Daily and seasonal spore dispersal by *Mycosphaerella pinodes* and development of mycosphaerella blight of field pea

Jin Xiu Zhang, W.G. Dilantha Fernando, and Allen G. Xue

Abstract: Daily and seasonal spore dispersal of *Mycosphaerella pinodes* (Berk & Bloxam) Vestergren and the relationship of spore dispersal to distance and disease severity were investigated in a pea field in western Canada during two consecutive years. Most ascospores were released in response to rain events, during the first 23–27 d after the inoculum source area was infested with naturally diseased pea residue, whereas most pycnidiospores were trapped during the first 20 d. For both ascospores and pycnidiospores, the highest peaks of spore release occurred during the first 14–20 d after infestation. Few spores were trapped after day 27 after infestation. Daily peaks of ascospore and pycnidiospore release occurred between 1700 and 0400 hours. Most ascospores were released 1–2 d after a rain event and the largest peak appeared the first day after rain. In contrast, most pycnidiospores were released on the same day as rain occurred or the following day. The release of both spore types was associated with rainfall events ≥2 mm during the first 27 d after infestation but not with rainfall events after 27 d. Ascospore density was negatively correlated with distance from the inoculum source ($r \leq -0.92$) and positively related to the disease severity ($r \geq 0.92$). Disease severity decreased with increasing distance from the inoculum source. The patterns of spore dispersal associated with rain events have practical applications in the disease forecasting and spraying of chemicals to control the disease.

Key words: field pea, mycosphaerella blight, rainfall, spore release.

Résumé : L'auteur a étudié la dispersion quotidienne et saisonnière du *Mycosphaerella pinodes* (Berk & Bloxam) Vestergren ainsi que la relation entre la dispersion des spores avec la distance et la sévérité de la maladie; cette étude a été conduite dans un champs de pois de l'ouest canadien, au cours de deux années consécutives. La plupart des ascospores ont été relâchées en réaction à la pluie, au cours des premiers 23–27 jours, une fois que la région source d'inoculum fut infestée avec des résidus de pois naturellement malades, alors que les pycnidiospores ont été captées au cours des 20 premiers jours. Pour les ascospores aussi bien que pour les pycnidiospores, le pic le plus élevé de relâchement des propagules est survenu du 14–20 jours après l'infestation. Après le 27 jour de l'infestation, peu de spores ont été capturées. Les pics quotidiens du relâchement des ascospores et des pycnidiospores sont survenus entre 17 h 00 et 04 h 00. La plupart des ascospores ont été relâchées 1–2 jours après la pluie et le pic le plus élevé est arrivé le premier jour après la pluie. Au contraire, la plupart des pycnidiospores ont été relâchées le jour même où la pluie est survenue, ou le jour suivant. Le relâchement des deux types de propagules est associé à des précipitation de pluie ≥2 mm, au cours des 27 premiers jours après l'infestation, mais non avec les précipitations survenues après le 27 jour. La densité des ascospores est négativement corrélée avec la distance de la source d'inoculum ($r \leq -0{,}92$) et positivement reliée à la sévérité de la maladie ($r \geq 0{,}92$). La sévérité de la maladie diminue avec la distance de la source d'inoculum. Les patrons de dispersion des spores, associés aux précipitations, trouvent une application pratique dans la prédiction des infestations et pour l'application des traitements chimiques destinés à maîtriser la maladie.

Mots clés : pois des champs, brûlure ascochitique, précipitation, relâchement de spores.

Introduction

Mycosphaerella blight, caused by *Mycosphaerella pinodes* (Berk & Bloxam) Vestergren (asexual stage *Ascochyta pinodes* L.K. Jones), is the most important disease of field pea (*Pisum sativum* L.) in western Canada (Rashid et al. 1997; Xue et al. 1996). Average yield losses caused by the disease in western Canada were estimated at 10%, and losses >50% have been reported (Xue et al. 1995). The pathogen produces pseudothecia in its sexual stage, discharging wind-borne ascospores, and pycnidia containing splash-dispersed pycnidiospores in the asexual stage (Gregory et al. 1959; Lawyer 1984). Both pycnidiospores splashed by rain and ascospores borne by wind are considered the primary sources for inoculum in the field (Lawyer 1984). In the Netherlands, ascospores may be discharged from pseudo-

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thecia on pea residues and infect volunteer plants in the fall, then overwinter to become the primary source of inoculum in the subsequent spring (Kerling 1949). A study in Australia (Carter and Moller 1961) demonstrated that ascospores can be released from pseudothecia in residue and senescent plant tissues of the current pea crop after flowering. Pseudothecia produced on dead parts of growing pea plants are usually abundant, while pycnidia are often scarce in lesions (Hare and Walker 1945). Temperature and moisture affect pycnidial formation and spore germination of *M. pinodes*. Optimum temperatures for pycnidial formation ranged from 20 to 28 °C (Lawyer 1984; Roger and Tivoli 1996*a*) and pycnidial numbers increase with high humidity (Kerling 1949; Lawyer 1984)

Many studies have been conducted on spore release and spore dispersal patterns in ascomyceteous fungi (Smith et al. 1997; Burt et al. 1998; Fernando et al. 2000; Guerin et al. 2001; Rossi et al. 2001; Mondal and Timmer 2002; Mondal et al. 2003). A study on *Mycosphaerella citri* showed that wetting of leaves by rainfall or irrigation triggers release of ascospores within 20–30 min (Mondal et al. 2003). Although longer wetting durations produce pseudothecia more quickly (Mondal and Timmer 2002), shorter wetting periods maximized production of pseudothecia by *M. citri*. Smith et al. (1997) reported that periods of alternate drying and wetting of diseased leaves, and changes in temperature, were more conducive to spore release of *Mycosphaerella fijiensis* on banana than continuous wetting and exposure to constant temperatures.

