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# Effect of flooding on the survival of *Leptosphaeria* spp. in canola stubble

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This study used a versatile temperature-control device to assess the effect of temperature (12–40°C) and duration (2–12 weeks) of flooding on the survival of *Leptosphaeria* spp. in canola (*Brassica napus*) stubble. Canola basal stems with blackleg symptoms were submerged in water in small glass jars containing 20 $cm^3$ soil on a thermogradient plate capable of simultaneously maintaining up to 96 independent temperature regimes. Flooded stems were sampled at 2-week intervals, surface-sterilized, and incubated on V8-juice agar for 10 days to recover the pathogen. Flooding for 2 weeks substantially reduced pathogen recovery relative to non-flooded controls and the pathogen was not recovered after 6 weeks of flooding, irrespective of temperature. The pathogen was eliminated slightly more rapidly at flooding temperatures >20°C than at 12–16°C. There was no difference between *Leptosphaeria maculans* and *L. biglobosa* in their ability to survive flooding. Stem tissues degraded most rapidly during the first 2 weeks of flooding, corresponding to a quick decline in pathogen survival during the same period. These results indicate that a paddy rice crop following winter rapeseed may minimize the impact of blackleg by eradicating the inoculum of *Leptosphaeria* spp. in stubble.

*Keywords: Brassica napus*, crop rotation, residue degradation, stem canker

## Introduction

Blackleg disease, caused by *Leptosphaeria maculans* and *L. biglobosa*, is a serious constraint to canola (also known as oilseed rape or rapeseed) production in many countries (West *et al.*, 2001; Fitt *et al.*, 2008), including Australia, Canada and most of Europe. However, in China, the disease is reportedly caused only by *L. biglobosa*, a less aggressive pathogen species (Fitt *et al.*, 2006). Worldwide, the two species coexist in many regions, with *L. maculans* typically causing cortical infection near the base of the stem (basal stem canker) and *L. biglobosa* resulting in more superficial stem lesions or pith damage (Johnson & Lewis, 1994). In western Canada, both species may be found in association with basal-stem canker symptoms, with *L. biglobosa* at lower frequencies (Dilmaghani *et al.*, 2009). Often, canola crops infected by *L. biglobosa* can still be harvested with minimal yield losses under most growing conditions (Fitt *et al.*, 2006). The difference in distribution and prevalence of the two *Leptosphaeria* spp. may be attributed to variation in climate, cultivar choice, cropping system, disease management practices, or interactions of all these factors (West *et al.*, 2001).

*Leptosphaeria* species do not survive in the soil (West *et al.*, 2001) but can reside for many years in infected crop residue in the field or on canola seed (Bailey *et al.*, 2003). There is concern in China about the potential introduction of *L. maculans* and its impact on oilseed rape production in the country (Chen *et al.*, 2010). In China, the production of oilseed rape is concentrated in several eastern and central provinces along the Yangtze River (Fitt *et al.*, 2008). In these areas, winter oilseed rape is often followed by a paddy rice crop, which is flooded for several weeks during spring and summer (Wu *et al.*, 2009) at temperatures ranging from 20 to 33°C (World Meteorological Organization, 2012). Because of the relatively warm temperatures and high rainfall during this period in these regions, cotton and corn may also be grown following winter oilseed rape. It has not been documented whether these unique flooding conditions can affect the survival of *L. maculans* and *L. biglobosa* inoculum, in the form of ascospores (Thürwächter *et al.*, 1999) or pycnidiospores (Ghanbarnia *et al.*, 2011), in rapeseed residue. If flooding negatively affects these pathogens, paddy rice may be recommended over other crops to mitigate the risk of blackleg.

Flooding can negatively affect the survival of plant pathogens. Moore (1949) found that sclerotia of *Sclerotinia sclerotiorum* decayed completely when flooded for 23–45 days, but also indicated that such conditions would not be easily satisfied with normal rainfall in the southern United States. Wet soil conditions tend to result in greater degradation of canola residue and this may reduce the longevity of the pathogen (Baird *et al.*, 1999; West *et al.*,

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2001). Conversely, *Leptosphaeria* spp. survive longer under dry conditions and flooding of infected canola stubble, even for relatively short periods, impairs the pathogen's ability to produce ascospores (Petrie, 1995). However, it is not known whether the pathogen is killed, nor what the optimum conditions to eliminate the pathogen are. Therefore, the objectives of this study were to: (i) assess the effect of flooding duration and optimum water temperature on survival of *Leptosphaeria* spp. in canola stubble under controlled conditions; (ii) identify the optimal range of temperature regimes during flooding for pathogen eradication; and (iii) assess potential differences in survival between the *Leptosphaeria* species.

