Developing Rainfall- and Temperature-Based Models to Describe Infection of Canola Under Field Conditions Caused by Pycnidiospores of *Leptosphaeria maculans*

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**ABSTRACT**


Blackleg, also known as Phoma stem canker, caused by *Leptosphaeria maculans* (*Phoma lingam*), is one of the most serious diseases of canola worldwide. In this study, the mean disease severity ($D_s$) and incidence ($D_i$) of canola cv. Westar plants infected at the cotyledon, three-leaf, and six-leaf stages by pycnidiospores of *L. maculans* were monitored in the greenhouse after infection of the plants under field conditions in two successive years and associated with meteorological data during infection time. Pearson’s correlation coefficient showed that total rainfall per week ($R$) was significantly correlated to $D_s$ on plants infected at the cotyledon, three-leaf, and six-leaf stages, and average maximum temperature per week ($T_{max}$) only showed significant correlation with plants infected at the cotyledon and six-leaf stages. These results also indicated that there is correlation between $D_i$ and $R$ for plants infected at all three growth stages. A nonlinear model was developed to evaluate the combined effects of $R$ and $T_{max}$ on $D_s$. The best model comprised monomolecular function and $β$ probability density function for plants infected at the above three growth stages. Parameters, including maximum potential for $D_s$ at a given rainfall ($d_{max}$), rate of changes with respect to rainfall ($k$), constant of integration ($B$), maximum potential for $D_s$ with respect to $T_{max}$ ($e$), rate of increase with increasing $T_{max}$ to optimum ($n$), and rate of decrease as $T_{max}$ increased and passed the optimum $T_{max}$ ($p$), were estimated for plants infected at the above three growth stages. The effect of plant growth stage was characterized by differences in the upper limit parameter $a$. This parameter was greater for the plants infected at the cotyledon stage than for plants infected at the other two stages. The estimate of parameter $k$ was the same for the plants infected at the cotyledon and three-leaf stages. This parameter was much lower for the plants infected at the six-leaf stage compared with the other two stages. The logistic model could describe the disease incidence with respect to $R$ slightly better than the other two models in the plants infected at all three growth stages. Based on the model, upper-limit estimate (−$d_{max}$) was ±100, 94, and 88.8% in the plants infected at cotyledon, three-leaf, and six-leaf stages, respectively. $D_i$ increased until rainfall reached ±18, 10, and 13 mm/week and became constant in the plants at cotyledon, three-leaf, and six-leaf stages, respectively. Effects of plant growth stage on the rate of change with respect to $R$ (parameter $k$) were lower in the plants infected at cotyledon than at the other two stages. The accuracy of the nonlinear models suggests that they could be used to develop a comprehensive model to evaluate epemics of blackleg based on pycnidiospores as sources of inoculum. However, additional years of data collection should improve model fit and evaluation of introduced models and contribute to the development of a more robust predictive model.

Additional keywords: *Brassica napus*.

Blackleg, caused by *Leptosphaeria maculans* (Desm.) Ces & De Not (anamorph: *Phoma lingam* (Tode) Desm.), is one of the most serious diseases of canola worldwide (10,41). The pathogen over-winters on crop residue in the form of pseudothecia (3,41) or pycnidia and mycelia (14). In the spring, ascospores or pycnidiospores are discharged and act as primary inoculum in western Canada (14,41). Petrie (31) showed that the pattern of ascospore release strongly depends on rain events. In a study in western Canada, Guo and Fernando (13) showed that the peak ascospore dispersal occurred several hours after a rainfall ≥2 mm and persisted for 3 days. Rain-splash dispersal of pycnidiospores of *L. maculans* was studied by Travadon et al. (37). They showed that dispersal pattern of pycnidiospores of *L. maculans* produced by simulated rain in controlled conditions matched the spatial dispersal of pycnidiospores. The effect of temperature was studied on rate of germination, length of germ tube, time of inoculation to penetration, and successful infection of pycnidiospores and ascospores in this pathosystem (5,16,17,37–39). For example, it was observed that, as the temperature increased from 5 to 20°C, the percentage of ascospores and pycnidiospores germination, length of germ tube, and rate of germination increased (16,17). Vanniasingham and Gilligan (38) mentioned that the probability of successful inoculation with pycnidiospores increased with in-
increasing temperature from 10 to 25°C. The effect of relative humidity on blackleg incidence was examined by Wood and Barbetti (43). They indicated that disease incidence rose from 36 to 98%, after cotyledon inoculation with 1 to 50 ascospores, as the duration of high relative humidity (100%) was increased from 0 to 120 h. However, when 50 to 10^5 pycnidiospores were used, the disease incidence was affected very little by duration of high humidity and was 18 to 32%. Guo and Fernando (13) also indicated the importance of relative humidity in the release of ascospores in western Canada.

