Plant Pathology Bulletin 12:133-136, 2003

Overwintering of *Erwinia rhapontici*, Causal Agent of Pink Seed of Pea, on the Canadian Prairies

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Accepted for publication: April 30, 2003.

ABSTRACT

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.

A 2-year field study demonstrated that *Erwinia rhapontici*, causal agent of pink seed of pea, survived Canadian prairie winters on infected seeds and stems of pea (*Pisum sativum* L.), regardless of burial depth at 0 or 6 cm. For the samples on the soil surface (0 cm), the average rate of survival of *E. rhapontici* was reduced to 88% for infected seeds and 70% for infected stems after the winter (March) and a further drastic reduction in survival of the pathogen was observed during the spring (March to May) period, when soil temperatures increased to above freezing and soil moisture was abundant. A similar trend of survival was observed for samples buried at the depth of 6 cm. The study suggests that *E. rhapontici* in infected pea seeds or stems can be an important source of inoculum for pink seed of dry peas and other legume crops under Canadian prairie conditions.

Key words: *Erwinia rhapontici*, survival, pink seed of pea, *Pisum sativum*.

Erwinia rhapontici (Millard) Burkholder is the causal agent of pink seed of pea (*Pisum sativum* L.) [2,11]. The disease results in pink or pinkish-brown lesions on pods and seeds, and reduces seed size and quality. *E. rhapontici* also causes pink seed of common bean (*Phaseolus vulgaris* L.) [1], durum wheat (*Triticum durum* Desf.) [6], and common wheat (*T. aestivum* L.) [3,9]. It is not known whether *E. rhapontici* can survive the winter in western Canada. The purpose of this study was to determine the survival and overwintering ability of *E. rhapontici* on naturally infected seeds and stems of pea under Canadian prairie conditions.

Field experiments were conducted during the winters of 2000 and 2001 at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada. Pink pea seeds infected by *E. rhapontici* were collected from a bulk sample of a commercial crop, cultivar Delta, near Vulcan, Alberta, Canada. Infected pea stems were obtained from field-grown plants arising from pink seeds, cultivar Delta. The infection of stem tissues of each plant was verified by excising a 2 cm segment from each end of the stem, surface sterilising in 70% ethanol for 90 sec, air-drying on paper towel, and plating onto potato dextrose agar (PDA) in Petri dishes. The stem segments were incubated on PDA for 3 days at 20℃ and examined for presence of *E. rhapontici* by the method described by Huang *et al.* [2]. The remaining portion of those stems that were positive for *E. rhapontici* at both ends, were cut into 2-cm long segments and used for the study. The infection of seeds was verified using the same surface sterilisation and plating procedure described for the stems.

Infected pea seeds or stems were sealed in nylon mesh bags (50 cm long x 30 cm wide) divided into 100 compartments with 1 seed or stem segment per compartment, resulting in a separation distance of approximately 2.5 cm between samples. Bags containing healthy seeds or stems of pea, cultivar Delta, were used as controls. The field experiment was set up during the first week of November by placing the bags of seeds or stems on the soil surface (depth of 0 cm) or burying at the depth of 6 cm. Treatments were arranged in a split-plot design with three replicates, using burial depth as main treatment and sampling date as sub-treatment. Bags on the soil surface were fastened to the ground with metal stakes to prevent movement from wind. For the bags on the soil surface, seeds and stems were retrieved and tested for viability and presence of *E. rhapontici* during the first week of November, January, March and May, using the surface sterilisation and plating methods described above. For bags buried at the depth of 6 cm, seeds and stems were retrieved and tested using the same methods, during the first week of November and the following May and no samples were retrieved during January and March due to frozen ground.

For each sample, the percent of seeds and stems with viable *E. rhapontici* was determined. For the treatments of pink seeds and stems, the data at the beginning and end of each time interval were compared using Student's *t*-test, to determine whether significant changes in viability of *E. rhapontici* had occurred. All statistical analyses were conducted using SAS/STAT® software [10]. Weather data for the duration of the experiments in 2000 and 2001 were collected at the weather station of the Lethbridge Research Centre situated approximately 300 m from the experimental sites. The data included soil temperature at a depth of 5 cm, measured daily at 4:00 PM, and total daily precipitation of rain and snow, as water equivalent.

Results of the field experiments in 2000 and 2001 showed that *E. rhapontici* survived the prairie winter on infected pea seeds (Table 1) and stems (Table 2) in both years, regardless of burial depth. In the 2000 experiment, for example, the viability of *E. rhapontici* on peas seeds placed on the soil surface (burial depth of 0 cm) decreased from 100% before winter (November 2000) to 99% after winter (March 2001) and to 47% during spring (May 2001) (Table 1). The viability of *E. rhapontici* on peas stems placed on the soil surface decreased from 100% before winter (November 2000) to 79% after winter (March 2001) and to 59% during spring (May 2001) (Table 2). The reduction in viability of *E. rhapontici* on infected seeds or stems placed on the soil surface (burial depth of 0 cm) was most drastic ($P<0.05$) during the interval of March to May in both years (Tables 1 and 2). For samples buried at the depth of 6 cm in the 2000 experiment, the viability of *E. rhapontici* decreased from 100% before winter (November) to 86% in the spring (May) for infected pea seeds (Table 1) and from 100% before winter (November) to 33% in the spring (May) for infected pea stems (Table 2). The trend of survival and overwintering ability of *E. rhapontici* on infected pea seeds and stems in the 2001 experiment was similar to that observed in 2000 (Tables 1, 2).

