	9	21.2	7	46.2	5
October	76.4	6	2.6	9	11.0	8	44.1	3
November	37.6	0	8.6	2	57.0	0	33.7	0
December	16.8	0	7.4	0	40.6	0	27.4	0
Annual	383.6	48	218.0	78	428.9	45	445.8	36
Wagga Wagga, New South Wales								
January	13.2	0	69.4	0	40.2	0	40.1	0
February	46.8	0	1.8	0	54.6	0	39.7	0
March	6.6	0	10.6	0	23.8	0	43.0	0
April	14.6	1	17.4	6	46.0	0	41.3	1
May	4.6	9	4.6	15	52.4	3	51.2	6
June	69.0	6	39.4	18	19.4	10	49.7	10
July	65.0	8	49.2	14	38.2	17	55.0	14
August	56.4	14	7.6	22	22.2	8	50.8	11
September	85.0	5	20.0	10	7.4	12	49.6	7
October	77.6	2	3.8	0	14.6	5	57.7	2.0
November	44.8	0	34.0	0	73.0	0	43.7	0
December	29.4	0	9.4	0	74.6	0	44.8	0
Annual	513.0	45	267.2	86	466.4	55	566.4	50

[A] The number of frost days for the long term average was determined by the mean number of days that the minimum temperature was equal to or lower than 2°C over the period of 1957 to 2008

Table 2 Phenotypic traits and release dates of commercial cultivars used to estimate yield loss caused by *P. syringae* pv. *syringae* during 2006

Cultivar	Leaf type	Height	Maturity	Seed type	Flower colour	Flowering time	Lodging resistance at harvest	Year of release
Bundi	Semi-leafless	Semi-dwarf	Early	White	White	Early	Good	2007
Dundale	Conventional	Tall	Mid	Dun	Purple	Early	Poor	1970
Dunwa	Conventional	Tall	Mid	Dun	Purple	Mid	Poor	2003
Excell	Semi-leafless	Semi-dwarf	Early-Mid	Blue	White	Early	Excellent	2000
Helena	Conventional	Tall	Mid	Dun	Purple	Mid	Poor	2000
Kaspa	Semi-leafless	Semi-dwarf	Mid	Dun	Pink	Late	Good	2003
Moonlight	Semi-leafless	Tall	Mid	White	White	Mid	Fair	2003
Morgan	Semi-leafless	Tall	Late	Dun	Purple	Late	Fair	1998
Parafield	Conventional	Tall	Mid	Dun	Purple	Mid	Poor	1999
Snowpeak	Semi-leafless	Semi-dwarf	Mid	White	White	Early	Good	2001
Sturt	Conventional	Tall	Mid	White	White	Mid	Poor	2006

Disease severity within each plot was assessed on the 18 September 2006 using the 0 to 9 scale described in Table 3. Grain yield was determined at plot maturity by recording grain weight from each plot following harvest with a self-propelled Hege plot harvester on the 4 December 2006.

To determine the cause of bacterial blight symptoms a representative, inoculated plot of each cultivar was tested for the causal agent present. Plants with symptoms were arbitrarily selected and infected material was removed and placed in a separate plastic zip lock bag for each cultivar and promptly returned to the laboratory for testing. Bacterial isolation and identification was then conducted as described above

Statistical analysis

All statistical analyses were conducted using Genstat 11th Edition. Analysis of variance was conducted on the grain yield data. For analysis of the disease score a Friedman Sum Rank test (Friedman 1937) was conducted. Regression analysis was conducted to relate yield to disease score for each plot.

Field screening of pea cultivars for their reaction to *P. syringae* pv. *syringae*

Sixty six advanced field pea breeding lines from Pulse Breeding Australia were screened at Horsham for resistance to bacterial blight caused by *P. syringae* pv. *syringae* using the same site as the field experiment described previously. Field pea stubble naturally infected with *P. syringae* pv. *syringae* was spread at a rate of 2,500 kg/ha on 28 June 2006 on all plots following the sowing of uninoculated seed on 17 May 2006. The experimental layout was a non-replicated design with checks. The checks consisted of 12 commercial cultivars, distributed randomly throughout the experimental design,

Table 3 Disease scale used for assessment of bacterial blight severity in field plots at Horsham during 2006

Disease score	Description
0	No disease present
1	<5% plants with leaf and stem lesions less than 10 mm in size
2	5% to 25% diseased plants with leaf and stem lesions less than 10 mm in size
3	25% to 50% of plants with leaf and stem lesions less than 10 mm in size
4	< 50% of plants with lesions less than 10 mm in size and <5% of plants with lesions larger than 10 mm in size
5	> 50% of plants with lesions less than 10 mm in size and <5% of plants with lesions larger than 10 mm in size
6	100% of plants with lesions less than 10 mm in size and 5% to 10% of plants with lesions larger than 10 mm in size
7	Dead patches in plot developing, with lesions larger than 10 mm in size on most plants
8	Up to 80% plant death
9	80% to 100% plant death with few remaining survivors

and replicated at least twice. Agronomic details, disease and yield assessment were as described for the previous field experiment. The cause of disease symptoms was determined within a representative plot of each cultivar and breeding line, also as described above for the previous field experiment. Likewise, data were analysed as described above.

