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Differential responses to pea bacterial blight in stems, leaves and pods under glasshouse and field conditions

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Abstract

Resistance to pea bacterial blight (*Pseudomonas syringae* pv. *pisi*) in different plant parts was assessed in 19 *Pisum sativum* cultivars and landraces, carrying race-specific resistance genes (R-genes) and two *Pisum abyssinicum* accessions carrying race-nonspecific resistance. Stems, leaves and pods were inoculated with seven races of *P. s.* pv. *pisi* under glasshouse conditions. For both race-specific and nonspecific resistance, a resistant response in the stem was not always associated with resistance in leaf and pod. Race-specific genes conferred stem resistance consistently, however, there was variability in the responses of leaves and pods which depended on the matching R-gene and A-gene (avirulence gene in the pathogen) combination. R2 generally conferred resistance in all plant parts. R3 or R4 singly did not confer complete resistance in leaf and pod, however, R3 in combination with R2 or R4 enhanced leaf and pod resistance. Race-nonspecific resistance conferred stem resistance to all races, leaf and pod resistance to races 2, 5 and 7 and variable reactions in leaves and pods to races 1, 3, 4 and 6.

Disease expression was also studied in the field under autumn/winter conditions. *P. sativum* cultivar, Kelvedon Wonder (with no R genes), and two *P. abyssinicum* accessions, were inoculated with the most frequent races in Europe under field conditions (2, 4 and 6). Kelvedon Wonder was very susceptible to all three races, whereas *P. abyssinicum* was much less affected. The combination of disease resistance with frost tolerance in *P. abyssinicum* enabled plants to survive through the winter. A breeding strategy combining race-nonspecific resistance derived from *P. abyssinicum* with race-specific R-genes should provide durable resistance under severe disease pressure.

Introduction

Studies on resistance to pea bacterial blight (*Pseudomonas syringae* pv. *pisi)* have focused on race-specific resistance. The presence of race-specific resistance genes (R-genes) is common both in commercial cultivars and *Pisum* germplasm. There are seven races of *P. s.* pv. *pisi* currently recognized and interaction of races and cultivars is controlled by a gene-for-gene relationship (Taylor et al., 1989; Bevan et al., 1995).

Tests for resistance have usually been done by a stem inoculation procedure (Malik et al., 1987). This method allows sequential multiple inoculations with different races on the same plant and avoids the risk of contamination associated with spray inoculation of leaves. It was thought that the response in stems was likely to be the same for other plant parts. However, in stem inoculation of a cultivar carrying the resistance gene R3 with race 3, it was observed that when the inoculation was very close to the stipule, sometimes a water-soaked susceptible response developed which spread into the stipule, while the stem showed a necrotic resistant response (J.R. Bevan, pers. comm.). This indicated that stem and stipule (leaf) tissues may express a differential response to the pathogen. There is

some evidence in another host/pathogen combination (*Phaseolus vulgaris*/*P. s.* pv. *phaseolicola*) for differential responses in leaves and pods (Hill et al., 1972).

In addition to the race-specific genes in *Pisum*, race-nonspecific resistance has been identified in *Pisum abyssinicum* (Schmit et al., 1993; Elvira-Recuenco and Taylor, 1998). This confers a quantitative stem resistance to all races of the pathogen including race 6, for which there are no known resistant *P. sativum* cultivars. *P. abyssinicum* is a traditional crop in the highlands of Ethiopia and is adapted to low temperatures (Vavilov, 1992).

The effects of environmental factors on disease expression are important in the epidemiology of pea blight. Frost damage increases susceptibility of plant tissue to the pathogen (Boelema, 1972). Disease severity is greater in winter sown than in spring sown peas for both winter and spring cultivars (Mansfield et al., 1997). Factors that may be involved are high soil moisture which influences transmission of the pathogen from seed to seedling (Skoric, 1927; Roberts, 1992), frost and hail damage which favours entry of the pathogen (Young and Dye, 1970; Roberts et al., 1995) and wind blown rain which favours dissemination of the pathogen (Stead and Pemberton, 1987).

Because of the possibility of differential responses between stem and leaf tissue, it was decided to investigate the full range of responses of both race-specific and race-nonspecific resistance to all known races of *P. s.* pv. *pisi* in stems, leaves and pods under controlled glasshouse conditions. Investigations were also done in the field under autumn/winter conditions, when environmental factors (rain, frost, etc.) would be likely to predispose plants to maximum disease expression.