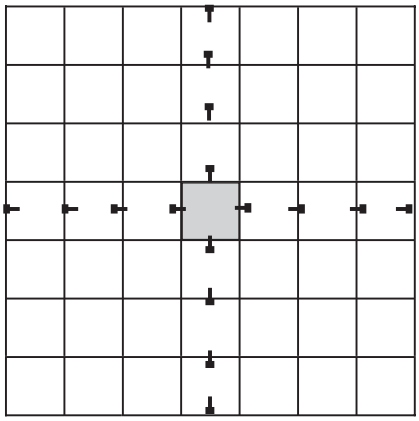
Roger and Tivoli (1996*b*) observed seasonal patterns of spore release by *M. pinodes* using a Hirst spore trap in the field in France. However, little information is available on daily and seasonal dynamics of these spore types, and spore dispersal patterns during rainfall events and dry days in Canada. In addition, little is known about how spore dispersal affects disease in a field. These data are needed for forecasting disease occurrence and developing management strategies. Therefore, the objectives of this study were to determine (*i*) the daily and seasonal patterns of ascospore and pycnidiospore dispersal of *M. pinodes* in western Canadian environmental conditions, (*ii*) spore dispersal patterns during rainfall events and dry days, (*iii*) disease progress over time, and (*iv*) the relationship of spore dispersal and disease severity to distance from the inoculum source.

Materials and methods

Preparation of inoculum

An isolate (MB100) of *M. pinodes* from a blighted field pea plant at the Agriculture and Agri-Food Canada, Morden Research Center (AAFC-MRC) in Morden, Manitoba, in 1999 was used for inoculum. The isolate was cultured on potato dextrose agar (Becton Dickinson and Company, Sparks, Maryland, USA) at 20 °C under a 14-h photoperiod of cool-white fluorescent light. Pycnidiospores were washed from the surface of 14-d-old cultures with sterile water containing 0.05% Tween 20. The resulting spore suspension was filtered through two layers of cheesecloth and adjusted to 10^5 pycnidiospores·mL^{-1} for inoculation in the field. Pea plants (*Pisum sativum* 'Profi') at the 4- to 5-node stage (approximately 2 weeks after planting) in the field were inoculated at a rate of 0.5 mL of the spore suspension per plant using a DeVilbiss model 15 atomizer (DeVilbiss Co., Somerset, Pennsylvania). Disease was allowed to develop in inoculated plants, and at the end of the season, symptomatic plant residue was collected, stored at 3 °C, and used to infest the inoculum source area in 2000. Similarly, symptomatic residues in the inoculum source area were collected at the end of the season in 2000 for inoculation in 2001.

Fig. 1. Schematic diagram of the field layout with spore traps, for spore dispersal and disease assessment. The T-bars indicate sites at which the 16 Rotorod spore samplers were placed. The central shaded square is the inoculum source area infested with diseased pea residue. The 64 nodes of subsquares were the sampling sites for disease assessment.



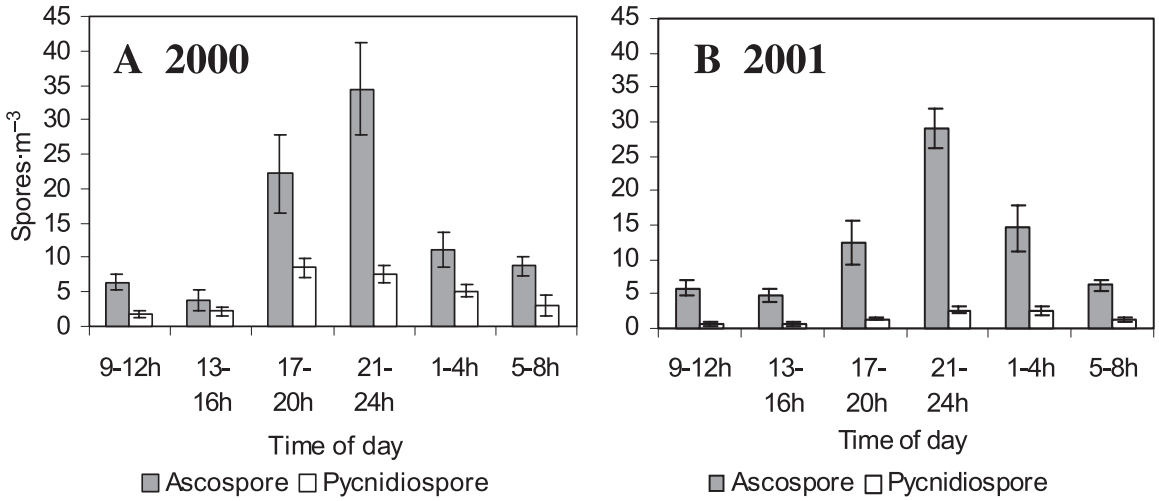
Field site

Experiments were conducted in two separate 42 m × 42 m field plots at AAFC-MRC in 2000 and 2001. The fields selected in both years had never been sown to pea and were separated by at least 500 m. The fields were also separated by at least 300 m from other pea fields to prevent local inoculum from affecting the experiment. 'Profi' was sown in rows spaced 17 cm apart in the experimental fields on 4 May 2000 and 15 May 2001. Diseased pea residue (3 kg) was spread in the central 6 m × 6 m square of the field as an inoculum source on 8 June in both years when pea seedlings were at the 4- to 5-node stage (Fig. 1).

