Pycnidiospores of *Leptosphaeria maculans* as primary inoculum and their infection on canola at different growth stages to develop a predictive model

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Abstract

Leptosphaeria maculans causes blackleg disease of canola (*Brassica napus* L.). Canola plants at cotyledon, 3-leaf and 6-leaf stages were placed for one week in a blackleg-infested canola field during the summers of 2004 and 2005. The plants were then returned to the greenhouse and grown to maturity to rate disease severity (DS) on infected plants. Very high numbers of pycnidiospores were trapped on spore traps each week. Examination of stubble also showed that pycnidia were the predominant spore-bearing structures. Two-year old blackleg-infected stubble buried in mesh bags and re-isolated from field also showed a significantly higher number of pycnidia being formed on stubble. Results confirmed that pycnidiospores can be considered as primary inoculum. There were no significant differences in DS at different growth stages. The results indicated that the disease severity can be modeled for plants infected at three different stages with regard to pycnidiospores as primary inoculum and epidemiologically important environmental factors. Among environmental factors only rainfall was strongly associated with DS. The model to predict the DS (Y) of plants infected at cotyledon stage was based on total rainfall (X) per week was $Y=-0.004X^2 + 0.2X + 1.45$ ($R^2=0.63$). The models for plants infected at 3-leaf and 6- leaf stages were $Y=-0.003X^2 + 0.15X + 1.13$ ($R^2=0.79$) and $Y=-0.0014X^2 + 0.1X + 0.69$ ($R^2=0.55$) respectively. There was a linear relationship between pycnidiospores concentration (m^3/h) and total rainfall received per week.

Key words: canola, blackleg, *Leptosphaeria maculans*, modeling, pycnidiospores, growth stage

Introduction

Several diseases cause significant yield losses and remain a constant threat to oilseed production. Blackleg, caused by *Leptospheria maculans* (Desm) Ces & de Not (anamorph:*Phoma lingam*)(Tode ex Fr.)(Desm) is a devastating disease of *Brassica* species in many areas of the world (West et al , 2001). Knowledge on the epidemiology of blackleg is essential to the development of disease control practices. Some studies have been done on different epidemiological aspects of blackleg (Xi et al, 1991; Hall 1992; Zhou et al, 1999; West et al, 2001; Guo et al., 2005) on oilseed rape. Also, some studies indicating the importance of ascospores in dispersal of disease as primary inoculum have been done in Europe, Australia and Canada (McGee 1977; Hall, 1992; Guo and Fernando, 2005; West et al.2001) but only a few reports have mentioned the role of pycnidiospores as primary inoculum (Hall, 1992 and Williams, 1992). There are also a few models to predict the ascospore release at the beginning of the growing season (Salam, et al, 2007) There is also a study to introduce models to predict latent period and pycnidia production based on environmental factors in controlled conditions (Vannisingham and Gilligan, 1989). Barbetti (1979) studied the role of pycnidiospors of *L. maculans* in the spread of blackleg in rape using artificial inoculation in isolated fields. To our knowledge, there is no study on the role of pycnidiospores as primary inoculum under field conditions and investigation on influence of important environmental factors on predicting the disease severity based on pycnidiospores as primary inoculum. Therefore the objectives of this work are (1) to investigate the role of pycnidiospores as primary inoculum and (2) develop a model to predict the disease severity of blackleg of canola on different stages of growth.

Materials and methods

During the summer seasons of 2004 and 2005, Plants at cotyledon, 3-leaf and 6-leaf stages were each placed for one week in a blackleg-infested canola field in Carman, MB. Placements of pots were carried out for a total of 5 weeks for the first and 10 weeks for the second year. The plants were then returned to the greenhouse and grown to maturity to rate disease severity on infected plants. Stem disease severity (DS) was assessed using a 0-5 scale at maturity. Spores were trapped using a 7-day Burkard spore trap and 5 Rotorod spore traps. To examine formation of pseudothecia or pycnidia on stubble as primary inoculum, one and two-year-old stubble were put in mesh bags and placed in fields. Also, infected stubbles were selected from the field each week to examine for the presence of pycnidia or pseudothecia. The weather records of daily and hourly precipitation, temperature, relative humidity and wind speed were accessed through Carman weather station located in Carman. Several environmental weather factors; total rainfall per week, average minimum and maximum temperature, average relative humidity, and wind speed were used to develop a model to predict the DS on *B. napus* (canola).The relationship between total rainfall per week, temperature, wind speed was described using multiple regression procedures. Linear and quadratic effects of rainfall and temperature (average, minimum and maximum) and also relative humidity and temperature and their interactions on DS were tested. Multiple regression procedures were done separately between rainfall and relative humidity with temperature. Parameters not significantly different from zero (P<0.05) were omitted from the equation unless higher-degree terms of the corresponding variable were associated with significant parameters. This stepwise procedure was conducted until a simple model with the highest coefficient of determination (R2) was derived. The coefficient of determination and the pattern and distribution of residuals for each year were examined and the coefficient for combined data calculated.

