Epidemiology / Épidémiologie

Estimating inoculum production relative to percent leaf area infected and environmental conditions required for stimulating release of *Entomosporium mespili* conidia on *Amelanchier alnifolia*

Q.A. Holtslag, W.R. Remphrey, W.G.D. Fernando, R.G. St-Pierre, and R. Dill-Macky

Abstract: *Amelanchier alnifolia*, commonly known as saskatoon, is a fruit-bearing shrub native to the Canadian Prairies. Currently, production is limited by the fungal pathogen *Entomosporium mespili*. It was found that there is a positive linear relationship between the relative concentration of *E. mespili* conidia (*X*) and the percent of saskatoon leaf area that is infected (*Y*), as represented by the equation $Y = 0.0534X$. In a field study, initial disease symptoms appeared 5 d after the first precipitation event that occurred 1 d or more after the date of flowering. A splash-dispersed-conidia trap was constructed and used to trap conidia within the canopy of a saskatoon plant and, in both years of this study, conidia were first trapped on the 184th day of the year, which coincided with the exponential increase of percent leaf area infected. Conidia were primarily released during the first 2 h of precipitation events. Information on inoculum production and release will eventually be integrated into a dynamic disease-forecasting model for entomosporium leaf and berry spot caused by *E. mespili* on saskatoon.

Key words: entomosporium leaf and berry spot, *Entomosporium mespili*, inoculum production, percent leaf area infected, conidia release, *Amelanchier alnifolia*.

Résumé : L'*Amelanchier alnifolia*, connu sous le nom d'amélanchier ou petite poire, est un arbuste fruitier originaire des Prairies canadiennes. Présentement, le champignon pathogène *Entomosporium mespili* en restreint la production. Une relation linéaire positive a été trouvée entre la concentration relative de conidies d'*E. mespili* (*X*) et le pourcentage de la surface foliaire infectée des amélanchiers (*Y*); cette relation est représentée par l'équation $Y = 0{,}0534X$. Lors d'une étude sur le terrain, les premiers symptômes de la maladie sont apparus 5 d après la première précipitation qui a suivi d'au moins 1 d la date de floraison. Un piège à conidies dispersées par éclaboussure a été construit et employé pour capturer les conidies à l'intérieur du feuillage d'amélanchiers et, lors des deux années de cette étude, les conidies ont d'abord été capturées au 184^{e} jour de l'année, lequel a coïncidé avec un accroissement exponentiel du pourcentage de la surface foliaire infectée. Les conidies étaient principalement relâchées au cours des deux premières heures des précipitations. L'information sur la production et le relâchement d'inoculum sera éventuellement intégrée à un modèle dynamique de prévision de la maladie pour la tache des feuilles et des fruits causée par l'*E. mespili* sur l'amélanchier.

Mots clés : tache des feuilles et des fruits, *Entomosporium mespili*, production d'inoculum, pourcentage de la surface foliaire infectée, relâchement de conidies, *Amelanchier alnifolia*.

Introduction

Amelanchier alnifolia Nutt., more commonly known as saskatoon, is a native Canadian shrub that produces an abundant and staple fruit crop (St-Pierre 1997). After the cultivar 'Smoky' was released in 1952, it became the dominant cultivar in commercial production, amassing roughly

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85% of the hectarage planted by 1990 (St-Pierre 1997). However, since 1990, more hectares have been planted with the cultivar 'Northline'. 'Northline' may have been planted more frequently because it has greater resistance to entomosporium leaf and berry spot than 'Smoky' (Ronald et al. 2001). Other popular cultivars that help make up the roughly 1200 ha of production throughout the Prairie provinces are 'Thiessen', 'Honeywood', 'Pembina', 'Martin', and 'Regent'.