Effects of weather variables have been studied in the blackleg—canola pathosystem as a basis for predicting disease severity, disease incidence, pseudothecia maturity, and ascospore showers (12,30,33,34). For example, in a study in the United Kingdom, Gladders and Symonds (12) tried to relate rainfall to stem canker incidence based on combined effects of temperature and humidity. Peres and Poisson (30) used a combination of the date of first sporangium collection and weather variables (amount of rain, number of rain-days, temperature, and number of hours of relative humidity >90%) to generate a preliminary model to predict the risk of stem canker Salam et al. (33,34) introduced models to predict pseudothecia maturity and ascospore shower in relation to blackleg disease in canola based on temperature and rainfall. To our knowledge, a simple model to describe blackleg severity or incidence of canola plants infected by pycnidiospores of blackleg in relation to the combined effects of rainfall and temperature has not been used in this pathosystem. Application of these kinds of models to empirical data from controlled conditions has been studied on different pathogens (7,44). For example, Carisse et al. (7) used a nonlinear model to study the influence of temperature and wetness duration on infection of strawberry leaves by *Mycosphaerella fragariae* under controlled conditions. However, reports on fitting field data and interpreting the response have not been published and are novel in the blackleg—canola pathosystem. In this study, two previously introduced equations describing disease severity of different pathogens as a function of temperature (6,9,25,28,44) were incorporated into general disease progress models such as monomolecular, logistic, and Gompertz (6,25) to produce an equation to evaluate the combined effects of moisture and temperature factors on Phoma stem canker severity and incidence. Therefore, the objectives of this study were to (i) develop a nonlinear model to describe mean blackleg severity based on combined effects of moisture factors and temperature and (ii) introduce a weather-based model to describe disease incidence.

**MATERIALS AND METHODS**

**Field experiment and disease assessment.** A 2-year study was conducted to compare the mean blackleg severity and the disease incidence of the canola plants infected under field conditions at three growth stages using *Brassica napus* cv. Westar. The experiment was carried out in a 50-by-60-m plot from 1 June to 7 July 2004 for 5 weeks and from 27 May to 10 August 2005 for 10 weeks at the Carman Research Station, Carman, Manitoba, Canada. Ten plants at cotyledon (GS 1.0), three-leaf (GS 1.03), and six-leaf growth stages (GS 1.06) were each placed in a blackleg-infested canola field for 1 week. The field contained stubble pieces left from blackleg-infected canola plants from previous years. Cv. Westar, susceptible to pathogenicity group 2 (PG2), was used for both years to ensure infection of plants. The plants were then returned to the greenhouse after 1 week and left to grow until maturity. Stem disease severity and incidence (*Ds* and *Di*, respectively) were evaluated at the pod-filling stage of canola (pods turn yellow). Stem *Ds* was recorded using a 0-to-5 scale (20). *Di* was evaluated based on the percentage of infected plants to total plants each week.

**Determination of source of inoculum.** Three steps were used to determine the source of inoculum in the local area of investigation. In the first approach, blackleg-infected stubble pieces were chosen randomly from the field each week during the experiment in 2004 and 2005 to examine for the presence of pseudocarpi or pseudothecia using a dissecting microscope (x65; WILD M3Z, Heerbrugg, Switzerland). The pseudocarpi and pseudothecia were recognized based on the shape and size of the fructification body (35). The mean weekly density of fructification bodies was calculated as described previously (11). In the second step, five rotorod impact spore samplers (Aerobiology Company, Nepean, Ontario, Canada) were set up in a diagonal orientation within the experimental plot to measure ascospore concentrations based on daily dispersal in the local area. Four rotorod samplers were set up 15 m from each corner and the last was set up in the middle of the field. The samplers were placed 30 cm above ground. A CR10 datalogger (Campbell Scientific, Logan, UT) was programmed to operate the rotorods for 5 min/h. In addition, a 7-day Burkard spore trap (Burkard Scientific Ltd., Uxbridge, Middlesex, UK) was set up in the center of the plot to trap ascospores locally and those probably blown from other areas. Ascospore concentration was measured as previously described (11). Because ascospore showers usually start in the first or second week of June in western Canada (13,31), spore sampling started in the middle of May. Finally, trap plants (all three growth stages) were used in the infested blackleg area to confirm the development of disease.

**Data analysis.** A generalized randomized complete block design with growth stages (three different stages) of plant as the treatment and week as the block was performed to assess blackleg severity. Ten plants were observed for each growth stage treatment in each week. There were 5 replicate weeks (blocks) in year one and 10 replicate weeks in year two, for 15 replicate weeks in total. Analysis of variance was performed using PC SAS (SAS Institute, Cary, NC) with PROC MIXED. Year, treatment, and their interaction were considered to be fixed effects. Week within year and interaction of treatment with week within year were considered to be random effects. The error term for testing treatment effects is the interaction of treatment and week within year. Square root and arcsine square root transformations were performed for the data of mean *Ds* and *Di* due to slight departure of the residuals from normality. Means were separated by t test (least significant difference) for the mean *Ds* and *Di*. For *Di*, the percentage of affected plants per 10 plants in each batch was analyzed as in a model similar to that used for blackleg severity but there was only one observation per batch. Also, a randomized complete block design with fruiting bodies (pseudocarpi and pseudothecia) as treatment and week as the block was performed to compare the presence of main fruiting bodies in the local area. Thirty-five stubble pieces were observed each week. Correlation analysis between weather variables, the total rainfall per week (*R*), mean temperature per week (*T*), average minimum and maximum temperature per week (*Tmin* and *Tmax*), mean relative humidity per week (*RH*), and mean blackleg severity and incidence was performed using PROC CORR procedure of SAS (version 9.1). Variance inflation factor (VIF) was measured to assess multicollinearity between independent variables. A value of VIF >10 was regarded as multicollinearity. The correlations of the variables with mean *Ds* and *Di* of blackleg were used as a guide in developing the models. Mean *Ds* and *Di* data were fit to the models, using the Statistical Analysis System nonlinear regression procedure NLIN with the Guass-Newton method of iteration (version 9.1; SAS Institute). The best model fit was selected based on lower Akaiki’s Information Criterion (AIC) and mean square error (MSE), higher pseudocoefficient of determination (*R^2*), as well as a plot of residuals versus predicted values of *Ds* or *Di* (6,25).