Results

Primary inoculum

To examine the primary inoculum in disease, three different approaches were used.

Table 1- The number of pycnidia and Pseudothecia counted from 1-year-old stubble in 2004 and 2005.

Year	Stubble	Pycnidia	Im. Pseudothecia	M. Psuedothecia
2004	100	8950	3	0
2005	200	19436	15	1

Table2 -The number of pycnidia and pseudothecia counted from 2-year-old stubbles

Year	Stubble	Pycnidia	Im. Pseudothecia	M. Psuedothecia
2005	200	14351	35	5

Table3-The number of pycnidiospores and ascopores counted from Burkhard and roror-rod sporetrap

Year	Pycnidiospore	Ascospore
2004	2321	1
2005	3846	3

Development of the model

The results showed that DS of all three plant stages are significantly associated with the total rainfall/week. Minimum, maximum and average temperature, and wind speed did not show any relationship with the DS. R^2 was 0.63, 0.79 and 0.55 for models describing DS (Y) based on the total rainfall per week at cotyledon, 3-Leaf and 6-Leaf stage respectively. The model for all three stages is linear in 2004 and quadratic in 2005. In general, the combined model of both years indicated that DS increases as total rainfall/week (X) increases till 10 to15mm but it shows a constant trend or declines after 15mm rainfall (Fig 1 - 3). Model for three different stages of canola in 2004, 2005 and combined model for these two years are shown in table 4.

Table 5. Introduced models to predict mean DS for plants infected at different stages in canola cv. Westar

Growth Stage		Model
	Model in 2004	$Y= 0.19X + 1.69$ (R^2=0.93)
Cotyledon	Model in 2005	$Y= - 0.0018X^2 +0.12X + 1.29$ (R^2=0.80)
	Final Model	$Y=-0.004X^2 + 0.2X +1.45$ (R^2=0.63)
	Model in 2004	$Y=0.13X + 1.05$ (R^2=0.92)
3-Leaf	Model in 2005	$Y=-0.002X^2 +0.11X+1.28$ (R^2=0.71)
	Final Model	$Y=-0.003X^2 + 0.15X + 1.13$ (R^2=0.79)
	Model in 2004	$Y=0.067X + 0.98$ (R^2=0.56)
6-Leaf	Model in 2005	$Y= -0.0017X^2 + 0.12X + 0.29$ (R^2=0.57)
	Final Model	$Y=-0.0014X^2 +0.1X + 0.69$ (R^2=0. 55)

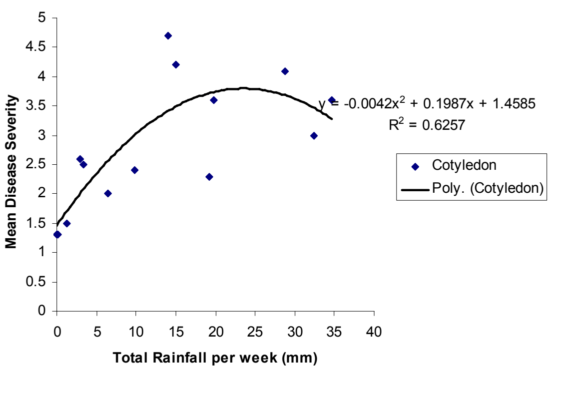

Fig.1- The relationship between mean DS and total rainfall/wk in plants infected at cotyledon stage in years 2004 and 2005 (combined data).

