Development of an Infection Model for *Cercospora carotae* on Carrot Based on Temperature and Leaf Wetness Duration

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This research was funded by the Natural Sciences and Engineering Research Council of Canada and Conseil des Recherches en Pêche et en Agro-alimentaire du Québec.

We thank Dr. D. Cloutier for his suggestions on statistical analysis.

Accepted for publication 25 June 1990 (submitted for electronic processing).

ABSTRACT

Carisse, O., and Kushalappa, A. C. 1990. Development of an infection model for *Cercospora carotae* on carrot based on temperature and leaf wetness duration. Phytopathology 80:1233-1238.

Leaves of carrot (*Daucus carota* L. var. *Sativa* DC.), cv. Dagger, were inoculated with a conidial suspension (10^4 conidia/ml) of *Cercospora carotae* and incubated at various combinations of temperatures (16–32 C) and leaf wetness durations (12–96 hr). Infection was quantified by counting the number of lesions per inoculated leaf. In general, the lesion number increased with an increase in temperature and wetness duration, except at 32 C, where the lesion number decreased with an increase in wetness duration. Plants incubated at 16 and 32 C developed only 8% of the total number of lesions (all temperatures and wetness durations). A polynomial model (using arcsin $\sqrt{Y}$ transformation) and the Richards function were evaluated for reliability in describing infection as a function of temperature and leaf wetness duration. The Richards model fit the data better and was more relevant biologically.

Additional keywords: disease forecast, quantitative epidemiology.

Leaf blight induced by *Cercospora carotae* (Pass.) Solh. is a major foliar disease of carrots in the organic soil region of Quebec. Blight was present in 99 and 91% of the commercial carrot fields surveyed in southwestern Montreal in 1988 and 1989, respectively (4). The fungus may attack any aerial part of the plant and induces dark brown circular lesions. Blighted leaves weaken the petiole and reduce the grip required for mechanical harvesters to pull the carrots. Carrots not pulled by the harvester remained in the soil and contributed to yield loss. In commercial carrot fields the disease is controlled by weekly applications of protectant fungicides. An integrated pest management program for southwestern Montreal recommends that fungicide applications be initiated at 50% disease incidence for late sown carrots (14) and the subsequent applications at 7- to 10-day intervals. Although several protectant fungicides provide satisfactory control of the disease, very little is known about judicious scheduling of fungicide applications. Quantitative studies on the influence of environmental factors on disease development are required to improve Cercospora blight management. Both temperature and leaf wetness play a critical role in the infection process of foliar diseases (1,3,7,10,13). In 1944, Hooker (11) investigated the effect of temperature on Cercospora blight development for a wet period of 48 hr, but the combined effect of temperature and leaf wetness duration on infection has not been studied for Cercospora blight of carrots. A quantitative model relating temperature and leaf wetness duration to infection is necessary for the development of a forecast model to time fungicide application and to improve Cercospora blight management.

The objective of this study was to develop a mathematical model based on temperature and leaf wetness duration to predict infection of carrot leaves by *C. carotae*.

MATERIALS AND METHODS

Plant production. Carrot plants were seeded in 13-cm-diameter pots with a 3:1 (v/v) mixture of organic soil (27–30% organic matter) and perlite. Fertilizer was applied every 2 days in the following manner: first and fifth weeks with 200 ppm of 10-52-10 (N-P-K) mixture; second, third, and fourth weeks with 200 ppm of 15-15-18 mixture. Plants were grown in a growth chamber maintained at 20 C with 12 hr of light/day (200 $\mu Em^{-2}s^{-1}$) regime. The cultivar Dagger was used throughout the experiment because it is moderately susceptible to *C. carotae* (Arcelin and Kushalappa, *unpublished*) and is popular with Quebec growers. Plants used for inoculum production were grown in a similar way.

