Plant Pathology Bulletin 13: 261-266, 2004

Impact of Pink Seed of Pea Caused by *Erwinia rhapontici* in Canada

Hung-Chang Huang[1,2] and R. Scott Erickson[1]

[1] Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, Lethbridge, Alberta, T1J 4B1 Canada

[2] Corresponding author, E-mail: huangh@agr.gc.ca; Fax: 0021-403-382-3156

Accepted for publication: September 8, 2004.

ABSTRACT

Huang, H. C. and Erickson, R. S. 2004. Impact of pink seed of pea caused by *Erwinia rhapontici* in Canada. Plant Pathol. Bull. 13: 261-266.

A field study was conducted during 2000-2002 at Lethbridge, Alberta, Canada to determine the impact of pink seed of pea caused by the bacterial pathogen, *Erwinia rhapontici*, on seedling emergence, seedling vigor, seed quality, and seed yield. Seeds of peas, cv. Delta, from commercial seed lots were used for each year of the study. Use of *E. rhapontici*-infected seeds for planting resulted in an average of 33% reduction ($P<0.05$) in seedling emergence and a 44% reduction ($P<0.05$) in seedling vigor (height), compared to the use of healthy seeds (control). Injury of pea plants by gentle abrasion with a stiff wire brush at the young pod stage, followed immediately by sprinkle irrigation, led to significantly ($P<0.05$) higher frequencies of infection by *E. rhapontici* in harvested seed, lower 1000-kernel weights, and lower seed yields, compared to uninjured controls. The 3-year field study concludes that use of *E. rhapontici*-infected seeds for planting reduced seedling emergence, seedling vigor and seed yields of pea. The possibilities for managing this seedborne disease of pea are discussed.

Key words : Pea, *Pisum sativum*, pink seed, *Erwinia rhapontici*, seed germination, seedling vigor, seed quality, seed yield

INTRODUCTION

Dry pea (*Pisum sativum* L.) is an important field crop in Canada. The majority of Canadian pea production is located in the three Prairie Provinces of Alberta, Saskatchewan, and Manitoba. With the emphasis on crop diversification of western Canadian agriculture in recent years, the total production area of dry pea for these three provinces has increased dramatically from 74,500 ha in 1985, to 297,000 ha in 1988, and to 835,100 ha in 1999 (3,4).

Erwinia rhapontici (Millard) Burkholder is an opportunistic bacterial pathogen that causes pink discoloration of seeds in field crops (10). Host crops reported in Canada and USA include legumes such as pea (11,15), bean (*Phaseolus vulgaris* L.) (9), lentil (*Lens culinaris* Medik.) (8) and chickpea (*Cicer arietinum* L.) (8), as well as cereals such as wheat (*Triticum aestivum* L. and *Triticum durum* Desf.) (2,5,6,12,13). Despite the increasing frequency of reports of pink seed discoloration on field crops in Canada, no information exists regarding the effect of this disease on crop productivity. The purpose of this study was to provide vital new information on pink seed of pea by determining the importance of seedborne inoculum (infected seeds) on seedling emergence, seedling vigor, seed quality, and seed yield.

MATERIALS AND METHODS

Source of seeds and establishment of field experiment

Seeds of dry pea, cv. Delta, were obtained from a commercial field near Vulcan, Alberta, Canada that had an outbreak of pink seed in 1999 following hail damage to the crop. Seeds were sorted into categories of pink and non-pink, and three subsamples of 100 seeds from each category were surface sterilized in 70% ethanol for 90 seconds, air-dried, and plated onto potato dextrose agar (PDA) (Difco; Detroit, Michigan, USA) in Petri dishes. The presence of *Erwinia rhapontici* was determined by production of characteristic pink, soluble pigment on PDA within 48 hours. The frequency of *E. rhapontici* in pink seeds was 100%, whereas the frequency in non-pink seeds was less than 1%. The sorted seed samples, pink and non-pink, were stored in a cold room (4℃) until used for the field experiments.