Saskatoon production is currently limited by entomosporium leaf and berry spot, a disease caused by the fungal pathogen *Entomosporium mespili* (DC.) Sacc. (Lange and Bains 1994). *Entomosporium mespili* is spread primarily by means of asexual conidia, which are produced and overwinter in acervuli that are located at the center of lesions found on young branch tissue, infected leaves, and infected fruit that remain on or around the plant (Horie and Kobayashi 1980; Stathis and Plakidas 1959). The distinctive conidium consists of an apical cell, a basal cell, and three lateral cells (Mims et al. 2000). A slender appendage is commonly found on the apical and lateral cells. The production of conidiospores was shown to cause rupture of the acervuli surface and permit release of the conidia contained within (Ronald and St-Pierre 2002). Wind and rain splash are the critical components in epidemic development of many diseases (Madden 1997), and although no specific studies were conducted, Lange and Bains (1995) have observed that heavy rainfall is the most important environmental factor contributing to entomosporium leaf and berry spot in saskatoon orchards. Once released, conidia of *E. mespili* can germinate within 6 to 18 h and then form appressoria, which penetrate the cuticle of leaves (Baudoin 1986; Ronald and St-Pierre 2002; van der Zwet and Stroo 1985). Disease symptoms first appear on leaves as small brown spots that develop yellow halos. The spots may eventually coalesce and cause the entire leaf to become yellow and fall prematurely (Bains 2000). Besides the direct damaging effect *E. mespili* lesions have on fruit quality, fruit quantity can suffer when photosynthetic area is lost to lesions and early defoliation of the plant (Horie and Kobayashi 1979; St-Pierre 1997).

Polycyclic diseases, like entomosporium leaf and berry spot, can be efficiently suppressed by reducing initial inoculum and (or) by limiting potentially rapid rates of inoculum increase (Fry 1982). Thompson and Jenkins (1985) showed that a reduction in lesion area results in a lower number of *Colletotrichum lagenarium* (Pass.) conidia produced from infected leaves of *Cucumis sativus* L. Disease-forecasting models based on leaf-wetness duration and temperature (Grove 2002; Monroe et al. 1997) can be integrated with a fungicide spray program to help reduce disease. A similar disease-forecasting model based on leaf-wetness duration and temperature for *E. mespili* on saskatoon was developed (Holtslag et al. 2003). The accuracy of this forecasting model would be improved if information on inoculum production and release were included. There are many studies that have examined the epidemiology of fungal spore release in relation to environmental factors (Paulitz 1996; Pinkerton et al. 1998). The objectives of this study were: (1) to relate the percent of saskatoon leaf area infected to the relative concentration of *E. mespili* conidia released from the infected tissue and to develop a regression equation for this relationship, (2) to identify when *E. mespili* conidia are released in relation to prevailing environmental conditions during the growing season, and (3) to determine if the amount of conidia released is related to the amount of rainfall during precipitation events.

Materials and methods

Leaf sampling and analysis

Three 'Smoky' saskatoon orchards were used as a source of leaf samples for this study. Sampling was done over a 2-year period (1999 and 2000). One orchard (Carman site 1) was located near Carman, Manitoba, and was not treated for control of *E. mespili* in either study year. A second orchard (Carman site 2) was located adjacent to Carman site 1 and received fungicide treatments during the 1999 growing season, although the treatments were relatively ineffective for controlling the disease. A third site, which also did not receive any fungicide treatments, was located at the University of Manitoba, Winnipeg, Manitoba. Data from this site were collected during the 2000 season.

Leaf samples from each orchard were collected once per week between the 152nd and 243rd day of the year. Five leaves per plant were randomly collected from each of 10 plants in each orchard during each sampling period. The five leaves from each plant were placed on a piece of blue construction paper and photographed with a digital camera. The mean percent leaf area infected (PLAI) of the samples was then determined by importing the pictures into the software program Assess for Windows (American Phytopathological Society Press, St. Paul, Minn.). Assess for Windows was selected over other programs because it did not require specialized equipment or software application expertise to detect diseased areas on infected plant tissue. The program first identifies the total leaf area and then assesses the PLAI by pixel color analysis.