Inoculation and treatments. An isolate of *C. carotae* was obtained from naturally infected carrot leaves collected in 1987 at the Agriculture Canada Experimental Farm in Sainte-Clotilde, Quebec. A monospore isolate of the fungus was cultured on carrot leaf infusion agar as previously described (8). To reduce loss of vigor, conidia were subcultured only once (6,16). Inoculum was prepared by flooding 12-day-old culture plates with a solution of 0.01% Tween 80 (v/v) in distilled water. The cultures were gently rubbed with a glass rod to dislodge the conidia. The conidial suspension was filtered through a 100-mesh sieve to remove mycelial fragments and adjusted to a concentration of 10^4 conidia/ml (8). At the sixth leaf stage (5-wk-old plants), the second and third true leaves from bottom were tagged. The first true leaf was not used because it usually senesced before symptoms developed. Both upper and lower surfaces of the tagged leaves were inoculated (2). Leaves were sprayed to near runoff using an artist air brush (Badger-350) operated at 100 KPa air pressure. Immediately after inoculation the plants were placed in a mist chamber kept at specific temperatures. A fine mist was produced using a humidifier (Sovereign, model 707ms-110) installed inside a growth chamber (Conviron, model 15E). To prevent short circuits, the top portion of the growth chamber, where the electrical components are located, was separated from the lower half with a plexiglass sheet. A set of five plants was allowed to dry in a growth chamber maintained at 20 C and relative humidity of less than 65 ± 5% after an exposure of 12, 24, 48, 72, and 96 hr of leaf wetness. Since the plants took less than 10 min to dry, this additional duration of leaf wetness was considered negligible and consequently was not included in the wetness period. This procedure was repeated for temperatures in the mist chamber of 16, 20, 24, 28, and 32 C. The entire experiment consisted of 25 temperature and wetness duration combinations. To minimize variation, the inoculation and the distribution of plants in the mist chamber was randomized for

the five wetness durations (five plants per wetness duration) within each temperature. The same growth and mist chambers were used for all temperatures, and the order in which the temperature were tested was random. Because the temperature treatments were tested over time (each temperature was tested using different inoculum suspension), the percentage of spore germination of the conidial suspension for each inoculation was estimated to determine if there was any variation due to the inoculum. The percentage of spore germination was estimated by spraying three water agar plates with the conidial suspension and counting the number of spores that had germinated after 3 hr. Ten days after inoculation, the number of lesions on each inoculated leaf was counted at 2-day intervals until all the lesions appeared (or two successive similar readings occurred). The highest number of lesions developed in each of the two leaves were added, and the total number per plant was used in the analysis. The entire experiment was conducted twice.

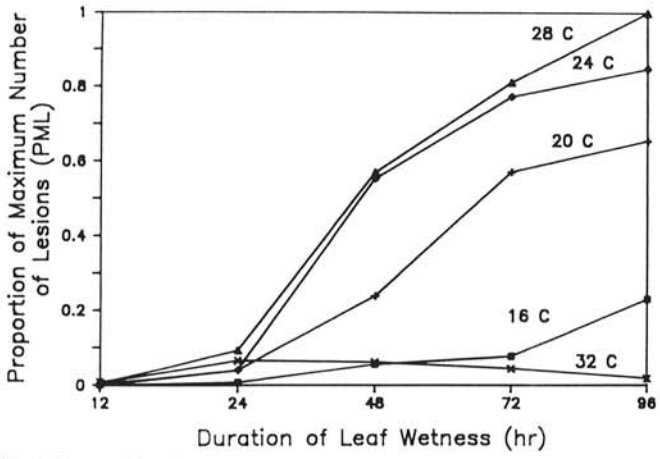

Fig. 1. Proportion of maximum number of lesions of *Cercospora carotae* observed on carrot leaves at various temperatures and leaf wetness durations. Each point was an average of observations made on 10 plants (2 experimental replications, 5 plants/replication, 2 leaves/plant).