## Materials and methods

### Plant and fungal collection

Thousands of canola basal stems with blackleg symptoms were collected from a plot of spring canola cv. Westar (susceptible) on the AAFC Research Farm near Melfort, Saskatchewan, Canada after the 2011 harvest, dried at room temperature for a month, and cut into *c.* 2-cm pieces. Only those with >50% diseased area on the cross-section at both ends were used for the study. Two diseased stem pieces were wrapped in a nylon pouch (Fig. 1a) and tied with a colour-coded string for easy sample identification during flooding treatments. To estimate stem degradation, additional stem pieces of random sizes were oven-dried at 60°C for 3 days to remove moisture, and 1-g samples (dry weight; DW) placed in pouches tied with a string of a different colour (Fig. 1a). A total of six pouches of each stem-piece type were placed in a 100-mL glass jar (a replicate) containing 20 $cm^3$ field soil, and then 'flooded' with 50 mL tap water (Fig. 1b). Jars with flooded stem pieces were placed in the wells of a custom-made thermogradient plate (TGP; Fig. 2).

The TGP is capable of simultaneously maintaining 96 different diurnal temperature cycles in individual cells (11·2 × 12·0 cm: diameter × depth) during a 24-h period (McLaughlin *et al.*, 1985). Each cell can operate at a constant temperature or diurnal temperature cycles. In the latter case, cell temperatures would decrease in 12 steps from maximum to minimum and then increase gradually to maximum within a 24-h cycle. The temperature control was monitored for a week prior to the trial, and deviations in all cells were < ±1°C. A 14-h light period was provided with cool white fluorescent tubes during 'daytimes', and the in-cell light intensity was 62 $\mu$mol $m^{-2}$ $s^{-1}$. The temperature in all cells was monitored throughout the trial.

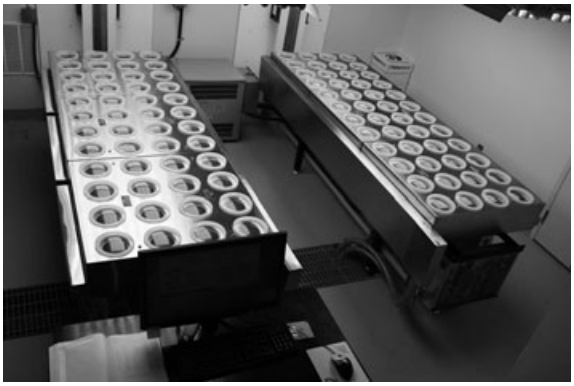

Figure 2 A thermogradient plate unit with 96 independently temperature-controlled wells.

### Effect of flooding under constant temperature conditions

Initially, eight constant temperatures: 12, 16, 20, 24, 28, 32, 36 and 40°C, were used to represent common and extreme temperature conditions in paddy rice production areas and diseased stem pieces were flooded for up to 12 weeks. One pouch of diseased stem pieces was removed from each jar biweekly for the assessment of pathogen survival. The pouch was rinsed with tap water for about 1 min to remove soil, and stem pieces then cut into *c.* 2- × 5-mm sections. The sections were surface-sterilized by placing in 70% ethanol for 5 s and 0·6% NaOCl for 60 s, then rinsed twice with sterilized water amended with lactic acid (1-mL 85% acid/L) to discourage bacterial growth. The surface-sterilized stem sections were dried on autoclaved paper towel for *c.* 1 h in a laminar flow hood, and 25 sections placed on V8 juice agar (200 mL V8 juice, 0·75 g $CaCO_3$, 15 g Difco agar in 1 L water) in a 9-cm Petri dish to form an experimental unit. The agar was amended with 100 ppm streptomycin and 1% Triton X-100 (Terochem) to reduce bacterial contamination and slow the growth rate of fungal colonies (Peng & Sutton, 1991) recovered from the stem pieces. The plates were sealed with Parafilm and incubated at 22°C under fluorescent light with a 12-h photoperiod. Pathogen survival was assessed based on the recovery of *Leptosphaeria* spp. from flood-treated stem pieces under a stereomicroscope; fungal colonies with pycnidia of *Phoma lingam* or pseudothecia of a *Leptosphaeria* sp. were considered positive identification of a blackleg pathogen. Non-flooded stem pieces, kept in paper bags at 20°C in darkness, were processed using the same surface-sterilization and isolation protocol described above as controls. The incidence of pathogen