**Model development.** To develop a model to describe *Ds* and *Di* based on combined effects of moisture and temperature factors, three steps were performed. In the first step, three nonlinear
models (Table 1)—monomolecular, logistic (LG), and Gompertz—were selected (6,25,28,29) to describe the $D_s$ as a function of moisture factors ($R$: rainfall or relative humidity). In these equations, upper asymptote, $d_{max}$ parameter (carrying capacity), corresponds to the maximum potential for $D_s$ at given rainfall. Rate parameter $k$ determines how rapidly disease changes with respect to rainfall and parameter $R$ is a constant of integration. In the second step, two models based on the exponential probability density function and the $\beta$ probability density function (Table 2), to describe the dependent variable ($Ds$) as a function of temperature ($T$), were selected (6,9,25,28,29). In this study, mean temperature per week, average maximum temperature per week, or average minimum temperature per week were considered as temperature factor ($T$). In these two equations, the parameter $a$ characterizes the maximum potential for $Ds$ with respect to $T$. The parameters $m$ and $g$ characterize the intrinsic rate of change and the optimal temperature $Ds$ with respect to $T$, respectively. Parameter $n$ describes how steeply $Ds$ increases with increasing $T$ up to optimum and the parameter $p$ describes how steeply $D_s$ decreases as $T$ increases past the optimum. Parameter $t = (T - T_{min})/(T_{max} - T_{min})$.

Temperature influences the maximum potential for mean $Ds$ ($d_{max}$) at a given moisture factor (9). The effect of temperature could be characterized by allowing parameter $d_{max}$ to vary with temperature. Therefore, the third and final step, which involved describing $Ds$ as a function of $R$ and $T$, $Ds = f(R,T)$, can be performed by a modified version of equations in Table 1 in which $d_{max}$ is a function of $T(1.7,9.44)$. Substituting $d_{max}$ in equations in Table 1 gives

$$Ds = f(R,T) = f(T)[1 - Bexp(-kR)]$$
$$Ds = f(R,T) = f(T)[1 + Bexp(-kR)]$$
$$Ds = f(R,T) = f(T)exp[Bexp(-kR)]$$

in which $f(T)$ is calculated by equations in Table 2. The six final models which describe the $Ds$ as a function of $R$ and $T$ can be seen in Table 3.

**RESULTS**

To determine the source of inoculum in the local area of investigation, three approaches were used. In the first, the pycnidia and pseudothecia density on stubble pieces were compared. The results showed that all of them harbored spore-bearing pycnidia in each week in 2004 and 2005 (data not shown). In the second method, two different types of spore samplers (Rotors) were used to trap ascospores. No ascospores were detected in most weeks and only very low concentrations of them (0.06 ascospore/cm²/h) were observed in weeks one and five in 2005. In the third method, a high level of blackleg incidence and subsequent disease development on the trap plants was observed (Table 4). These findings allowed us to conclude that the disease development on the trap plants could be attributed to the role of pycnidiospores as a main source of inoculum in these years.

Data for the mean $Ds$ and $Di$ for the plants infected at cotyledon, three-leaf, and six-leaf stages and weather variables were summarized in Table 4. Mean $Ds$ was significantly different among the plants infected at the three growth stages (data not shown). However, significant differences in $Di$ were only observed between the plants infected at six-leaf stage and the plants infected at the two other stages. Correlation analysis between $Ds$ and $Di$ and the previously mentioned weather variables was performed. The results showed that positive and significant Pearson’s correlations existed between the mean $Ds$ of blackleg for plants infected at three growth stages and selected weather variables in both years (Tables 5 and 6). In all three canola growth stages, correlations between $Ds$ of blackleg and total rainfall per week were significant ($p < 0.05$). The correlation value was 0.62, 0.53, and 0.30 in 2004 and 0.52, 0.31, and 0.49 in 2005 for plants infected at cotyledon, three-leaf, and six-leaf stages, respectively. However, significant correlation between relative humidity ($RH$, $RH_{min}$, and $RH_{max}$) and mean $Ds$ was only observed in 2005. A negative correlation also was observed between $Ds$ and temperature factors (mean temperature and average maximum temperature per week) in the plants infected at cotyledon and six-leaf stages. This correlation was observed in plants infected at three-leaf stage only in 2004. The correlation value between $Ds$ and $T_{max}$ was higher than $Ds$ and $T$ for the two plant growth stages in both years except for the plants infected at cotyledon stage, in which the value was slightly smaller for $T_{max}$ than $T$ (0.49 to 0.50) in 2004. The correlation between the remaining variables and the mean $Ds$ were either not significant or there was a correlation between them and $R$ (e.g., $RH$ correlated to $R$ in the plants infected at six-leaf stage) (data not shown). Therefore, total rainfall per week ($R$) and average maximum temperature per week ($T_{max}$) were used as independent variables to describe the mean $Ds$ of plants infected at cotyledon and six-leaf stages. Multi-collinearity between these two variables was not a cause for concern (VIF between them was <2 for the three plant growth stages tested). $T_{max}$ did not correlate with $Ds$ in the plants infected at three-leaf stage. Nevertheless, the data for $T_{max}$ was used to estimate parameters of the final model describing $Ds$ for the plants at this growth stage only for comparison with the models introduced for the other two stages.

**TABLE 2. Model expressing mean disease severity ($Ds$) as a function of temperature ($T$)**

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>$Ds = f(T) = a[1 - Bexp(-kR)]$</td>
</tr>
<tr>
<td>Model 2</td>
<td>$Ds = f(T) = a[1 + Bexp(-kR)]$</td>
</tr>
</tbody>
</table>

$^a$ Each of these models relates mean disease severity to temperature ($T$). Models 1 and 2 are based on exponential probability density function and $\beta$ probability density function, respectively.