Spore sampling

Daily spore density was used to observe seasonal spore dynamics. To obtain data on the daily density of both ascospores and pycnidiospores in the air, 16 Rotorod spore samplers (Aerobiology Research Laboratories, Nepean, Ontario) were placed around the inoculum source area on 8 June in both years (Fig. 1). Four samplers on each side of the inoculum source area (the easterly, southerly, westerly and northerly sides) were placed 0, 6, 12, and 18 m from each edge of the inoculum source area (Fig. 1). The eight samplers on the northerly and southerly sides, or the eight samplers on westerly and easterly sides of the inoculum source area were located on a line transect running north to south or east to west, respectively, across the field (Fig. 1). Samplers were fixed on steel poles with sampling heads positioned at 1 m above the soil surface. The motors were programmed to rotate at 2400 rpm for 5 min each hour. The rods for collect-

Fig. 2. Daily ascospore and pycnidiospore release pattern. Spores were collected in the inoculum source area by a Burkard spore sampler in 2000 and 2001. Error bars indicate SE of means (n = 25 in 2000, n = 27 in 2001; Duncan's multiple range test, P = 0.05).



ing spores were exchanged once every 24 h. Ascospores and pycnidiospores were identified based on their morphological characteristics. Spore density in the air at each sampling site was calculated for each sampling date and converted into number of ascospores or pycnidiospores per cubic metre of air based on a formula provided by the company manual (Aerobiology Research Laboratories): spores per m^3 = total spores/(rotations per minute × K × sampling period), where K is a conversion factor equal to 0.0197 for the Type I rods used in this study. Daily spore densities for 53 d in both 2000 and 2001 were analyzed for seasonal spore dynamics. Spore sampling was continued until 3 d before harvest.

A Burkard 24-h wind-oriented spore sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, England), with the orifice set 75 cm above the soil surface, was placed in the center of the inoculum source area in both years. Microscopic spore counts were converted to ascospores or pycnidiospores per cubic metre of air per hour. Data on hourly spore densities were collected daily for 25 d (n = 25) in 2000 and 27 d (n = 27) in 2001. To observe daily ascospore and pycnidiospore release patterns, mean hourly spore densities were calculated for six 4-h periods (by averaging the spore counts from each of the following 4-h periods: between 0800 and 1200, 1200 and 1600, 1600 and 2000, 2000 and 2400, 2400 and 0400, and 0400 and 0800 h). Duncan's multiple range test (SAS Institute Inc., Cary, North Carolina) was used for the separation of hourly mean ascospores and pycnidiospores. To investigate hourly dynamics of pycnidiospores and ascospores during dry and rain days, hourly spore density data were collected on 13 June (rain day), 14, 15, and 16 June in 2000, and 18 June (rain day), 19, 20 and 21 June in 2001.

Disease assessment

Disease severity assessment was initiated after the onset of symptoms on plants in the inoculum source area. Disease assessment was carried out twice a week until harvest, for a total of nine observations in each year. For each assessment, the field was divided into 49 squares, each being 6 m × 6 m. Each node of each square was used as a sampling site, resulting in a total of 64 sampling sites spaced 6 m apart (Fig. 1). The same 10 plants at each node were assessed on each assessment date. Percentage of leaf area with symptoms (LAS, including senescent area resulting from disease) was estimated visually using a 0–100% scale. The mean LAS of the 10 plants represented the disease severity at a sampling site. The mean LAS of 64 sites represented the mean disease severity of the field on each assessment date. To analyze the relationship of ascospore density to disease severity at different distances from the inoculated area, mean disease severity of plants at the sites where the Rotorod samplers were placed (Fig. 1) was calculated. The disease progress in response to rainfall was measured using the absolute rate of disease increase (dy/dt) which is defined as the change (dy) in disease (y) with an infinitesimal change (dt) in time (t) (Campbell and Madden 1990).

Relationship between ascospore density and distance from the inoculum source, and disease severity

Mean spore density at each of the four distances (0, 6, 12, and 18 m) from the inoculum source was calculated on each sampling date by averaging the spore density obtained from four spore samplers located at the north, east, south, and west sides of the inoculum source (Fig. 1). The Pearson's correlation coefficients between mean ascospore density (n = 4) and mean disease severity (n = 4) at different distances (n = 4) from the inoculum source were analyzed with statistical software SPSS version 13.0 (SPSS Inc., Chicago, Illinois).

Weather data

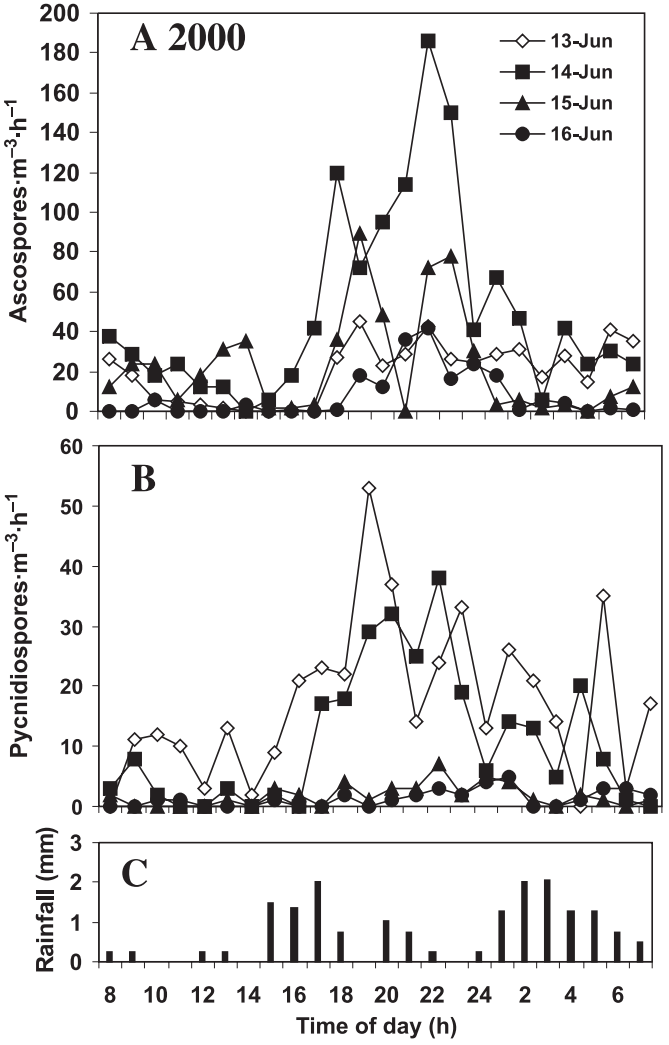
Hourly rainfall during the season was measured by a 0.025-cm rain gauge (Spectrum Technologies Inc., Plainfield, Illinois) installed in the field plot. The general temperature data were obtained from the Environment Canada, Morden Weather Station, Morden, Manitoba, Canada.