An irrigated field at the Agriculture and Agri-Food Canada Research Centre near Lethbridge, Alberta, Canada was used for the study in 2000, 2001, and 2002. For each year, the experiment was conducted in an area of the field that was fallowed in the previous season. Pea seeds were sorted into lots containing 0, 10, 50 and 100% pink seeds, and were planted in the field on 25 May 2000, 23 May 2001, and 31 May 2002, using a 4-row plot seeder. Plots consisted of four 5-m rows of 100 seeds per row, with rows spaced 0.23 m apart. There were six treatments for the experiment, including (1) healthy seed, plants not injured (control); (2) healthy seed, plants injured at young pod stage; (3) 10% pink seed, plants not injured; (4) 50% pink seed, plants not injured; (5) 100% pink seed, plants not injured; and (6) 100% pink seed, plants injured at young pod stage. Treatments were arranged in a randomized block design with four replicates in each treatment.

Data collection and plot maintenance

After all the seedlings had emerged and prior to the elongation stage, the number of emerged seedlings was counted for each plot. Unemerged seedlings were recovered from the soil, washed for 30 min in running water, air-dried, surface sterilized in 70% ethanol for 90 sec, and plated on PDA in Petri dishes for two to seven days to examine for presence of *E. rhapontici*. The height of emerged seedlings was determined by measuring ten seedlings in each row at 50-cm intervals.

From the late vegetative growth stage (mid-June) to the late pod-filling stage (mid-August), irrigation was provided as needed to maintain crop growth and development. Weeds were controlled during the growing season by hand weeding. For the treatments involving plant injury, pea plants were gently abraded with a stiff wire brush at the young pod stage and irrigated immediately after, to provide conditions conducive for development of pink seed. Wire brushes were sterilized between plots by washing for 90 sec in 70% ethanol and air-drying before re-use, to prevent cross-contamination.

At maturity (early September), plants were harvested using a Nurserymaster Elite 2000 plot combine (Wintersteiger, Ried im Innkreis, Austria). Seed samples were dried at 20℃ for 4 weeks, cleaned, and weighed to determine seed yield for each plot. The 1000-kernel weight of seeds for each plot was determined by counting the entire sample and dividing by the sample weight. The percentage of infection by *E. rhapontici* was determined for each plot by sorting seed samples into pink and non-pink seeds, confirming the correctness of the visual sorting by plating a 100-seed subsample as previously described, and calculating the resulting percentage of pink seeds.

Statistical analysis

Within each experiment, differences between treatments in percent seedling emergence, seedling height, seed yield of pea, percent infection of seed by *E. rhapontici*, and 1000-kernel weight, were analyzed for statistical significance using analysis of variance (ANOVA). Means were separated using Duncan's multiple range tests. All statistical analyses were conducted using SAS/STAT® computer software [14].

RESULTS

Seedling emergence and height

Seedling emergence was significantly ($P<0.05$) lower for the treatments of 50% pink seed and 100% pink seed compared to the healthy control in all three years (Table 1). For example, in 2002, the seedling emergence for the treatment of 100% pink seed was 36%, compared to 84% in the healthy control. Seedling emergence for the treatment of 10% pink seed was significantly lower than the healthy control in 2001, but not in 2000 or 2002. The frequency of *E. rhapontici* recovered from unemerged seeds was high (41-

Table 1. Effect of seed infection by *Erwinia rhapontici* on seedling emergence of dry pea (field experiments, 2000-2002).

Treatment[1]	Seedling emergence (%)[2]		
	2000	2001	2002
Healthy seed, non-injured (control)	62 a[3]	73 a	84 a
10% pink seed, non-injured	59 a	63 b	81 a
50% pink seed, non-injured	53 b	51 c	54 b
100% pink seed, non-injured	43 c	42 d	36 c
Healthy seed, injured	nd[4]	nd	nd
100% pink seed, injured	nd	nd	nd
Standard error	0.9	0.5	1.1

[1] Pea seeds cv. Delta obtained from a commercial grower in Vulcan, Alberta, Canada in 2000.
[2] Data on seedling emergence was collected at 3 wks after seeding.
[3] Means within each column followed by the same letter are not significantly different (Duncan's multiple range test; $P>0.05$).
[4] Data not determined, as emergence data was collected prior to plant injury.