Materials and methods

Glasshouse studies

Pea lines. The pea lines used are listed in Table 1. Peas were sown in compost in seed trays and grown at 20 °C ± 2 day/17 °C ± 2 night with supplementary lighting to give a 12-h day.

Bacterial isolates. Seven type strains, corresponding to the seven races of *P. s.* pv. *pisi* (Taylor et al., 1989; Bevan et al., 1995) were used for inoculation. In some of the experiments an additional isolate of each race was used (Table 2). For inoculum production, isolates were subcultured onto King's B medium (King et al., 1954) for 24–48 h at 25 °C. They were derived from one transfer of cultures stored either at −80 °C in broth (8 g l^{-1} nutrient broth, 150 ml l^{-1} glycerol) or freeze dried.

Stem inoculation. Pea seedlings were stem inoculated approximately 2 weeks after sowing (Malik et al., 1987). Isolates were scraped from the surface of the plate with the tip of a sterile entomological mounting pin and stabbed into the main stem at its junction with the stipules at the youngest node.

Leaf inoculation. This was done simultaneously with stem inoculation. Young leaves were wounded with the same entomological pin used for stem inoculation and sprayed with a bacterial suspension (ca. 10^9 cfu ml^{-1}) prepared in sterile $\frac{1}{4}$ strength Ringer's or sterile tap water. A wetting agent (Manoxol) was added to give a concentration of 0.025% in the bacterial suspensions which were sprayed at a low pressure to throughly wet the leaf surfaces. Inoculated plants were maintained in a mist chamber for 48 h after inoculation and then transferred to the glasshouse.

Pod inoculation. Young flat detached pods were placed on moist filter papers in Petri plates. Isolates were scrapped from the surface of culture plates with sterile entomological pins and pods stab inoculated (3–4 inoculations per pod). Petri plates with pods were stored in closed boxes at room temperature.

Inoculation responses. Stem and leaf responses were recorded 7–10 days after inoculation and pod reactions 5–7 days after inoculation. Responses were assigned to one of three categories: a typical susceptible response showed an area of water-soaking spreading from the site of inoculation, whereas a typical resistant response resulted in necrosis localized at the point of inoculation. Incomplete expression of resistance was characterized by a combination of resistant and susceptible symptoms: localized necrosis surrounded by a limited water-soaked area.

Field studies

Cultivar Kelvedon Wonder (susceptible to all races) and *P. abyssinicum* accessions JI2202 and JI1640 (race-nonspecific resistance) were used for these studies. They were sown in compost in seed trays

Table 1. Pea accessions and their resistance characteristics to *Pseudomonas syringae* pv. *pisi*

Accession number[a]	Designation	Stem resistance to races	Resistance genes[c]
Pisum sativum cultivars			
JI2430	Kelvedon Wonder[b]	None	None
JI2431	Early Onward[b]	2	R2
JI2432	Belinda[b]	1, 3, 7	R3
JI2435	Hurst's Greenshaft[b]	1, 4, 5, 7	R4, (R6)
JI2438	Partridge[b]	1, 3, 4, 5, 7	R3, R4
JI2436	Vinco[b]	1, 2, 3, 5, 7	R1, R2, R3, (R5)
JI2437	Sleaford Triumph[b]	1, 2, 4, 5, 7	R2, R4, (R5)
JI2439	Fortune[b]	1, 2, 3, 4, 5, 7	R2, R3, R4
ZP-0034	Frisson	1, 2, 3, 5, 7	R2, R3
	Jade	1, 3, 7	R3
	Shasta	1, 3, 7	R3
Pisum sativum landraces			
JI171	*P. sativum* (Ethiopia)	1, 2, 3, 4, 5, 7	R2, R3, R4
JI1577	*P. sativum* (China)	1, 3, 4, 5, 7	R3, R4
ZP-0101	*P. sativum* (Spain)	1, 3, 4, 5, 7	R3, R4
ZP-0103	*P. sativum* (Spain)	1, 3, 7	R3
ZP-0104	*P. sativum* (Spain)	1, 2, 3, 4, 5, 7	R2, R3, R4
ZP-0107	*P. sativum* (Spain)	1, 3, 7	R3
ZP-0110	*P. sativum* (Spain)	1, 2 , 3, 4, 5, 7	R2, R3, R4
ZP-0112	*P. sativum* (Spain)	1, 3, 4, 5, 7	R3, R4
Pisum abyssinicum			
JI1640	*P. abyssinicum* (Ehiopia)	1, 2, 3, 4, 5, 6, 7	Race-nonspecific
JI2202	*P. abyssinicum* (Ethiopia)	1, 2, 3, 4, 5, 6, 7	Race-nonspecific