Results

Daily dynamics of spore release

The mean hourly density of ascospores in the air varied from 3.5 to 50.8 m^{-3}, and the mean hourly density of

Fig. 3. Patterns of ascospore (A) and pycnidiospore (B) release during and after a rain event in 2000, and the hourly rainfalls during 13 June (C). Times of day indicate 24 h starting at 0800.



pycnidiospores varied from 1.1 to 8.2 m^{-3} in both years. In general, most ascospores or pycnidiospores were trapped between 1700 and 0400 hours in both years (Fig. 2). The largest peak of ascospore release occurred between 2100 and 2400, significantly differing from other periods of a day ($P < 0.01$) (Fig. 2). Fewer ascospores and pycnidiospores were trapped between 0500 and 1600 than during other periods (Fig. 2).

Daily patterns of spore dynamics were also observed during dry and rain days for ascospores and pycnidiospores collected with the Burkard spore sampler in both years. A total precipitation of 18 mm occurred between 0800 on 13 June and 0700 h on 14 June in 2000 (Fig. 3C). Characteristic changes in ascospore density were observed for the next 4 d starting with 13 June (Fig. 3A). During the rainfall on 13 June, the density of ascospores in the air was similar to 16 June (a dry day) from 0800 to 2400, but after 2400 and until 900 on 14 June, ascospore density remained higher (Fig. 3A) than that during the same period on 16 June. Greater densities of ascospores were observed after 1600 on 14 June, presumably in response to earlier rainfall (Fig. 3A). The highest density of ascospores appeared between 2000 and 2400 h on 14 June, 13–17 h after rain. The effects of the rainfall of 13 June on ascospore release persisted until 15 June, the second day after the rain, during which two higher peaks of ascospore release occurred between 1600 and 2400. Density of ascospores decreased greatly during 16 June, the third day after rain, during which only a small peak appeared between 2200 and 2400 (Fig. 3A). In 2001, similar patterns of ascospore release during dry and rain days were observed between 18 and 21 June (Fig. 4A) when it rained 12 mm between 0800 on 18 June and 0700 h on 19 June in 2001 (Fig. 4C). The largest peak of ascospore release appeared on 19 June, the first day after the rainfall (Fig. 4A). More ascospores were trapped during rainfall events and after rain in 2000 (Fig. 3A) than in 2001 (Fig. 4A).

In contrast to ascospore release, fewer pycnidiospores than ascospores were trapped during and after rainfall events. During the rainfall on 13 June 2000, pycnidiospore density remained higher than that on the other 3 d (Fig. 3B). Most pycnidiospores were trapped after 1600 on the rain day, and the highest peak occurred at 1900. Several pycnidiospore release events also appeared between 1800 and 0500 during the night of 14 June, presumably in response to this rainfall. Few pycnidiospores were trapped during 15 and 16 June, the second and third days after the rainfall (Fig. 3B). Similar patterns of daily pycnidiospore release were observed in 2001 (Fig. 4B). More pycnidiospores were trapped during rain events and after rain in 2000 (Fig. 3B) than in 2001 (Fig. 4B).

Seasonal dynamics of spore release

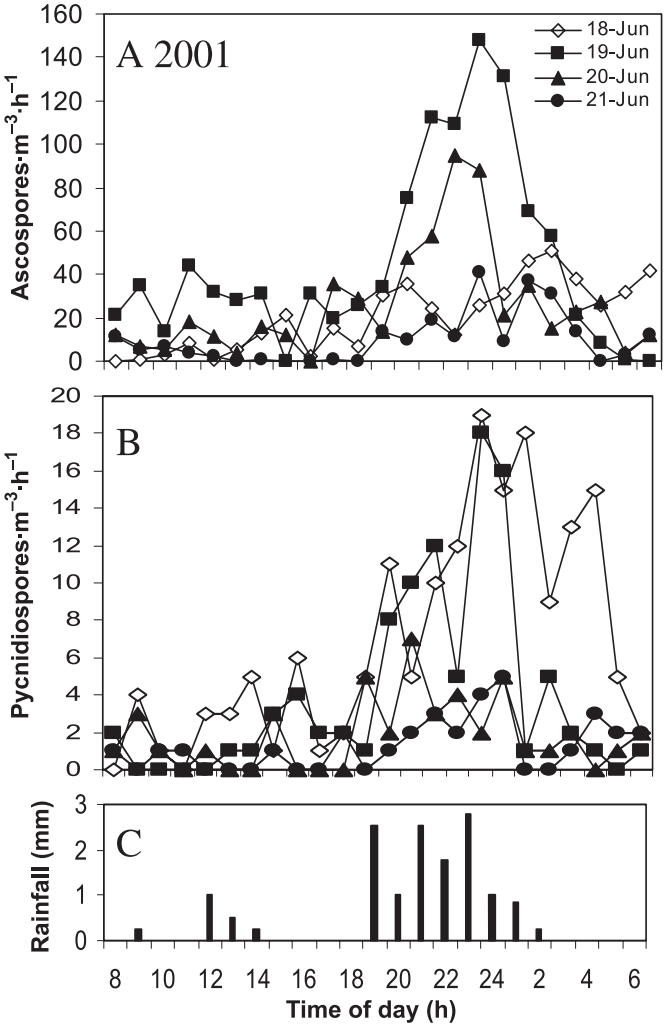
In 2000, pycnidiospores and ascospores had similar release patterns (Fig. 5A). Most ascospores were released during the first 23 d after the inoculum source area was infested with diseased residue, presumably in response to rain events during this period, while pycnidiospores were released within the first 20 d (Fig. 5A). Density of ascospores and pycnidiospores decreased over time. The largest peaks of ascospores and pycnidiospores appeared during the first 20 d after infestation. Few spores were observed in the air beyond 23 d after infestation even though rain events occurred later in the season (Fig. 5A). In 2001, similar patterns of spore release were observed (Fig. 5B) and most ascospores were released during the first 27 d after inoculation, presumably in response to rainfall during this period, whereas pycnidiospores were released during the first 19 d (Fig. 5B). The largest peaks of ascospores and pycnidiospores appeared during the first 14 d after infestation. Few spores were trapped after 27 d after infestation (Fig. 5B).