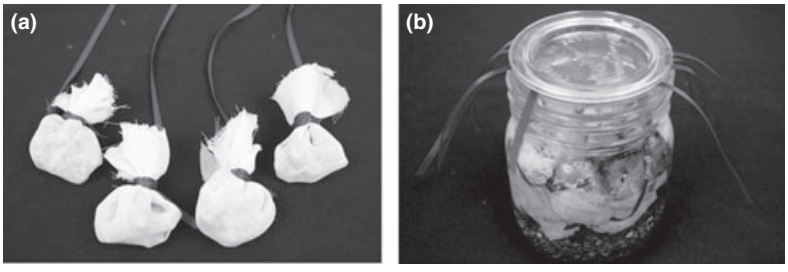


Figure 1 Pouches (a) of canola stubble pieces 'flooded' with 50 mL tap water in a 100-mL glass jar containing 20 $cm^3$ field soil (b).

recovery from the 25 stem sections incubated in a Petri plate (experimental unit) was calculated as the pathogen isolation frequency to represent pathogen survival.

A parallel study was conducted simultaneously under the same constant temperature conditions to assess the impact of flooding on degradation of stem tissues. At each of the 2-week sampling intervals for 12 weeks, a separate pouch of stem pieces (1 g DW) was removed and rinsed with tap water for 1 min. The flood-treated stem pieces were then transferred to small aluminium trays (7 × 2 cm: diameter × depth) and placed in an oven at 60°C for 3 days to dry. Pouches containing 1 g of stem pieces dried at 60°C for 2 days prior to the trial but not exposed to flooding were used as controls and dried with treated samples under the same conditions. Immediately after drying, the DW of the stem pieces from each individual pouch was recorded.

### Effect of flooding under variable temperature conditions

To further determine the reaction of *Leptosphaeria* spp. to variable flooding temperatures, diurnal temperature cycles were programmed on the TGP, based on the results from the constant-temperature trial. Four temperature regimes, i.e. 4/12, 12/20, 20/28 and 28/36°C, were used to represent common and extreme minimum/maximum temperature conditions in eastern and central China during spring and summer. The temperatures in each cell decreased in 12 steps from maximum to minimum and then increased gradually to the maximum within a 24-h cycle. This pattern of temperature change was intended to mimic daily temperature fluctuation in the natural environment. The temperature control was monitored throughout the trial and other conditions on TGP were the same as in the constant-temperature trial. To assess pathogen survival under flooding, replicated pouches of diseased stem pieces under varying temperature regimes were sampled at 1, 2, 4, 6 and 8 weeks after flooding, and processed similarly as described above to estimate pathogen survival. Non-flooded diseased stem pieces were used as controls.

### Characterization of *Leptosphaeria* spp. isolates from flood-treated stem pieces

To determine the fungal species isolated from flood-treated stem tissues, cultures grown on V8-juice agar for 2 weeks were flooded with water and scrubbed with a bent glass rod to dislodge the pycnidiospores. The concentration of the spore suspension was estimated using a haemocytometer and adjusted to $2 \times 10^7$ mL$^{-1}$. Canola cv. Westar, which has no known major resistance genes to *L. maculans*, was sown in a soilless planting mix (one part sand to 12 parts of a 1:2 sphagnum peat moss: vermiculite mixture amended with 1% (w/v) of 16-8-12 (N-P-K) and 0·2% of 0-20-0 controlled-release fertilizers) in 12-pack multiport seeding flats. At 7 days after planting, each cotyledon was wounded with a pipette tip and inoculated with 10 μL of pycnidiospore suspension of each isolate. As controls, additional plants were wounded and received a 10-μL droplet of sterile distilled water. Disease severity was assessed using a 0–9 scale (Williams, 1985) at 12 days after inoculation (El Hadrami *et al.*, 2010). Cotyledons of four plants were inoculated with each isolate and an average severity of 5 or greater indicated a virulent *L. maculans* isolate.