[a]JI numbers correspond to accession numbers in *Pisum* germplasm collection, John Innes Centre, Norwich, UK; ZP numbers correspond to accession numbers in the collection of 'Servicio de Investigación Agraria', 'Junta de Castilla y León', Valladolid, Spain.
[b]Differential cultivars (Bevan et al., 1995).
[c]Designation of resistance genes (Bevan et al., 1995); R-genes in parentheses partly proven.

in a glasshouse. Three weeks later they were transplanted to 25 cm diameter pots containing John Innes compost number 1 and placed in a series of 'cold frames' with removable glass covers at Wellesbourne, UK. Temperatures were expected to be similar to those in the open field but with some protection from the wind.

One week after transplanting the 'cold frames' were uncovered and 2 days later plants were either inoculated with the type strains of the races 2, 4 or 6 (Table 2) or uninoculated. Inoculation was done by spraying the plants with a bacterial suspension (ca. 10^9 cfu ml^{-1}) prepared as described above for glasshouse studies. Each treatment was represented by a block of 16 pots (4 pots per accession) distributed randomly. Treatments were separated by a minimum of 2.5 m. Disease symptoms and condition of the plants were recorded 1, 3, 5 and 8 weeks after inoculation. Meteorological data were also recorded.

Results

Glasshouse studies

Race-specific resistance. Stem inoculations with the seven races of *P. s.* pv. *pisi* consistently confirmed the reactions expected (Bevan et al., 1995) (Table 3). When the appropriate R-gene was present in the pea accession, the stem-inoculated race carrying the matching avirulence gene caused a resistant (hypersensitive) response. The only exception was race 4 where some partial stem susceptibility was observed.

Inoculation responses in leaves and pods in some cases differed from those in the stems (Tables 4–6). A susceptible response in the stem was always associated with a susceptible response in leaf and pod. However, a resistant response in the stem was associated with either a resistant or susceptible/partially susceptible response in leaf and pod. In general,

Table 2. Source and origin of the isolates of *Pseudomonas syringae* pv. *pisi*

Race	Isolate number HRI-W	Source[c]	Origin		
			Cultivar	Country	Year
1	299A[a]	ICPM 2955	Rondo	New Zealand	1970
1	1684	ICPM 3198	Unknown	India	1971
2	202[a]	ICPM 815	Unknown	USA	1944
2	4616	HRI-W	Solara	UK	1992
3	870A[a]	HRI-W	Martus (seed)	USA	1975
3	2191A	HRI-W	Rondo	Canada	1988
4	895A[a]	HRI-W	Martus (seed)	USA	1975
4	2817A	HRI-W	Unknown	Spain	1991
5	974B[a]	HRI-W	Puget (seed)	USA	1978
5	4012	HRI-W	Snowflake	Zimbabwe	1988
6	1704B[a]	MAFF	Stehgolt (seed)	France	1986
6	4129B	HRI-W	Stanton	UK	1990
7	2491A[a,b]	ICPM 5316	Unknown	Australia	1976
7	4409	HRI-W	Bikini	UK	1991

[a]Type strains.
[b]Selection from ICPM 5316 which was a mixture.
[c]HRI-W, Horticulture Research International, Wellesbourne, Warwickshire, UK.
ICPM, International Collection of Micro-organisms from Plants, Plant Diseases Division, DSRI, Auckland, New Zealand.
MAFF, Ministry of Agriculture, Fisheries and Food, Cambridge, UK.

Table 3. Gene-for-gene relationship between pea cultivars and races of *Pseudomonas syringae* pv. *pisi* (Bevan et al., 1995)

							Race/avirulence genes						
							1	2	3	4	5	6	7
							1	—	—	—	—	—	—
							—	2	—	—	2	—	2
							3	—	3	—	—	—	3
							4	—	—	4	4	—	4
							—	—	—	—	5[a]	—	—
	Resistance (R) genes						6[a]	—	—	—	6[a]	—	—
KelvedonWonder	—	—	—	—	—	—	S	S	S	S	S	S	S
Early Onward	—	2	—	—	—	—	S	R	S	S	R	S	R
Belinda	—	—	3	—	—	—	R	S	R	S	S	S	R
Hurst's Greenshaft	—	—	—	4	—	6[a]	R	S	S	R	R	S	R
Partridge	—	—	3	4	—	—	R	S	R	R	R	S	R
Sleaford Triumph	—	2	—	4	5[a]	—	R	R	S	R	R	S	R
Vinco	1	2	3	—	5[a]	—	R	R	R	S	R	S	R
Fortune	—	2	3	4	—	—	R	R	R	R	R	S	R