$^b$ Model parameters are maximum potential for disease severity at given temperature ($T$), rate of change of $Ds$ with respect to $T$ ($m$), optimal temperature ($g$), rate of change of $D_s$ as $T$ increases ($n$), and rate of change of $D_s$ as $T$ decreases ($p$).

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$Ds = f(R,T) = a[1 - Bexp(-kR)]$</td>
</tr>
<tr>
<td>2</td>
<td>$Ds = f(R,T) = a[1 + Bexp(-kR)]$</td>
</tr>
<tr>
<td>3</td>
<td>$Ds = f(R,T) = a[1 + Bexp(-kR)]$</td>
</tr>
<tr>
<td>4</td>
<td>$Ds = f(R,T) = a[1 - Bexp(-kR)]$</td>
</tr>
<tr>
<td>5</td>
<td>$Ds = f(R,T) = a[1 + Bexp(-kR)]$</td>
</tr>
</tbody>
</table>

$^a$ Model parameters are maximum potential for disease severity at given rainfall ($d_{max}$), constant of integration ($R$), rate of disease change with respect to increasing moisture factor ($k$), total rainfall per week ($R$), maximum potential for disease severity at given temperature ($T$), rate of change of $Ds$ with respect to $T$ ($m$), optimal temperature ($g$), rate of change of $Ds$ as $T$ increases ($n$), and rate of change of $Ds$ as $T$ decreases ($p$).

$^b$ Model parameters are maximum potential for disease severity or disease incidence at given rainfall ($d_{max}$), constant of integration ($B$), and rate of disease change with respect to increasing moisture factor ($k$).
Effects of rainfall and temperature on the mean $Ds$. The results of nonlinear regression analysis in the SAS program for six mentioned models are summarized in Table 7. To avoid over-parameterization, parameter $B$ for models 1, 3, and 5 was fixed to 0.7, 1.9, and 1.1, respectively, for the plants infected at cotyledon, three-leaf, and six-leaf stages based on the preliminary regression analysis. To use models 2, 4, and 6, estimates of $T_{\text{maxmin}}$ and $T_{\text{maxmax}}$ were also needed, which were not known before data collection. Based on Table 1, $T_{\text{maxmin}}$ was 17.4°C and $T_{\text{maxmax}}$ was 29.2°C. Therefore, the value of 17 and 29.5°C were used as $T_{\text{maxmin}}$ and $T_{\text{maxmax}}$, respectively. The results indicated that models 2, 4, and 6 described $Ds$ better than models 1, 3, and 5 for all three growth stages. The first group had higher $R^2$ and lower MSE and AIC (Table 7). The plot of residual versus predicted year week DSC DSL DSR DIC DIL DIR

Year Week DSC DSL DSR DIC DIL DIR

2004 1 4.2 2.9 1.8 100 100 80 15.0

2004 2 2.8 1.5 1.3 77 50 50 3.0

2004 3 4.7 2.9 1.9 100 100 90 14.0

2004 4 1.2 0.7 0.4 50 66 22 0.0

2004 5 2.4 1.8 1.8 100 80 90 7.0

2005 1 2.4 2.2 1.6 100 100 100 9.9

2005 2 3.6 2.6 3.4 100 100 100 19.8

2005 3 3.0 2.7 2.6 100 88 100 32.4

2005 4 1.3 0.7 0.4 70 44 44 0.2

2005 5 4.0 3.2 3.4 100 88 83.8

2005 6 4.1 2.7 2.1 100 90 66 28.8

2005 7 2.3 1.1 0.9 100 75 77 19.2

2005 8 3.6 2.4 2.6 100 100 100 34.6

2005 9 1.5 1.8 0.9 60 55 40 1.2

2005 10 2.0 2.4 0.7 60 88 66 6.4

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

0.62*** 0.53*** 0.30* 0.52*** 0.31* 0.49**

-0.50** -0.32* -0.25 -0.25 -0.24 -0.45**

-0.29* -0.11 -0.01 -0.14 -0.19 -0.31*

-0.49** -0.33* -0.27* -0.33** -0.25 -0.55**

-0.14 -0.11 0.15 0.50*** 0.32* 0.54***

-0.34* -0.26 0.01 0.36** 0.21* 0.44**

0.09 0.09 0.24 0.43*** 0.38* 0.39**

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

0.78* 0.79* 0.91** 0.79* 0.65* 0.68*

-0.17 -0.63 -0.60 -0.35 -0.56 -0.56

0.21 -0.11 -0.25 -0.77 -0.45 -0.34

-0.22 -0.58 -0.59 -0.53 -0.64 -0.71

-0.11 -0.19 0.02 0.89* 0.69* 0.80**

-0.34 -0.15 0.31 0.80* 0.42 0.62

-0.005 0.47 0.31 0.59 0.77* 0.58

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

$\text{Cotyledon Three-leaf Six-leaf}$

0.85, 0.84, and 0.85 for models 2, 4, and 6, respectively, in the plants infected at cotyledon, three-leaf, and six-leaf stage. However, MSE and AIC value was lower in model 2 compared with the other two models. These values were 0.35 and –19.4 for model 2 compared with 0.38 and –18.3 for model 4 and 0.36 and –19.1 for model 6. Overall, model 2 described the mean $Ds$ slightly better compared with models 4 and 6 in the plants infected at cotyledon stage. This model also could express $Ds$ better than five other models in the plants infected at three-leaf and six-leaf stages. Therefore, model 2 was selected to describe $Ds$ as a function of total rainfall per week ($R$) and average maximum temperature per week ($T_{\text{max}}$) in the plants infected at all three stages. The response surface for this model, $Ds = f(R,T) = a(R^2 + BT + C(T^2) + D(R^2T^2))$, on the plants infected at cotyledon stage, three-leaf, and six-leaf stages can be seen in Figures 1, 2, and 3, respectively.

