***Bremia lactucae* Infection Efficiency in Lettuce is Modulated by Temperature and Leaf Wetness Duration Under Quebec Field Conditions**

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Abstract

Fall, M. L., Van der Heyden, H., Beaulieu, C., and Carisse, O. 2015. *Bremia lactucae* infection efficiency in lettuce is modulated by temperature and leaf wetness duration under Quebec field conditions. *Plant Dis.* 99:1010-1019.

More than 80% of Canadian lettuce production is located in the province of Quebec. Yet most of our knowledge on the epidemiology of lettuce downy mildew (*Bremia lactucae*) is derived from controlled-condition experiments or field experiments conducted in subtropical climates and, thus, cannot readily be applied to Quebec lettuce production. The influence of temperature and leaf wetness duration on the infection efficiency (IE) of *B. lactucae* was studied for 4 years (2003, 2004, 2012, and 2013) under field and growth-chamber conditions. IE was defined as the ratio of the number of lesions/leaf to the airborne conidia concentration (ACC). *B. lactucae* ACC was measured with rotating-arm samplers three times/week. In addition, 72 lettuce trap plants/sampling day were exposed to the potential airborne *B. lactucae* inoculum and disease intensity was assessed after 7 days of incubation in greenhouse. Under growth-chamber conditions, an ACC of 1 conidium/m³ was sufficient to cause

1 lesion/leaf, and IE ranged from 0.25 to 1.00. Under field conditions, an ACC of 10 to 14 conidia/m³ was required to cause 1 lesion/leaf, and IE ranged from 0.02 to 0.10, except in 2004, when IE ranged from 0.03 to 1.00. IE increased with increasing leaf wetness duration but decreased with increasing temperature. Also, considering an observed average temperature range from 10 to 20°C in the area of Quebec, 2 h of leaf wetness was sufficient for infection by *B. lactucae*. Therefore, under Quebec lettuce production conditions, a leaf wetness period of 2 h and an ACC of 10 to 14 conidia/m³ can be used as risk indicators to facilitate disease management decisions. Also, under typical Quebec weather conditions, measuring both morning and evening leaf wetness events could be used to improve the reliability of leaf wetness duration as a downy mildew risk indicator. Further research is needed to validate these risk indicators for integration into management strategies.

Lettuce (*Lactuca sativa*) downy mildew, caused by the oomycete *Bremia lactucae* Regel, is a major threat in lettuce production worldwide (13). Spores of *B. lactucae* have lost the ability to form zoospores and germinate directly on a leaf surface (11). Thus, the term “conidia” is used in this study instead of “sporangia”, although the latter term is used commonly in the literature (7,21).

Despite the presence of the disease every year, outbreaks of lettuce downy mildew are typically sporadic and associated with specific environmental conditions (13). The potential risk of disease development and consequent yield losses also depend on the amount of *B. lactucae* conidia in lettuce fields. *B. lactucae* produces conidia that are adapted to aerial dispersal (6,26). They are produced when relative humidity (RH) is high and wind speed is low, and conidia release coincides with decreasing humidity and increasing temperature, conditions that generally occur in the morning (28). Viable conidia that are deposited on the leaf of a susceptible lettuce plant germinate and colonize the leaf, resulting in symptoms that are visible 8 to 14 days after the initiation of infection, depending on host susceptibility and environmental conditions (17,19).

Environmental factors such as temperature, RH, wind speed, solar radiation, and leaf wetness duration have been identified as factors that influence sporulation, dispersal, survival, and infection processes (6,20,24,28,29). Hence, conidia survival is greater at 23 than at 31°C (2 to 5 h) at an RH between 33 and 76% whereas, at an RH ≥90%, survival of conidia increases substantially (24,27). Wind speed plays a major role in the conidia dispersal process, and the amount of solar radiation affects the survival of the airborne conidia

(4,27). However, the most important factor for successful infection seems to be a duration of morning leaf wetness of at least 3 or 4 h (8,10,16,23). Therefore, many forecasting systems for lettuce downy mildew use morning leaf wetness duration as an indicator for occurrence of an infection period (10,16,18). In fact, according to the forecasting system developed by Scherm et al. (16) (and a modified version developed subsequently), infection by *B. lactucae* occurs on days when leaf wetness ends late in the morning (10:00 h) (16). The BREMCAS system developed in Quebec, Canada, by Kushalappa (10) considers a leaf wetness duration of 3 to 5 h after dawn (continuing until 10:00 h) to initiate fungicide application. These systems assume that sporulation is nocturnal, that conidia are released at dawn, and that infections occur in the morning (10,16). However, in a study conducted in Quebec, Carisse and Pilon (4) observed conidia release patterns during the afternoon on 21 and 17% of the days when spores were trapped. Moreover, Bhaskara Reddy et al. (2) observed that >50% of conidia survived a solar radiation dose of 10 MJ/m² over a period of 3 h and that, on overcast days, up to 80% of those conidia were viable after 9 h. Wu et al. (29) noted that a forecasting system using a threshold of morning leaf wetness lasting from 06:00 to 10:00 h missed some infection days, and suggested that a leaf wetness duration of 3 h may be long enough for conidia to germinate. Scherm and van Bruggen (17) also observed some infection under short wetness durations, such as 2 h. Therefore, some major epidemiological questions have arisen from these observations, including the following: What proportion of daily conidia is released in the morning (until 10:00 h)? Can a large amount of conidia released after 10:00 h infect lettuce later in the day in the region of Quebec? What is the minimum leaf wetness duration required for infection under field conditions in this region? What are the environmental factors that modulate infection events?