The results of the cotyledon assay were verified by confirming *L. maculans* isolates using a polymerase chain reaction (PCR) in a 25-μL reaction volume containing 4 ng DNA, 1× thermopol reaction buffer, 200 μM dNTP, 0·2 μM *L. maculans* species-specific forward and reverse primers (Mahuku *et al.*, 1996), 1·0 U long amplification *Taq* DNA polymerase and 15 μL nuclease-free sterile water (Applied Biosystems) in a 200-μL PCR tube. PCR amplification used the following thermal cycling parameters: initial denaturation at 95°C for 2 min, followed by 38 cycles of 95°C for 1 min, annealing at 58°C for 1 min, elongation at 72°C for 1 min and a final extension at 72°C for 10 min. PCR products were visualized on 1% (w/v) agarose gels (ABI) stained with GelGreen (0·05 μg mL$^{-1}$) after electrophoresis in 1 × TAE (tris–acetic acid–ethylenediamine tetraacetic acid, pH 8·0) buffer at 100V for 45 min for a single-banded diagnostic amplicon corresponding to the molecular size (377 bp) of *L. maculans* (Kaczmarek *et al.*, 2009).

### Data analysis

All experiments used a completely randomized design (CRD) with four replicates, and were conducted twice. SAS v.9.1 was used for statistical analysis. Data on the isolation frequency of pathogen and stem-tissue degradation from repeated trials showed homogeneity of virulence ($P$ = 0·5187–0·5242) based on the Bartlett test, and were therefore pooled prior to analyses. Logarithm transformation was used to improve the normality of incidence data. Effects of flooding temperature, duration and their interaction on pathogen isolation frequency were determined based on analysis of variance (ANOVA). Means were compared using the LSD test when treatment effects were significant ($P \leq 0·05$). When the interaction between flooding temperature and duration was significant, CONTRAST was used to compare the effect of flooding temperature over several ranges. Non-transformed data were used in results.

## Results

### Effect of flooding under constant temperature conditions

Throughout the trial period, temperature deviations in all cells were < ±1°C at any time and in general, deviations were corrected automatically within minutes. Under all temperature conditions, there was a significant reduction in the frequency of pathogen isolation from diseased stem pieces relative to non-flooded controls. After only 2 weeks of flooding, pathogen isolation was reduced substantially (Fig. 3). Although the pathogen was still recovered from some of the stem pieces after 4 weeks, it was generally at a low frequency and only at lower temperatures (12 and 16°C). After 6 weeks, the pathogen was no longer isolated, irrespective of temperature. In general, the temperature during flooding affected pathogen recovery, while flooding duration or its interaction with temperature had no impact (Table 1).

In all flooding temperature and duration treatments, there was a significant decrease in stem tissue DW relative to non-flooded controls (ANOVA, $P < 0·0001$). The reduction of DW was most rapid during the initial 2 weeks of flooding for most of the temperatures tested (Fig. 4), and the decreasing trend was less substantial in subsequent weeks, except under 36°C ($P$ = 0·05) which

resulted in the maximum DW reduction. Flooding duration and its interaction with temperature also affected degradation (ANOVA, $P < 0{\cdot}0001$), which, at longer flooding durations, was highest at 36°C (CONTRAST, $P = 0{\cdot}05$).

### Effect of flooding under variable temperature conditions

The pattern of pathogen isolation frequency from diseased stem pieces flooded under variable temperature regimes was similar to that under constant temperature conditions, except that the pathogen was no longer recovered after 3 weeks, regardless of temperature (Fig. 5). Pseudothecia were observed only occasionally on non-flooded stem tissues. Temperature and its interaction with flooding duration influenced pathogen recovery (Table 1), but flooding duration longer than 4 weeks generally killed the pathogen ($P = 0{\cdot}1968$). With 1 week of flooding, pathogen isolation frequency was slightly higher in the cold temperature regime (4/12°C) than at warm (20/28°C) and hot (28/36°C) temperatures (CONTRAST, $P \leq 0{\cdot}05$).

### Differentiation of *Leptosphaeria* spp. isolated from flood-treated stem tissues

Only six *Leptosphaeria* spp. isolates were recovered from flooded canola stem samples during the entire study. They were tested, along with an additional four isolates recovered from non-flooded stem tissues, on cotyledons of cv. Westar. Seven of these isolates, including four from flooded samples, were highly aggressive and caused the death of all inoculated cotyledons 12 days after treatment. The remaining three isolates (possibly *L. biglobosa*) showed relatively low aggressiveness, with an average disease severity >5. *Leptosphaeria maculans* species-specific primers amplified the DNA of those isolates that caused severe infection on Westar with a visible band at about 380 bp, but not that of other isolates which caused little infection on cotyledons.