Disease resistance was characterized primarily by differences in the upper limit of $Ds$ (parameter $a$). This parameter was great-

TABLE 5. Pearson’s correlation coefficient between weather variables and mean disease severity in 2004 and 2005

<table>
<thead>
<tr>
<th>Weather variables</th>
<th>Cotyledon</th>
<th>Three-leaf</th>
<th>Six-leaf</th>
<th>Cotyledon</th>
<th>Three-leaf</th>
<th>Six-leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total rainfall</td>
<td>0.62***</td>
<td>0.53***</td>
<td>0.30*</td>
<td>0.52***</td>
<td>0.31*</td>
<td>0.49**</td>
</tr>
<tr>
<td>Mean temperature</td>
<td>-0.50**</td>
<td>-0.32*</td>
<td>-0.25</td>
<td>-0.25</td>
<td>-0.24</td>
<td>-0.45**</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>-0.29*</td>
<td>-0.11</td>
<td>-0.01</td>
<td>-0.14</td>
<td>-0.19</td>
<td>-0.31*</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>-0.49**</td>
<td>-0.33*</td>
<td>-0.27*</td>
<td>-0.33**</td>
<td>-0.25</td>
<td>-0.55**</td>
</tr>
<tr>
<td>Mean $RH$</td>
<td>-0.14</td>
<td>-0.11</td>
<td>0.15</td>
<td>0.50***</td>
<td>0.32*</td>
<td>0.54***</td>
</tr>
<tr>
<td>Minimum $RH$</td>
<td>-0.34*</td>
<td>-0.26</td>
<td>0.01</td>
<td>0.36**</td>
<td>0.21*</td>
<td>0.44**</td>
</tr>
<tr>
<td>Maximum $RH$</td>
<td>0.09</td>
<td>0.09</td>
<td>0.24</td>
<td>0.43***</td>
<td>0.38*</td>
<td>0.39**</td>
</tr>
</tbody>
</table>

a Values followed by *, **, and *** are significant at 0.01, 0.001, and 0.0001, respectively.

$b RH = \text{mean relative humidity per week.}$
three growth stages are summarized in Table 8. These results imply that $Ds$ was increasing with lower slope with respect to $R$ in this growth stage. These results also showed that the value of parameter $p$ was significantly smaller for plants at the three-leaf stage ($p = 0.03$) compared with plants at the cotyledon and six-leaf stages. This was (as expected) due to lack of correlation of $Ds$ with average maximum temperature in the plant at three-leaf stage. The value for parameter $p$ was almost the same for plants at the cotyledon ($p = 0.16$) and six-leaf stage ($p = 0.17$), which means that $Ds$ for plants in both growth stages decreases with the same slope after it passes the optimum temperature.

**Effects of rainfall and temperature on $Di$.** Positive and significant correlation was also observed between total rainfall per week and blackleg incidence in the plants infected in all three growth stages (Table 6). These correlations were 0.78, 0.79, and 0.91 in 2004 and 0.79, 0.65, and 0.68 in 2005 for the plants infected at cotyledon, three-leaf, and six-leaf stages, respectively. The correlations between the remaining variables and $Di$ were either not significant or not reproducible (e.g., mean $RH$ per week correlated to $Di$ only in 2005). Therefore, only total rainfall per week was used for model development among all weather variables. The results of nonlinear regression of the three mentioned models to describe the blackleg incidence in the plants infected at three growth stages are summarized in Table 8. These results showed that the LG model could describe the $Di$ slightly better than the other two models in plants infected at all three growth stages. The pseudo-$R^2$ was higher and AIC and MSE were smaller for the LG model compared with the other two. The nonlinear regression analysis showed that the LG model could explain 80, 70, and 77% of blackleg incidence variation in the plants infected at cotyledon, three-leaf, and six-leaf stages, respectively. The blackleg incidence increased with increasing rainfall, then became constant (Fig. 4). $Di$ increased until rainfall reached $\approx 18, 10$, and 13 mm and became constant in the plants at cotyledon, three-leaf, and six-leaf stages, respectively. Upper limit of $Di$ ($d_{\text{max}}$) was higher in the plants infected at cotyledon stage than in the plants infected at the other two stages. Effects of plant growth stage on the rate of change with respect to $R$ (parameter $k$) were lower in the plants infected at cotyledon stage than in the plants infected at the other two stages. This value was $0.18, 0.37$, and 0.35 for the plants infected at cotyledon, three-leaf, and six-leaf stages, respectively.

**DISCUSSION**

The effects of total rainfall per week ($R$) and average maximum temperature ($T_{\text{max}}$) on the mean $Ds$ of plants infected at three growth stages are summarized in Table 8. These results implied that the LG model could describe the $Di$ slightly better than the other two models in plants infected at all three growth stages. The pseudo-$R^2$ was higher and AIC and MSE were smaller for the LG model compared with the other two. The nonlinear regression analysis showed that the LG model could explain 80, 70, and 77% of blackleg incidence variation in the plants infected at cotyledon, three-leaf, and six-leaf stages, respectively. The blackleg incidence increased with increasing rainfall, then became constant (Fig. 4). $Di$ increased until rainfall reached $\approx 18, 10$, and 13 mm and became constant in the plants at cotyledon, three-leaf, and six-leaf stages, respectively. Upper limit of $Di$ ($d_{\text{max}}$) was higher in the plants infected at cotyledon stage than in the plants infected at the other two stages. Effects of plant growth stage on the rate of change with respect to $R$ (parameter $k$) were lower in the plants infected at cotyledon stage than in the plants infected at the other two stages. This value was $0.18, 0.37$, and 0.35 for the plants infected at cotyledon, three-leaf, and six-leaf stages, respectively.