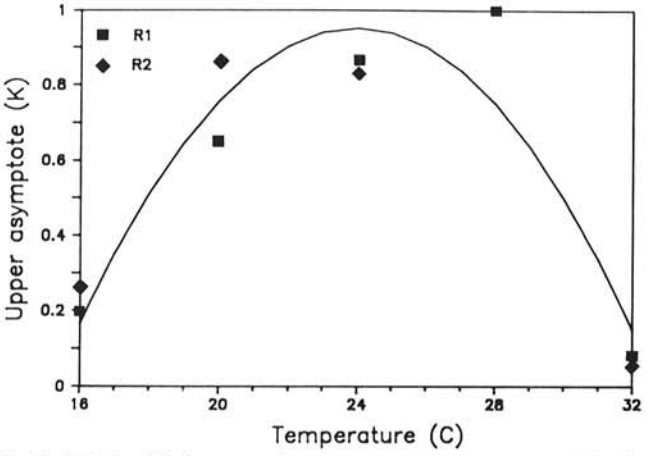

Fig. 2. Relationship between the upper asymptote parameter (K) of the Richards function and temperature for the pooled data of the two experimental replications. The value of K was calculated using equation 5 (see text). The ■ and ◆ refer to the data of experimental replications 1 and 2, respectively.

Data analysis and model development. Data were expressed as proportion of the maximum number of lesions (PML) derived as:

$$PML = \frac{\text{observed number of lesions}}{\text{maximum number of lesions observed}} \quad (1)$$

Both polynomial and Richards functions were used to describe the effect of temperature (T) and leaf wetness duration (W) on the PML. Models were evaluated based on the following criteria: significance of the estimated parameters, coefficient of determination, R^2 (i.e., goodness of fit between observed and predicted values of PML), and analysis of residuals.

An F-test was used to determine whether the percentage of spore germination of inoculum significantly varied between inoculations and to determine whether the results of the two experimental replications could be pooled. Both functions were fitted to the data using linear (PROC GLM) and nonlinear (PROC NLIN) modeling procedures for polynomial and Richards functions, respectively, in the Statistical Analysis System (SAS) program (18).

The polynomial model used was:

$$PML = \sin^2 f(T, W) \quad (2)$$

which can be rewritten as:

$$\arcsin \sqrt{PML} = f(T, W) \quad (3)$$

where arcsin is the inverse sine function of $\sqrt{PML}$, and $f(T, W)$ are linear combinations of temperatures and wetness duration. The model was fitted to the pooled data. All possible combinations of T and W terms were tested for the significance of the estimated parameters and coefficients of determination as described by Grove, et al (10).

The Richards model used was of the form:

$$PML = K(1 + e^{-rW})^{1/(1-m)} \quad (4)$$

where PML is the proportion of maximum number of lesions, K is the maximum possible PML at a given temperature (upper asymptote), r is the rate parameter, W is the duration of leaf wetness, and m is the shape parameter. A high r value indicates a rapid increase in PML with increase in wetness duration. Values of m near 2 give logistic-type curves, values near 1 give Gompertz-type curves, and values near 0 produce monomolecular-type curves. The function was fitted separately to the data of each replicate, the averaged data, and the pooled data in four steps using the general methodology outlined by Lalancette et al (15).

TABLE 1. Polynomial regression of the asymptote parameter (K) of the Richards[z] function on temperature

Model	DF error	Sum of square error	R^2	F P-value	Estimate/P-value b_0	b_1	b_2
Rep. 1	2	0.1118	0.83	0.169	−6.192	0.593	−0.012
					0.106	0.0895	0.088
Rep. 2	2	0.1144	0.83	0.169	−6.134	0.595	−0.012
					0.110	0.090	0.088
Avg.	2	0.1127	0.83	0.169	−6.163	0.594	−0.012
					0.107	0.089	0.088
Pooled	7	0.2289	0.83	0.002	−6.163	0.594	−0.012
					0.001	0.001	0.001

[z]Richards function; $PML = K(1 + e^{-rW})^{1/(1-m)}$ see text, equation 4.

First, an equation for predicting the upper asymptote of *PML* (K) was derived by regressing the maximum *PML* observed at each temperature against the second-order polynomial of temperature (T):

$$K = B_0 + B_1T + B_2T^2 \quad (5)$$

In the second step, the no-intercept version of the Richards function (Eq. 4) was fitted to the data for each temperature separately.

$$PML_t = K(1 + e^{-rW})^{1/(1-m)} \quad (6)$$

where PML_t is the proportion of maximum number of lesions for a given temperature (t) and $K = B_0 + B_1T + B_2T^2$ (Eq. 5). The values of r and m were estimated using nonlinear regression. The minimum wetness period of 12 hr (less than 1% infection was obtained for all temperatures) was subtracted from each wetness period so that the curves would begin at the origin (i.e. $W = 0$). The initial range of starting values was minimized by trying different ranges of starting points as recommended by Davis and Ku (9). A range of m-values were tested for the best fit over all temperatures.

