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A.G. Xue et P.A. Burnett

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# Evaluation of interactions between *Rhynchosporium secalis* and *Pyrenophora teres* on barley

Allen G. Xue<sup>1</sup> and Peter A. Burnett<sup>2</sup>

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Interactions between *Rhynchosporium secalis* and *Pyrenophora teres* were investigated on barley (*Hordeum vulgare*) seedlings grown in a greenhouse and growth chambers. Following mixed inoculations, the two pathogens colonized the same leaf simultaneously, but the leaf area with symptoms (LAS) was less than that produced by either of the two pathogens alone at the same inoculum concentration. On plants inoculated with the mixed inocula, LAS induced by *R. secalis* was reduced by a greater amount than LAS induced by *P. teres*. The predominance of *P. teres* over *R. secalis* was observed even when inoculations with *R. secalis* either preceded or followed the inoculation with *P. teres* by 24 h. Antagonism occurred when inoculum densities were  $10^3$ - $10^4$  spores mL<sup>-1</sup> for each pathogen, wetting periods were 24-28 h, and incubation temperature was above 12°C.

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Les interactions entre le *Rhynchosporium secalis* et le *Pyrenophora teres* ont été étudiées sur des plantules d'orge (*Hordeum vulgare*) cultivées dans une serre et dans des chambres de croissance. À la suite d'inoculations mixtes, les deux agents pathogènes ont colonisé la même feuille simultanément, mais la surface foliaire portant des symptômes était moindre que celle produite par l'un ou l'autre des deux agents pathogènes utilisés seuls à la même concentration d'inoculum. Sur les plantes inoculées avec les inoculums mixtes, la surface foliaire portant des symptômes induits par le *R. secalis* était grandement inférieure à celle induite par le *P. teres*. La prédominance du *P. teres* sur le *R. secalis* a même été observée quand les inoculations avec le *R. secalis* précédait ou suivait l'inoculation avec le *P. teres* par 24 h. Un antagonisme s'est produit quand les concentrations d'inoculum étaient de  $10^3$ - $10^4$  spores mL<sup>-1</sup> pour chaque agent pathogène, avec les durées d'humectation de 24-48 h et une température d'incubation supérieure à 12°C.

## INTRODUCTION

*Rhynchosporium secalis* (Oudem.) J.J. Davis, the causal agent of scald, and *Pyrenophora teres* (Died.) Drechs., the causal agent of net blotch, are the two

most common pathogens of barley (*Hordeum vulgare* L.) in the world (Mathre 1982; Shipton *et al.* 1974). Both organisms are commonly present on barley leaves and the diseases they induce are frequently observed on the same plant in a field (Xue

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1. Agriculture and Agri-Food Canada, Agri-Food Diversification Research Centre, Unit 100-101 Route 100, Morden, Manitoba, Canada R6M 1Y5
  2. Agriculture and Agri-Food Canada, Lacombe Research Centre, Bag Service 5000, Lacombe, Alberta, Canada T0C 1S0. Contribution No. 769. Agriculture and Agri-Food Canada Research Centre, Lacombe, Alberta

*et al.* 1994). Numerous studies have shown that both *R. secalis* and *P. teres* may cause large reductions in yield and grain quality of barley (Shipton *et al.* 1974). However, no information is available on the occurrence and importance of interactions between the two pathogens. Cook and Baker (1983) stated that when two or more plant pathogens attack the same plant organ, the organisms may interact antagonistically, synergistically, or exhibit neutralism toward each other. These interactions, therefore, may significantly influence the development of diseases on host plants. This study was conducted to examine the interactions between *R. secalis* and *P. teres* on barley leaves, and to determine the impact of biotic and abiotic factors on the occurrence of the interactions.

## MATERIALS AND METHODS

### Fungal isolates and inoculum production

Single conidial isolates coded LRS9205 (*R. secalis*) and LPT9207 (*P. teres*) were used in all the experiments. These isolates were obtained from naturally infected barley cv. Harrington, grown at the Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe (52°15' N 113°30' W), Alberta, in 1992. Cultures of LRS9205 were maintained at 4°C on wheat germ agar (WGA) (Xue *et al.* 1991) and LPT9207 on V-8 juice agar (V8).