**TABLE 7.** Parameter estimates and other statistics for six nonlinear models to describe the mean blackleg severity as a function of total rainfall per week ($R$) and average maximum temperature per week ($T_{\text{max}}$) in canola cv. Westara

<table>
<thead>
<tr>
<th>Model</th>
<th>GS</th>
<th>MSE</th>
<th>$R^2$</th>
<th>AIC</th>
<th>$a$</th>
<th>$n$</th>
<th>$p$</th>
<th>$k$</th>
<th>$m$</th>
<th>$g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 0</td>
<td>0.41</td>
<td>0.72</td>
<td>11.7</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.25</td>
<td>0.16</td>
<td>18.75</td>
</tr>
<tr>
<td>1, 03</td>
<td>0.19</td>
<td>0.69</td>
<td>22.3</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.40</td>
<td>0.17</td>
<td>20.31</td>
</tr>
<tr>
<td>1, 06</td>
<td>0.16</td>
<td>0.83</td>
<td>24.9</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.12</td>
<td>0.04</td>
<td>18.67</td>
</tr>
<tr>
<td>2, 0</td>
<td>0.35</td>
<td>0.85</td>
<td>19.4</td>
<td>4.29</td>
<td>0.07</td>
<td>0.16</td>
<td>0.15</td>
<td>0.20</td>
<td>0.16</td>
<td>18.78</td>
</tr>
<tr>
<td>1, 03</td>
<td>0.08</td>
<td>0.92</td>
<td>40.3</td>
<td>2.77</td>
<td>0.01</td>
<td>0.03</td>
<td>0.07</td>
<td>0.24</td>
<td>0.04</td>
<td>18.78</td>
</tr>
<tr>
<td>1, 06</td>
<td>0.08</td>
<td>0.95</td>
<td>40.1</td>
<td>2.65</td>
<td>0.19</td>
<td>0.17</td>
<td>0.17</td>
<td>0.04</td>
<td>0.04</td>
<td>18.78</td>
</tr>
<tr>
<td>3, 0</td>
<td>0.41</td>
<td>0.72</td>
<td>11.6</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.39</td>
<td>0.16</td>
<td>18.78</td>
</tr>
<tr>
<td>1, 03</td>
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<td>0.68</td>
<td>22.1</td>
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<td>...</td>
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<td>0.59</td>
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</tr>
<tr>
<td>1, 06</td>
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<td>0.81</td>
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<td>...</td>
<td>...</td>
<td>...</td>
<td>0.19</td>
<td>0.06</td>
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<td>0.84</td>
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<td>4.26</td>
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<td>0.16</td>
<td>0.30</td>
<td>0.13</td>
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</tr>
<tr>
<td>1, 03</td>
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<td>0.91</td>
<td>38.6</td>
<td>2.76</td>
<td>0.006</td>
<td>0.03</td>
<td>0.07</td>
<td>0.39</td>
<td>0.11</td>
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<tr>
<td>1, 06</td>
<td>0.08</td>
<td>0.95</td>
<td>40.1</td>
<td>2.39</td>
<td>0.20</td>
<td>0.17</td>
<td>0.18</td>
<td>0.08</td>
<td>0.04</td>
<td>...</td>
</tr>
<tr>
<td>5, 0</td>
<td>0.41</td>
<td>0.72</td>
<td>11.8</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.32</td>
<td>0.16</td>
<td>18.78</td>
</tr>
<tr>
<td>1, 03</td>
<td>0.19</td>
<td>0.69</td>
<td>22.2</td>
<td>...</td>
<td>...</td>
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<td>...</td>
<td>0.48</td>
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</tr>
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<td>1, 06</td>
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<td>0.82</td>
<td>23.9</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.15</td>
<td>0.05</td>
<td>18.82</td>
</tr>
<tr>
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<td>0.85</td>
<td>19.1</td>
<td>4.26</td>
<td>0.07</td>
<td>0.16</td>
<td>0.16</td>
<td>0.25</td>
<td>0.11</td>
<td>...</td>
</tr>
<tr>
<td>1, 03</td>
<td>0.08</td>
<td>0.92</td>
<td>39.6</td>
<td>2.76</td>
<td>0.01</td>
<td>0.03</td>
<td>0.06</td>
<td>0.30</td>
<td>0.09</td>
<td>...</td>
</tr>
<tr>
<td>1, 06</td>
<td>0.08</td>
<td>0.94</td>
<td>39.6</td>
<td>2.45</td>
<td>0.19</td>
<td>0.17</td>
<td>0.17</td>
<td>0.06</td>
<td>0.04</td>
<td>...</td>
</tr>
</tbody>
</table>

* Model parameters are maximum potential for disease severity ($Ds$) at given temperature $T$ ($a$), rate of change of $Ds$ with respect to $T$ ($n$), rate of change of $Ds$ as $T$ increases ($p$), rate of disease change with respect to increasing moisture factor ($k$). Mean square error (MSE), pseudo-coefficient of determination ($R^2$), and Akaike’s Information Criterion (AIC) are presented for each model.