Extraction of conidia

Following PLAI determination, each sample of five leaves was placed in a beaker with 10 mL of water and several small glass beads to stimulate conidia release during a period of agitation. The beakers and their contents were agitated on a flat-top oscillator for 1 h. A preliminary study determined that 1 h was adequate to induce maximum conidia release from infected leaf tissue. Following agitation, an aliquot was extracted with a pipette and the conidial concentration was quantified on a hemacytometer. The relative concentration of conidia (RCC) in each sample was determined by averaging the number of conidia observed from six 1-mm^2 grids on the hemacytometer.

Measuring conidia release

An Adcon Telemetry (Klosterneuburg, Austria) weather-monitoring station was used at the Winnipeg orchard to quantify precipitation during the study. These weather stations, which are maintained by the Agrometeorological Center of Excellence in Manitoba, provide environmental-variable monitoring with real-time and site-specific microclimate measurements.

During the 1999 field season, Vaseline®-coated microscope slides served to detect conidia release. Several saskatoon plants in the Winnipeg orchard that had a history of entomosporium leaf and berry spot were selected on the 152nd day of the year for this study. Two Vaseline-coated microscope slides were hung parallel to and at a height of 1 m from the ground from randomly selected branches on these plants just prior to precipitation events. After rainfall ceased, the slides were collected for analysis. A light microscope (magnification, × 40) was employed to locate and identify conidia on the microscope slides.

A trap designed to capture splash-dispersed conidia was placed in the Winnipeg orchard on day 182 of year 2001 and day 121 of year 2002. In both years, conidia were trapped until day 243. The trap was placed in the periphery of the saskatoon plant canopy to ensure the greatest capture of runoff rainwater from leaves (Fig. 1). In a saskatoon plant that measured 3.5 m in height, the trap was placed 1 m from the ground and 30 cm in from the outer edge of the canopy. The trap rotated the position of a test tube each hour, using a battery-powered electric step motor. For every hour during the precipitation event, test tubes containing rainwater were retrieved and the number of conidia per milliliter of rainwater was estimated on a hemacytometer.

Measuring phenological development and PLAI at the Winnipeg orchard

Monitoring of disease symptoms began in the Winnipeg orchard when 50% of the overwintering buds reached the stage of bud break. A bud was considered dormant until it began to swell, separating its outer scales and revealing pubescent hairs that had a silvery appearance. The phenological stage of flowering was defined as the time when an estimated 50% of the inflorescences, which had expanded from overwintering buds, had one open flower. A weekly estimate of PLAI in the orchard, as described above, was determined between the 152nd and 243rd day of the year by selecting five leaves from each of 10 randomly selected plants soon after disease symptoms in the orchard were first observed.

Data analysis

The RCC and PLAI data were analyzed with the software program Statistix for Windows (Analytical Software, Tallahassee, Fla.). For each sample site and year, the correlation between the RCC and PLAI was compared with Pearson's correlation coefficients. An *F* test was performed to determine if combining the data means from each sampling site and year was justified (Grove et al. 1985) and a combined analysis was conducted where appropriate. The interaction between the combined RCC and PLAI data was illustrated with scatter plot and regression analysis. A Pearson's correlation analysis was calculated for the number of conidia per milliliter of trapped rainwater and the total amount of rainfall during a precipitation event. For both study years, regression of PLAI in the orchard versus day of the year was calculated, and the relationship was plotted and visually compared with precipitation versus trapped conidia data. The presence of conidia in 1 h increments after the start of each precipitation event was assessed.

Fig. 1. The splash-dispersed-conidia trap positioned 1 m from the ground in the periphery of a saskatoon plant canopy (A). Power for the electrical step motor in the trap was supplied by a 12-V deep-cycle battery (B), which was recharged by a 30-W solar panel (C).