Survival of *P. syringae* pv. *syringae* on field pea stubble

The survival of *P. syringae* pv. *syringae* on field pea stubble was studied using the method described by Hollaway and Bretag (1997). Stubble naturally infected with bacterial blight, caused by *P. syringae* pv. *syringae,* was collected during January 2005 from a field pea crop (cv. Kaspa) near Rupanyup in the Wimmera region of Victoria. The crop was harvested during December 2004.

Infected pea stubble (5 g) was placed into plastic mesh bags (mesh size 3×2 mm; bag size 15×15 cm) which were stapled closed and placed in the field. The mesh bags were either pegged and left at the soil surface or buried at 10 cm below ground level.

The experiment was conducted twice at each of two sites using randomised block designs. The first site was at the Plant Breeding Centre of the Victorian Department of Primary Industries (friable grey clay, soil pH 8.6) near Horsham and was established on 2 March 2005 and 10 February 2006. The second site was at the Wagga Wagga Agricultural Institute, New South Wales Department of Primary Industries (red-brown earth, soil pH 5.0) near Wagga Wagga in the Riverina region of New South Wales and was established on 21 February 2005 and 13 February 2006.

Each treatment was replicated five times with ten bags of field pea stubble (5 buried, 5 surface) and removed from the field after 0, 10, 14, 18, 22, 26, 30, 34, 38, 66 and 118 weeks. The content of each bag was tested for the presence of viable *P. syringae* pv. *syringae* as described below. Monthly rainfall data and frost days for the Horsham and Wagga Wagga weather stations were obtained from the Bureau of Meteorology (Table 1).

Upon removal from the field excess soil was shaken from each bag and any plant matter attached was also removed. The bags were opened, stubble residues removed, placed into a 500 ml Schott bottle with 100 ml of sterile distilled water and left overnight to soak at 21°C±4°C . The resulting suspensions were serially diluted with sterile distilled water and using a glass rod, 100 µl of each of the dilutions 1:1, 1:10, 1:100 and 1:1000 were spread onto sucrose-nutrient agar supplemented with boric acid, cefuroxime, cycloheximide and cephalexin (SNAC) as used by Hollaway and Bretag (1995a). Isolation and identification methods were then conducted as described previously in the section, "Cause of bacterial blight in symptomatic field pea crops".

Results

Cause of bacterial blight in symptomatic field pea crops

P. syringae pv. *syringae* and/or *P. syringae* pv. *pisi* were isolated from all 40 crops with symptoms of bacterial blight sampled from south-eastern Australia during 2005 (Table 4). *P. syringae* pv. *syringae* was the sole cause of bacterial blight identified in 40% of crops, *P. syringae* pv. *pisi* the sole cause in 47.5%, while both pathovars of *P. syringae* were detected in 12.5% of crops.

Disease scores and yield loss in pea cultivars infected with *P. syringae* pv. *syringae*

In the presence of *P. syringae* pv. *syringae* infected pea residues all eleven commercial cultivars evaluated in the field showed symptoms of bacterial blight and suffered grain yield loss relative to the uninoculated plots in which only minimal symptoms of bacterial blight developed (Table 5). Within the cultivars there were reductions in grain yield ranging from 13% in cv. Sturt to 94% in cv. Moonlight.

With regard to disease score there were significant treatment, variety and treatment by variety interactions observed (Table 5). Within the varieties two distinct groups were identified; a resistant group, with a disease score less than 4 in the presence of inoculum and a susceptible group with a disease score of greater than 6 in the presence of inoculum. The median disease scores of the resistant and susceptible groups in the presence of inoculum were 3 and 7, respectively. Similarly, in the presence of inoculum the mean grain yield of the resistant and susceptible groups was 1.82 t/ha and 0.04 t/ha, respectively. The average yield loss of the resistant cultivars in the presence of infected stubble was 23% and within the susceptible group it was 75%.