The rate parameter (r) obtained separately for each temperature was regressed against the second-order polynomial of temperature:

$$r = B_0 + B_1T + B_2T^2 \quad (7)$$

where T is temperature and B_0, B_1, and B_2 are regression coefficients.

The final step, modeling *PML* as a function of T and W, was achieved by substituting the equation predicting the upper asymptote (K) (Eq. 5), the equation predicting the rate parameter (r) (Eq. 6), and the m value in the Richards function. The function was then fitted to the data for all temperatures by the nonlinear regression procedures as follows:

$$PML = B_0 + B_1T + B_2T^2 (1 + e^{-(B_0W + B_1TW + B_2T^2W)})^{1/(1-m)} \quad (8)$$

RESULTS

In general, infection occurred after 12 hr of leaf wetness and increased with increasing wetness duration (Fig. 1). Maximum number of lesions was reached at wetness duration of 96 hr at 16, 20, 24, and 28 C . At 32 C, infection increased from 12 to 24 hr of leaf wetness, then decreased, resulting in very low levels of infection for all wetness periods. The curves for infection against wetness duration at 20, 24, and 28 C were sigmoid. Lesion number increased slowly between 12 and 24 hr of leaf wetness at these temperatures. However, the number of lesions increased rapidly between leaf wetness durations of 48 and 72 hr (Fig. 1).

The percentage of spore germination of the inoculum varied from 88 to 92%, and the F-test indicated no significant difference between inoculations ($P > 0.05$). Therefore, the effect of inoculum associated with the temperature treatments (different inoculum was used for each temperature) was considered negligible, and data for all temperatures were pooled. The F-test indicated no significant difference between the two experimental replications ($P > 0.05$). Therefore, some analyses were performed on pooled data.

Polynomial model. The influence of temperature and leaf

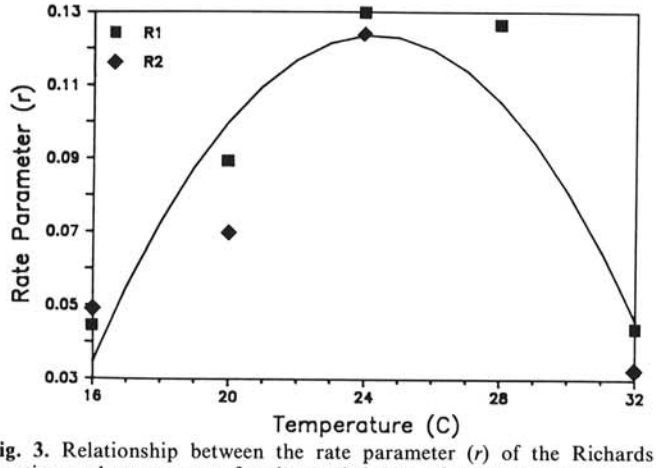

Fig. 3. Relationship between the rate parameter (r) of the Richards function and temperature for the pooled data of the two experimental replications. The value of r was calculated using equation 7 (see text). The ■ and ◆ refer to the data of experimental replications 1 and 2, respectively.