One way of improving lettuce downy mildew forecasting systems is to consider the amount of inoculum available locally, in addition to leaf wetness duration (16). For example, for the oomycete *Phytophthora infestans*, spore sampling coupled with a disease-forecasting

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Accepted for publication 31 January 2015.

<http://dx.doi.org/10.1094/PDIS-05-14-0548-RE>
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system is useful for targeting the optimal time to apply a disease-control product (5,14). For *Botrytis* leaf blight of onion, which is caused by *Botrytis squamosa*, the use of a fungicide spray program based on a threshold of 10 to 15 conidia/m³ made it possible to reduce fungicide application by 75 and 56%, depending on years and trials (3). Carisse and Phillon (4) also observed that fluctuations in the numbers of airborne conidia of *Bremia lactucae* followed fluctuations in disease severity.

The life cycle of *B. lactucae* can be divided into four distinct stages: sporulation, conidia release, conidia survival, and infection. Each of these processes has been studied independently (10,16,25). However, to study the combined effects of these processes, infection efficiency (IE), defined as the ratio of the number of lesions/leaf to the airborne conidia concentration (ACC), should be considered. Indeed, IE combines components derived from the pathogen (sporulation and conidia release) with components derived from the host-pathogen interaction (infection of leaves). Therefore, IE is probably influenced by environmental factors such as leaf wetness, solar radiation, and temperature. Despite the large amount of scientific information available on lettuce downy mildew, no field studies have focused on *B. lactucae* IE and the ways in which environmental factors influence IE. Therefore, the aim of this research was to study the influence of environmental factors on the IE of *B. lactucae* under Quebec weather conditions. Specifically, the study objectives were to (i) assess the daily percentage of conidia that is released outside the morning period (06:00 to 10:00 h) and the reliability of using morning leaf wetness duration as a risk indicator under Quebec weather conditions, (ii) examine the relationship between the ACC of *B. lactucae* and the number of lesions/leaf (IE) under growth-chamber and field conditions, and (iii) assess the influence of selected weather parameters on IE.

Materials and Methods

Data collected: Pattern of daily ACC and relationship between leaf wetness duration and pattern of ACC. The experiment was conducted between 1 July and 20 September 1997, 1998, 2003, and 2004. Each year, a plot of 'Ithaca' lettuce was established in an organic soil at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, QC (latitude 45°10'N, longitude 73°40'W). Lettuce plants produced in a greenhouse by Les Serres Lefort (Ste-Clotilde, QC, Canada), were transplanted 0.3 m apart in the rows, with 0.35 m between rows. The plots measured 30 by 30 m, for a total of 33 rows and 5,676 plants. A 7-day volumetric spore sampler (Burkard Manufacturing Co, Rickmansworth, Hertfordshire, UK) placed in the center of the plot was used to monitor ACC (4). The sampler was adjusted to sample air at 10 liters/min. Impaction tapes were coated with a thin layer of silicone grease before they were placed in the sampler. Conidia counts were performed with a microscope at ×250 magnification and converted to conidia per cubic meter of air as follows: (number of conidia/rod × 1,000 liters/m³/h)/(10 liters/min × 60 min/h × 1 h) (4).

Relationship between ACC and number of lesions/leaf (IE), and the influence of selected weather parameters on IE.
Growth-chamber trial. In a growth chamber (PGC20 growth chamber; Conviron, Winnipeg, MB, Canada), 20 lettuce plants (Ithaca) that had been produced in a greenhouse and had reached the six- to eight-leaf stage were placed 0.15 m apart in the chamber. Ten lettuce leaves with sporulating lesions were placed 1.25 m above the lettuce plants on a perforated shelf so that the plants were infected by falling airborne conidia in a manner similar to infection under field conditions (Fig. 1). The amount of inoculum was not controlled over the course of this experiment but, for each trial, an effort was made to provide the same number and size of sporulating lesions. To promote infection, the plants were held for 15 h in the dark, then for 9 h in light (growth-chamber fluorescent lamps, 150 μmol/m²/s) at 18°C and 100% RH, with a leaf wetness period of 6 h. After 24 h, the sporulating leaves were removed and the growth chamber was maintained at 18°C and 90% RH for 6 days, with a daily cycle of 9 h of darkness and 15 h of light. A rotating-arm spore sampler (Compagnie de Recherche Phytodata, Inc., Sherrington, QC, Canada) placed 0.5 m above the lettuce plants was used to monitor ACC in the growth

chamber. The sampler was operated for 17.6 h per 24-h sampling period (alternating between 11 min on and 4 min off), and the effective air-sampling rate was 21 liters/min. Conidia caught on the sampling surfaces (rods) were counted within 24 h after sampling with a microscope at ×250 magnification. The number of conidia/rod was converted to conidia per cubic meter as follows: (number of conidia/rod × 1,000 liters/m³/h)/(21 liters/min/rod × 60 min/h × 17.6 h). This experiment was repeated 30 times. The number of lesions/leaf was assessed on all plants in the growth chamber by randomly selecting five leaves on each plant.