In general, in both years, a peak release of pycnidiospores occurring during the first 20 d was associated with rainfall ≥2 mm that occurred during the current day, whereas a peak release of ascospores occurring during the first 23 d was associated with rainfall ≥2 mm of the previous day (Figs. 5A and 5B). These results were consistent with daily observations (Figs. 3 and 4), further proving that the release of two spore types was in response to rainfall events.

Development of disease

Disease symptoms were initially observed on plants in the inoculum source area 15 and 22 d after infestation in 2000

Fig. 4. The patterns of ascospore (A) and pycnidiospore (B) release during and after a rain event in 2001, and the hourly rainfall during 18 June (C). Times of day indicate 24 h starting 0800.



(Fig. 6A) and 2001 (Fig. 6C), respectively. Disease severity in the field increased over time in both years (Figs. 6A and 6C). However, the disease severity was greater in 2000 than in 2001 (Figs. 6A and 6C).

The rate of disease increase was related to rainfall during the disease epidemics in both years. In 2000, rainfall during the first 10 d after infestation presumably provided humidity for initiating the disease by day 15 after infestation (Figs. 6A and B). The rainfall between days 16 and 41 presumably provided humidity for disease development so that the amount of disease (Fig. 6A) and the rate of disease increase (Fig. 6B) continued to increase during this period. Although disease increased between days 42 and 48, the rate of disease increase did not, presumably because no rain event occurred during this period. Rainfall between days 49 and 51 resulted in the largest rate of disease increase in the late season (Fig. 6B). In 2001, the disease progress (Fig. 6C) and the rate of disease increase (Fig. 6D) remained at low levels during the first 36 d, which was presumably associated with a lack of rainfall events during this period. Rainfall totaling 25 mm between days 37 and 40 was associated with an increase in disease development (Fig. 6C) and in the rate of disease increase during this period (Fig. 6D). The high rainfall amount between days 48 and 50 also allowed the disease to increase substantially at the end of the season in 2001 (Figs. 6C and 6D).

Relationships between ascospore density, disease severity, and distance from the inoculum source

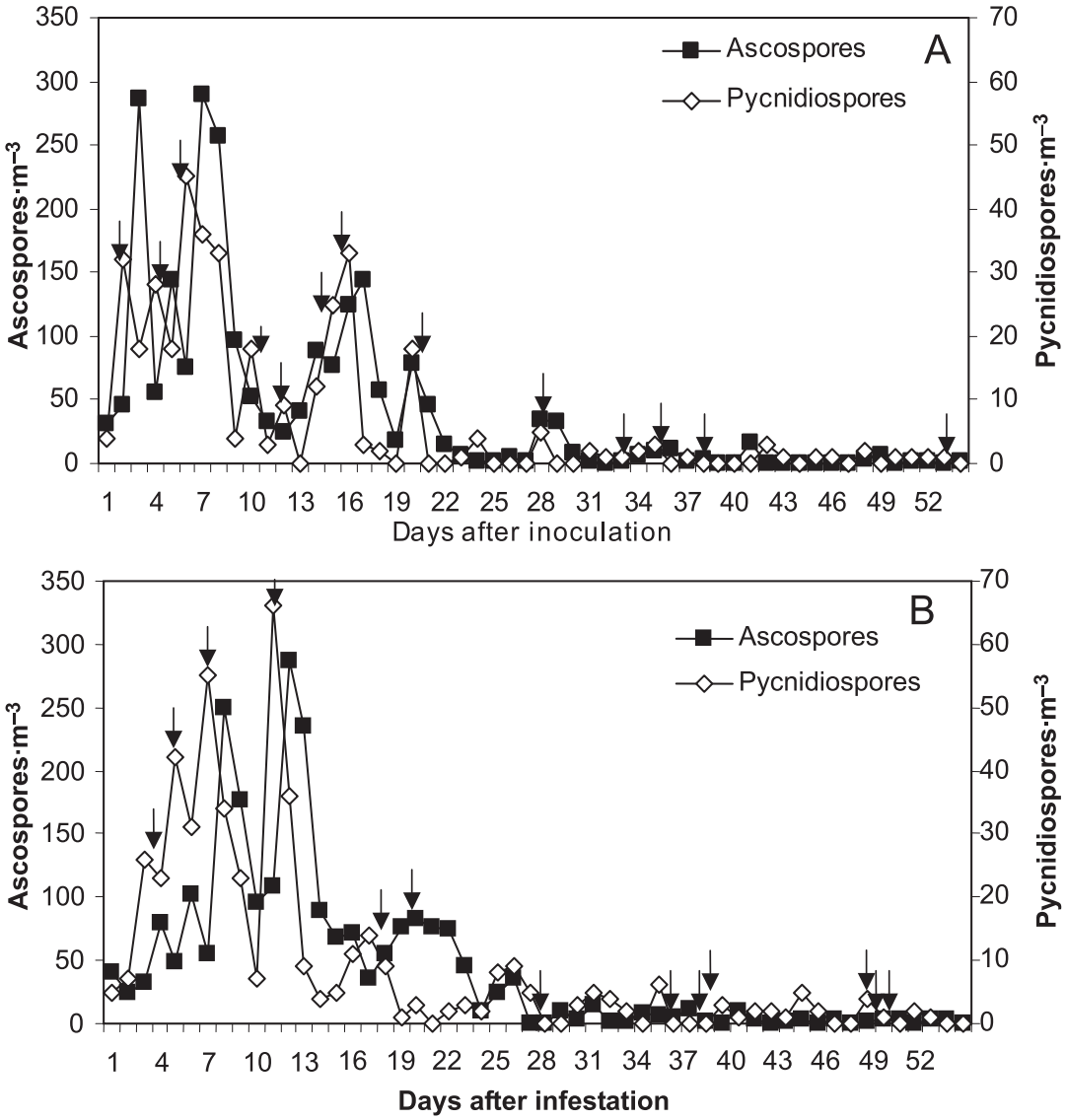
Ascospore density (n = 4) was negatively correlated to the distance (n = 4) from the inoculum source in 2000 (r = –0.98, P = 0.034) and 2001 (r = –0.92, P = 0.042) based on a correlation test (Fig. 7), suggesting that ascospore density significantly decreased with the increase in distance from the inoculum source. Disease severity (n = 4) at varying distances from the inoculum source was positively related to the ascospore density (Figs. 8A and 8B) in 2000 (r = 0.98, P = 0.016) and in 2001 (r = 0.92, P = 0.041), indicating that disease severity in the field was significantly caused by inoculum arising from the inoculum source area. The relationships between pycnidiospore density, disease severity, and distance from the inoculum source were not subjected to regression analysis because of insufficient spore density.