Inoculum of *R. secalis* was produced in petri dishes on WGA supplemented with 100 µL L<sup>-1</sup> of streptomycin (Xue and Hall 1991). Conidia from 21-d-old cultures were harvested by flooding the cultures with distilled water containing 0.01 % Tween 20 (polyoxyethylene sorbitan) and rubbing gently with a sterile, latex-tipped glass rod to dislodge spores. The resulting spore suspension was filtered through two layers of cheesecloth and adjusted to the desired concentration with the aid of a haemocytometer.

Inoculum of *P. teres* was produced by growing the fungus on V8 in petri dishes with 100 µL L<sup>-1</sup> of streptomycin. The petri dishes were incubated for 10 d at 17 ± 1°C, with a 14-h photoperiod under cool white fluorescent lamps. Conidial suspensions for inoculations were prepared using the same method described for *R. secalis*.

### Growth of barley in greenhouse and growth chambers

Pedigree seeds of the barley cultivars Harrington, Klages, Diamond, Leduc, Empress and Johnston were used in the interaction studies. The susceptibility of these cultivars to *R. secalis* and *P. teres* is shown in Table 1.

Groups of five barley plants were grown in 14-cm-diam plastic pots containing a soil:perlite:peat moss mixture (1:1:1,v:v:v) in a greenhouse maintained at 23 ± 1°C during the day and 20 ± 1°C during the

**Table 1. Reaction of six barley cultivars to *Rhynchosporium secalis* (Rs) and *Pyrenophora teres* (Pt) alone and in combination**

Cultivar	Reaction to pathogen <sup>†</sup>		LAS <sup>‡</sup> (%)					Contrast among means (t value)				
			Mixed inoculum			Rs	Pt					
	Rs	Pt	Total	(1)	(2)			(3)	(4)	(5)	(1) vs (2)	(1) vs (4)
Harrington	S	S	6 b <sup>§</sup>	39 a	45 a	56 a	67 a	8.63**	6.83**	4.23**	1.10	3.32*
Klages	S	S	13 a	34 ab	47 a	61 a	67 a	4.27**	7.74**	4.41**	1.45	2.32*
Diamond	MR	MR	2 bc	23 bc	25 b	47 ab	46 b	4.40**	11.23**	4.02**	4.40**	4.60**
Leduc	MR	MR	1 c	11 c	12 b	44 ab	42 c	3.69**	13.57**	4.97**	9.70**	4.86**
Empress	R	S	4 bc	38 ab	42 a	19 b	67 b	14.60**	1.40	4.56**	2.14	4.98**
Johnston	R	S	3 bc	45 a	48 a	2 c	64 ab	19.19**	0.22	5.01**	11.90**	3.71**

<sup>†</sup> S: susceptible; R: resistant; MR: moderately resistant.

<sup>‡</sup> LAS: Leaf area with symptoms.

<sup>§</sup> Means followed by the same letter within a column are not significantly different at  $P \leq 0.05$  (LSD).

\* = significant at  $P \leq 0.05$ ; \*\* = significant at  $P \leq 0.01$ .

night. Plants were watered twice weekly from the base. The potting mixture contained sufficient nutrients to support healthy plant growth for the course of the experiments. Supplemental light was provided by 400 W metal halide lamps to ensure a 14-h photoperiod and a light intensity of  $150 \mu\text{mol m}^{-2}\text{s}^{-1}$ .