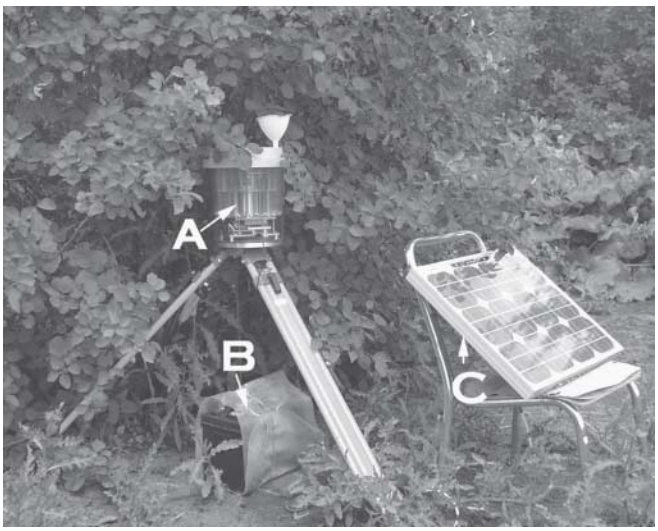
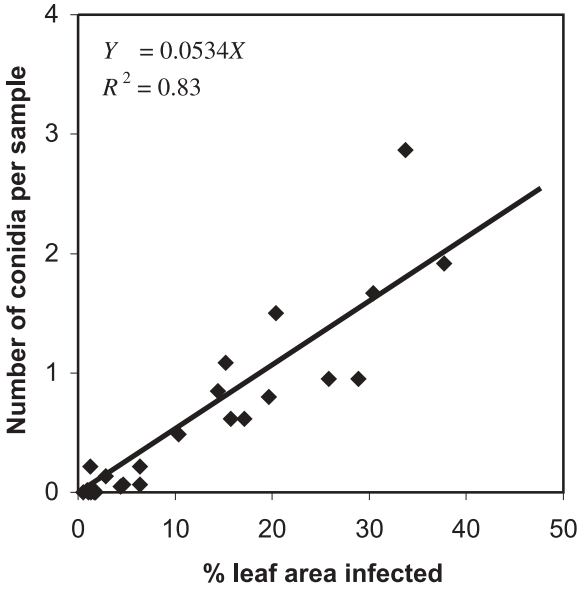


Fig. 2. Regression analysis between the percent leaf area infected on saskatoon and the relative concentration of *Entomosporium mespili* conidia. Data were collected at Winnipeg and Carman sites 1 and 2 in 1999 and 2000.



Results

Relationship of RCC to mean PLAI

When the RCC was compared against PLAI, the Pearson's correlation coefficients (*r*) were 0.73 for 1999 and 0.83 for 2000, at Carman site 1, 0.72 at Carman site 2 in 1999, and 0.69 at Winnipeg in 2000. The main effects of study site and year were not significant ($P = 0.05$), so the data were combined for further analysis. Regression eq. 1 best describes the positive linear relationship between the

Fig. 3. Precipitation (A), number of conidia of *Entomosporium mespili* per milliliter of rainwater (B), and mean percent leaf area infected on saskatoon (C) in the Winnipeg orchard in 2001. Indicated in the figure are the flowering date (*X*, day 133 of the year), the first rainfall event 1 d or more after the date of flowering (*Y*, day 135 of the year), and the first observed disease symptoms (*Z*, day 140 of the year). A regression curve with a coefficient of determination (R^2) was used to illustrate PLAI increase.

