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Lack of host specificity of strains of *Erwinia rhapontici*, causal agent of pink seed of pulse and cereal crops

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Abstract. *Erwinia rhapontici* is the causal agent of pink seed and soft rot diseases of several crops. Laboratory and field experiments were conducted to study the host specificity of strains of *E. rhapontici* collected from diseased seeds of pea, bean, lentil, chickpea, wheat, and canola or from infested field soil in western Canada. For the growth chamber experiments, plants of pea, bean, lentil and chickpea were inoculated with each strain of *E. rhapontici* by injection of bacterial suspension (1×10^9 cfu/mL) into young pods at 0.1 mL/pod, whereas developing heads of wheat were injured by abrading with a wire brush and inoculated by spraying of bacterial suspension at 20 mL/plant. Results showed that the *E. rhapontici* strains were not host specific, since all of the strains could infect each of the host crops tested, regardless of the origin of strains. The frequency of infected seeds was high (>50%) for most strain by crop combinations. Field experiments conducted in 2003 and 2004 revealed that the inoculum of *E. rhapontici* on infected pea seeds was readily transmitted to neighboring crops of durum wheat, spring wheat, and common bean, if the crops were injured by abrading with a wire brush at the early pod formation stage. The impact of the lack of host specificity on management of the pink seed disease caused by *E. rhapontici* is discussed.

Keywords: *Erwinia rhapontici*; Host specificity; Pink seed disease; Strain differentiation.

INTRODUCTION

Erwinia rhapontici (Millard) Burkholder is a bacterial pathogen that causes a variety of plant diseases, including pink seed of cereal and pulse crops, as well as soft rots of horticultural crops. Examples of diseases of horticultural crops caused by *E. rhapontici* include soft rot of wasabi (*Eutrema wasabi* Maxim.) (Goto and Matsumoto, 1986), crown rot of rhubarb (*Rheum rhaponticum* L.) (Millard, 1924; Metcalfe, 1940; Letal, 1976), soft rot of onion (*Allium cepa* L.) (Ohuchi et al., 1983) and others (Huang et al., 2003b). Pink seed disease is found in crops such as pea (*Pisum sativum* L.) (Huang et al., 1990; Schroeder et al., 2002), common bean (*Phaseolus vulgaris* L.) (Huang et al., 2002), lentil (*Lens culinaris* Medik.) (Huang et al., 2003a), chickpea (*Cicer arietinum* L.) (Huang et al., 2003a), common wheat (*Triticum aestivum* L.) (Howe and Simmonds, 1937; Campbell, 1958; Roberts, 1974; Forster and Bradbury, 1990) and durum wheat (*Triticum durum* Desf.) (McMullen et al., 1984). The pink discoloration of seed observed in instances of this disease is attributable to production of pigments called ferrorosamines by the pathogen (Feistner et al., 1983). *Erwinia rhapontici* is an opportunistic pathogen that depends on plant injury for initiation of infection (Huang et al., 2003b; Huang and Erickson, 2004).

Pink seed disease has potential negative impacts on the production and marketability of crops. For example, Huang and Erickson (2004) reported that planting pink seeds of pea infected by *E. rhapontici* resulted in reductions in seed yield, seed size, seedling emergence, and seedling vigor. McMullen et al. (1984) reported that when durum wheat kernels infected with *E. rhapontici* were milled, the resulting semolina had a pink discoloration, and was therefore unsuitable for pasta production.

A 2-year field study on the overwintering of *E. rhapontici* under Canadian prairie conditions showed that the pathogen survived winters on infected seeds and stems of pea regardless of burial depth at 0 or 6 cm (Huang and Erickson, 2003), and therefore such infected seeds or stems can serve as a source of inoculum for infection of crops in the subsequent growing season. However, no information exists to indicate whether strains of *E. rhapontici* from one host crop can infect a different host crop. The increased use of pulse-wheat rotations in North America in recent years raises concerns regarding the possibility of transmission of the bacterial pathogen *E. rhapontici* from pulse crops to wheat, a major cereal crop in Canada and the USA. The purpose of this study was to determine whether or not strains of *E. rhapontici* from pea, bean, lentil, chickpea, wheat, canola or soil are host-specific under controlled conditions in a growth chamber, and under field conditions.