Discussion

Canada is one of the largest pea producers and exporters in the world and mycosphaerella blight is the most important disease affecting pea production. We observed the daily and seasonal patterns of spore dispersal of *M. pinodes* during dry and rainy days in Canada. Unlike the previous research in France (Roger and Tivoli 1996*b*), naturally infested plant residue was used in the current study as the inoculum source to simulate ascospore and pycnidiospore dispersal patterns that would more closely reflect spore dynamics in a natural field. Although seasonal dynamics of spores were observed in France (Roger and Tivoli 1996*b*), we studied spore dispersal during and after rain events and analyzed the daily spore release patterns. In addition, we analyzed the relationship of spore dispersal to dispersal distance and disease severity. We found that the peaks of ascospore and pycnidiospore dispersal during the season occurred within the first 20 d after the plants were inoculated with infested residue and was associated with rain events. The largest dispersal peaks of both types of spores were during the night within 1 to 2 d after a rain event ≥2 mm. Ascospore density was positively correlated to disease severity and negatively to the distance from the inoculum source. These new results would be important for understanding the epidemiology of mycosphaerella blight.

Temporal patterns of *M. pinodes* ascospore and pycnidiospore release in the field showed a similar seasonal periodicity in the two study years, 2000 and 2001, in western Canada. The majority of both spore types were trapped during the first 27 d (seedling stage) after the inoculum source area was infested with diseased residue and few were trapped late in the season. These results do not agree with the results of Roger and Tivoli (1996*b*) and Banniza and Vandenberg (2003), who showed that ascospores and pycnidiospores were discharged throughout the growing season, with the peak release for both spore types toward the end of season. These contrasting results might be due to the

Fig. 5. Seasonal patterns of ascospore and pycnidiospore release in 2000 (A) and 2001 (B). Day 1 was 8 June in both years. Arrows indicate the days with rainfall ≥2 mm.



use of different forms of inoculum sources. Roger and Tivoli (1996*b*) employed barley grains infested by the fungus as an inoculum source to investigate spore disposal patterns in France. They infested the plot with infected grains in March and found fruiting bodies (pycnidia and pseudothecia) after a month. Ascospores were trapped on slides until the end of May, 2 months after the plot was infested with artificially infested barley grains. Obviously, the fungus needed a period to produce pycnidia and pseudothecia, and then spores in their study. This may result in a different spore release pattern, especially at the early stage, than that seen in our study. In addition, once the fungus colonized the barley grain, fruiting bodies could be continuously produced because grains are an ideal nutritional source compared with the dead residue. Production of fruiting bodies on grains may last throughout the whole season until nutrients were used up. Therefore, it is not surprising that more ascospores were trapped in the late season in their study. However, we employed infected pea residue collected from the previous season. Most pycnidia and pseudothecia had matured in the residue before being used for infestation of the inoculum source area in the spring. As soon as the diseased residue was applied to the inoculum source area, both types of spores started to be released when it rained, leading to many spores being trapped at the early stage. Unlike on barley grain, the pathogen could not continuously produce fruiting bodies on the dead residue after spores from those fruiting bodies that had overwintered had been released, resulting in a lack of spores in the late season. Besides different forms of inoculum source, different times of plant infestation with inoculum may also be a factor resulting in differences of spore dispersal patterns in these different studies. In investigating the influence of plant injury on development of *M. pinodes*, Banniza and Vandenberg (2003) sowed pea in early May and infested plots with infected wheat grains in early July, which is the early flowering stage of pea in Canada. Spores were trapped in early August from these plots. A late infestation of plots with artificially infested wheat grains

Fig. 6. Disease progress (A and C) and the rate of disease increase (B and D) over time in 2000 (A and B) and 2001 (C and D).