Field trial. In 2003, 2004, 2012, and 2013, a plot of Ithaca lettuce was established in an organic soil at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, QC. Lettuce plants produced in a commercial greenhouse by Les Serres Lefort were transplanted 0.3 m apart in the rows, with 0.35 m between rows. The plots measured 30 by 30 m, for a total of 33 rows and 5,676 plants. In the lettuce plots each year, 72 lettuce trap plants (Ithaca) were placed randomly in the field each Tuesday, Wednesday, and Thursday for 24 h (total of 216 trap plants/week). The trap plants were divided into four trays (0.135 by 0.07 m and 0.04 m in depth) containing 18 plants each. The trap plants placed in the field over the course of the study were always at the same growth stage (five- to seven-leaf stage) and were produced in a greenhouse by Les Serres Lefort (Ste-Clotilde, QC, Canada). After 24 h of potential exposure to airborne *B. lactucae* inoculum in the field, the trap plants were incubated in a greenhouse for 7 days under 90% RH and a temperature between 18 and 24°C. After 7 days of incubation, disease severity was assessed as the number of lesions on five randomly selected leaves/plant, excluding leaves that had emerged after exposure in the field. Trap plants were exposed to the potential inoculum release 47 times in 2003, 60 times in 2004, 24 times in 2012, and 23 times in 2013 (for a total of 154 times). While the trap plants were exposed to naturally occurring airborne *B. lactucae* inoculum, ACC was monitored with three rotating-arm spore samplers with retracting sampling heads (Compagnie de Recherche Phytodata Inc.). Each sampler was supported 1.5 m off the ground by a pole, and the three poles were positioned in the center of the plot in a triangular pattern. Conidia counts were performed with a microscope at ×250 magnification and converted to conidia per cubic meter of air as follows: (number of conidia/rod × 1,000 liters/m³/h)/(21 liters/min/rod × 60 min/h × 4.5 h).

Measurement of environmental variables. Leaf wetness duration was assessed every minute by means of an electrical-impedance leaf-wetness sensor (Model 237; Campbell Scientific, Edmonton, AB, Canada) placed at the height of the lettuce leaves. Air temperature (°C) and RH (%) were monitored using WatchDog data loggers (Spectrum Technologies, Aurora, IL) located near the spore samplers. Weather variables were monitored every 30 min, and hourly averages were used in the analyses. Temperature and RH probes were placed in a white shelter 1.5 m above the ground. Solar radiation data were obtained from an Environment Canada weather station located approximately 200 m from the plots.

Fig. 1. Diagram illustrating the growth-chamber set-up for evaluating infection of lettuce plants by the downy mildew pathogen, *Bremia lactucae*. 1, Sporulating lettuce leaves; 2, *Bremia lactucae* airborne inoculum sampler (Compagnie de Recherche Phytodata Inc., Sherrington, QC, Canada); and 3, healthy Ithaca lettuce plants.

Data analysis. First, normality of the data was tested using the Shapiro-Wilk test and then, if necessary, logarithmic transformation was done to improve normality. All statistical tests were performed in R version 3.0.0, as described below.

Pattern of daily ACC and relationship between leaf wetness duration and pattern of ACC. *Airborne conidia pattern.* Graphical representations of daily ACC released between 06:00 and 10:00 h as a function of day of the year in 1997, 1998, 2003, and 2004 were prepared. Scherm and van Bruggen (16) observed that *B. lactucae* conidia release in coastal California was initiated at sunrise (06:00 h) and that infection occurred within a 3- to 4-h leaf wetness duration after sunrise. Each graphical representation for this study shows the daily ACC released during the period of 06:00 to 10:00 h in comparison with the total daily ACC for a 24-h sampling period, as well as the proportion of daily ACC that was released between 06:00 and 10:00 h. For each year, the percentages of daily conidia released during and outside the period of 06:00 to 10:00 h were calculated. The percentages of conidia released between 06:00 and 14:00 h were also calculated for each year.

Relationship between leaf wetness duration and airborne conidia pattern. A graphical representation of ACC and number of leaf wetness events as a function of time (4-h intervals) was prepared for the data from all 4 years (1997, 1998, 2003, and 2004). A period of at least three consecutive hours of leaf wetness was considered a leaf wetness event (10,16,23). According to the hypothesis of Scherm and van Bruggen (17), regardless of the amount of inoculum, infection occurs on days when leaf wetness ends late in the morning (between 06:00 and 10:00 h). Thus, time was expressed in 4-h intervals. Fig. 2 shows the number of leaf wetness events in comparison with the ACC during the same period. The percentage of leaf wetness events that occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 h were also calculated.