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MATERIALS AND METHODS

Twelve strains of the pink seed pathogen, *E. rhapontici* collected in western Canada were assessed for pathogenicity on common bean, pea, lentil, chickpea, spring wheat, and durum wheat, under environmentally controlled conditions. The sources and locations of collection of these strains are listed in Table 1. Seeds of bean cv. US1140, pea cv. SS2, chickpea cvs. Myles and Sanford, lentil cv. Laird, spring wheat cv. Fielder, and durum wheat cv. Kyle, were planted in Cornell peat-lite mix (Boodley and Sheldrake, 1977) in 15 cm-diameter plastic pots, and were kept in a growth chamber at 20°C/18°C; 16-h day/8-h night, until the plants reached the early pod-filling stage for bean, pea, lentil and chickpea, or the booting stage for wheat. For the inoculations on bean, pea, lentil, and chickpea, each strain was inoculated into 30 pods from 6 plants using the method of Huang et al. (1990). Each pod was inoculated with 0.1 mL of bacterial suspension (10^9 cfu mL^{-1}), by injection through the mid-rib at the basal end. The same number of uninoculated and water-inoculated pods served as controls. Plants were kept in the growth chamber until maturity, and seeds were harvested and plated onto potato dextrose agar (PDA) (Difco, Detroit, Michigan, USA) at room temperature (20 ± 2°C) for 3 days to determine the presence or absence of *E. rhapontici*, using the method of Huang et al. (1990) [i.e., observation of culture characteristics such as pigment production]. Treatments were arranged in a completely randomized design. For spring wheat and durum wheat, similar experiments were conducted, except that the plants were inoculated by spraying 20 mL/plant of bacterial suspension (10^9 cfu mL^{-1}), onto developing heads that had been injured by lightly stroking with a sterilized wire brush. For each crop and strain, the frequency of seeds infected by *E. rhapontici* was calculated. The experiments were performed twice for each crop.

Field experiments were conducted during 2003 and 2004 at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada, to determine whether *E. rhapontici* on infected pea seeds could be transmitted to adjacent durum wheat, spring wheat, and common bean. Pea seeds cv. Delta were obtained from a commercial field near Vulcan, Alberta, Canada that had high incidence of pink seed following a hailstorm. Seeds were sorted into the categories of pink and non-pink, and three sub-samples of 100 seeds from each category were surface sterilized in 70% ethanol for 90 s, air-dried on paper towel, incubated on PDA in Petri dishes at 20°C for 3 days, and examined for presence of *E. rhapontici* by the method described by Huang et al. (1990). The frequency of *E. rhapontici* in samples of non-pink seeds from this field was less than 1%, whereas the frequency in pink seeds was 100%. Seed samples were stored in a cold room at 4°C until used for the field experiments.

Field plots were established in late May of each year, in an area of an irrigated field that was fallowed during the previous growing season. For experiment 1, each plot consisted of 4 rows of healthy beans (cv. US1140) on the south side of the plot, 4 rows of peas (cv. Delta, healthy or infected with *E. rhapontici*) in the middle of the plot, and 4 rows of healthy wheat (spring cv. Fielder) on the north side of the plot. For experiment 2, the plots were the same, except that the beans were cv. AC Skipper, and the wheat was durum cv. Kyle. Both experiments were conducted in both years. For all crops in each experiment, row length was 5 m and row spacing was 22 cm. Treatments were arranged in a randomized block design with 6 replicates. Plots were maintained until the pea plants reached the young pod stage (mid-July), and the wheat and bean plants in each plot were injured by gently abrading with a sterilized wire brush. Plots with non-injured wheat and bean plants were used as controls. At maturity (early

Table 1. Source and location of *Erwinia rhapontici* strains used for the study.

Strain of *E. rhapontici*	Source (host)	Host cultivar	Location
LRC 8251	Bean	Othello	Bow Island, Alberta
LRC 8252	Bean	US1140	Bow Island, Alberta
LRC 8253	Bean	Viva	Bow Island, Alberta
LRC 8289	Bean	Othello	Carman, Manitoba
LRC 8345	Canola	Hyola 401	Bow Island, Alberta
LRC 8266	Chickpea	Myles	Beechy, Saskatchewan
LRC 8265	Lentil	Laird	Bladworth, Saskatchewan
LRC 733	Pea	Marrowfat	Grassy Lake, Alberta
LRC 965	Pea	Radley	Saskatoon, Saskatchewan
LRC 7954	Pea	Trapper	Lethbridge, Alberta
LRC 1076	Soil	-	Lethbridge, Alberta
LRC 8314	Wheat	Unknown	Ponteix, Saskatchewan

September) the plots were harvested with a Nurserymaster Elite 2000 plot combine (Wintersteiger, Ried im Innkreis, Austria). The frequency of infection of bean, pea, and wheat seeds by *E. rhapontici* was determined for each plot by sorting seed samples into pink and non-pink seeds, and confirming the accuracy of visual sorting by plating a subsample of 100 seeds from each plot as described previously.