Weather data collected during the experiments in 2000 and 2001 indicated that the soil temperature measured at a depth of 5 cm ranged from near freezing to the high twenties

Table 1. Overwintering of *Erwinia rhapontici* on infected pea seeds in southern Alberta (Field experiments, 2000 and 2001)

Treatment [1]	Burial Depth	% seeds with viable *E. rhapontici*			
		Nov.	Jan.	Mar.	May
Experiment I (2000)					
Pink	0 cm	100 a[2]	100 a	99 a	47 b
Healthy	0 cm	0	0	0	1
Pink	6 cm	100 a	-	-	86 b
Healthy	6 cm	0	-	-	4
Experiment II (2001)					
Pink	0 cm	100 a	82 b	77 b	52 c
Healthy	0 cm	0	0	0	0
Pink	6 cm	100 a	-	-	80 b
Healthy	6 cm	0	-	-	1

[1] Pea seeds, cultivar Delta, were from an infected crop grown in 2000 by Markert Seeds Ltd., Vulcan, Alberta, Canada.
[2] Means within a row followed by the same letter are not significantly different at $P=0.05$ level (Student's *t*-test).

Table 2. Overwintering of *Erwinia rhapontici* on pea stems in southern Alberta (Field experiments, 2000 and 2001)

Treatment [1]	Burial Depth	% seeds with viable *E. rhapontici*			
		Nov.	Jan.	Mar.	May
Experiment I (2000)					
Pink	0 cm	100 a[2]	87 a	79 a	59 b
Healthy	0 cm	0	0	0	0
Pink	6 cm	100 a	-	-	33 b
Healthy	6 cm	0	-	-	2
Experiment II (2001)					
Pink	0 cm	100 a	63 b	61 b	17 c
Healthy	0 cm	0	0	0	0
Pink	6 cm	100 a	-	-	28 b
Healthy	6 cm	0	-	-	0

[1] Pea stems, cultivar Delta, were from plants originating from infected seed from Markert Seeds Ltd., Vulcan, Alberta, Canada.
[2] Means within a row followed by the same letter are not significantly different at $P=0.05$ level (Student's *t*-test).

(-2℃ to 29℃) in the fall (September through November), remained near freezing (-10℃ to 8℃) during the winter (December through February), and returned to above freezing (-6℃ to 29℃) during the spring (March through May). Precipitation was moderate in the fall in 2000, with daily amounts ranging from 0 to 23 mm, but less abundant in the fall of 2001, with daily amounts ranging from 0 to 5 mm. There was little precipitation during the winter of both years, and precipitation was most frequent in the spring of both years, with daily amounts ranging from 0 to 21 mm.

This study concludes that *E. rhapontici* can survive the Canadian prairie winter on infected pea seeds and stubble, and is present in the field during the spring season. The pathogen survived well in infected tissues during the winter period at burial depths of 0 and 6 cm. The rapid decline of *E. rhapontici* in infected seeds or stems during the spring period (March to May) in both years may be associated with the increase of soil temperature to above freezing and the increase of soil moisture from melting frost and frequent precipitation, thereby increasing the activity of soil microorganisms antagonistic to *E. rhapontici*.

In addition to causing pink seed of pea [2,11], bean [1], common wheat [3,9], and durum wheat [6], *E. rhapontici* also causes root rot or crown rot or soft rot on other plants such as citrus fruits [13], hyacinth [12], onion [8], rhubarb [5,7], and wasabi [4]. Results of preliminary studies showed that strains of *E. rhapontici* from pea were pathogenic to bean and vice versa (H. C. Huang *et al.*, unpublished). The wide host range of *E. rhapontici*, its lack of host specificity, and its ability to survive the harsh Canadian winters all suggest that the common practice of using pulse-wheat rotations in western Canada may not offer effective control of the pink seed disease.

ACKNOWLEDGMENTS

The authors thank the Saskatchewan Pulse Crop Development Board (Project No. AGR0108), the Alberta Pulse Growers Commission (Project No. 01-P4), and the Agriculture and Agri-Food Canada Matching Investment Initiative, for financial support. This is LRC Contribution No. 387- 03002.

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摘 要

黃鴻章[1,2]、R. S. Erickson [1]. 2003. 豌豆粉紅種子的病原細菌 (*Erwinia rhapontici*) 在加拿大西部草原的越冬能力. 植病會刊 12:133-136. ([1.] 加拿大農部 Lethbridge 研究中心；[2.] 聯絡作者：電子郵件 huangh@agr.gc.ca，傳眞：+0021-403-382-3156)

從兩年的田間試驗結果顯示，造成豌豆粉紅色種子的病原細菌 (*Erwinia rhapontici*) 能夠在加拿大西部草原越冬。將受*E. rhapontici*侵染的豌豆種子或莖稈置於地表，或埋藏於6 cm 深的土中，以測定其越冬能力。結果將豌豆病種子置於地表時，此病原細菌平均存活百分率由處理初期 (11 月) 的 100 %，經一冬天 (翌年 3 月) 降至 88 %，而將莖稈置於地表時，此病原細菌平均存活百分率由處理初期 (11月) 的100%，經一冬天 (翌年3 月) 降至 70%。在春天期間 (3 - 5 月) 由於氣溫回升至冰點以上，且土壤水分充足，乃造成此病原細菌存活的下降率更爲急速。將病種子或莖稈埋於 6 cm 深的土中，此病原細菌存活的下降趨勢與置於地表的材料類似。由這兩年的試驗結果顯示受*E. rhapontici* 危害的豌豆種子或殘餘莖稈，在田中有可能成爲來年新種豆科作物或其他非豆科寄主的主要感染源。

關鍵詞：*Erwinia rhapontici*、越冬、存活、碗豆種子粉紅病害、*Pisum sativum*