S, susceptible response to stem inoculation.
R, resistant response to stem inoculation.
[a]Resistance genes 5 and 6 only partly proven.

responses in leaves and pods were similar although in some cases pods tended to be more susceptibile. Responses for the different combinations of accessions and races tested are shown in relation of their matching R/A genes.

When accessions Belinda, Jade, Shasta, ZP-0103 and ZP-0107, which carry only R3, were challenged with races 1, 3 or 7 carrying the avirulence gene A3, resistance was expressed completely in stems and partially in leaves and pods (Table 4).

Table 4. Inoculation responses to races of *Pseudomonas syringae* pv. *pisi* in different plant parts (stem, leaf and pod) of pea accessions carrying the race-specific gene R3 singly or in combination with other R-genes

Pea accession (Resistance gene)	Race	R/A[a]	Inoculation response[b]		
			Stem	Leaf	Pod
Belinda (R3)	1	3	R	R/S	R/S
	3	3	R	R/S	R/S
	7	3	R	R/S	R/S
Jade (R3)	1	3	R	R/S	R/S
	3	3	R	R/S	R/S
	7	3	R	R/S	R/S
Shasta (R3)	1	3	R	R/S	R/S
	3	3	R	R/S	R
	7	3	R	R/S	R/S
ZP-0103 (R3)	1	3	R	R	NT
	3	3	R*	R/S	NT
	7	3	R	R/S	NT
ZP-0107 (R3)	1	3	R	R/S	NT
	3	3	R	S*	NT
	7	3	R	R/S	NT
Frisson (R2 + R3)	1	3	R	R*	NT
	2	2	R	R	NT
	3	3	R	R/S	NT
	5	2	R	R	NT
	7	2 + 3	R	R	NT
Partridge (R3 + R4)	1	3 + 4	R	R	R
	3	3	R	R/S	R/S
	4	4	R	R/S	R/S
	5	4	R	R*	R*
	7	3 + 4	R	R*	R
ZP-0101 (R3 + R4)	1	3 + 4	R	R	NT
	3	3	R	R/S	NT
	4	4	R	R/S	NT
	5	4	R	R	NT
	7	3 + 4	R	R	NT
ZP-0112 (R3 + R4)	1	3 + 4	R	R	NT
	3	3	R	R/S	NT
	4	4	R	S	NT
	5	4	R	R*	NT
	7	3 + 4	R	R	NT
JI1577 (R3 + R4)	1	3 + 4	R	R	R
	3	3	R	R/S	R/S
	4	4	R	R/S	S
	5	4	R	R/S	R/S
	7	3 + 4	R	R*	R*
Fortune (R2 + R3 + R4)	1	3 + 4	R	R	R
	2	2	R	R	R
	3	3	R	R/S	R/S
	4	4	R	R/S	S
	5	2 + 4	R	R	R
	7	2 + 3 + 4	R	R	R/S
ZP-0104 (R2 + R3 + R4)	1	3 + 4	R	R	NT
	2	2	R*	R/S	NT
	3	3	R	R/S	NT
	4	4	R*	S	NT
	5	2 + 4	R	R	NT
	7	2 + 3 + 4	R	R	NT
ZP-0110 (R2 + R3 + R4)	1	3 + 4	R	R	NT
	2	2	R	R/S	NT
	3	3	R	S*	NT
	4	4	R	S*	NT
	5	2 + 4	R	R*	NT
	7	2 + 3 + 4	R	R	NT
JI171 (R2 + R3 + R4)	1	3 + 4	R	R	R
	2	2	R	R	R
	3	3	R	R*	R
	4	4	R	R/S	R/S
	5	2 + 4	R	R	R
	7	2 + 3 + 4	R	R	R
Vinco (R1 + R2+ R3 + R5)	1	1 + 3	R	R	R
	2	2	R	R	R
	3	3	R	R	R/S
	5	2 + 5	R	R	R*
	7	2 + 3	R	R	R

[a]R/A, matching resistance/avirulence genes in pea accessions and *P. s.* pv. *pisi* races.
[b]R, uniformly resistant. Resistant category includes typical resistant responses (necrosis) and some intermediate responses (necrosis and limited watersoaking).
S, uniformly susceptible.
R/S, variable response.
R*, predominantly resistant response.
S*, predominantly susceptible response.
NT, not tested.