### Inoculation

Plants were inoculated at Zadoks growth stage 15 (Zadoks *et al.* 1974), which occurred 16-18 d after seeding. Spore suspensions of *R. secalis* and *P. teres* were prepared and adjusted to  $5 \times 10^3$  conidia  $\text{mL}^{-1}$ . Mixed inocula of *R. secalis* and *P. teres* were prepared by taking half volume of each pathogen from previously prepared spore suspensions of  $10^4$  conidia  $\text{mL}^{-1}$  per pathogen ( $5 \times 10^3$  conidia  $\text{mL}^{-1}$  of each pathogen). The inocula were sprayed on whole plants at a rate of 0.5 mL  $\text{plant}^{-1}$  using a DeVilbiss model 15 atomizer (The DeVilbiss Co., Somerset, Pennsylvania). After allowing the inocula to dry for 30 min, the plants were transferred to a humidity chamber at  $20 \pm 1^\circ\text{C}$  for a period of 48 h, then returned to the greenhouse bench or growth chambers. The saturated atmosphere in the humidity chamber was maintained by a mist produced by an ultrasonic humidifier and monitored with a hygrothermograph. Treatments were arranged in a randomized complete block design with four replicate pots. Four pots of cv. Harrington sprayed with distilled water plus 0.01 % Tween 20 were included with each experiment as checks against extraneous airborne inocula. All experiments were repeated two times.

### Factors affecting interactions between *R. secalis* and *P. teres*

The relationship between inoculation sequence, inoculum concentration, wetting period and incubation temperature, and interactions between *R. secalis* and *P. teres* was examined in five experiments using barley cv. Harrington. In all the experiments, plants were inoculated with the combination of the two pathogens (1:1, vol:vol). Plants inoculated with each pathogen individually were included as checks. Conidial suspensions used for inoculations were adjusted to  $5 \times 10^3$  spores  $\text{mL}^{-1}$  or  $5 \times 10^3$  conidia  $\text{mL}^{-1}$  of each pathogen in mixed inocula, except in experiments to observe the effect of inoculum concentration.

### Inoculation sequence and inoculum concentration

To determine the effect of inoculation sequence, five different sequences of treatment with *R. secalis* (Rs), *P. teres* (Pt), or the mixed inocula of the two pathogens were carried out. The respective combinations were coded Rs-Rs, Rs-Pt, Pt-Pt, Pt-Rs, and (Rs+Pt)-(Rs+Pt) (Table 2). Following the first inoculation, plants were placed in the humidity chamber for 24-h and then moved out. Surface moisture was evaporated from the plants within 15 min with a fan at room temperature. The second inoculation was done immediately after surface moisture evaporation. These plants were returned to the humidity chamber for another 24-h wetting period. Upon completion of the second wetting period, plants were dried for 15 min with the fan and transferred to the greenhouse bench.

**Table 2. Effect of inoculation sequence on the interactions between *Rhynchosporium secalis* (Rs) and *Pyrenophora teres* (Pt) on barley**

Inoculation sequence	LAS <sup>‡</sup> (%)		Total
	Rs	Pt	
Rs - Rs	64.2 a <sup>†</sup>	-	64.2 b
Rs - Pt	9.9 b	42.9 b	52.8 c
Pt - Pt	-	74.0 a	74.0 a
Pt - Rs	1.2 c	52.0 b	53.6 c
(Rs+Pt) - (Rs+Pt)	3.6 c	32.5 c	36.1 d

<sup>‡</sup> LAS: Leaf area with symptoms.

<sup>†</sup> Means within a column followed by the same letter are not significantly different at  $P \leq 0.05$  (LSD).

The effect of different concentrations of inocula on interactions between the pathogens was tested using five concentrations of inocula ( $10^2$ ,  $10^3$ ,  $5 \times 10^3$ ,  $10^4$  and  $10^5$  spores  $\text{mL}^{-1}$  pathogen $^{-1}$ ). Inoculated plants were kept 48 h in a humidity chamber and returned to the greenhouse bench as described above.

#### Wetting period and temperature

The effects of wetting period and temperature were studied in three experiments. In the first experiment, inoculated plants were placed in the humidity chamber for 0.5-48 h and returned to the greenhouse bench for disease development. In the second experiment, inoculated plants were exposed to a wetting period of 48 h and then transferred to growth chambers adjusted to 5, 10, 17 and  $25 \pm 1^\circ\text{C}$ . Each chamber was operated with a 14-h photoperiod and a photosynthetic photon flux density of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  provided by fluorescent and incandescent lamps. In the third experiment, the combination of four wetting periods (12, 24, 36 or 48 h) and three temperatures (10, 17 and  $25^\circ\text{C}$ ) was studied. After each wetting period, inoculated plants were dried for 15 min with a fan and placed in each of the three temperature-controlled chambers.