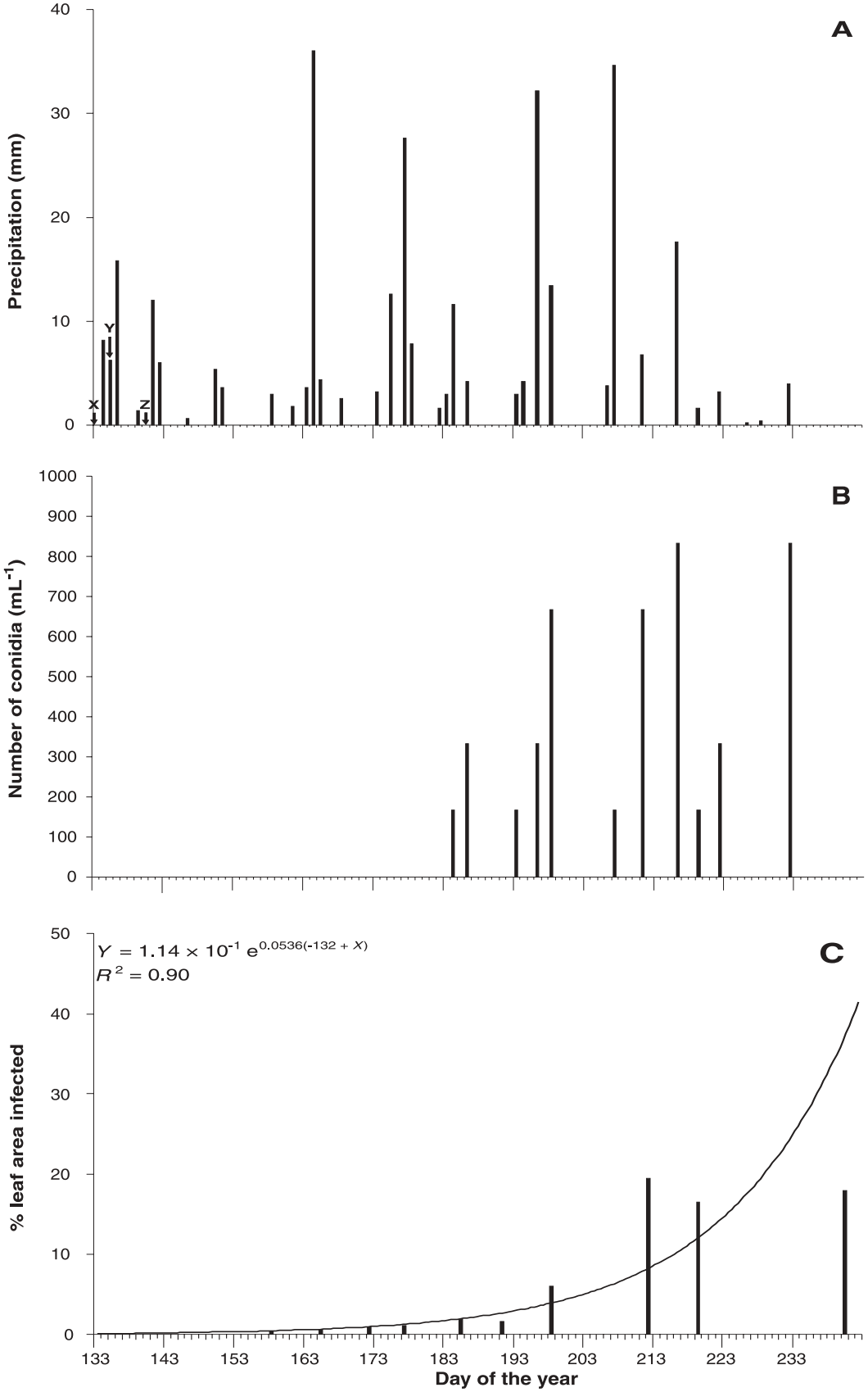