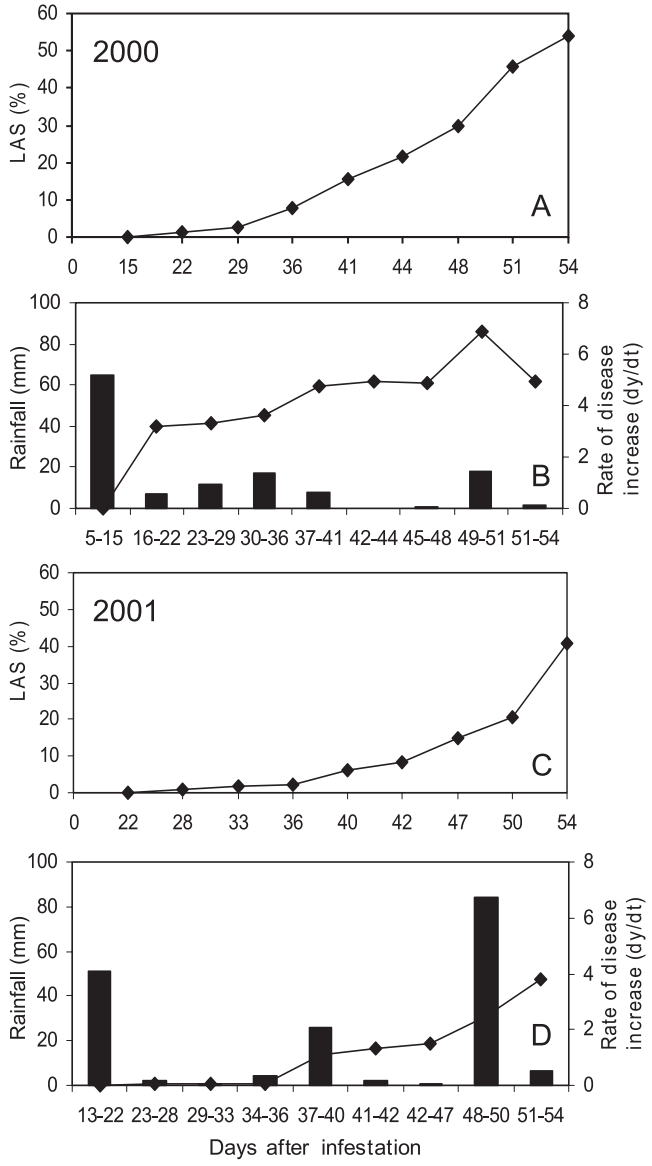


Fig. 7. The gradient of ascospore density over distance from the inoculum source area in 2000 (A) and 2001 (B).

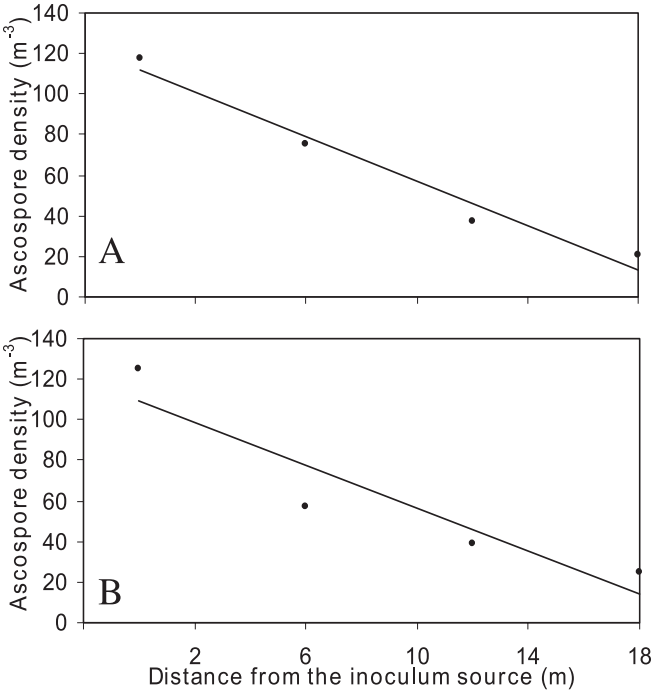
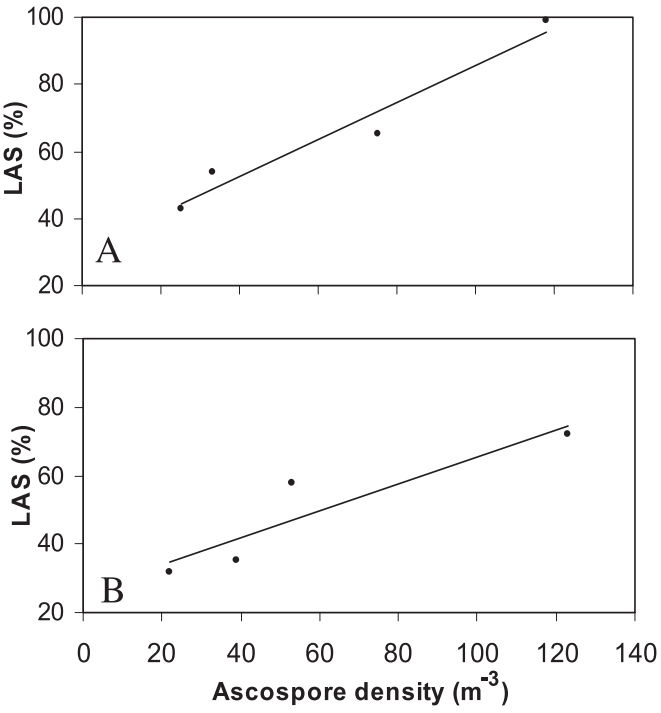


Fig. 8. Relationship between mean leaf area with symptoms (LAS) and the mean ascospore density at four distances from the inoculum source area (0, 6, 12, and 18 m) in 2000 (A) and 2001 (B).



changed spore dispersal patterns and resulted in a late season spore release, which was not seen in our results. A second factor may have been the differences in environmental factors in France compared with those in western Canada. Previous studies have shown that temperature has significant effects on the number of pseudothecia and pycnidia of *M. pinodes* in vitro (Gregory 1968; Lawyer 1984; Roger and Tivoli 1996*a*). The temperatures required for pycnidial formation of *M. pinodes* range from 5 to 28 °C (Gregory 1968; Lawyer 1984; Roger and Tivoli 1996*a*) with the optimum temperatures being 20 to 24 °C (Lawyer 1984). More pseudothecia were produced at 20 °C than at 15 °C in vitro (Roger and Tivoli 1996*a*). No information was reported on the optimum temperature for production of pseudothecia. In France, Roger and Tivoli (1996*b*) reported that more ascospores and pycnidiospores were trapped when daily mean temperatures ranged from approximately 16 to 22 °C in the field (Roger and Tivoli 1996*b*). However, fewer spores were trapped after June in both years in our experiments in western Canada, when temperatures fluctuated between 6 and 33 °C and daily mean temperatures were more extreme, ranging from 12 to 27 °C, than in France. Daily mean temperatures at the experimental site in France seemed to be closer to optimum temperatures for pycnidial formation than

those at our experimental site in western Canada. This suggests that temperatures during epidemics in western Canada might also limit the formation of fruiting bodies and thus amount of spore release, but further observations are needed.