To assess the reliability of using a leaf wetness duration of 3 to 4 h as an indicator of infection by *B. lactucae* under Quebec weather conditions, a contingency table was calculated based on a 3-h threshold for leaf wetness duration. Each sampling date (24 h) in 2003 and 2004 was divided into six 4-h periods (02:00 to 06:00, 06:00 to 10:00, 10:00 to 14:00, 14:00 to 18:00, 18:00 to 22:00, and 22:00 to 02:00 h). For the contingency table, three periods of the day were considered. The period between 06:00 and 10:00 h was considered the standard in accordance with the Scherm and van Bruggen

hypothesis (17). The periods between 10:00 and 14:00 h and 18:00 and 22:00 h were also taken into account because the ACC and number of leaf wetness events were considerable during those periods (Fig. 2). For each of these three periods, a leaf wetness duration ≥ 3 h was considered to be a prediction of the presence of lettuce downy mildew (P+) on trap plants, and a leaf wetness duration < 3 h was considered to be a prediction of the absence of this disease (P-). For each sampling day, the presence of downy mildew on trap plants was indicated by O+ (observed) and the absence of disease was indicated by O- (not observed). Therefore, there were four possible outcomes. First, disease was predicted (P+) and observed (O+). This situation was termed a “true positive” (TP). Second, disease was not predicted (P-) and was not observed (O-), termed a “true negative” (TN). Third, disease was predicted (P+) but was not observed (O-), termed a “false positive” (FP). Fourth, disease was not predicted (P-) but was observed (O+), termed a “false negative” (FN). The proportion of true positives was considered the indicator sensitivity [TP/(TP + FN)]. The proportion of true negatives was considered the indicator specificity [TN/(TN + FP)]. Overall accuracy was calculated as the number of correct assessments (TN + TP) divided by the number of all assessments (TP + TN + FP + FN). The likelihood ratio of positive predictions (LR+) was estimated by $LR+ = \text{sensitivity} / (1 - \text{specificity})$, and the likelihood ratio of negative predictions (LR-) was estimated by $LR- = (1 - \text{sensitivity}) / \text{specificity}$ (9,12).

Relationship between ACC and number of lesions/leaf.

Growth-chamber trial. A scatter plot was prepared to represent the number of lesions/leaf as a function of ACC. A linear function was fitted to the data, as follows:

$$y = \beta_0 + \beta_1 x \quad (1)$$

where y is the number of lesions/leaf, x is the ACC, β_0 is the intercept parameter, and β_1 is the slope.

Field trial. Scatter plots representing the number of lesions/leaf as a function of ACC were prepared for the data from 2003, 2004, 2012, and 2013 separately and for the data from all four of these years. A three-parameter sigmoid model was fitted to these data as follows:

$$y = \frac{a}{1 + e^{-\left(\frac{x - x_0}{b}\right)}} \quad (2)$$

Fig. 2. Number of lettuce leaf wetness events (NLWE) and *Bremia lactucae* airborne conidia concentration (ACC) as a function of 4-h intervals. A period of at least three consecutive hours of leaf wetness was considered a leaf wetness event (10,16,24). This study was done in field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, in Quebec, Canada in 1997, 1998, 2003, and 2004.

Fig. 3. Daily airborne conidia concentration (ACC) of *Bremia lactucae* as a function of day of the year (where day 1 is 1 January) in **A**, 1997; **B**, 1998; **C**, 2003; and **D**, 2004. The term “Total daily ACC” represents the ACC for a 24-h sampling period. This study was done in lettuce crops at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada.

Table 1. Contingency table for assessment of the reliability of using a leaf wetness duration of 3 to 4 h as an indicator of infection of the lettuce plants by *Bremia lactucae* under field conditions in field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada

Infection period ^a	Observed	Predicted		Total	Overall accuracy ^b	Sensitivity ^b	Specificity ^b	LR+ ^c	LR– ^c
		P+	P–						
06:00–10:00 h (T1)	O+	15	23	38	0.545	0.395	0.882	3.00	0.69
	O–	2	15	17	...	...	...	...	...
	Total	17	38	55	...	...	...	...	...
10:00–14:00 h (T2)	O+	2	36	38	0.317	0.053	0.941	0.89	1.01
	O–	1	16	17	...	...	...	...	...
	Total	3	52	55	...	...	...	...	...
18:00–22:00 h (T3)	O+	5	33	38	0.382	0.132	0.941	2.24	0.92
	O–	1	16	17	...	...	...	...	...
	Total	6	49	55	...	...	...	...	...
T1 + T3	O+	26	12	38	0.709	0.684	0.765	2.91	0.41
	O–	4	13	17	...	...	...	...	...
	Total	30	25	55	...	...	...	...	...
	O+	26	12	38	...	...	...	...	...
	O–	4	13	17	...	...	...	...	...
	Total	30	25	55	...	...	...	...	...

^a For each of the infection periods T1, T2, T3, and T1 + T3, a leaf wetness duration of ≥ 3 h was considered to be a prediction of the presence of downy mildew (P+) on lettuce trap plants, and a leaf wetness duration < 3 h was considered a prediction of the absence of downy mildew (P–). For each sampling day, the presence of lesions on trap plants was indicated by O+ (observed), and the absence of lesions was indicated by O– (not observed).

^b The proportion of true positives (O+ and P+) was regarded as a measure of sensitivity, the proportion of true negatives (O– and P–) as specificity, and the overall accuracy was calculated as the number of correct assessments (O+ and P+, and O– and P–) divided by the number of all assessments (9).