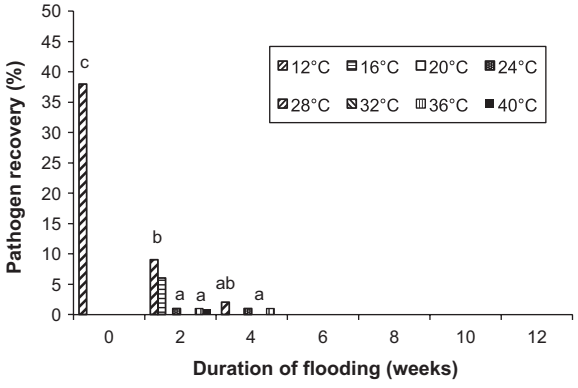


Figure 3 Recovery of blackleg pathogen (*Leptosphaeria* sp.) in canola stubble flooded under constant temperature conditions for 0–12 weeks. The pathogen isolation at 0 weeks was from non-flooded stem pieces. Treatment means with the same letter do not differ ($P = 0{\cdot}05$).

Table 1 Summary of statistical analysis – effect of flooding temperature and duration on survival of blackleg pathogens (*Leptosphaeria* spp.) in canola stubble

| | Constant T | | Variable T | |
|---|---|---|---|---|
| Factors | d.f. | *P*-value | d.f. | *P*-value |
| Temperature (T) | 8 | <0·0001 | 4 | <0·0001 |
| Duration (D) | 5 | 0·6999 | 4 | 0·1968 |
| T × D | 40 | 0·9999 | 16 | <0·0001 |

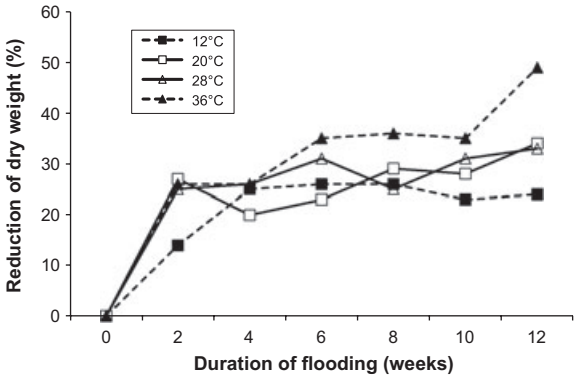


Figure 4 Canola stubble dry weight reduction (%) after exposure to different flooding conditions (temperature and duration), relative to non-flooded stem tissues.

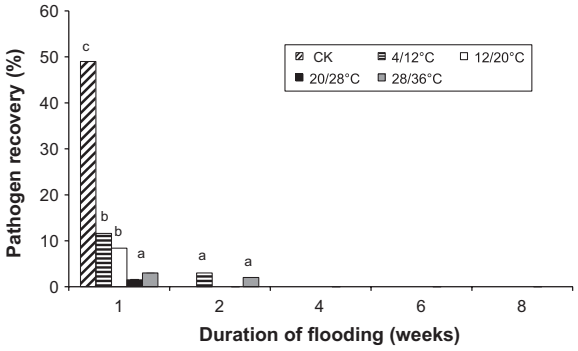


Figure 5 Recovery of blackleg pathogen (*Leptosphaeria* sp.) in canola stubble flooded under variable (min/max) temperature regimes. Treatment means with the same letter do not differ ($P = 0{\cdot}05$). CK, non-flooded control stem pieces.

## Discussion

This study demonstrates that flooding rapidly reduces the survival of the blackleg pathogen in canola stubble. Both *L. maculans* and *L. biglobosa* may be eliminated after 6 weeks under a wide range of flooding-temperature conditions. This disease is generally monocyclic (Hall, 1992); the early infection of cotyledons by ascospores or

conidia from rapeseed residues (Huang *et al.*, 2007; Khangura *et al.*, 2007; Ghanbarnia *et al.*, 2009, 2011) tends to cause the most severe symptoms and crop yield losses (Khangura & Barbetti, 2002). Suppression or elimination of the initial inoculum reduces the incidence of early infection and consequently alleviates the disease impact. Throughout this study, pseudothecia or pycnidia were never observed on any of the treated stem tissues. A previous study also found that ascospore production by *L. maculans* was reduced substantially after only 1 day of flooding of infested stubble (Petrie, 1995). Taken together, it is clear that flooding in paddy rice fields could be detrimental to *L. maculans* or *L. biglobosa*.