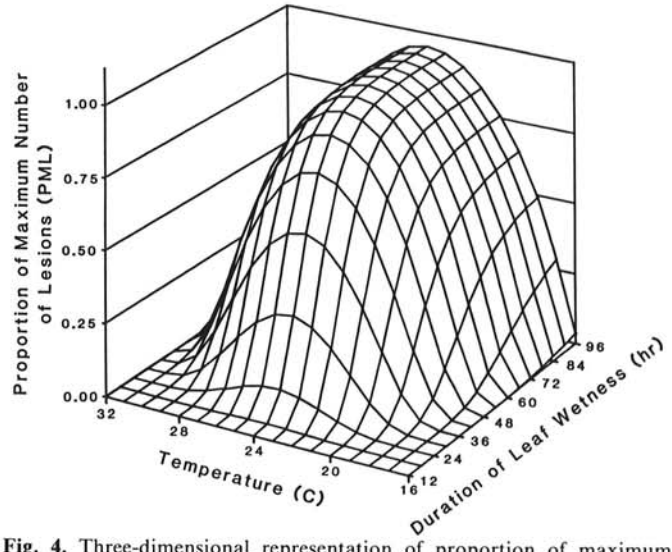

Fig. 4. Three-dimensional representation of proportion of maximum number of lesions predicted by the Richards model as a function of temperature and leaf wetness duration. The predicted values were calculated using the Richards model (Eq. 10, see text) derived from the pooled data of the two experimental replications.

TABLE 2. Polynomial regression of the rate parameter (r) of the Richards[z] function on temperature

Model	DF error	Sum of square error	R^2	F P-value	Estimate/P-value b_0	b_1	b_2
Rep. 1	2	0.0006	0.92	0.081	−0.660	0.064	−0.001
					0.052	0.041	0.042
Rep. 2	2	0.0013	0.80	0.198	−0.596	0.059	−0.001
					0.131	0.104	0.104
Avg.	2	0.0001	0.95	0.048	−0.457	0.047	−0.001
					0.035	0.024	0.024
Pooled	7	0.0024	0.83	0.002	−0.642	0.063	−0.001
					0.001	0.001	0.001

[z]Richards function; $PML = K(1 + e^{-rW})^{1/(1-m)}$ see text, equation 4.

wetness duration on infection of carrot by *C. carotae* was described by the following equation:

$$\text{Arcsin}\sqrt{PML} = B_0 - B_2W^2 + B_3T^2W + B_4T^2W^2 + B_5T^3W \quad (9)$$

The polynomial model accounted for 82% of the variation in proportion of maximum number of lesions for the pooled data. The intercept (B_0) was significantly different from zero, and all parameter estimates were significant ($P < 0.05$). The model indicated a linear and quadratic relationship between W and PML within the range of W studied. Interactions between T^2 and W, T^2 and W^2, and between T^3 and W were found to be significant. The model did not predict the initial lag phase in infection at wetness duration of less than 24 hr. The model overestimated infection at wetness duration less than 48 hr for all temperatures and underestimated infection at wetness duration of 48 hr for temperatures of 24 to 28 C. At 32 C it overestimated infection for all leaf wetness durations. Inspection of residual plots against temperature and against wetness duration revealed that residuals followed a nonrandom pattern of distribution at temperatures of 20, 28, and 32 C (at all wetness durations) and for wetness durations of 24, 72, and 96 hr (at all temperatures). The distribution pattern of residuals suggested a poorly specified model and a lack of precision in predicting low levels of infection (5,18).

Richards model: Estimation of model parameters. The maximum PML observed at each temperature (K) was highest at 20, 24, and 28 C and lowest at 16 and 32 C (Fig. 2). When K was regressed against temperature, the quadratic function (Eq. 5) described 83% of the variation in K for the two replicates, the pooled data, and the averaged data. The model fit to the