^c LR+ and LR– = positive likelihood ratio and negative likelihood ratio, respectively; LR+ = sensitivity/(1 – specificity) and LR– = (1 – sensitivity)/specificity (12).

where y is the number of lesions/leaf, a is the asymptote, x_0 is the ACC at the inflection point where the number of lesions/leaf is 50% of the asymptote, and b is the slope of the linear portion of the curve. The model appropriateness and goodness of fit to the data were assessed using an adjusted R^2 and a concordance correlation coefficient (12).

Relationship between IE and weather parameters under field conditions. Correlations among IE and selected weather variables were analyzed using Spearman's rank correlation matrix in R (Hmisc package 3.4.0). For each sampling date, the average temperature (TPa), maximum solar radiation (SR), and leaf wetness duration (LWD) were considered.

Binary recursive partitioning in the R tree package was used to build a classification tree for IE (15). This process successively split the data along coordinate axes of explanatory variables so that, at any node, the split which maximally distinguished the response variable in the left and right branches was selected. Each explanatory variable (TPa, SR, and LWD) was assessed in turn, and the variable explaining the greatest amount of deviance in IE was selected. The smallest permitted node was 10, and the node was not partitioned if the within deviance was ≤ 0.01 of the root node (15). The two explanatory variables explaining the greatest amount of deviance in IE were used to plot the classification tree using the partition.tree function. To construct the partition plot, IE was transformed into a binary variable where IE values of ≥ 0.1 (1 lesion/leaf/10 conidia/m³) were coded as "Is" (lesion) or "nls" (no lesion). IE values between 0.056 and 0.099 were rounded to 0.1.

Results

Pattern of daily ACC and relationship between leaf wetness duration and pattern of ACC. Most conidia were released outside the period between 06:00 and 10:00 h (Figs. 2 and 3). On 91, 95, 90, and 92% of the sampling days, conidia released between 06:00 and 10:00 h accounted for $<50\%$ of the conidia released daily in 1997, 1998, 2003, and 2004, respectively (Fig. 3). The maximum ACC was 12,236, 2,136, 2,885, and 3,707 in 1997, 1998, 2003, and 2004, respectively (Fig. 3). The percentage of conidia released between 06:00 and 14:00 h was 70, 76, 73, and 71% of the total conidia released daily in 1997, 1998, 2003, and 2004, respectively (Fig. 2).

In 1997, 76.6, 6.4, and 17.0% of all leaf wetness events occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 h, respectively (Fig. 2). In 1998, 90, 3.3, and 6.7% of all leaf wetness events occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 h, respectively. In 2003, 79, 14, and 7% of all leaf wetness events occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 h, respectively. In 2004, 79, 6.5, and 14.5% of all leaf wetness events occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 h, respectively. According to the data from all 4 years together, 80% of all leaf wetness events occurred between 22:00 and 06:00 h, 7% between 06:00 and 10:00 h, 13% between 10:00 and 22:00 h, and 8.5% between 18:00 and 22:00 h. In addition, the greatest daily ACCs were detected between 06:00 and 14:00 h (Fig. 2).

The overall accuracy of using a leaf wetness duration ≥ 3 h as a lettuce downy mildew risk indicator was greater when morning (06:00 to 10:00 h) leaf wetness was used than when evening (18:00 to 22:00 h) leaf wetness was used (accuracy of 0.545 versus 0.382). Using only morning leaf wetness led to 39.5% of downy mildew infections being predicted (sensitivity = 0.395). In contrast, using both morning and evening leaf wetness as a downy mildew risk indicator led to 68.4% of downy mildew infections being predicted. Also, using both morning and evening leaf wetness did not increase the likelihood ratio of positive prediction of downy mildew but decreased the likelihood ratio of negative prediction of downy mildew (Table 1). However, using leaf wetness events between 10:00 and 14:00 h (Table 1, T2), morning leaf wetness events (between 06:00 and 10:00 h; Table 1, T1), and evening leaf wetness events (between 18:00 and 22:00 h; Table 1, T3) did not improve the accuracy and sensitivity of the downy mildew risk indicator compared with using the combination of morning leaf wetness and evening leaf wetness (Table 1, T1 + T3).

Relationship between ACC and number of lesions/leaf. Growth-chamber trial. The number of lesions/leaf was directly proportional to ACC (Fig. 4). An ACC of 1 conidium/m³ was sufficient to cause 1 lesion/leaf based on the regression equation shown in Figure 4.

Fig. 4. Relationship between the number of downy mildew lesions/lettuce leaf and *Bremia lactucae* airborne conidia concentration under controlled conditions in a growth chamber (refer to main text for details on growth-chamber conditions and lettuce inoculation protocol). This study was done in Quebec, Canada. The solid straight line represents the fitted linear function and dotted lines represent the confidence interval. Each data point represents the mean number of lesion per leaf and the airborne conidia concentration.