#### Disease assessment and statistical analysis

The severity of scald and net blotch was estimated for each inoculated leaf using a scale in which the percentage values are leaf area with symptoms (0 = 0%, 1 = 1-5%, 2 = 6-10%, 3 = 11-20%, 4 = 21-30%, 5 = 31-50%, 6 = 51-75%, and 7 = 76-100%). The scale was modified from that of Horsfall and Cowling (1978). Disease severity scores were converted to percentage of leaf area with symptoms (LAS) from Eq. [1].

Inoculated plants were rated for disease severity on the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> leaves 14 d after inoculation. Analysis of variance was conducted using Statistical Analysis System (SAS) (Cody and Smith 1991) and treatment means were separated by the

least significant difference (LSD) test or by a Student's *t* test at a probability level of 0.05.

## RESULTS AND DISCUSSION

### Interactions between *R. secalis* and *P. teres*

Both *R. secalis* and *P. teres* developed simultaneously on the same leaf after inoculation with the mixed inocula. The appearance of scald and net blotch when they developed simultaneously on the same leaf was slightly changed from their appearance when they developed separately. Scald lesions were often changed from the normal eye-shape to an irregular shape, and did not show the typical dark brown margin when in contact with necrotic areas caused by *P. teres*. Net blotch development was also retarded in the presence of scald and lesions tended to be shorter in length. On all the cultivars inoculated with the mixed inocula, more net blotch developed than did scald. The proportions of leaf area colonized by scald and net blotch were on average 4.8% and 31.5%, respectively. Contrast between the two diseased proportions was significant at  $P < 0.001$ . In most instances, the total LAS were reduced when both pathogens were present on the leaves compared with the effect of each pathogen individually (Table 1). From this standpoint, we suggest that the interactions between the two pathogens were antagonistic. Depending on resistance to the individual pathogens, the cultivars used in this study had a different impact on the occurrence and the intensity of the interactions. When both pathogens were applied simultaneously, cv. Diamond and Leduc, which are moderately resistant to both pathogens, showed significant reduction ( $P < 0.01$ ) in total LAS compared to inoculations with each pathogen individually. On cv. Klages and Harrington, which are susceptible to both pathogens, and cv. Empress and Johnston, which were resistant to *R. secalis* but susceptible to *P. teres* (Table 1), the reductions in total LAS were significant ( $P < 0.05$ ) only when

$$\text{LAS} = \frac{\sum \left( \begin{array}{l} \text{median value} \\ \text{in a category} \end{array} \times \begin{array}{l} \text{number of leaves} \\ \text{in the category} \end{array} \right)}{\text{total number of leaves}} \quad [1]$$

compared to that from the inoculation with *P. teres* alone (Table 1).

The LAS on plants inoculated twice consecutively with *R. secalis* or with *P. teres* were significantly greater than those on plants with sequential inoculation of one pathogen followed by another (Table 2). The lowest LAS was obtained on plants inoculated twice consecutively with the mixed inocula. Diseases induced on plants inoculated twice consecutively with mixed inocula or with sequential inoculations of one pathogen followed by another had less scald than net blotch. The fewest symptoms of scald were observed on plants when *R. secalis* was applied 24 h after the inoculation with *P. teres*. We observed that the scald pathogen colonized only unaffected tissues. If *P. teres* was already present on a leaf, *R. secalis* usually did not develop, or if it did, it was only on the lower portion of the leaf, which was not usually affected by net blotch.