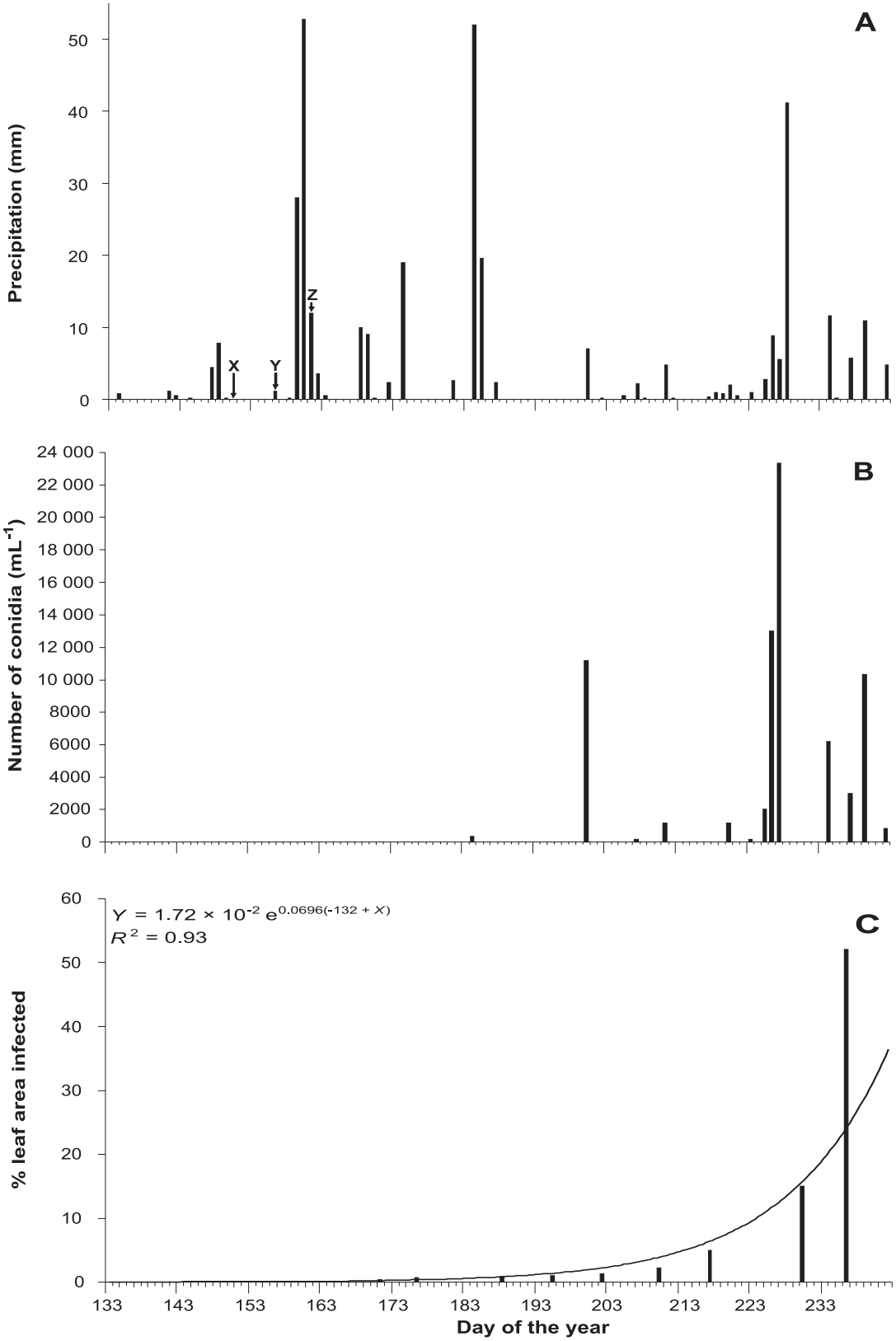