In each experiment each year, differences between treatments in frequency of infection of seed by *E. rhapontici* in each crop were statistically analysed using analysis of variance (ANOVA), and means were separated using Duncan's multiple range tests. SAS/STAT™ computer software, version 8.2, was used for the statistical analyses (SAS Insitute, 1999).

RESULTS

In the growth chamber experiments, testing of the strains of *E. rhapontici* from bean, canola, chickpea, lentil, pea, wheat, and soil showed that none of the strains were host specific, since they could infect all of the tested crops of bean, chickpea, lentil, and wheat, regardless of the origin of the strains (Table 2). The frequency of infection of seeds by *E. rhapontici* was high (>50%) for most of the strains on most of the crops. Although none of the strains was consistently more virulent than the others, some variation in the susceptibility of crops was observed. For example, the frequency of infected seeds for chickpea ranged from 94-100% for cv. Myles, and from 92-100% for cv. Sanford, compared to a frequency of 28-67% for wheat cv. Kyle (Table 2). Lentil was also very susceptible to *E. rhapontici*, with the frequency of infected seeds ranging from 88-100%.

Results of the field experiments revealed that *E. rhapontici* in naturally infected pea seeds can spread onto adjacent spring wheat, durum wheat, and common bean (Table 3). For all crops and years except bean in 2003, the rate of transmission of *E. rhapontici* was significantly ($p<0.05$) higher for the treatments of plant injury, compared to the uninjured controls. For example, the frequency of durum wheat cv. Kyle seeds infected by *E. rhapontici* in 2003 was 16% for injured plants grown adjacent to pink peas (diseased seeds used as a source of inoculum), 12% for injured plants grown adjacent to healthy peas, 4% for non-injured plants grown adjacent to pink peas, and 1% for non-injured plants grown adjacent to healthy peas (Table 2). Although transmission of *E. rhapontici* from infected pea plants to common bean seeds was not observed in the 2003 field experiment, it was observed in the 2004 field experiment.

DISCUSSION

This study demonstrates for the first time that the strains of *E. rhapontici* from pea, bean, chickpea, lentil, canola,

Table 2. Infection of cereal and pulse crops by strains of *Erwinia rhapontici* (growth chamber experiments).

Strain of *E. rhapontici* (source)	Frequency of seed infection (%)						
	Bean cv. US1140[1]	Chickpea cv. Myles[1]	Chickpea cv. Sanford[1]	Lentil cv. Laird[1]	Pea cv. SS2[1]	Wheat cv. Fielder[2]	Wheat cv. Kyle[2]
LRC 8251 (bean)	60	100	100	93	62	59	51
LRC 8252 (bean)	69	96	100	96	54	51	46
LRC 8253 (bean)	63	100	92	88	43	53	42
LRC 8289 (bean)	62	100	100	100	65	58	28
LRC 8345 (canola)	73	97	97	89	54	68	47
LRC 8266 (chickpea)	61	100	100	94	70	69	67
LRC 8265 (lentil)	66	97	100	94	45	76	50
LRC 733 (pea)	58	100	97	100	61	52	44
LRC 965 (pea)	70	94	100	93	64	60	47
LRC 7954 (pea)	61	100	100	100	54	69	43
LRC 1076 (soil)	58	100	94	91	43	68	38
LRC 8314 (wheat)	56	100	96	95	69	66	63

[1]Isolates were inoculated into 30 pods by injection of 0.1 mL pod^{-1} of bacterial suspension, 10^9 cfu mL^{-1}.
[2]Isolates were inoculated onto 30 heads by spray of bacterial suspension, 10^9 cfu mL^{-1}, to the runoff point.

Table 3. Spread of *Erwinia rhapontici* from infected pea to bean and wheat (field experiments, 2003-2004).

Experiment 1	Frequency of seed infection (%)					
Treatment	Beans cv. US1140		Peas cv. Delta		Wheat cv. Fielder	
	2003	2004	2003	2004	2003	2004
Injured[1], pink[2]	0 a[3]	8 a	17 a	15 a	14 a	9 a
Non-injured, pink	0 a	0 b	4 b	3 b	3 b	1 b
Injured, non-pink	0 a	5 a	15 a	18 a	9 a	10 a
Non-injured, non-pink	0 a	0 b	3 b	2 b	2 b	2 b
Standard error	0.0	1.1	1.3	1.8	1.4	1.5
Experiment 2	Frequency of seed infection (%)					
Treatment	Beans cv. AC Skipper		Peas cv. Delta		Wheat cv. Kyle	
	2003	2004	2003	2004	2003	2004
Injured[1], pink[2]	0 a[3]	4 a	14 a	18 a	16 a	14 a
Non-injured, pink	0 a	1 b	1 b	2 b	4 b	2 b
Injured, non-pink	0 a	6 a	14 a	12 a	12 a	11 a
Non-injured, non-pink	0 a	0 b	2 b	1 b	1 b	3 b
Standard error	0.0	0.8	1.6	2.1	1.0	1.3