Our data shows that rainfall was associated with the spore release events under our field conditions during the first 27 d (during June) after inoculation in both years, but later (during July), fewer pycnidiospores and ascospores were trapped even after a rain event. There are two factors that might contribute to this dynamic pattern. First, most ascospores might have been released in June, presumably in response to rain events, so that few ascospores remained in the pseudothecia by July. Second, the pea plants were short with thin canopies in June in western Canada. All disease residues in the inoculum source area were thus directly exposed to rain. However, by July plants had developed a dense canopy, which might have intercepted rain and affected the movement of spores released from diseased stubble in the inoculum source area, reducing the amount of spores trapped by the plant canopy. The disease, when initiated by air-borne ascospores, may become distributed over a large area (Lawyer 1984). Pycnidiospores, on the other hand, may result in a more localized disease spread. Even when large numbers of pycnidiospores are released during rainfall, most will be splashed locally onto the lower leaves, causing a localized disease pattern (Lawyer 1984). The effects of plant canopy structure on spore dispersal have also been observed in other pathosystems (Hirst and Stedman 1971). Overall, ascospores and pycnidiospores released from residue in the inoculum source area before July probably constituted most of the primary inoculum of mycosphaerella blight in the field.

We observed that the peak ascospore release occurred 1 to 2 d after rain, while more pycnidiospores were trapped during the same day that it rained. The timing differences for the release of both spore types may be caused by different mechanisms. In general, ascospores are discharged by the development of pressure in pseudothecia usually resulting from their hydration. When pseudothecia are covered by free water during rain, the asci hydrate and swell, and the pressure is released by an expulsion of the pseudothecial contents, resulting in ascospore release (Lawyer 1984; Roger et al. 1999). A similar pattern of ascospore release has also been observed in other ascomycete fungi (Hare and Walker 1945; Allard et al. 1992; Paulitz 1996; Fernando et al. 1997). We found that fewer pycnidiospores were trapped during dry days than ascospores. This was consistent with observations by Allard et al. (1992) that pycnidiospore dispersal occurred only during rainfall and remained limited, whereas ascospores could be transmitted longer distances by wind and result in secondary spread of the disease. Previous studies under controlled conditions demonstrated that temperature and moisture significantly affect pycnidial formation and spore germination of *M. pinodes* (Roger and Tivoli 1996*a*). In the field, these fruiting bodies are formed first at the base of the plant and then move progressively upwards (Lawyer 1984). In a greenhouse study, longer wetting periods were required for pycnidial formation when temperature was unfavorable for pycnidial formation (Roger and Tivoli 1996*a*). However, in France, Roger and Tivoli (1996*b*) observed dispersal of both pycnidiospores and ascospores of *M. pinodes* during the whole season.

Our study showed that ascospore and pycnidiospore release occurred mainly during night. This pattern is similar to ascospore release of *Calonectria nivalis* on oat (Sanderson 1970) and *Gibberella zeae* on wheat (Paulitz 1996). Because ascospore release needs high moisture, the higher moisture during the night may provide the pathogen with an adaptive advantage. Roger and Tivoli (1996*a*) showed that light also had a significant impact on the perithecial production of *M. pinodes*. Light might play a role for spore release, but no further experimental evidence has been reported.

Rainfall not only affected spore release but also disease development in the field. In our work, the rainfall on days 48 and 49 in 2000 may have increased the rate of disease increase to a maximum. Similarly, the rainfall between days 38 and 51 may have continued to cause the rapid development of disease during the middle and late periods of the epidemic in 2001. The disease occurred later in 2001 than in 2000, probably because of a dry period after the field inoculation. Roger et al. (1999) studied the effects of interrupted wet periods on the development of mycosphaerella blight on pea seedlings under controlled conditions. They found that germ tubes of pycnidiospores did not penetrate the plants when a 6- to 12-h wet period was followed by a dry period. In this case, however, pycnidiospores could survive a dry period of up to 21 d after inoculation. When wet conditions were restored, a large number of pycnidiospores was still viable, causing a high level of disease. These observations suggest that dry periods may be the key factor limiting disease development.

Humidity in the field may be a major factor limiting the formation of pycnidia in western Canada. In our experimental fields, the weather was dry during the early stages of the epidemic in 2001 and disease remained low. Although there was more rainfall during the early stages of the epidemic in 2000, the leaf surface may have soon become dry in the field because the plant canopy was not dense. However, 'Profi' developed a dense canopy later in the season; thus surfaces of plants and the ground maintained a longer wet period after rain because of the shade from the canopy. This was favorable for disease progress. It has been shown in other studies that disease develops rapidly during periods of wet weather and moderate temperature (Wallen 1974; Roger et al. 1999).

The observations from this research may have practical applications for improving disease control strategies. Because most of *M. pinodes* spores from residue were released during early stages of crop growth and may have constituted the primary inoculum of epidemics of foliar mycosphaerella blight in western Canada, turning the infected pea residue under may reduce the amount of the primary inoculum for newly planted adjacent pea fields. A decrease in ascospore density and disease severity over distance suggests that planting field pea at some distance from former pea fields could reduce the risk of disease occurrence in the next season. The close association of rain with spore release and disease development suggests that peas should be surveyed after rains to determine the need for a fungicide application. More pycnidiospores and ascospores were dispersed from the source within 1 to 2 d after a rain event in this research, and thus disease developed more quickly after a rain event. This suggests that if the disease needs to be controlled, a

fungicide should be applied as soon as possible after a rain event to protect against fungal infection.

A variety with a dense canopy such as 'Profi' results in a favorable microclimate with higher humidity for disease development in the field, suggesting that a variety with a thinner canopy should be planted in field pea production areas with higher levels of inoculum. The dispersal and release of either ascospores or pycnidiospores were closely associated with rain events, suggesting that a forecasting model might be developed by combining data of rainfall and pea physiology to help growers to take advantage of disease management through fungicide applications, but also to refrain from fungicide use if it is not really warranted. This will help growers increase their net profit.

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