### Effects of inoculum concentration, wetting period and incubation temperature

Neither scald nor net blotch developed on plants inoculated with spore suspensions lower than  $10^2$  conidia  $\text{mL}^{-1}$ . Both diseases were observed and the total LAS increased markedly with the increase of inoculum concentration from  $10^2$  to  $10^5$  conidia  $\text{mL}^{-1}$  for each pathogen. The total LAS induced by the mixed inocula were significantly lower than those induced by each pathogen alone at the inoculum concentrations of  $10^3$  to  $5 \times 10^3$ , but not at  $10^2$  or above  $10^4$  conidia  $\text{mL}^{-1}$  pathogen $^{-1}$ . As the inoculum concentration increased to  $10^5$  conidia  $\text{mL}^{-1}$ , plants receiving either mixed inocula or each pathogen alone withered and leaf necroses caused by the individual pathogens could not be differentiated (Table 3).

**Table 3. Effects of inoculum concentration, wetting period and incubation temperature on the interactions between *Rhynchosporium secalis* (Rs) and *Pyrenophora teres* (Pt) on barley**

Factor	LAS <sup>ψ</sup> (%)					Contrast among means (t value)				
	Mixed inoculum			Rs	Pt	(1) vs (2)	(1) vs (4)	(2) vs (5)	(3) vs (4)	(3) vs (5)
	Rs	Pt	Total							
	(1)	(2)	(3)	(4)	(5)					
<i>Inoculum concentration (spores <math>\text{mL}^{-1}</math> pathogen<math>^{-1}</math>)</i>										
$1 \times 10^2$	1	2	3	3	2	1.22*	2.65*	0.61	0.05	0.85
$1 \times 10^3$	4	10	14	39	17	2.46*	14.41**	2.79*	8.02**	1.01*
$5 \times 10^3$	10	30	40	52	57	6.88**	20.46**	5.40**	6.38**	3.47*
$1 \times 10^4$	15	37	52	52	58	7.30**	9.40**	7.02**	0.23	2.52*
$1 \times 10^5$	x <sup>†</sup>	x	88	88	88	- <sup>‡</sup>	-	-	0.01	0
<i>Wetting period (h)<sup>ε</sup></i>										
0.5	0	0	0	0	0	-	-	-	-	-
4	0	0	0	1	0	-	1.00	-	1.00	-
8	0	5	5	1	5	4.01*	4.50*	0.15	3.67	0.15
12	1	3	4	3	28	2.90	2.90	7.45*	0.49	7.45*
24	4	17	21	42	57	3.94	8.74*	10.30**	4.95*	9.22*
36	9	18	27	58	52	3.10	8.55*	6.70*	5.20*	8.25*
48	7	28	35	59	63	4.89*	22.70**	8.45*	10.78**	13.71**
<i>Incubation temperature (°C)<sup>ε</sup></i>										
5	5	2	7	5	13	2.24	0.18	4.57**	2.06	2.87*
10	10	12	22	35	37	1.40	5.41*	5.49**	2.97*	5.62**
17	14	17	31	56	46	2.03	13.60**	9.00**	10.60**	5.86**
25	3	20	23	37	55	6.47**	11.80**	11.53**	4.21*	11.19**

<sup>ψ</sup> LAS: Leaf area with symptoms.

<sup>ε</sup> Conidial suspensions used for inoculations were adjusted to  $5 \times 10^3$  spores  $\text{mL}^{-1}$  or  $5 \times 10^3$  conidia  $\text{mL}^{-1}$  of each pathogen in mixed inocula.

<sup>†</sup> Plants withered, leaf necroses caused by individual pathogens can not be differentiated.

\* = significant at  $P \leq 0.05$ ; \*\* = significant at  $P \leq 0.01$ .

<sup>‡</sup> Student's t test cannot be applied.