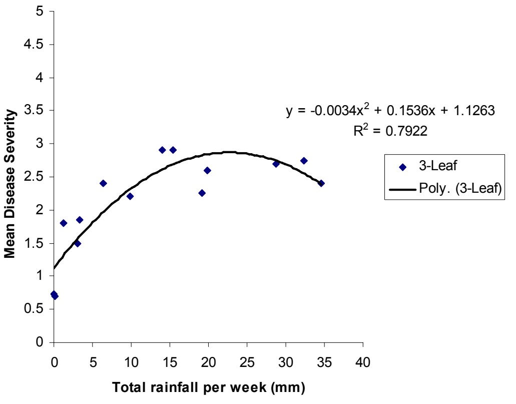

Fig.2- The relationship between DS and total rainfall/wk in plants infected at 3-Leaf stage in years 2004 and 2005 (combined data).

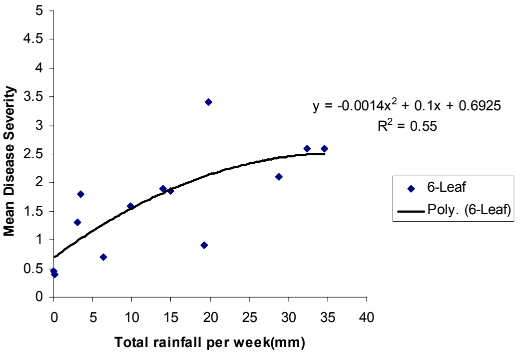

Fig.3- The relationship between DS and total rainfall/wk in plants infected in 6-Leaf stage in years 2004 and 2005 (combined data).

These results also demonstrated that there is a linear relationship between total rainfall/wk and pycnidiospore concentration (m^3/hr). The coefficient of determination was 0.82, 0.84 and 0.61 in 2004, 2005 and combined data for two years respectively (Fig.4).

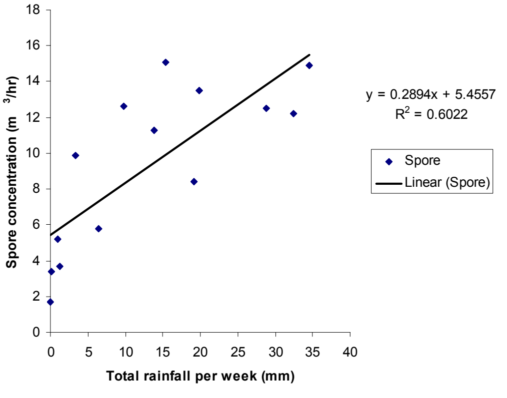

Figure 4. Relationship between spore concentration (m^3/hr) and total rainfall/wk for years 2004 and 2005.

Discussion

Our three-way study convincingly showed that pycnidiospores can be primary inoculum in blackleg disease of canola. Williams (1992) mentioned about the role of pycnidiospore as primary inoculum. Hall (1992) concluded that pycnidia could play a role as primary and secondary inoculum. He also mentioned that if the environmental condition is suitable, they can contribute to epidemics of the disease. Guo and Fernando (2005) illustrated that difference in ascospore and pycnidiospore release in western Canada in the beginning of the growing season is only 5 days. It confirms that both pycnidiospores and ascospores can be primary inoculum. To predict the DS, total precipitation per week with minimum, maximum and average temperature were used in multiple regression analysis separately. The results showed that mean disease severity can be explained by total precipitation per week and temperature is not significantly important (Fig.1, 2 and 3). However, Vanniaingham and Gilligan (1989) mentioned that temperature can contribute in germination and sporulation of pycnidia but this relationship is quadratic. In fact, at 15-25˚C, spore germination is constant. That is why there was no relationship between temperatures and DS seen in our study. The relationship of the mean DS with total rainfall/wk is linear whenever the rainfall is less than 15 mm but this relationship converted to quadratic equation when the rainfall is more than 15 mm. The quadratic trend of DS in relationship with rain intensity was shown by Madden (1997). The biology of *L. maculans* showed that dispersal of pycnidiospores is associated to rainfall. On the other hand, rainfall has a linear relationship with spore concentration (Fig. 4). This means that with increase in total rainfall per week spore concentration increases (Madden, 1997). The quadratic nature of the relationship of mean DS with total rainfall/wk can be due to spore removal from the source (Pycnidia) and spore wash-off from infection site (leaf) (Madden 1997). This may explain why disease severity is constant or decrease as rainfall and spore concentration in turn increases.

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