When the accession Frisson, carrying R3 in combination with other R-genes (R2+R3), was challenged with races 1 (A3), 2 (A2), 3 (A3), 5 (A2) or 7 (A2 + A3), resistance was complete in stems and leaves (pods were not tested) for the matching combinations R2/A2 and R2 + R3/A2 + A3 (Table 4).

When accessions Partridge, ZP-0101, ZP-0112 and JI1577, carrying R3 + R4, were challenged with races 1 (A3 + A4), 3 (A3), 4 (A4), 5 (A4) or 7 (A3 + A4), resistance expression was generally complete in stems, leaves and pods for the matching combination R3 + R4/A3 + A4 (Table 4). This differs from the response when R3/A3 and R4/A4 were acting separately and suggests an additive resistance effect when both R3 + R4 are operating.

When accessions Fortune, ZP-0104, ZP-0110 and JI171, carrying R2 + R3 + R4, were challenged with races 1 (A3 + A4), 2 (A2), 3 (A3), 4 (A4), 5 (A2 + A4) or 7 (A2+A3+A4), resistance expression in stems was generally complete for all the R/A gene combinations. However, resistance in leaves and pods was more complete when there was more than one R gene operating (Table 4).

When Vinco, carrying R1 + R2 + R3 + R5, was challenged with races 1 (A1 + A3), 2 (A2), 3 (A3), 5 (A2 + A5) or 7 (A2 + A3), resistance expression was complete in stems, leaves and pods except in pods to race 3 and 5 (Table 4).

When Early Onward, which does not carry R3 and carries R2, was challenged with races 2 (A2), 5 (A2) or 7 (A2), resistance was complete in stems, leaves and pods except in leaves and pods to race 7 (Table 5).

Table 5. Inoculation responses to races of *Pseudomonas syringae* pv. *pisi* in different plant parts (stem, leaf and pod) of pea accessions carrying race-specific genes other than R3

Pea accession (Resistance gene)	Race	R/A[a]	Inoculation response[b]		
			Stem	Leaf	Pod
Early Onward (R2)	2	2	R	R	R
	5	2	R	R	R
	7	2	R	R*	R/S
Hurst's Greenshaft (R4 + R6)	1	4 + 6	R	R	R
	4	4	R	R/S	R/S
	5	4 + 6	R	R/S	R/S
	7	4	R	R/S	R/S
Sleaford Triumph (R2 + R4 + R5)	1	4	R	R*	R/S
	2	2	R	R	R
	4	4	R	R/S	S
	5	2 + 4 + 5	R	R	R
	7	2 + 4	R	R	R/S

[a]R/A, matching resistance/avirulence genes in pea accessions and *P. s.* pv. *pisi* races.
[b]R, uniformly resistant.
S, uniformly susceptible.
R/S, variable response.
R*, predominantly resistant response.

When Hurst's Greenshaft (R4 + R6) was challenged with races 1 (A4 + A6), 4 (A4), 5 (A4 + A6) or 7 (A4), resistance expression was complete in stems, leaves and pods to race 1 and partial in leaves and pods to races 4, 5 and 7 (Table 5).

When Sleaford Triumph (R2 + R4 + R5) was challenged with races 1 (A4), 2 (A2), 4 (A4), 5 (A2 + A4 + A5) or 7 (A2 + A4), resistance was complete in stems and not complete in leaves and pods for the combination R4/A4. When R4 was present in combination with R5 and/or R2 resistance was enhanced (Table 5).

Race-nonspecific resistance. When *P. abyssinicum* accessions (JI2202 and JI1640) carrying race-nonspecific resistance were challenged with races 2, 5 or 7, resistance expression was generally complete in stems, leaves and pods with the exception of JI1640, which showed partial resistance in the pod with race 2. Resistance to races 1, 3, 4 and 6 was complete in the stems but only partial in leaves and pods to races 1 and 6 and leaves and pods were generally susceptible to races 3 and 4 (Table 6).