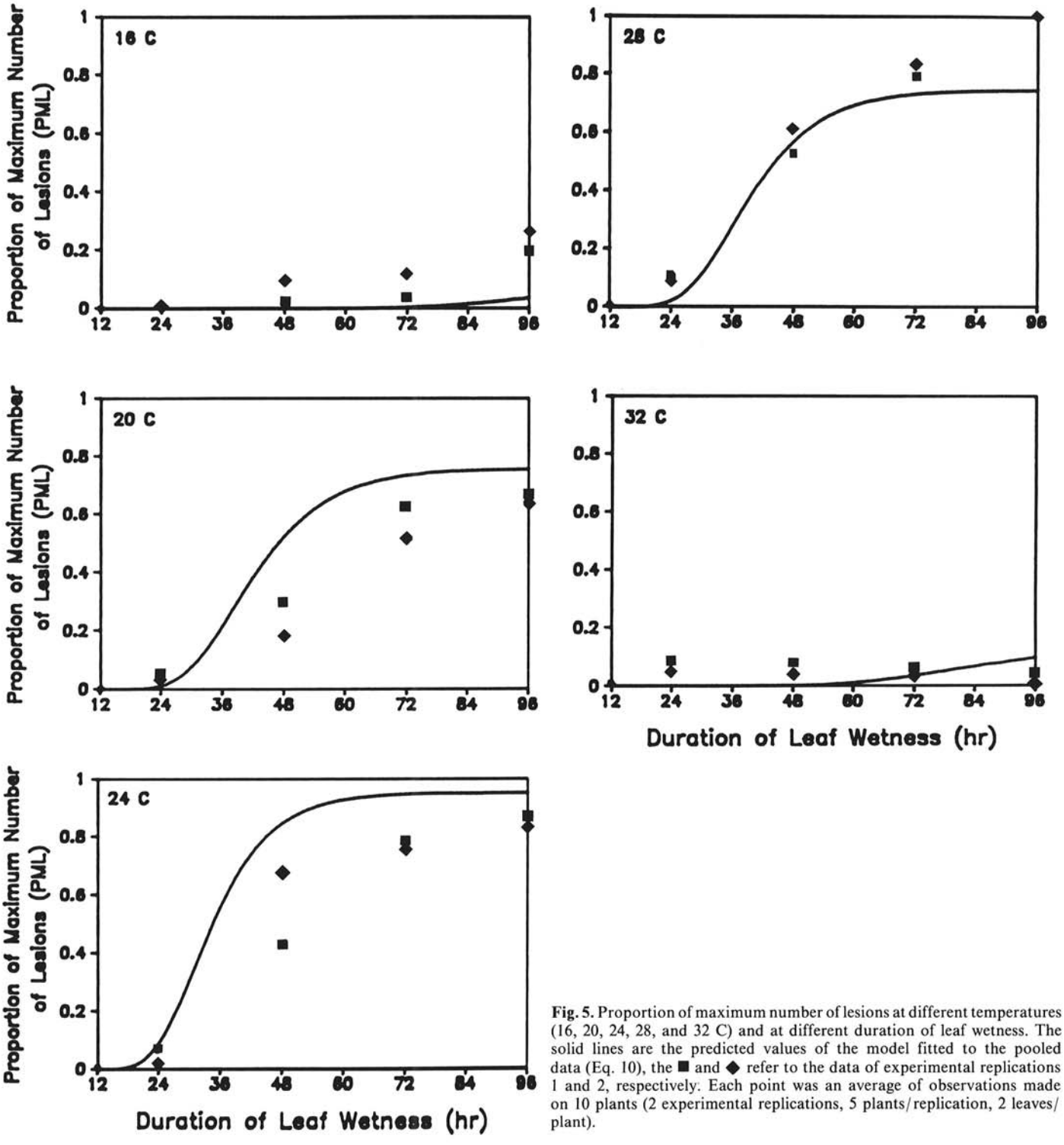

Fig. 5. Proportion of maximum number of lesions at different temperatures (16, 20, 24, 28, and 32 C) and at different duration of leaf wetness. The solid lines are the predicted values of the model fitted to the pooled data (Eq. 10), the ■ and ◆ refer to the data of experimental replications 1 and 2, respectively. Each point was an average of observations made on 10 plants (2 experimental replications, 5 plants/replication, 2 leaves/plant).

replicates and the averaged data, however, have insignificant parameters estimates (B_0, B_1, B_2) (Table 1).

The rate parameter (r) was the highest at 24 and 28 C and the lowest at 16 and 32 C (Fig. 3). When r was regressed against temperature, the quadratic function (Eq. 6) described 91, 80, 95, and 83% of the variation in r for the two replications, the averaged data, and the pooled data, respectively (Table 2). Intercepts (B_0) were significantly different from zero for the averaged and pooled data but not for the experimental replications. Although the replicates and averaged data produced a high R^2 for the prediction of K and r parameters, the F-statistics were much less significant than for the pooled data (Tables 1 and 2). However, with only two degrees of freedom, an F-test is not very powerful for determining significant relationships (19). Values of the shape parameter (m) for each temperature varied from 1.0 to 1.4 for all models. A final m value of 1.02, which produces a Gompertz-type curve, was chosen because it provided the best fit for all temperatures.