The idea of using flooding to eliminate plant pathogen inoculum is not new. Moore (1949) assessed flooding as a means to destroy sclerotia of *S. sclerotiorum* and found that sclerotia decayed in submerged soils after 4–6 weeks. Stover (1954) reported up to 85% eradication of *Fusarium oxysporium* f. sp. *cubense* inoculum after *c.* 6 weeks of flooding. These fungal pathogens produce resistant structures such as sclerotia and chlamydospores that can survive for years under natural soil conditions. In contrast, the blackleg pathogen colonizes rapeseed stubble in the form of mycelium, pseudothecia and pycnidia (Hall, 1992), structures less tolerant of adverse conditions than are sclerotia or chlamydospores. Therefore, it was not surprising that, in the current study, the blackleg pathogen was largely eliminated after 4 weeks of flooding. Flooding temperature also affected the survival of *F. oxysporium* f. sp. *cubense*; at 13°C, the pathogen survived 10–20 times longer than at 24–36°C (Stover, 1954). Although pathogen isolation frequency was higher on stem tissues flooded under cool temperatures (12°C constant or 4/12°C variable) than at >20°C constant temperature or >20/28°C variable temperatures after 1–2 weeks, all treatments reduced pathogen incidence by more than 80% relative to non-flooded controls. This indicated that flooding rapidly inactivates blackleg pathogens in canola stubble at a wide range of temperatures.

The DW data indicated rapid degradation of flooded canola stem tissues. Although the process was affected by temperature and flooding duration, as implied in other studies (Baird *et al.*, 1999; West *et al.*, 2001), the rate of decomposition (reduction in DW) was quickest during the first 2 weeks of flooding. This rapid stem-tissue degradation coincided with the low pathogen recovery observed after 2 weeks of flooding, implying the two events are related. In dry summers or cold winters, blackleg pathogens remained viable in canola residues for many years in western Australia (Khangura & Barbetti, 2002) and intact debris continues to support *L. maculans*. Conversely, in an environment where wet soils are prevalent and the debris is decomposed more, the pathogen is less capable of producing inoculum (Baird *et al.*, 1999). Based on the present data, degradation of rapeseed stubble would be expected to occur rapidly in most paddy rice fields under a range of temperature conditions, especially during the hot summer season. Thus, the probability of the pathogen surviving and producing ascospores for the winter rapeseed crop would be low.

The present study also indicates that flooding temperature is a factor contributing to pathogen mortality; higher temperatures tend to eliminate the pathogen in canola stem tissues more rapidly. Variable temperatures were used to mimic the pattern of daily temperature change under natural conditions, and the effect was similar to that of constant temperatures, with extremely low pathogen survival after 1 week of flooding at 20/28°C and 28/36°C. This reinforces the view that higher flooding temperatures eliminate the blackleg pathogen in canola residues more effectively than lower temperatures. In the regions of China where paddy rice often follows winter rapeseed, the average low and high temperatures during late spring and summer range from 20 to 25°C and 28 to 33°C, respectively (World Meteorological Organization, 2012). Based on the present results, these temperatures during flooding are optimal for degradation of rapeseed stubble and inactivation of blackleg pathogen inoculum. Therefore, if blackleg disease is a concern in any of these areas, crop rotation with paddy rice should be recommended over a dry-land crop following winter rapeseed to reduce pathogen inoculum. In contrast, flooding duration appears to be a less important factor for pathogen survival, because there was little recovery of the pathogen from flood-treated stem tissues after 4 weeks. Petrie (1995) pointed out that a flooding duration of less than 6 days might still allow the blackleg pathogen to resume inoculum production on stubble. In this present study, pseudothecia or pycnidia were not observed on stem tissues after more than 1 week of flooding. This result possibly implies that the conditions in paddy rice fields are sufficient to prevent *L. maculans* or *L. biglobosa* from producing ascospores or pycnidiospores as initial inoculum, probably contributing to reduced blackleg severity on rapeseed (Lô-Pelzer *et al.*, 2009).