Table 2. Estimated three-parameter sigmoidal regression models describing the relationship between the number of downy mildew lesions/leaf and *Bremia lactucae* airborne conidia concentration under field conditions in field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada

Year	a^a	p^b	b^c	p^d	x_0^e	p^f
2003	22.96 (0.80)	<0.0001	39.76 (2.03)	<0.0001	122.80 (3.85)	<0.0001
2004	20.24 (0.48)	<0.0001	35.46 (2.14)	<0.0001	117.47 (3.35)	<0.0001
2012	12.39 (3.16)	0.0008	18.99 (4.78)	0.0007	118.34 (6.97)	<0.0001
2013	15.73 (0.38)	<0.0001	71.43 (6.45)	<0.0001	121.62 (6.80)	<0.0001
2003 + 2004 + 2012 + 2013	19.29 (0.36)	<0.0001	31.42 (1.60)	<0.0001	116.75 (2.43)	<0.0001

^a Asymptote of the three-parameter sigmoid model, number in parentheses = standard error.

^b Probability that a is significantly different from 0 at $\alpha = 0.05$.

^c Slope of the linear portion of the curve.

^d Probability that b is significantly different from 0 at $\alpha = 0.05$.

^e Airborne conidia concentration at the inflection point where the number of lesions/leaf = 50% of the asymptote.

^f Probability that x_0 is significantly different from 0 at $\alpha = 0.05$.

Fig. 5. Relationship between the number of downy mildew lesions/lettuce leaf and *Bremia lactucae* airborne conidia concentration under field conditions in **A**, 2003; **B**, 2004; **C**, 2012; and **D**, 2013. A three-parameter sigmoidal model was fitted with the same shape parameter for each year (see equation 2 in the main text). CCC = concordance correlation coefficient, a measure of model appropriateness and goodness of fit (12), and R^2_{adj} = adjusted coefficient of determination. The study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada. Each data point represents the mean number of lesion per leaf and the airborne conidia concentration.

Fig. 6. Relationship between the number of downy mildew lesions/lettuce leaf and *Bremia lactucae* airborne conidia concentration under field conditions for the data pooled from 4 years (2003, 2004, 2012, and 2013). A three-parameter sigmoid model was fitted to the data (see equation 2 in the main text). CCC = concordance correlation coefficient, a measure of model appropriateness and goodness of fit (12), and R^2_{adj} = adjusted coefficient of determination. The study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada. Each data point represents the mean number of lesion per leaf and the airborne conidia concentration.

Field trial. The x_0 , which is an estimate of the ACC at the inflection point that corresponds to the number of lesions/leaf at 50% of the maximum (asymptote), was 122.80 ± 3.85 , $117.46 (\pm 3.35)$, 118.33 ± 6.97 , and 121.62 ± 6.80 conidia/m³ in 2003, 2004, 2012, and 2013, respectively (Table 2; Fig. 5). When the data from all 4 years were pooled, 116.75 ± 2.43 conidia/m³ were required to achieve 50% of the maximum number of lesions/leaf (20 lesions) (Table 2; Fig. 6). According to a sigmoidal regression model (Fig. 6), an ACC of 14 conidia/m³ was sufficient to cause 1 lesion/leaf. The sigmoidal model for all 4 years in Figure 6 consisted of three phases: a quasilinear phase between 0 and 15 lesions/leaf, in which the number of lesions/leaf was proportional to the ACC; a deceleration phase between 15 and 20 lesions/leaf; and a stationary phase of about 20 lesions/leaf, in which the number of lesions did not change even though the ACC increased (Fig. 6).

Relationship between IE and selected weather parameters. Under controlled conditions in the growth-chamber trials, IE ranged from 0.250 to 1.000; whereas, under field conditions, IE ranged from

Fig. 7. *Bremia lactucae* infection efficiency (IE) on plants of Ithaca lettuce under growth-chamber conditions and field conditions at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada, with $n = 30$ plants in the growth-chamber trial, and $n = 154$ for trap plants in the field trials. IE was defined as the ratio of the number of lesions/leaf to the airborne conidia concentration (see the main text for details on the growth-chamber conditions and the lettuce inoculation protocol). The “whiskers” of the box plots represent the lower quartile, “+” sign represents the mean value, and horizontal line inside box plots represents the median value.

0.034 to 0.100 in 2003, 0.033 to 1.000 in 2004, 0.000 to 0.100 in 2012, and 0.000 to 0.023 in 2013 (Fig. 7). Some weather parameters had significant associations with *B. lactucae* IE under field conditions. For all 4 years, IE increased as LWD increased (Table 3) whereas, for all years except 2003, IE decreased as daily TPa increased; in 2003, there was no significant association between the TPa and IE (Table 3). Overall, the TPa association with IE was weak (correlation coefficients of 0.00, -0.06 , -0.62 , and -0.06 in 2003, 2004, 2012, and 2013, respectively), and SR did not show significant association with IE except in 2004 (correlation coefficient was -0.60 and $P = 0.04$; Table 3). LWD was associated significantly with IE measured in all years, and TPa had limited association with IE (Table 3; Fig. 8). There was an exponential relationship between IE and LWD, with 120 min (2 h) of leaf wetness being sufficient to cause an IE of 0.1 (Fig. 8). Under field conditions, 2 h of LWD and an ACC of 10 conidia/m³ were needed to cause 1 lesion/leaf as long as the TPa was $<20^{\circ}\text{C}$ (Fig. 9). A leaf wetness duration of ≥ 180 min (3 h) caused at least 1 lesion per leaf even at temperatures between 20 and 25°C . Temperatures $\geq 26^{\circ}\text{C}$ were not tested in this study (Fig. 9).