Table 4 Number of field pea crops with symptoms of bacterial blight from which *P. syringae* pv. *pisi* (*Psp*) and/or *P. syringae* pv. *syringae* (*Pss*) were detected during 2005 in New South Wales, Victoria and South Australia

Location	*n*	*Pss* only	*Psp* only	*Pss* and *Psp*
New South Wales	21	6	10	5
Victoria	11	6	5	0
South Australia	8	4	4	0
Total	40	16	19	5

Table 5 Means of disease scores and grain yields recorded from uninoculated (nil) and inoculated plots of eleven commercial cultivars from a field trial in Horsham, 2006

Cultivar	Treatment				Reaction Group
	Nil	Stubble	Seed & stubble	Mean	
Disease score (0–9, where 0=resistant and 9=very susceptible)					
Sturt	0 a[A]	2 b	4 cd	2 a[C]	Resistant
Dunwa	0 a	2 b	3 bc	2 a	Resistant
Parafield	0 a	2 b	3 bc	1 a	Resistant
Helena	0 a	2 b	1 ab	1 a	Resistant
Dundale	0 a	2 b	3 bc	2 a	Resistant
Morgan	0 a	2 b	3 bc	2 a	Resistant
Snowpeak	2 b	8 de	7 e	6 b	Susceptible
Kaspa	0 a	7 e	6 e	4 b	Susceptible
Bundi	0 a	7 e	7 e	5 b	Susceptible
Excell	1 ab	8 de	8 de	5 b	Susceptible
Moonlight	1 ab	7 e	7 e	5 b	Susceptible
Mean	0 a[B]	4 b	5 c		
l.s.d (0.05): cultivar=1.0; treatment=0.5; cultivar x treatment=1.7					
Grain yield (t/ha)					
Sturt	3.1	2.7	1.8	2.5 a	Resistant
Dunwa	2.1	1.8	1.5	1.8 a	Resistant
Parafield	2.0	1.5	1.2	1.6 a	Resistant
Helena	2.8	2.1	2.8	2.6 a	Resistant
Dundale	3.0	2.1	2.1	2.4 a	Resistant
Morgan	2.1	1.4	1.7	1.7 a	Resistant
Snowpeak	0.7	0.4	0.0	0.4 b	Susceptible
Kaspa	1.3	0.6	0.9	0.9 b	Susceptible
Bundi	1.9	0.2	0.4	0.8 b	Susceptible
Excell	1.3	0.1	0.0	0.4 b	Susceptible
Moonlight	1.8	0.1	0.0	0.1 b	Susceptible
Mean	2.0 a	1.2 b	1.1 b		
l.s.d (0.05): cultivar=0.53; treatment=0.27; cultivar x treatment=*n.s*					

[A] Treatment by variety means within the disease scores followed by the same letter are not significantly different ($P<0.05$), as calculated from the least significant difference (l.s.d) using a cultivar by treatment comparison. Means calculated from three replicates per treatment

[B] Treatment means followed by the same letter are not significantly different ($P<0.05$) as calculated from the l.s.d using a treatment comparison. Means calculated from the three replicates of each cultivar for a single treatment

[C] Cultivar means within the same column followed by the same letter are not significantly different ($P<0.05$) as calculated from the l.s.d using a cultivar comparison. Means calculated from the three replicates of each treatment for a single cultivar

A linear regression model relating yield to disease score was fitted to individual plot data. There was a significant negative linear effect of disease on yield ($P<0.001$). The model for yield was:

$$\text{Yield}\,(\text{t/ha}) = 2.35 - 0.283 \times \text{disease score}$$

The overall variance explained from this model was 65%.

Testing of symptomatic plants from the field trial confirmed in all cases that *P. syringae* pv. *syringae* was the cause of the bacterial blight epidemic in the field.

Evaluation of breeding lines for resistance to *P. syringae* pv. *syringae*

The 66 breeding lines evaluated varied significantly ($p=0.006$) in disease severity and corresponding grain yields ($p=<0.001$) (data not shown). They could be grouped into resistant and susceptible categories (Table 6) based on disease score (0–5 and 6–9 respectively) and yield (>0.3 t/ha and<0.29 t/ha respectively).

A linear regression model relating yield to disease score was fitted to individual plot data. There was a significant negative linear effect of disease on yield ($P<0.001$). The model for yield was:

$$\text{Yield}(\text{t/ha}) = 1.41 - 0.186 \times \text{disease score}$$

The overall variance explained from this model was 69%.

Survival of *P. syringae* pv. *syringae* on field pea stubble

P. syringae pv. *syringae* could not be detected on naturally infected field pea stubble monitored in the field after 30 weeks in either of the two studies each conducted at two locations (Horsham, Victoria or Wagga Wagga, New South Wales) regardless of whether the stubble was buried or remained on the soil surface (Table 7).