[1] Plants were injured by gently abrading with a wire brush at the young pod stage.
[2] Naturally infected (pink) pea seeds were obtained from a commercial field and used for the study.
[3] Means within each column followed by the same letter are not significantly different (Duncan's multiple range test; $p>0.05$).

wheat and soil, are not host-specific, and are capable of infecting a wide range of crop plants, including great northern bean cv. US1140, desi chickpea cv. Myles, kabuli chickpea cv. Sanford, lentil cv. Laird, pea cv. SS2, spring wheat cv. Fielder, and durum wheat cv. Kyle, regardless of the host origin of the pathogen. The lack of host specificity among strains of *E. rhapontici* is further proven in the field experiments, as the pathogen on naturally infected pea seeds can readily spread onto adjacent plants of great northern bean, spring wheat and durum wheat in the field.

The indoor and field studies further confirm previous reports that plant injury at the seed formation stage is critical for *E. rhapontici* to gain entrance into host plants and cause formation of pink seeds (Huang et al., 2003b; Huang and Erickson, 2004). This strongly suggests that *E. rhapontici* is an opportunistic pathogen, and that its dissemination may be limited by the occurrence of circumstances that cause plant injury. Wounding of plant tissue due to wind or hail damage, or insect damage, likely provides opportunities for *E. rhapontici* to infect and spread in the field. Other factors such as temperature or humidity within the crop canopy could also be important in dissemination of *E. rhapontici* in the field. The observation that infection can occur on injured plants that are adjacent to infected plants, suggests that movement of the pathogen occurs within a certain area. The actual mechanism of dispersal remains to be determined, but may be related to movement of insects or splashing of droplets during irrigation.

A previous study indicates that *E. rhapontici* can survive Canadian winters in infected seeds and crop debris (Huang and Erickson, 2003). The lack of host specificity observed in this study suggests that the inoculum of *E. rhapontici* from infected pea seeds or stems can serve as a potential source of inoculum for other crops including wheat. Since pulse crops such as peas are important crops for rotation with wheat on the North American prairies (Biederbeck et al., 1999), the transmission of *E. rhapontici* from infected pea seeds to wheat crop observed in this study raises new concerns about the appropriateness of using peas in a rotation sequence with wheat in areas where the pink seed pathogen is prevalent.

Huang and Erickson (2004) reported that planting pea seeds infected by *E. rhapontici* can have serious impact, including losses in stand establishment, seedling vigor, seed yield, and seed quality. In addition, a study on durum wheat infected with *E. rhapontici* showed that the milled wheat kernels resulted in pink semolina that was unsuitable for producing pasta (McMullen et al., 1984). Since the cereal and pulse crops used in this study are

used worldwide as sources of food and food products for people and animals, and since the effect of consumption of seeds infected by *E. rhapontici* on the health of humans and livestock is still largely unknown, further research is needed on the quality and safety of crops infected by pink seed disease.

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豆類及禾本科作物粉紅種子病病原菌 *Erwinia rhapontici* 不具寄主專一性

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Erwinia rhapontici 係造成作物粉紅種子與軟腐病的病原菌，本病原菌菌株分離自碗豆、菜豆、扁豆、雞豆、小麥、油菜或加拿大西部罹病田田土，並於室內與田間測試其寄主專一性。在室內生長箱試驗時，碗豆、菜豆、扁豆及雞豆的幼嫩豆莢以每莢注射接種 0.1 毫升病原細菌胞子懸浮液 (1×10^9 cfu/ml) 之量接種所有測試菌株，而小麥的孕穗期幼穗則先以鐵刷擦傷，再以噴霧接種方式每株接種 20 毫升細菌胞子懸浮液。結果所有的菌株均可危害所有測試作物，無關菌株來源，且大部分菌株危害種子比率超過 50%，顯示本病原菌不具寄主專一性。於 2003 至 2004 年的田間試驗結果亦顯示，當健康的硬粒小麥、春小麥及菜豆在幼穗或果莢形成期以鐵刷刮傷後，緊臨於以罹病種子栽植的罹病碗豆時，罹病株上之接種源可傳播並危害周遭的健康植株。對於由不具寄主專一性的 *E. rhapontici* 所引起的粉紅種子病在病害管理上所造成的衝擊，將於文中一併討論。

關鍵詞：粉紅種子病；*Erwinia rhapontici*；寄主專一性；菌株特異性。