88%) in the treatments of 50% pink seed and 100% pink seed, but low (0-4%) in the treatments of 10% pink seed and non-pink seed (healthy control). Thus, planting 100% pink seeds resulted in an average of 33% reduction in seedling emergence (Table 1).

Seedling height was significantly (P<0.05) lower for the treatments of 50% pink seed and 100% pink seed compared to the healthy control in all three years (Table 2). Seedling height for the treatment of 10% pink seed was significantly lower than the healthy control in 2001 and 2002, but not in 2000. For example, the average height of seedlings in the healthy control in 2002 was 9.4 cm, compared to 8.1, 6.7, and 4.2 cm for the treatments of 10%, 50% and 100% pink seed, respectively. Therefore, planting 100% pink seeds resulted in an average of 44% reduction in seedling height (Table 2).

Seed quality and yield

The frequency of harvested pea seeds infected by *E. rhapontici* was significantly (P<0.05) higher for the injured treatments, compared to the non-injured treatments, regardless of whether the seed used for planting was healthy, 10% pink, 50% pink or 100% pink (Table 3). For the injured treatments, the frequency of infected seeds ranged from 17 to 42%, compared to a range of 1 to 14% for the uninjured treatments. In addition, the 1000-kernel weight of harvested pea seeds was significantly lower for the injured treatments in 2000 and 2002, compared to the non-injured treatments (Table 3). Seed weights for the injured treatments were 18, 17 and 21% lower than for the uninjured treatments, in the years 2000, 2001, and 2002, respectively. Thus, injury of pea plants in the presence of *E. rhapontici* resulted in an average of 374% increase in frequency of harvested pink seeds, and an average of 19% reduction in seed weight (Table 1).

Pea seed yield was significantly (P<0.05) higher for the non-injured treatments, compared to the injured treatments in 2001 and 2002, but not 2000 (Table 4). For example, in 2002, the pea seed yield for the healthy, non-injured control was 2342 kg/ha, whereas the yields for the treatments of healthy seed (injured) and 100% pink seed (injured) were 1337 and 1185 kg/ha, respectively. Over all three years, injury of pea plants in the presence of *E. rhapontici* resulted in an average yield reduction of 44% (Table 4).

Table 2. Effect of seed infection by *Erwinia rhapontici* on seedling vigor (height) of dry pea (field experiments, 2000-2002).

Treatment[1]	Seedling height (cm)[2]		
	2000	2001	2002
Healthy seed, non-injured (control)	7.9 a[3]	8.3 a	9.4 a
10% pink seed, non-injured	7.5 ab	7.7 b	8.1 b
50% pink seed, non-injured	6.6 bc	5.7 c	6.7 c
100% pink seed, non-injured	5.5 d	4.8 d	4.2 d
Healthy seed, injured	nd[4]	nd	nd
100% pink seed, injured	nd	nd	nd
Standard error	0.2	0.1	0.2

[1] Pea seeds cv. Delta obtained from a commercial grower in Vulcan, Alberta, Canada in 2000.
[2] Data on seedling emergence was collected at 3 wks after seeding.
[3] Means within each column followed by the same letter are not significantly different (Duncan's multiple range test; P>0.05).
[4] Data not determined, as height data was collected prior to plant injury.

Table 3. Effect of seed infection by *Erwinia rhapontici* and plant injury on seed quality of dry pea (field experiments, 2000-2002).

Treatment[1]	Infected seeds (%)			Seed weight (g/1000 kernels)		
	2000	2001	2002	2000	2001	2002
Healthy seed, injured[2]	20 a[3]	23 a	38 a	203 a	203 a	197 a
100% pink seed, injured	17 a	19 a	42 a	192 a	224 b	206 a
Healthy seed, non-injured (control)	2 b	4 b	11 b	239 b	240 bc	265 b
100% pink seed, non-injured	1 b	5 b	14 b	239 b	251 bc	262 b
50% pink seed, non-injured	1 b	7 b	10 b	242 b	263 c	241 b
10% pink seed, non-injured	1 b	3 b	9 b	244 b	269 c	254 b
Standard error	0.9	0.8	1.3	2	5	7

[1] Pea seeds cv. Delta obtained from a commercial grower in Vulcan, Alberta, Canada in 2000.
[2] Injury was caused by gently abrading plants with a wire brush at the early pod stage.
[3] Means within each column followed by the same letter are not significantly different (Duncan's multiple range test; P>0.05).