Table 6 Severity of bacterial blight in advanced breeding lines and commercial cultivars of field peas grown in the presence of stubble infected with *P. syringae* pv. *syringae* in the field at Horsham, 2006

Bacterial blight disease score	
0–5	6–9
Resistant	Susceptible
[a]Parafield	[a]Yarrum
[a]Dundale	[a]Moonlight
[a]Helena	[a]Kaspa
[a]Morgan	[a]Excell
[a]Dunwa	[a]Snowpeak
[a]Sturt	01-309-6
[a]Bundi	97-446*2
00-226-5	01-230-14
97-033-1-6	01-303-3
97-360-*5-11-2	97-031-6-6
96-286*1-9	96-262*1
01-186-6	97-017*2-8
96-286*1-16	01-260-6
01-478-2	01-271-1
01-503-8	01-323-3
01-511-7	00-160-6
99-228*12	01-503-7
97-015*2-8	97-033*4
96-286*1-11	99-104*2
01-246-13	01-269-6
01-255-7	97-031-6-10
01-256-10	01-278-5
97-031-6-3	01-271-4
00-257*6	99-098*4
99-098*3	99-228*5
97-015-4D-11	97-031-6-M-P
01-228-2	97-015*2-5
01-323-9	01-126-7
	01-136-3
	01-261-4
	01-255-5
	01-230-27
	01-032-8
	99-246*12
	01-019-6
	01-227-5
	98-309*6
	01-226-6
	01-227-20
	01-136-4
	89-036*3-6
	97-031-6-M-Pi
	01-226-3
	01-284-2
	98-309*1
	01-322-12
	01-253-13
	01-230-5
	01-226-2
	96P403-2

[a] Denotes a variety used as a control and replicated in the field experiment

Discussion

This study shows that *P. syringae* pv. *syringae* can be an important cause of bacterial blight in field pea and is the first to quantify yield losses due to this pathogen. Variation between field pea cultivars in their reactions to *P. syringae* pv. *syringae* was demonstrated which has implications for cultivar selection and plant breeding. Previously *P. syringae* pv. *syringae* was not considered a serious pathogen of field pea (Lawyer and Chun 2001), but results of these studies showed that *P. syringae* pv. *syringae* can cause significant crop loss in susceptible cultivars.

In 40% of crops with symptoms of bacterial blight, *P. syringae* pv. *syringae* was the causative agent during 2005. Although *P. syringae* pv. *syringae* was known to be widespread in field pea crops within Victoria during the 1980s (Wimalajeewa and Nancarrow 1984; Clarke 1990) it has not been considered an important cause of bacterial blight epidemics (Hollaway et al. 2007). The following possibilities exist for the increased importance of *P. syringae* pv. *syringae* as a cause of bacterial blight in field peas: 1) an increased prevalence of the pathogen in field pea crops; 2) the environment has become more conducive for development of bacterial blight caused by this pathogen; and/or 3) new field pea cultivars are more susceptible to this pathogen than older cultivars.

Results of these studies implies that the increased prevalence of bacterial blight caused by *P. syringae* pv. *syringae* may be associated with the adoption of cultivars susceptible to this pathogen. Prior to 2000 the dominant field pea cultivar in south-eastern Australia was Dundale, which was shown to be moderately resistant to *P. syringae* pv. *syringae* in our studies. Since 2000, growers have adopted new higher yielding cultivars such as cv. Kaspa, Excell and Snowpeak, which have been shown in this study to be more susceptible to bacterial blight caused by *P. syringae* pv. *syringae* than the conventional cultivar Dundale. The five most *P. syringae* pv. *syringae* susceptible cultivars identified in this field study were all released after 1999. As bacterial blight caused by *P. syringae* pv. *syringae* has been regarded as a minor disease of field peas, there has been no selection applied within breeding programs during the development of these cultivars.

Results from the Horsham field experiments showed that yield losses due to *P. syringae* pv. *syringae* can be as high as 94% in cv. Moonlight. Eleven commercial cultivars could be separated into two groups: resistant cultivars with a disease score <5 and susceptible varieties with a score >5. The yield loss in the resistant group was approximately 23% whereas in the susceptible group the loss was approximately 75% and this is the first report quantifying losses due to bacterial blight caused by *P. syringae* pv. *syringae* in field pea. The susceptible group consists of cvs.