* Numbers in parentheses correspond to the standard error.
growth stages of *B. napus* cv. Westar were defined. To describe $Ds$ as a function of $R$, three known models (monomolecular, LG, and Gompertz) were employed. Weibull and Richards models are more flexible than the three above models used in this study due to their shape parameter, which can accommodate a wide variety of disease. However, using models with a higher number of parameters when the sample sizes are small causes inaccuracy in the parameter estimation or overparameterization. The accuracy of the introduced model for describing $Ds$ in the plants infected at cotyledon, three-leaf, and six-leaf stages based on the combined effects of the two above variables was high. Because pseudo-coefficient of determination ($R^2$) value is not always reliable in estimating goodness-of-fit for nonlinear models (25), other statistics such as AIC, MSE, and plots of residuals and estimates intervals were used. Reasonable estimates of parameters and their standard errors were also considered to choose appropriate models. Based on these five criteria, the results indicated that a significant proportion of variability in $Ds$ was accounted for by the independent variables of $R$ and $T_{\text{max}}$. The mean $Ds$ of plants infected at all three growth stages increased with increase in the total rainfall per week. At rainfall values totaling $>15$ mm/week, $Ds$ became constant in the plants infected at cotyledon and three-leaf stages. Unlike the above two growth stages, $Ds$ increased exponentially when $R$ exceeded $15$ mm in plants infected at the six-leaf stage. However, the rate of increase of $Ds$ with respect to $R$ (parameter $k$) is much less than in plants infected at the two other stages (this value is 0.04 for plants infected at the six-leaf stage compared with 0.2 and 0.24 for plants infected at the cotyledon and three-leaf stages, respectively). Increasing mean $Ds$ with increasing precipitation can be attributed to increase in splash dispersal of pycnidiospores (14,37,42,43). The correlation between pycnidiospores dispersal and rainfall events has been observed in other pathogens producing rain-splashed spores (2,3). However, the constant level of mean $Ds$ whenever total rainfall per week exceeded $15$ mm may be due to spore removal from the source (pycnidia) and spore wash-off from infection sites and shaking caused by the impact of the drops (8,21,24). It was also shown that germination of pycnidiospores of *L. maculans* under controlled conditions was strongly inhibited by a high density of the spore suspension (38). Based on this finding, concentration of pycnidiospores on the plants with a higher number of leaves and larger leaf area will be less than those plants with a lower number. This might be the reason that $Ds$ in plants infected at the six-leaf stage increased constantly after rainfall exceeded $15$ mm compared with plants at the other two stages.

Parameter $a$ explained the resistance of the plants infected at different growth stages to *L. maculans*. This parameter was higher in the plants infected at cotyledon stage than plants infected at the other two stages. This can be attributed to the larger size of the plants at six-leaf stage compared with cotyledon stage, because the fungus takes longer to grow from the leaf to the crown in larger plants than in smaller (younger) plants (31); or it may be explained by age-related resistance (ARR), as was observed in *Arabidopsis* in response to *Pseudomonas syringae* pv. *tomato* (19,32).

Average maximum temperature also showed a negative relationship with the mean $Ds$ of the plants infected at cotyledon and six-leaf stages in both years. $Ds$ decreased slightly at $>22$ and $18^\circ \text{C}$ for the plants infected at cotyledon and six-leaf stages (except at temperatures between 18 and 20°C for plants infected at the six-leaf stage, in which the slope was high). However, no correlation was observed between this weather variable and $Ds$ for plants infected at the three-leaf stage. A number of studies showed that the optimum temperature for canker developments (ascospore as main source of inoculum) was at temperatures of 20 to $24^\circ \text{C}$ and development of disease decreased at 4 to $8^\circ \text{C}$ and did not develop at 28 to $30^\circ \text{C}$ (4,14,23,40). Li et al. (22) indicated that crown canker severity of the Australian cultivars inoculated with pycnidiospores of virulent isolates of blackleg at different growth stages under controlled conditions were higher at a temperature regime of 18 and $24^\circ \text{C}$ (night and day, respectively) compared with 11 and $18^\circ \text{C}$. Also, Sosnowski et al. (36) reported that the greatest number of leaf lesions was observed at a temperature regime of 18 and 15°C (day and night) in different Australian cultivars inoculated with pycnidiospores of virulent isolates of blackleg under controlled conditions. They also mentioned that the highest canker development was at a temperature regime of 23 and $20^\circ \text{C}$, Vanniasingham and Gilligan (38) showed

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**Fig. 2.** Response surface for model 2, disease severity ($Ds$) = $2.77^{0.03}(1 - r)^{-0.04}[1 - 0.7 \exp(-0.24R)]$, describing the influence of total rainfall per week ($R$) and average maximum temperature per week ($T_{\text{max}}$) on the mean blackleg severity of the canola plants infected at three-leaf stage.

**Fig. 3.** Response surface for model 2, disease severity ($Ds$) = $2.65^{0.19}(1 - r)^{-0.17}[1 - 0.7 \exp(-0.04R)]$, describing the influence of total rainfall per week ($R$) and average maximum temperature per week ($T_{\text{max}}$) on the mean blackleg severity of the canola plants infected at six-leaf stage.
that the optimum temperature for germination of pycnidiospores and production of pycnidia of *L. maculans* was 20 to 25°C. In another study, they showed that the rating of pycnidioспорe germination at 25°C was slightly lower than at 20°C. The *T* max value for development of *Ds* for plants infected at the six-leaf stage was underestimated. It could partly be explained by the fact that a constant temperature regime was employed in growth cabinets (22) compared with fluctuating temperature under field conditions in our study (*T* max in most warm weeks exceeded 30°C). Nevertheless, underestimation of *T* max for development of *Ds* for plants infected at the six-leaf stage and lack of correlation of this variable with development of *Ds* for plants infected at the three-leaf stage imply that more detailed data sets are needed to precisely evaluate the effect of this variable on the mean *Ds* under field conditions.