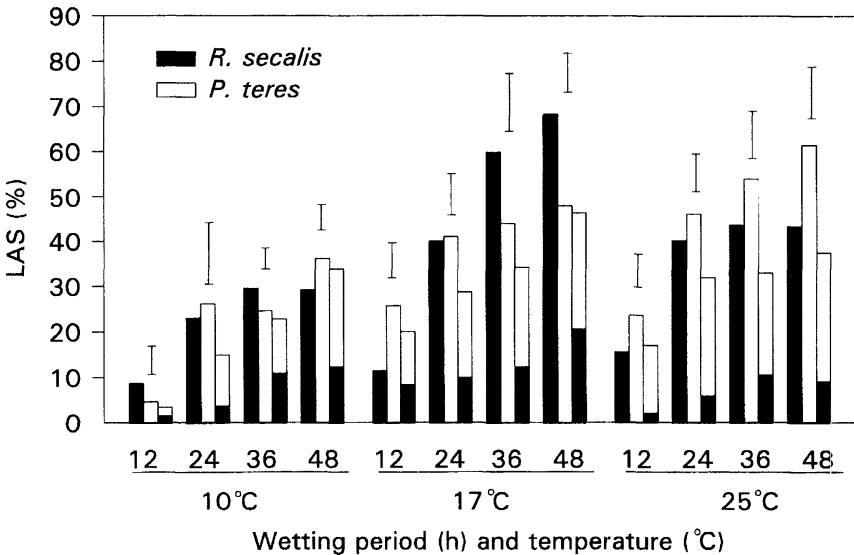
At the incubation temperature of  $23 \pm 1^\circ\text{C}$ , no scald developed on plants inoculated with *R. secalis* alone when the wetting period was  $< 1$  h nor on plants inoculated with the mixed inocula when the wetting period was  $\leq 8$  h. Net blotch was not observed on plants inoculated with either *P. teres* alone or the combination of *P. teres* and *R. secalis* when the wetting period was  $< 4$  h. Both scald and net blotch were observed and LAS increased with the increase of wetting period from 4 to 48 h. When the wetting period was in the range of 12-48 h, the total LAS of plants inoculated with mixed inocula were significantly lower ( $P < 0.05$ ) than that of plants inoculated with an individual pathogen. The reduction in total LAS was greatest when the wetting period was 48 h (Table 3).

Regardless of whether the inoculation of plants was with each pathogen alone or with a combination of the two organisms, severity of scald increased with the increase of temperature from  $5$  to  $17^\circ\text{C}$ , and severity of net blotch increased with the increase of temperature from  $5$  to  $25^\circ\text{C}$ , when the wetting period was 48 h. When the temperature increased from  $10$

to  $25^\circ\text{C}$ , the total LAS of plants inoculated with the mixed inocula were significantly lower ( $P < 0.05$ ) compared to that of plants inoculated with each pathogen separately. On plants inoculated with the mixed inocula, the greatest reduction for scald was at  $17^\circ\text{C}$ , and for net blotch at  $25^\circ\text{C}$  (Table 3).

The combined effects of wetting period and incubation temperature on severity of the diseases and on interactions of the two pathogens are shown in Figure 1. At all the temperatures tested, scald and net blotch increased with the increase of wetting period from 12 to 48 h. On plants inoculated with either *R. secalis* or *P. teres* alone, the maximum severity of scald and net blotch was achieved with a 48 h wetting period, at temperatures of  $17$  and  $25^\circ\text{C}$ , respectively. Under each of the treatment combinations of wetting period and temperature, plants inoculated with the mixed inocula developed less scald compared with net blotch. The total LAS on these plants were similar or smaller than those affected by each pathogen separately.

Although interactions among pathogenic microorganisms have been reported



**Figure 1. Combined effects of wetting period and incubation temperature on the interactions between *Rhynchosporium secalis* and *Pyrenophora teres* on leaves of barley cv. Harrington.** Simple and stacked columns represent single and combined inoculations, respectively. Vertical bars indicate LSD values at  $P \leq 0.05$ . LAS: Leaf area with symptoms.

on barley and other cereal crops (da Luz and Bergstrom 1987; Madariaga and Scharen 1986; Pauvert *et al.* 1978; Simkin and Wheeler 1974; Spadafora and Cole 1987), no study has been done on factors affecting their interactions. It seems possible that certain biotic and abiotic factors may affect the expression of any possible interactions among the pathogenic organisms. Further studies are needed to verify the occurrence of the antagonistic interactions in a field situation where both pathogens are present and to determine the possible effect of such interactions on disease development and yield reductions.

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