Discussion

The majority of Canadian lettuce production is located in the province of Quebec (1) and, each year, downy mildew caused by *B. lactucae* occurs in this area (4). However, information on the epidemiology of *B. lactucae* is derived primarily from controlled-condition experiments or field experiments conducted in subtropical climates and, therefore, cannot readily be applied to Quebec lettuce production (2,13,17–20,23,24,27). Even though considerable efforts have been made to develop downy-mildew-resistant lettuce cultivars, no cultivar is resistant at all growth stages (seeding, young plant, and mature plant) and to all races of *B. lactucae* to avoid losses for growers (22). Therefore, efficient control of lettuce downy mildew currently depends on a good understanding of the epidemiology of *B. lactucae* and on implementing appropriate control measures. Moreover, lettuce is a short-season crop (45 to 55 days in Quebec) and, on farms with successive plantings of lettuce each season, as in Quebec, the potential risk of downy mildew and consequent yield losses is affected by inoculum build-up over the season. Therefore, the main purpose of this study was to investigate the relationship between *B. lactucae* airborne inoculum and downy mildew severity under Quebec weather conditions, and the ways in which environmental factors influence this relationship.

In Quebec lettuce production systems, the assumption (as a result of studies on the disease in other climates and regions) that infection by *B. lactucae* occurs only between 06:00 and 10:00 h has meant that

Table 3. Correlation matrix for infection efficiency (IE) of the lettuce downy mildew pathogen *Bremia lactucae*, average daily air temperature (TPa), solar radiation (SR), and leaf wetness duration (LWD)^a

Year	Variable	TPa ($^{\circ}\text{C}$) ^b	SR (KJ/m^2) ^b	LWD (min) ^b
2003	IE	0.00	-0.03	0.76^{***}
	LWD	0.00	-0.17	...
	SR	0.26	...	...
2004	IE	-0.06^{*}	0.25	0.66^{***}
	LWD	-0.05^{*}	0.25	...
	SR	0.04	...	...
2012	IE	-0.62^{*}	-0.60^{*}	0.72^{***}
	LWD	-0.28^{**f}	-0.49	...
	SR	0.36	...	...
2013	IE	-0.06^{*}	-0.21	0.68^{***}
	LWD	-0.39	-0.35	...
	SR	-0.04^{*}	...	...
2003 + 2004 + 2012 + 2013	IE	-0.48^{*}	0.19	0.84^{***}
	LWD	-0.33	0.06	...
	SR	-0.16^{*}	...	...

^a This study was done in field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada. IE = the ratio of the number of lesions/leaf to the airborne conidia concentration. Refer to main text for details on measurement of the variables TPa, SR, and LWD.

^b Asterisks: ***, **, and * indicate $P < 0.001$, 0.01, and 0.05, respectively.

>50% of the conidia released daily have not been considered. These conidia would need only to survive until the next leaf wetness event to infect lettuce leaves. Thus, under favorable weather conditions, there is a strong probability that infection can occur after 10:00 h. In this context, favorable conditions refer to leaf wetness, which is

the most important variable associated with lettuce downy mildew infection (10,20). According to data analyzed in this study for 1997, 1998, 2003, and 2004, 80% of leaf wetness events occurred between 22:00 and 06:00 h, 7% between 06:00 and 10:00 h, and 13% between 10:00 and 22:00 h. Thus, infection by *B. lactucae* could

Fig. 8. Relationship between *Bremia lactucae* infection efficiency (IE) and lettuce leaf wetness duration under field conditions for the data pooled from 4 years (2003, 2004, 2012, and 2013) together. An exponential growth function was fit to the data. IE was defined as the ratio between the number of downy mildew lesions/leaf and airborne conidia concentration. CCC = concordance correlation coefficient, a measure of model appropriateness and goodness of fit (12), and R^2_{adj} = adjusted coefficient of determination. The study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada. Each data point represents the mean number of lesion per leaf and the airborne conidia concentration.

Fig. 9. Occurrence of lettuce downy mildew (*Bremia lactucae*) lesions (ls) and lack of lesions (nls = no-lesion events) based on leaf wetness duration and average daily air temperature. Data from 4 years (2003, 2004, 2012, and 2013) were pooled. *B. lactucae* infection efficiency (IE) was defined as the ratio of the number of lesions/leaf to airborne conidia concentration. IE was transformed to a binary variable where IE values ≥ 0.100 (1 lesion/leaf/10 conidia/m³) were coded as ls and IE values ≤ 0.055 were coded as nls. IE values between 0.056 and 0.099 were rounded to 0.1. The study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada, with $n = 154$ trap plants in each field trial. The vertical line represents a threshold based on the leaf wetness duration, which distinguished the ls and nls events. The horizontal line represents a threshold based on the average daily temperature, which distinguished the ls and nls events.

occur at any time of the day. Also, using only morning leaf wetness as a lettuce downy mildew risk indicator led to 60% of downy mildew infections being missed. To improve the accuracy and sensitivity of leaf wetness events as a risk indicator of lettuce downy mildew, both morning and evening leaf wetness events should be considered. The results also suggest that, under Quebec weather conditions, infection by *B. lactucae* occurs mostly during the morning and evening. Similar observations have been made in subtropical climates, where conidia were released mostly at dawn and leaves were infected primarily in the morning (10,16). However, Hovius et al. (8) observed that, under Ontario (Canada) weather conditions, lettuce downy mildew did not always develop despite multiple leaf wetness events. Therefore, the limiting factor might be not only leaf wetness duration but also ACC. Scherm et al. (16) suggested that the availability of inoculum must be considered for predicting lettuce downy mildew. Hence, in addition to LWD, ACC should be considered, provided that the relationship between ACC and the severity of infection is known.