Fig. 4. Precipitation (A), number of conidia of *Entomosporium mespili* per milliliter of rainwater (B), and mean percent leaf area infected on saskatoon (C) in the Winnipeg orchard in 2002. Indicated in the figure are the flowering date (*X*, day 149 of the year), the first rainfall event 1 d or more after the date of flowering (*Y*, day 156 of the year), and the first observed disease symptoms (*Z*, day 161 of the year). A regression curve with a coefficient of determination (R^2) was used to illustrate PLAI increase.



percent of saskatoon leaf area infected and the relative concentration of *E. mespili* conidia (Fig. 2).

$$[1] \qquad Y = 0.0534X$$

where Y is the RCC and X represents PLAI. The coefficient of determination (R^2) for this equation was 0.83.

Precipitation and conidia release

Flowering began on day 133 of year 2001 and day 150 of year 2002. Between flowering and day 243, there were 36 precipitation events in 2001 for a total of 293 mm, and 38 events in 2002 for a total of 328 mm (Figs. 3A and 4A). Despite the fact that no conidia were trapped at the beginning of the flowering period, the first disease symptoms appeared 5 d after the first precipitation event that occurred 1 d or more after the date of flowering. This observation suggests that initial conidia release must have occurred as early as day 135 in year 2001 and day 156 in year 2002 at the Winnipeg site, based on a 5-d incubation period. Conidia were first trapped on day 184, and continued to be trapped until day 243 in both 2001 and 2002 (Figs. 3B and 4B). The correlation between the number of conidia per milliliter of trapped rainwater and the total amount of rainwater collected during each precipitation event was not significant in 2001, but was in 2002 ($r = 0.74$, $P = 0.05$). Considerably fewer conidia were trapped during precipitation events in 2001 than in 2002. Conidia were trapped primarily in the first 2 h after the start of rainfall. There were only two events, both in 2002, in which conidia were released for several hours beyond the first 2 h of rainfall. These two events yielded the highest number of conidia during the entire season.

For each year of the study, conidia were trapped only during precipitation events that occurred after the PLAI began to increase exponentially (Figs. 3C and 4C). In general, the relationship between PLAI and the day of the year was captured by eqs. 2 and 3, in 2001 and 2002, respectively:

$$[2] \qquad Y = 1.14 \times 10^{-1}\ e^{0.0536(-132 + X)}$$

$$[3] \qquad Y = 1.72 \times 10^{-2}\ e^{0.0696(-132 + X)}$$

where Y is PLAI and X represents the day of the year. The coefficients of determination for the regression equations were 0.90 in 2001 and 0.93 in 2002.

Discussion

Combinations of leaf-wetness duration and temperature that favor entomosporium leaf and berry spot development on saskatoon leaves were previously identified (Holtslag et al. 2003). However, the presence of ideal weather conditions for disease development would be of little value to forecast a possible disease outbreak if inoculum was not present. For example, inoculum availability is an important factor in prediction of disease epidemics for both monocyclic and polycyclic pathogens (Fry 1982; Specht and Griffin 1988).

Results from the inoculum-production study indicated that the relative concentration of *E. mespili* conidia is strongly correlated with the percent of saskatoon leaf area infected. Therefore, an assessment of PLAI at the end of the growing season could provide an estimate of the inoculum overwintering in an orchard. Estimates of inoculum levels going into the winter have been valuable for implementing an early spring fungicide spray program to control pathogens like *Venturia inaequalis* (Cooke) G. Winter, on apple trees (Boone 1971). A similar system for disease control would benefit saskatoon producers because *E. mespili*, like *V. inaequalis* on apples, infects young saskatoon leaves in early spring when they are most susceptible to infection (Holtslag et al. 2003; Ronald et al. 2001).

Knowledge of inoculum potential within an orchard is an important part of understanding the epidemiology of a pathogen; however, it is also important to know when conidia dissemination occurs relative to prevailing environmental conditions. In a preliminary study, a Burkard 7-day volumetric spore trap (Burkard Manufacturing Co. Ltd., Hertfordshire, England) was used throughout the growing season to confirm that air currents do not disseminate conidia of *E. mespili* (unpublished data). Ascospores may be vectored by wind in the early part of the season, although no study, including this one, has conclusively shown this phenomenon to occur in saskatoon orchards. More research is required in this area. Results from both the Vaseline-coated microscope slides and the splash-dispersed-conidia trap showed that *E. mespili* conidia are dispersed by rain-splash during precipitation events. Madden et al. (1996) found that as rain intensity increased, the total number of *Colletotrichum acutatum* Simmonds conidia in rain-splash droplets also increased. Provided more rainwater is collected during periods of heavy rainfall, it may be expected that more *E. mespili* inoculum will be trapped as the total amount of precipitation increases. This phenomenon was observed in the second year of the present study. The lack of a correlation between the number of conidia trapped and the amount of precipitation in the first year of this study can be explained by examining the interval of time between precipitation events that caused conidia to be disseminated. For instance, there was a long period in the middle of the growing season (days 188 to 199 of the year) that did not have a precipitation event in 2002, whereas rainfalls occurred no more than 6 d apart in the middle of season 2001. The increased time between precipitation events allows acervuli to mature; thus, more mature conidia could potentially be released from previously infected plant tissue during future precipitation events. van der Zwet and Stroo (1985) indicated that it takes 2 to 4 weeks from the time of infection until mature conidia of *Entomosporium maculatum* Lév. exude from acervuli on pear leaves. Thus, a quantitative relationship between the time of rain events and the numbers of conidia trapped is not unexpected, because of the dynamics of the conidia production process. It was also observed in our study that a dry period of two or more hours between precipitation events is required to stimulate conidia release. A dry period may help to desiccate the surface of mature acervuli. Rainwater would then be able to wash conidia from the surface of the exposed acervuli (Ronald and St-Pierre 2002).