Table 6. Inoculation responses to races of *Pseudomonas syringae* pv. *pisi* in different plant parts (stem, leaf and pod) of pea accessions carrying race-nonspecific resistance

Pea accession (Resistance gene)	Race	Inoculation response[a]		
		Stem	Leaf	Pod
JI2202	1	R	R/S	R/S
	2	R	R	R
	3	R	S*	S
	4	R	S*	S
	5	R	R	R
	6	R	R/S	R/S
	7	R	R	R
JI1640	1	R	R/S	R/S
	2	R	R	R/S
	3	R	R/S	R/S
	4	R	S*	S
	5	R	R	R
	6	R	S*	R/S
	7	R	R	R

[a]R, uniformly resistant. Resistant category includes typical resistant responses (necrosis) and some intermediate responses (necrosis and limited watersoaking).
S, uniformly susceptible.
R/S, variable response.
S*, predominantly susceptible response.

Table 7. Disease development, frost damage and survival under winter conditions of the pea cultivar Kelvedon Wonder and the two *Pisum abyssinicum* accessions (JI2202 and JI1640) 1, 3, 5 and 8 weeks after inoculation with *Pseudomonas syringae* pv. *pisi* races 2, 4 and 6

Treatment	1 week disease symptoms	3 weeks[a]		5 weeks[b]		8 weeks[c]		
		Disease symptoms	Frost damage[d]	Disease symptoms	Frost damage[d]	Disease symptoms	Frost damage[d]	Plant survival
K. Wonder								
Uninoculated	−	−	None	−	Slight	−	Severe	Alive
Race 2	−	++		++		+++		Dead
Race 4	(+)	+++		+++		+++		Dead
Race 6	−	++		++		+++		Dead
P. abyssinicum								
Uninoculated	−	−	None	−	None	−	Slight	Alive
Race 2	−	(+)		(+)		+		Alive
Race 4	−	+		+		++		Alive
Race 6	−	(+)		(+)		+		Alive

[a]Including 5 frost days; [b]10 frost days; [c]12 frost days.
[d]Frost damage only recorded on uninoculated plants (due to the difficulty of distinguishing frost damage in the presence of disease symptoms).
−, no symptoms.
(+), few water-soaked lesions on lower leaves, mainly healthy.
+, discrete water-soaked lesions on stipules.
++, fan shaped water-soaked lesions on stipules and leaves, moderately severe.
+++, extensive fan shaped water-soaked lesions on stipules and leaves, severe (more than 50% leaf damage).

Aggressiveness of isolates. Based only on a visual assessment, the lesion size surrounding the inoculation points was similar for isolates of the same race, however, isolates of race 4 caused larger lesions than other races, both in stems and leaves.

Field studies

Symptoms were first observed in Kelvedon Wonder with race 4 only one week after inoculation (Table 7). Three weeks after inoculation, disease development was moderately severe in Kelvedon Wonder inoculated with races 2 and 6 and very severe with race 4. By comparison *P. abyssinicum* accessions showed only a few small lesions with races 2 and 6 and slightly larger lesions with race 4 (Figure 1). Eight weeks after inoculation, all the inoculated Kelvedon Wonder plants were dead whereas *P. abyssinicum* plants were alive and in good condition, although plants inoculated with race 4 showed some stem snapping. Uninoculated controls of both Kelvedon Wonder and *P. abyssinicum* showed no evidence of disease symptoms. Frost damage was more severe in Kelvedon Wonder than in *P. abyssinicum*. At least some of the *P. abyssinicum* plants survived through the winter to flower and produced seed.

Discussion

The experiments reported here confirmed the earlier observation of a differential response between stems and leaves/pods in the expression of resistance to *P. s.* pv. *pisi* races (J.R. Bevan, pers. comm.). This does not appear to be a commonly reported phenomenon although a differential response in different plant parts has also been reported in bean (*P. vulgaris*) with the bacterial pathogen *P. s.* pv. *phaseolicola* (Hill et al., 1972). In the present study, with both race-specific and race-nonspecific resistance, a resistant response in the stem was not always associated with resistance in leaf and pod. Race-specific genes conferred resistance in the stem in a consistent manner but variability in leaf and pod responses depended on the specific matching combination of R-genes and A-genes. R2 conferred resistance in all plant parts (stem, leaf and pod) in the majority of the accessions tested, however, two of the Spanish landraces (ZP-0104 and ZP-0110) showed partial resistance in leaves and pods to race 2 and cv. Early Onward showed partial resistance to race 7, indicating that expression of R2 was not always complete. R3 or R4 singly did not confer resistance in leaves or pods, however, R3 in combination with R4 had a positive additive effect.