Table 4. Effect of seed infection by *Erwinia rhapontici* and plant injury on seed yield of dry pea (field experiments, 2000-2002).

Treatment[1]	Seed yield (kg/ha)[2]		
	2000	2001	2002
Healthy seed, injured[2]	1123 a[3]	1153 a	1337 a
100% pink seed, injured	1133 a	1249 a	1185 a
Healthy seed, non-injured (control)	1765 a	2224 b	2342 b
100% pink seed, non-injured	1448 a	2168 b	2245 b
50% pink seed, non-injured	1466 a	2692 b	1987 b
10% pink seed, non-injured	2002 a	2654 b	2214 b
Standard error	62	112	123

[1] Pea seeds cv. Delta obtained from a commercial grower in Vulcan, Alberta, Canada in 2000.
[2] Injury was caused by gently abrading plants with a wire brush at the early pod stage.
[3] Means within each column followed by the same letter are not significantly different (Duncan's multiple range test; $P>0.05$).

DISCUSSION

This study demonstrates for the first time that pink seed of pea caused by *E. rhapontici* has negative impacts on seedling emergence, seedling vigor, seed quality, and seed yield. Although the disease is not as commonly occurring in Canada as some other pea diseases such as mycosphaerella blight (*Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.) [(1)], the fact that it negatively affects stand establishment, seed yield and seed quality of dry pea suggests that appropriate methods are required for the management of this seedborne disease. The evidence from this study suggests that it is important to use healthy seeds for planting and avoid using *E. rhapontici*-infected pea seeds. Since the pathogen can survive the winter in infected crop debris [(7)], and since preliminary research indicates a lack of host specificity among *E. rhapontici* isolates (Huang and Erickson, unpublished), a rotation using non-host crops may be helpful in preventing the disease.

The findings in this study indicate that plant injury and a subsequent period of high moisture are major factors that predispose dry pea to development of pink seed disease. In nature, these injuries could potentially arise from damage by hail, wind, insects, or other means. Since *E. rhapontici* is opportunistic in nature, and depends on plant injury to gain entrance into plant tissues, it would be feasible to protect all of these types of injury to the crop by preemptive colonization of injury sites by non-pathogenic or beneficial microorganisms. This biological control approach for protection of injured plants against *E. rhapontici* warrants further study.

In this study, a high occurrence of pink seeds was observed in injured pea plants originating from healthy seed. This suggests that *E. rhapontici* can move within a crop. The mechanism for this dispersal is not clear at present, but possible means could include splashing of water from rainfall or sprinkle irrigation, or transfer by pest or non-pest insects. More research is needed to investigate these possibilities, and if insects are found to play a role in transmission of pink seed, then control of the appropriate insect vectors may be another means for controlling the disease.

In addition to the agronomic impacts identified in this study, there may be other negative consequences associated with pink seed caused by *E. rhapontici*. It is not presently known whether infection of seeds by *E. rhapontici* results in adverse health effects when such seed is consumed by humans or livestock. Since the host range of *E. rhapontici* includes crops such as wheat and legumes, which are intended for both human consumption and livestock feed, it seems prudent to investigate whether this is the case.

ACKNOWLEDGEMENTS

The financial supports of the Alberta Pulse Growers Commission, Project No. 01-P4, the Saskatchewan Pulse Crop Development Board, Project No. AGR 0108, and the Agriculture and Agri-Food Canada Matching Investment Initiative fund are gratefully acknowledged. This is LRC Contribution No. 387-04026.