Although no conidia were trapped around the time of flowering, the first symptoms of infection appeared 5 d after the first rain event that occurred 1 d or more after flowering in both years in this study. This might be explained by

the observation that rainwater falling prior to 1 d after the phenological event of flowering is shed by gravity from unopened leaves. At this point in the season, most leaves are not opened enough to capture and retain rainwater as it moves from leaf to leaf. Disease symptoms first appeared 43 and 23 d before conidia were first trapped in 2001 and 2002, respectively, indicating that conidia were released before inoculum levels in the orchard were large enough to be trapped. Further work is required to identify the earliest date that *E. mespili* conidia can be released during the growing season.

During the course of the study, *E. mespili* conidia were primarily released during the first 2-h period of precipitation events, with only two instances where conidia were trapped beyond the first 2 h. One possible explanation for the short period of conidia release is that the conidia may have been washed past the point of collection on the trap during the first 2 h of precipitation. To verify that conidia are only disseminated in the first 2 h of precipitation events, the height of the trap could be lowered in future studies to allow more infected plant canopy to hang over the collection point on the trap. The placement of the trap may have also influenced why fewer conidia were trapped in the 2001 season. It is possible that, by chance, less infected material was located above the collection point on the trap during 2001. Another possible reason for limited conidia movement beyond the first 2 h of a precipitation event may be associated with the appendages on the conidium. Mims et al. (2000) suggested that these appendages may help to attach the conidium to the leaf surface, limiting their movement from the source of dissemination.

The first sign of exponential PLAI increase was associated with the first instance that conidia were trapped. The exponential equations developed in this study adequately described the increase in PLAI throughout most of each season. However, despite the fact that the R^2 values were quite high, the accuracy of both models decreased after the 228th day of the year. Disease development at the end of the season appeared to correspond with the number of rainfall events that occurred during that period. In the drier late-season period in 2001, actual PLAI was less than the predicted amount. This may have been the result of fewer conidia-release events. In contrast, the amount of PLAI in 2002 was greater than the predicted PLAI and this may be the result of more conidia-release events during that period. These results indicate the need for a study to investigate the timing of rain events relative to disease development throughout the growing season.

In summary, it was shown that the percent of leaf area infected can be used to estimate the production of *E. mespili* conidia in a saskatoon orchard with a regression analysis. This relationship may provide an estimate of the overwintering inoculum available to initiate disease in the spring. A splash-dispersed-conidia trap was used to show that *E. mespili* conidia are released primarily during the first 2-h period of precipitation events and that the number of conidia per milliliter of rainwater collected is not entirely dependent upon the total amount of rainfall during a precipitation event. Saskatoon plants are first infected by conidia that are disseminated by precipitation events occurring 1 d or more after the phenological event of flowering. Conidia were initially trapped on the 184th day of the year, which coincided with the start of exponential PLAI increase, and continued to be trapped until day 243. Multiple late-season rain events can promote disease-level increase by stimulating conidia release.

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