Table 7 Percentage of mesh bags (n=5) containing field pea stubble naturally infected with *P. syringae* pv. *syringae* from which viable *P. syringae* pv. *syringae* could be isolated when placed on the soil surface or buried at Horsham or Wagga Wagga for 2 years with studies commencing in March/February 2005 and in February 2006

Location	Year	Survival period (weeks)										
		0	10	14	18	22	26	30	34	38	66	118
Horsham, Victoria												
soil surface	2005	100	60	60	0	40	40	20	0	0	0	0
	2006	100	80	40	40	20	20	0	0	0	0	0
buried	2005	100	100	20	30	40	20	0	0	0	0	0
	2006	100	100	80	60	20	0	0	0	0	0	0
Wagga Wagga, New South Wales												
soil surface	2005	100	60	40	20	40	0	0	0	0	0	0
	2006	100	60	60	40	40	0	0	0	0	0	0
buried	2005	100	20	60	20	20	0	0	0	0	0	0
	2006	100	80	40	20	20	0	0	0	0	0	0

Snowpeak, Kaspa, Bundi, Excell and Moonlight. These cultivars are all semi-leafless and semi-dwarf types except for cv. Moonlight. These phenotypic traits may be linked to *P. syringae* pv. *syringae* susceptibility; however further studies are required to test this hypothesis.

The variability between field pea cultivars and advanced breeding lines in their reactions to *P. syringae* pv. *syringae* suggests that it should be possible for pea breeders to develop resistant cultivars and avoid the release of more susceptible cultivars. For the purpose of screening early generation breeding lines, when limited seed is available, this study has indicated that it is possible to screen for bacterial blight in the field. Of the 66 lines evaluated, 45 were rated as susceptible and 21 as resistant based on the disease scores applied relative to known control lines. Based on the results of this study the use of either a disease score or grain yield assessment would be appropriate to categorise breeding lines. However, the use of a disease score may be more suitable as it would allow for the use of un-replicated small plots and require fewer resources than replicated plots for yield assessment.

Within this study only a single isolate of *P. syringae* pv. *syringae* was used and consequently the varieties were categorised based on their reaction to this single bacterial isolate. Further work needs to be undertaken to determine if pathogenic variability exists within the *P. syringae* pv. *syringae* population as it does within the *P. syringae* pv. *pisi* population (Bevan et al. 1995). Therefore, screening of field pea breeding material should be undertaken with some caution until the pathogenic variability of *P. syringae* pv. *syringae* populations are established.

For a disease screening nursery to be successful, we believe that early sowing is critical for good disease development. There are many reports in the literature of the association of early sowing with the development of bacterial blight caused by *P. syringae* pv. *pisi* (see Hollaway et al. 2007). The field experiments reported in this study were sown early in the season relative to sowing dates recommended for the Horsham area, increasing the likelihood of an epidemic developing.

It is also likely that the high level of disease development within the field was assisted by the above average number of frosts that occurred during the 2006 growing season at Horsham (Table 1). *P. syringae* pv. *syringae*, like *P. syringae* pv. *pisi,* is an ice nucleating bacterium and there are many reports linking frost with increased occurrence and severity of bacterial blight caused by *P. syringae* pv. *pisi* (see Hollaway et al. 2007).

Results of this study have shown that infected stubble can be an important source of inoculum of *P. syringae* pv. *syringae* but only poses a significant risk to the following year's pea crop and not crops in later years. This is in contrast to the findings of Hollaway and Bretag (1997) who reported that *P. syringae* pv. *pisi* poses a risk to subsequent field pea crops for 2 years as *P. syringae* pv. *pisi* survived for 78 weeks on stubble on the soil surface. This suggests that *P. syringae* pv. *syringae* does not withstand environmental conditions as well as *P. syringae* pv. *pisi*.

Although artificially infected seed was used in this study, there is limited knowledge of the importance of seed infection in the epidemiology of bacterial blight caused by *P. syringae* pv. *syringae* in field pea. This knowledge gap should be addressed.

In light of this study's findings Australian field pea breeding programs should take this pathogen into consideration for future cultivar development to decrease the risk of commercialising new cultivars susceptible to bacterial blight. A glasshouse test should also be developed to help identify

both *P. syringae* pv. *syringae* resistant and susceptible breeding lines to further improve the resistance of field pea to bacterial blight. Glasshouse tests could also be used to determine the nature and extent of pathogenic variability in different isolates of *P. syringae* pv. *syringae*. This information will enable pea breeders to develop new cultivars with improved resistance to bacterial blight caused by *P. syringae* pv. *syringae*. Furthermore, growers need to be reminded of the importance of crop rotation to reduce inoculum levels and avoid growing very susceptible cultivars in bacterial blight-prone areas.

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