*Di* also correlated with total rainfall per week as expected. For three plant growth stages, *Di* increased with increase in total rainfall per week. *Di* then became constant when rainfall reached 20, 15, and 10 mm in plants infected at cotyledon, three-leaf, and six-leaf stages, respectively. The upper asymptote of *Di* of the plants infected at cotyledon stage (100%) was higher than the plants infected at the two other stages. It is interesting that *Di* is ≈60, 45, and 30% in the plants infected at cotyledon, three-leaf, and six-leaf stages, respectively, before rainfall begins. Constant mean relative humidity (70 to 93%) during most weeks in both years which may cause release of pycnidiospore (13,41) and subsequent direct contact of plant leaves with the sources of inoculum (14) could probably explain infection of the plants before rain events.

Introducing a nonlinear weather-based model to predict mean *Ds* and *Di* under field conditions based on pycnidiospores of *L. maculans* as primary inoculum is an important accomplishment of this research. These models showed that the timing of infection in relation to the growth stage of the plant is an important factor in development of severe stem canker. This study also confirmed the main effect of rainfall in development of disease caused by pycnidiospores of *L. maculans*. The results of this research could be used to develop a comprehensive model to evaluate epidemics of blackleg based on both ascospores and pycnidiospores as sources of inoculum. Additional years of data collection should improve model fit and evaluation of introduced models and contribute to the development of a more robust predictive model.

**ACKNOWLEDGMENTS**

We thank NSERC, Canada and ARDI Manitoba for its financial contributions to carry out the work; and P. Parks and A. Iverson for their assistance in the field.

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**Table 8. Parameter estimates and other statistics for three nonlinear models to describe blackleg incidence as a function of total rainfall per week (*R*) in canola cv. Westara**

<table>
<thead>
<tr>
<th>Model</th>
<th>GS</th>
<th>MSE</th>
<th>$R^2$</th>
<th>AIC</th>
<th>$d_{\text{max}}$</th>
<th>$B$</th>
<th>$K$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomolecular</td>
<td>1, 0</td>
<td>85.04</td>
<td>0.79</td>
<td>62.8</td>
<td>102.7 (5.7)</td>
<td>0.43 (0.06)</td>
<td>–0.13 (0.06)</td>
</tr>
<tr>
<td></td>
<td>1, 03</td>
<td>187.6</td>
<td>0.67</td>
<td>73.9</td>
<td>94.6 (5.6)</td>
<td>0.51 (0.10)</td>
<td>–0.25 (0.14)</td>
</tr>
<tr>
<td></td>
<td>1, 06</td>
<td>179.1</td>
<td>0.77</td>
<td>73.3</td>
<td>89.8 (5.9)</td>
<td>0.66 (0.09)</td>
<td>–0.20 (0.09)</td>
</tr>
<tr>
<td>Logistic</td>
<td>1, 0</td>
<td>83.2</td>
<td>0.80</td>
<td>62.5</td>
<td>102 (4.8)</td>
<td>0.73 (0.15)</td>
<td>0.17 (0.07)</td>
</tr>
<tr>
<td></td>
<td>1, 03</td>
<td>178.3</td>
<td>0.70</td>
<td>73.2</td>
<td>94.5 (4.9)</td>
<td>1.03 (0.38)</td>
<td>0.37 (0.19)</td>
</tr>
<tr>
<td></td>
<td>1, 06</td>
<td>178.0</td>
<td>0.77</td>
<td>73.1</td>
<td>88.9 (5.2)</td>
<td>1.75 (0.67)</td>
<td>–0.31 (0.15)</td>
</tr>
<tr>
<td>Gompertz</td>
<td>1, 0</td>
<td>82.2</td>
<td>0.79</td>
<td>62.7</td>
<td>102.3 (5.2)</td>
<td>0.56 (0.06)</td>
<td>–0.15 (0.09)</td>
</tr>
<tr>
<td></td>
<td>1, 03</td>
<td>182.4</td>
<td>0.68</td>
<td>73.5</td>
<td>94.5 (5.2)</td>
<td>0.71 (0.19)</td>
<td>–0.31 (0.16)</td>
</tr>
<tr>
<td></td>
<td>1, 06</td>
<td>178.0</td>
<td>0.77</td>
<td>73.1</td>
<td>89.2 (5.4)</td>
<td>1.05 (0.26)</td>
<td>–0.27 (0.12)</td>
</tr>
</tbody>
</table>

* Logistic model parameters are: maximum potential for disease severity or incidence at given rainfall ($d_{\text{max}}$), constant of integration ($B$), and rate of disease change with respect to increasing moisture factor ($k$). Mean square error (MSE), pseudo-coefficient of determination ($R^2$), and Akaike’s Information Criterion (AIC) are presented for each model.
* Growth stages: cotyledon stage (1, 0), three-leaf stage (1, 03), and six-leaf stage (1, 06).
* Numbers in parentheses correspond to the standard error.

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**Fig. 4.** Models disease incidence (*Di*) = *f*(R) = 102[1 + [0.74(exp[–0.18R])]]. *Di* = *f*(R) = 94.45[1 + [1.04(exp[–0.36R])]]. and *Di* = *f*(R) = 88.85[1 + [1.77(exp[–0.35R])]], describing the influence of total rainfall per week (*R*) on the predicted blackleg incidence of canola plants infected at cotyledon (dashed line), three-leaf (solid line), and six-leaf (dotted line) stages. Symbols represent observed mean of blackleg incidence for 15 weeks in 2 years for the plants infected at cotyledon (■), three-leaf (▲), and six-leaf (●) stages.

**LITERATURE CITED**