The research by Baird *et al.* (1999) showed that the thickness of stem tissue affects rate of decomposition; thicker stubble remains intact for a longer time and thus may allow longer survival of the blackleg pathogen. Caution should be exercised when extrapolating the data because diseased stem tissues used in the current study were from spring canola, which can be thinner than those of typical winter rapeseed plants because of a much shorter growing season (West *et al.*, 2001). To verify this, infested stubble from winter rapeseed could be tested under similar conditions. Additionally, although the thermogradient plate provided reliable temperature control for the current experiments, field trials in winter rapeseed/paddy rice versus dry-land crop rotations would provide invaluable validation. In some cases, light tillage may be used to help break up stubble of winter rapeseed to make the flooding more effective at degrading smaller residue pieces (Baird *et al.*, 1999). Throughout the study, non-flooded stem sections generally did not show 100%

pathogen recovery and sometimes the isolation rate was even below 50%. This may have been the result of the use of stem tissues that were not fully colonized by the pathogen. This shortcoming was alleviated somewhat by selecting more severely diseased stem tissues in later trials.

Because of the extremely low recovery of *Leptosphaeria* isolates from flood-treated stem samples, the results were inconclusive in terms of relative survival between *L. biglobosa* and *L. maculans* under these flooding conditions. In an earlier study, however, both species were found coexisting in diseased canola cv. Westar stems in western Canada (Dilmaghani *et al.*, 2009). Furthermore, testing of 334 basal stem canker samples from 2010 commercial canola fields in western Canada using a multiplex PCR method by the Canadian Food Inspection Agency indicated an approximate ratio of 3:1 for *L. maculans* (62) and *L. biglobosa* (199) (unpublished internal report). It is highly possible that both species were present in the stem samples used in the current study. The observation that none of the samples produced any *Leptosphaeria* sp. colony after 6 weeks of flooding indicates that the treatment is possibly effective against both *L. maculans* and *L. biglobosa*. Planting paddy rice after winter rapeseed should also reduce the survival of *L. biglobosa*, which causes sporadic blackleg problems on certain rapeseed cultivars in China (Fitt *et al.*, 2008; Chen *et al.*, 2010). However, the flooding treatment did not reduce the aggressiveness of *L. maculans* relative to isolates from non-flooded tissues (data not shown); all *L. maculans* isolates caused severe infection on cv. Westar. Flooding can cause rapid disintegration of canola stubble and limit the survival of both *L. maculans* and *L. biglobosa*. Although this information may be relevant to parts of the Canadian prairies where spring flooding is also frequent, especially along the Red River valley of Manitoba, it provides direct support for the use of paddy rice in rotation with winter rapeseed in areas where blackleg is a problem and paddy rice is an option to reduce the initial pathogen inoculum and disease impact. Conditions in paddy rice fields (extensive periods of flooding at temperatures >20°C) are generally conducive for eliminating most pathogen inoculum within a season, thus substantially mitigating the risk of blackleg disease.

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## References

Bailey KL, Gossen BD, Gugel RK, Morrall RAA, 2003. *Diseases of Field Crops in Canada*. 3rd edn. Saskatoon, Canada: The Canadian Phytopathological Society, University Extension Press, University of Saskatchewan.

Baird RE, Phillips DV, Mullinix BG, Alt PJ, 1999. Relative longevity of *Leptosphaeria maculans* and associated mycobiota on canola debris. *Phytoprotection* **80**, 1–11.

Chen GY, Wu CP, Li B, Su H, Zhen SZ, An YL, 2010. Detection of *Leptosphaeria maculans* from imported canola seeds. *Journal of Plant Diseases and Protection* **117**, 173–6.

Dilmaghani A, Balesdent MH, Didier JP *et al.*, 2009. The *Leptosphaeria maculans – Leptosphaeria biglobosa* species complex in the American continent. *Plant Pathology* **58**, 1044–58.

El Hadrami A, Fernando WGD, Daayf F, 2010. Variations in relative humidity modulate *Leptosphaeria* spp. pathogenicity and interfere with canola mechanisms of defence. *European Journal of Plant Pathology* **126**, 187–202.

Fitt BDL, Brun H, Barbetti MJ, Rimmer SR, 2006. Worldwide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *European Journal of Plant Pathology* **114**, 3–15.

Fitt BDL, Hu BC, Li ZQ *et al.*, 2008. Strategies to prevent spread *of Leptosphaeria maculans* (phoma stem canker) onto oilseed rape crops in China: costs and benefits. *Plant Pathology* **57**, 652–64.