The nonlinear regression equation derived by fitting the Richards function (Eq. 8) to the data yielded high coefficients of determination for all models ($R^2 = 0.90$, 0.91, 0.88, and 89% for first replication, second replications, averaged data, and pooled data, respectively).

The nonlinear relationship between proportion of maximum number of lesions, temperature, and leaf wetness duration derived from the pooled data was:

$$PML = K\,(1 + e^{-(0.642W + 0.063TW - 0.00137T^2W)})^{1/1-1.02)} \quad (10)$$

where $K = -6.1633 + 0.5941T - 0.0124T^2$, and W is the leaf wetness duration less 12 hr. The three-dimensional response surface for equation 10 is given in Figure 4. The residual plots exhibited a random pattern of distribution for all temperatures and wetness durations. Normal plot of residuals of the model fitted on both data of the first and second experiments indicated good prediction at most levels of infection, even though the model slightly overestimated infection at 20 and 24 C and underestimated infection at 16 C (Fig. 5).

DISCUSSION

Although previous studies (11,20) of *C. carotae* did not quantify the relationship of temperature and leaf wetness duration with infection, they are useful for comparison. Hooker (11) observed that infection of carrots by *C. carotae* increased progressively with temperature for a wet period of 48 hr. He obtained significant increase in infection between 16 and 20 C and between 20 and 24 C. Infection was not significantly different between 24 and 28 C. Our results revealed a similar trend, an optimum range of 20–28 C for infection.

The trends for infection as a function of wetness duration derived by the Richards model were in accord with what was observed. These results are comparable with those of Venus (21), who compared the Richards function to the polynomial model and concluded that the choice of the model should be based not only on statistical criteria but on the biological relations as well.

Various approaches have been proposed to describe infection as a function of temperature and leaf wetness duration. Polynomial models are often used to describe the effect of environmental factors on infection (3). They are used because of the flexibility of the curves given by the degree of polynomial. These functions are mathematically simple, and methods of curve-fitting are available (18,19). However, polynomial functions have little biological meaning and fit is limited by the degree of polynomial that can be used (21). The Richards function is based on curves describing biological processes, and the parameters (K, r, m) completely describe the curve (17). However, the function is more difficult to use and direct estimation of the parameters is not always possible, particularly when the estimated parameters are highly correlated (9). Imhoff, et al (12) were unable to obtain a significant relationship between the asymptote and temperature or between the rate parameter and temperature for the prediction of bean rust (*Uromyces phaseoli*) urediospore germination. Lalancette, et al (15) found a significant relationship between the asymptote and temperature and between the rate parameter and temperature in predicting the effect of temperature and duration of leaf wetness on infection efficiency of *Plasmopara viticola* on American grape. The linearized version of the Richards function was used, and the parameter values (K, r) were predicted from temperature using regression equations. The model proposed here is similar to the two examples discussed above except that the Richards function was not linearized. A significant relationship was observed between the asymptote parameter and temperature and between rate parameter and temperature. Theoretically, the model should allow accurate prediction of infection. However, because both the asymptote and the rate parameter were expressed as a second-order polynomial of temperature, the equation should not be applied to temperatures outside the range used to derive the estimates. The temperature range considered in the experiment was wide compared to that normally occurring in the field (in Quebec) when blight is present. Furthermore, low infection at the extreme temperatures indicated that the biologically relevant temperature range was studied.

The temperature and leaf wetness duration critically influenced the infection process of *C. carotae*. A minimum of 24 hr of leaf wetness at temperatures ranging between 20 and 28 C was necessary to induce severe infection in growth chambers. Such long periods of leaf wetness rarely occur in the field in Quebec. Other studies on infection are needed to explain the development of disease under field conditions. The effect of high relative humidity and interrupted leaf wetness may have to be considered in further studies on Cercospora blight infection.

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