Under controlled growth-chamber conditions in this study, the number of lesions/leaf was directly proportional to ACC, and an ACC of 1 conidium/m³ was sufficient to cause 1 lesion/leaf. Under field conditions, however, the number of lesions/leaf was proportional to ACC up to 15 lesions/leaf, and the maximum number of observed lesions/leaf was 20, probably reflecting the limited amount of healthy leaf area available at such inoculum density. An ACC of 14 conidia/m³ was required to cause 1 lesion/leaf in the field. For *Botrytis* leaf blight of onion, the number of lesions/leaf was directly proportional to *Botrytis squamosa* ACC, and a threshold of 10 to 15 conidia/m³ was used successfully to rationalize fungicide applications (3,26). Similarly, the results of this study suggest that a *Bremia lactucae* ACC of 14 conidia/m³ may be a threshold to time fungicide applications for lettuce downy mildew management in this region. However, to improve the reliability of this threshold, it will be imperative to understand how environmental factors influence infection of lettuce by *B. lactucae*, for which the IE should provide a reliable response.

In this study, IE increased with increasing LWD but decreased with increasing TPa, although the TPa association with IE was weak (correlation coefficients of 0.00, -0.06, -0.62, and -0.06 in 2003, 2004, 2012, and 2013, respectively). These results are supported by the findings of Scherm and van Bruggen (17,19) and Wu et al. (27,29), who reported that an increase in infection by *B. lactucae* resulted from increasing LWD. Wu et al. (27) also concluded that the effect of temperature was minor. Moreover, this study found that the amount of SR recorded in the Quebec region did not have a significant association with IE, except in 2004 ($P = 0.04$). These results differ from results reported by Wu et al. (27), who found that a dose of 0.5 MJ/m² was sufficient to kill most of the *B. lactucae* conidia, whereas the SR registered over the course of this study ranged from 0.5 and 3.8 MJ/m². Similarly, Bhaskara Reddy et al. (2) found that $\geq 50\%$ of *B. lactucae* spores survived an SR dose of 10 MJ/m². In summary, LWD seemed to explain most of the variation in IE, and TPa had a minor effect on IE.

The IE of *B. lactucae* in this study increased exponentially with increasing LWD. To achieve an IE of 0.1 (1 lesion/10 conidia/m³), 120 min of leaf wetness was sufficient. Thus, at an average temperature of 20°C, 2 h of leaf wetness was sufficient to cause infection by *B. lactucae*. These results diverge from those reported by Scherm and van Bruggen (19), who established a minimum LWD of 4 h at temperatures between 5 and 20°C for successful infection in California. Therefore, in Quebec lettuce production systems, it may not be sufficient to consider an LWD of 3 to 5 h after dawn as a lettuce downy mildew risk indicator to initiate fungicide applications. Moreover, an LWD of >180 min enabled at least 1 lesion/leaf to develop, even at temperatures between 20 and 26°C.

This study examined the relationship between the ACC of *B. lactucae*, the number of lesions/leaf, and selected weather parameters that may significantly influence IE. The results showed that, under controlled conditions, an ACC of 1 conidium/m³ was sufficient to cause 1 lesion/leaf whereas, under field conditions, an ACC of 10 to 14 conidia/m³ was required to cause the same disease severity. Consequently, in addition to monitoring leaf wetness periods,

real-time monitoring of ACC should help improve lettuce downy mildew control measures for growers. Infection by *B. lactucae* can occur at any time of the day if there is a leaf wetness duration of ≥ 2 h. This study also revealed that, under Quebec weather conditions, both morning and evening leaf wetness events should be used to improve the reliability of LWD as a lettuce downy mildew risk indicator.

To the authors' knowledge, this is the first study to focus on the IE of *B. lactucae* under field conditions, and how environmental factors can influence IE. This study lays the groundwork for future use of an ACC of 10 to 14 conidia/m³ and an LWD of 2 h as risk indicators for determining the optimal time to apply downy mildew fungicide. In the Quebec lettuce production area, airborne conidia of *B. lactucae* could be monitored seasonally to assess the usefulness of these risk indicators. A similar risk indicator has been used successfully in onion crops in the same area to manage *Botrytis* leaf blight (3). Nevertheless, further research should be done to validate these risk indicators and assess how they can be integrated into management strategies for lettuce downy mildew in this region.

Acknowledgments

We thank all of the summer students involved in this study; and A. Lavasseur, A. Lefebvre, and R. Bacon for their technical support and advice. This work was supported financially by the Prime-Vert program (sous-volet 11.1) of the Quebec Department of Agriculture, Fisheries and Food (MAPAQ), by Agriculture and Agri-Food Canada, and by Compagnie de Recherche Phytodata Inc.

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