Ghanbarnia K, Fernando WGD, Crow G, 2009. Developing rainfall- and temperature-based models to describe infection of canola under field conditions caused by pycnidiospores of *Leptosphaeria maculans*. *Phytopathology* **99**, 879–86.

Ghanbarnia K, Fernando DWG, Crow G, 2011. Comparison of disease severity and incidence at different growth stages of naturally infected canola plants under field conditions by pycnidiospores of *Phoma lingam* as a main source of inoculum. *Canadian Journal of Plant Pathology* **33**, 355–63.

Hall R, 1992. Epidemiology of blackleg of oilseed rape. *Canadian Journal of Plant Pathology* **14**, 46–55.

Huang YJ, Liu Z, West JS, Todd AD, Hall AM, Fitt BDL, 2007. Effects of temperature and rainfall on date of release of ascospores of *Leptosphaeria maculans* (phoma stem canker) from winter oilseed rape (*Brassica napus*) debris in the UK. *Annals of Applied Biology* **151**, 99–111.

Johnson RD, Lewis BG, 1994. Variation in host range, systemic infection and epidemiology of *Leptosphaeria maculans*. *Plant Pathology* **43**, 269–77.

Kaczmarek J, Jedryczka M, Fitt BD, Lucas JA, Latunde-Dada AO, 2009. Analyses of air samples for ascospores of *Leptosphaeria maculans* and *L. biglobosa* by light microscopy and molecular techniques. *Journal of Applied Genetics* **250**, 411–9.

Khangura RK, Barbetti MJ, 2002. Efficacy of impact to manage blackleg (*Leptosphaeria maculans*) in canola. *Australian Journal of Agricultural Research* **53**, 311–21.

Khangura R, Speijers J, Barbetti MJ, Salam MU, Diggle AJ, 2007. Epidemiology of blackleg (*Leptosphaeria maculans*) of canola (*Brassica napus*) in relation to maturation of pseudothecia and discharge of ascospores in Western Australia. *Phytopathology* **97**, 1011–21.

Lô-Pelzer E, Aubertot JN, David O, Jeuffroy MH, Bousset L, 2009. Relationship between severity of blackleg (*Leptosphaeria maculans/L. biglobosa* species complex) and subsequent primary inoculum production on oilseed rape stubble. *Plant Pathology* **58**, 61–70.

Mahuku GS, Hall R, Goodwin PH, 1996. Co-infection and induction of systemic acquired resistance by weakly and highly virulent isolates of *Leptosphaeria maculans* in oilseed rape. *Physiological and Molecular Plant Pathology* **49**, 61–72.

McLaughlin NB, Bowes GG, Thomas AG, Dyck FB, Lindsay TM, Wise RF, 1985. A new design for a seed germinator with 100 independently temperature-controlled cells. *Weed Research* **25**, 161–73.

Moore WD, 1949. Flooding as a means of destroying the sclerotia of *Sclerotinia sclerotiorum*. *Phytopathology* **39**, 920–7.

Peng G, Sutton JC, 1991. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. *Canadian Journal of Plant Pathology* **13**, 247–57.

Petrie GA, 1995. Long-term survival and sporulation of *Leptosphaeria maculans* (blackleg) on naturally-infected rapeseed/canola stubble in Saskatchewan. *Canadian Plant Disease Survey* **75**, 23–4.

Stover RH, 1954. Flood – fallowing for eradication of *Fusarium oxysporium* f. *cubense*: some factors involved in fungus survival. *Soil Science* **77**, 401–14.

Thürwächter F, Garbe V, Hoppe HH, 1999. Ascospore discharge, leaf infestation, and variations in pathogenicity as criteria to predict impact of *Leptosphaeria maculans* on oilseed rape. *Journal of Phytopathology* **147**, 215–22.

West JS, Kharbanda PD, Barbetti MJ, Fitt BDL, 2001. Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. *Plant Pathology* **50**, 10–27.

Williams PH, 1985. Crucifer genetics cooperative. *Plant Molecular Biology Reporter* **3**, 129–44.

World Meteorological Organization, 2012. Climatological information – Wuhan, China. [http://www.worldweather.org/001/c00351.htm]. Accessed 21 December 2012.

Wu J, Cheng X, Xiao H, Wang H, Yang L, Ellis EC, 2009. Agricultural landscape change in China's Yangtze Delta, 1942–2002: a case study. *Agriculture, Ecosystems and Environment* **129**, 523–33.; accepted 12 December 2012