Figure 1. Inoculation responses under autumn/winter conditions of: Kelvedon Wonder inoculated with race 4 of *Pseudomonas syringae* pv. *pisi* (top left), *P. abyssinicum* inoculated with race 4 (top right), Kelvedon Wonder inoculated with race 6 (bottom left) and *P. abyssinicum* inoculated with race 6 (bottom right).

Resistance was complete when combinations of two or more R-genes were challenged by races carrying two or more matching A-genes (e.g. R2 + R3/A2 + A3, R3 + R4/A3 + A4, etc).

Hill et al. (1972) reported three single dominant genes controlling separately leaf and pod resistance and expression of systemic chlorosis. F2 populations derived from crosses involving resistant and susceptible cultivars showed the same range of disease responses as exhibited by the parents. In the present study, no pea accession which was resistant in the leaf or pod was susceptible in the stem. Stem resistance was sometimes associated with leaf/pod susceptibility but leaf/pod resistance was never found to be associated with stem susceptibility. In limited studies of the inheritance of stem and leaf resistance (Elvira-Recuenco and Taylor, unpublished), there was no evidence of separate genetic control of resistance in the different tissues. What is more likely is that some component necessary for the expression of resistance may be lacking or not expressed in leaf and pod tissues. A similar observation on the failure of resistance expression in a particular plant tissue is given in a very recent study by Hermanns et al. (2002). These authors have shown that in the gene-for-gene relationship between *Arabidopsis* and *Peronospora parasitica*, R genes and components of the signalling pathway necessary for resistance are expressed in both leaves and roots, but they are only functional in the leaves. The implication that some other factor is necessary for resistance induction does not exclude the possibility of genetic control. The differential expression to *P. s.* pv. *pisi* in stems and leaves could provide a model system for the study of resistance induction.

Stem resistance to races 2, 3 and 4 is controlled by single dominant genes: R2, R3 and R4, respectively (Taylor et al., 1989; Bevan et al., 1995). However, deviations from the expected single gene segregation have been seen in some F2 families of the crosses Partridge × Early Onward for stem resistance to races 3 and 4, and Vinco × Hurst's Greenshaft for stem resistance to races 2 and 4 (Taylor et al., 1989; Hunter, 1996). An incomplete dominance of the resistant alleles or the existence of modifiers could play a role in these responses.

In stem inoculations with race 4 under glasshouse conditions, the size of the lesions was bigger than with other races and some slight susceptibility was observed in the stem even in the presence of R4. The aggressiveness of race 4 in stems and leaves was also confirmed in field experiments. This suggests that the interaction of host cultivar and pathogen race may be influenced by factors other than the R/A gene combination.

Race-nonspecific resistance did not confer resistance in leaves and pods to all races. *P. abyssinicum* accessions were uniformly resistant in the stems to all races, in leaves and pods to races 2, 5 and 7 and partially resistant in leaves and pods to races 1, 3, 4 and 6. The pattern of resistance in stems and leaves/pods to race 2 was the same as that shown by the race-specific combination R2/A2. This suggests that *P. abysissinicum* may also carry a 'R2-like' specificity.

Expression of race-nonspecific resistance in *P. abyssinicum* under field conditions was stronger than under glasshouse conditions. In particular, resistance to race 6 in leaves was better than expected, although race 4 caused moderately severe damage. The combination of race-nonspecific resistance with frost tolerance in *P. abyssinicum* was probably responsible for its survival under winter conditions. By comparison, the extreme disease susceptibility shown by the *P. sativum* cultivar Kelvedon Wonder was enhanced by its susceptibility to frost damage. On present evidence, there is a good case for the use of race-nonspecific resistance in the breeding of pea cultivars, specifically for winter conditions, where susceptibility to this disease is a major limiting factor. For spring sown cultivars, where the disease is less damaging, race-nonspecific resistance would also be useful in managing race 6. There are no known race-specific R-genes to race 6 and this race has been increasing in frequency in recent years (Schmit, 1991; Reeves et al., 1996). The demonstration of additive effects by various race-specific R-genes also suggests the possibility of combining race-nonspecific resistance with one or more race-specific genes. In order of importance these would be: R4, R2 + R4, R3 + R4, R2 + R3 + R4. The transfer of race-nonspecific resistance from *P. abyssinicum* to a *P. sativum* background with the possibility for the selection of R2 + R3 + R4 has been described (Elvira-Recuenco and Taylor, 1998).