LITERATURE CITED

1. Bailey, K. L., Gossen, B. D., Gugel, R. K., and Morrall, R. A. A. (eds.) 2003. Diseases of Field Crops in Canada, 3rd edition. Saskatoon, Saskatchewan: University Extension Press, University of Saskatchewan. 290 p.
2. Campbell, W. P. 1958. A cause of pink seeds in wheat. Plant Dis. Reptr. 42:1272.
3. Canada Grains Council. 1988. Canadian Grains Industry Statistical Handbook 88. Winnipeg: Canada Grains Council.
4. Canada Grains Council. 1999. Canadian Grains Industry Statistical Handbook 99. Winnipeg: Canada Grains Council.
5. Forster, R. L., and Bradbury, J. F. 1990. Pink seed of wheat caused by *Erwinia rhapontici* in Idaho. Plant Dis. 74:81.
6. Howe, E. T., and Simmonds, P. M. 1937. Bacterial pink blotch of wheat. Page 6 *in*: I. L. Conners, J. H. Graigie,

and T. C. Vanterpool (eds.), Proceedings of The Canadian Phytopathological Society. Seventh Session. June 28th to 30th, 1937, Ottawa, Canada.

7. Huang, H. C., and Erickson, R. S. 2003. Overwintering of *Erwinia rhapontici*, causal agent of pink seed of pea, on the Canadian prairies. Plant Pathol. Bull. 12:133-136.
8. Huang, H. C., Erickson, R. S., Yanke, L. J., Hsieh, T. F., and Morrall, R.A.A. 2003. First report of pink seed of lentil and chickpea caused by *Erwinia rhapontici* in Canada. Plant Dis. 87:1398.
9. Huang, H. C., Erickson, R. S., Yanke, L. J., Muendel, H.-H., and Hsieh, T. F. 2002. First report of pink seed of common bean caused by *Erwinia rhapontici*. Plant Dis. 86: 921.
10. Huang, H. C., Hsieh, T. F., and Erickson, R. S. 2003. Biology and epidemiology of *Erwinia rhapontici*, causal agent of pink seed and crown rot of plants. Plant Pathol. Bull. 12:69-76.
11. Huang, H. C, Phillippe, R. C., and Phillippe, L. M. 1990. Pink seed of pea: A new disease caused by *Erwinia rhapontici*. Can. J. Plant Pathol. 12:445-448.
12. McMullen, M. P., Stack, R. W., Miller, J. D., Bromel, M. C., and Youngs, V. L. 1984. *Erwinia rhapontici*, a bacterium causing pink wheat kernels. Proc. North Dakota Academy Sci. Grand Forks, N. D., USA. 38:78.
13. Roberts, P. 1974. *Erwinia rhapontici* (Millard) Burkholder associated with pink grain of wheat. J. Appl. Bact. 37:353-358.
14. SAS Insitute Inc. 1989. SAS/STAT™ User's Guide, Version 6, 4th Ed. SAS Institute Inc., Cary, North Carolina. 1686 pp.
15. Schroeder, B. K., Lupien, S. L., and Dugan, F. M. 2002. First report of pink seed of pea caused by *Erwinia rhapontici* in the United States. Plant Dis. 86:188.

摘 要

黃鴻章[1,2]、Erickson, R. S.[1] 2004. 加拿大豌豆粉紅種子病 (*Erwinia rhapontici*) 對豌豆之影響. 植病會刊 13：261-266. ([1.] 加拿大農業及農業食品部 Lethbridge 研究中心；[2.] 聯絡作者，電子郵件：huangh@agr.gc.ca ；傳真：+1-403-382-3156)

豌豆粉紅種子病 (pink seed) 係由 *Erwinia rhapontici* 所引起。將採自加拿大商業栽培田的豌豆 (Delta 品種) 種子，在田間進行健康種子與罹病種子間的差異性比較。自西元 2000 至 2002 年連續三年的田間試驗結果顯示，罹病種子的出苗率與幼苗高度均分別較健康種子低約 33% 與 44%。本研究亦證明植株受傷是 *E. rhapontici* 侵害豌豆的主要機制。植株於開花初期經過鐵刷處理後，所生產的種子罹粉紅種子病的百分率高、種子千粒重降低，導致產量亦隨之降低。三年田間試驗證實使用遭受 *E. rhapontici* 侵害的種子，會降低碗豆出苗率、植株生長高度及種子產量等。因此，採用健康種子是防治本病的首要關鍵。

關鍵詞：豌豆、粉紅種子病、種子出苗率、種子產量、健康種子