References

Bevan JR, Taylor JD, Crute IR, Hunter PJ and Vivian A (1995) Genetics of specific resistance in pea (*Pisum sativum*) cultivars to seven races of *Pseudomonas syringae* pv. *pisi*. Plant Pathology 44: 98–108

Boelema BH (1972) Bacterial blight (*Pseudomonas pisi* Sackett) of peas in South Africa with special reference to frost as predisposing factor. Mededelingen Landbouwhogeschool, Wageningen, the Netherlands 72: 1–87

Elvira-Recuenco M and Taylor JD (1998) Inheritance of potential race-nonspecific resistance derived from *Pisum abyssinicum* for the control of pea bacterial blight (*Pseudomonas syringae* pv. *pisi)*. Proceedings of the 3rd European Conference on Grain Legumes, Valladolid, Spain, p 234

Hermanns M, Slusarenko AJ and Schlaich NL (2002) Organ-specificity in plant disease is determined independently of R-gene signalling. Abstract. 6th Conference of the European Foundation for Plant Pathology (EFFP), Prague, 8–14 September 2002

Hill K, Coyne DP and Schuster ML (1972) Leaf, pod and systemic chlorosis reactions in *Phaseolus vulgaris* to halo blight controlled by different genes. Journal of the American Society of Horticultural Science 97: 494–498

Hunter PJ (1996) Study of isoenzyme and RAPD markers for genetic mapping in *Pisum* and *Phaseolus*. Ph.D. Thesis, School of Biological Sciences, University of Birmingham

King EO, Ward MK and Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescin. Journal of Laboratory Clinical Medicine 44: 301–307

Malik AN, Vivian A and Taylor JD (1987) Isolation and partial characterization of three classes of mutant in *Pseudomonas syringae* pv. *pisi* with altered behaviour towards their host *Pisum sativum*. Journal of General Microbiology 133: 2393–2399

Mansfield PJ, Wilson DW, Heath MC and Saunders PJ (1997) Development of pea bacterial blight caused by *Pseudomonas syringae* pv. *pisi* in winter and spring cultivars of combining peas (*Pisum sativum*) with different sowing dates. Annals of Applied Biology 131: 245–258

Reeves JC, Hutchins JD and Simpkins SA (1996) The incidence of races of *Pseudomonas syringae* pv. *pisi* in UK pea (*Pisum sativum*) seed stocks, 1987–1994. Plant Varieties and Seeds 9: 1–8

Roberts SJ (1992) Effect of soil moisture on the transmission of pea bacterial blight (*Pseudomonas syringae* pv. *pisi*) from seed to seedling. Plant Pathology 41: 136–140

Roberts SJ, Phelps K, Mckeown BM, Heath, MC and Cockerell V (1995) Effect of pea bacterial blight (*Pseudomonas syringae* pv. *pisi*) on the yield of spring sown combining peas (*Pisum sativum*). Annals of Applied Biology 126: 61–73

Schmit J (1991) Races of *Pseudomonas syringae* pv. *pisi*. Occurrence in France and host specificity towards spring and winter cultivars of protein peas. Proceedings of the 4th International Working Group on *Pseudomonas syringae* Pathovars, pp 256–262

Schmit J, Taylor JD and Roberts SJ (1993) Sources of resistance to pea bacterial blight (*Pseudomonas syringae* pv. *pisi*) in pea germplasm. Abstracts of the 6th International Congress of Plant Pathology, Montreal, p 180

Skoric V (1927) Bacterial blight of pea: Overwintering, dissemination, and pathological histology. Phytopathology 17: 611–627

Stead DE and Pemberton AW (1987) Recent problems with *Pseudomonas syringae* pv. *pisi* in the UK. EPPO Bulletin 17: 291–294

Taylor JD, Bevan JR, Crute IR and Reader SL (1989) Genetic relationship between races of *Pseudomonas syringae* pv. *pisi* and cultivars of *Pisum sativum*. Plant Pathology 38: 364–375

Vavilov NI (1992) Origin and Geography of Cultivated Plants. Cambridge University Press, Cambridge (Translated from the Russian by A. Filatenko)

Young JM and Dye DW (1970) Bacterial blight of peas caused by *Pseudomonas pisi* Sackett, 1916 in New Zealand. New Zealand Journal of Agricultural Research 13: 315–324