Influence of Interrupted Wet Periods, Relative Humidity, and Temperature on Infection of Carrots by *Cercospora carotae*

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ABSTRACT

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Carrot leaves were inoculated with a conidial suspension (10^4 conidia per milliliter) of *Cercospora carotae*. Then they were subjected to interrupted and continuous wet periods of various durations and to several combinations of relative humidity (RH, 84–100%) and temperature (16–32 C) with and without an initial wet period of 6 h. Number of lesions per leaf decreased with increasing length of dry period for dry periods greater than 3 h. However, a dry period of 3 h with initial and final wet periods of 24 and 12 h, respectively, resulted in more lesions per plant than the corresponding continuous wet period (39 h). The number of lesions increased with an increase in initial wet period duration for a fixed dry interruption period of 6 h. For all temperatures, very few lesions developed at 84% RH. However, the number of lesions increased rapidly with increase in percent RH greater than 84%. In general, the plants exposed to an initial wet period of 6 h developed more lesions than those exposed to RH only. The number of lesions per plant was transformed to proportion of those at a continuous wet period and to proportion of maximum number of lesions for experiments on interrupted wet period and RH, respectively. Polynomial models were used to describe the effects of dry period durations, initial wet period durations, and combinations of RH and temperature on infection.

Additional keywords: disease forecast, quantitative epidemiology.

The fungus *Cercospora carotae* (Pass.) Solheim is found in almost all carrot fields (organic soil) in Quebec and is very common in Ontario (3,17). The fungus also is present in the United States (18). The fungus attacks only the aerial parts of the plant. The economic loss due to this fungus occurs during mechanical harvesting when the diseased leaves and petioles break off easily, making it difficult to pull the roots from the ground. In Quebec, Cercospora blight is controlled by weekly applications of protectant fungicides. However, not all of these fungicide applications are needed and the best time to initiate fungicide applications is not yet established. The combined effects of constant temperature and continuous leaf wetness on infection of carrots (*Daucus carota* L. var. *sativa* Hoffm.) by *C. carotae* have been studied, and a mathematical model to predict infection as a function of temperature and leaf wetness duration has been established (9). In carrot fields in Quebec, long periods of leaf wetness rarely occur; in spite of this, the disease often reaches epidemic levels. The presence of disease when only short periods of leaf wetness are available suggests that interrupted leaf wetness or periods of high humidity may be sufficient for spore germination and penetration, as was reported for other pathogens (1,2,4,10,11). The *Cercospora* species are known for their tolerance to drying (12). In addition, studies on *C. beticola* (13,14) indicated that nocturnal wetting and diurnal drying may be more favorable for spore germination and penetration than continuous wetting.

When infection models based on continuous wetness periods are used for disease prediction, it becomes difficult to interpret the results of cyclic wet-dry-wet periods. In such cases the two wet periods can be considered as one continuous wet period if the pathogen growth has momentarily stopped during the dry period and resumed with wet conditions. However, if the pathogen continues to grow or if the dry period has a detrimental effect on pathogen growth, the wet periods interrupted by a dry period should be considered as one continuous wet period corrected for the effect of the dry period.

In carrot fields, when the rows are almost covered by the carrot leaves, a microclimate with long periods of high relative humidity (RH) could occur in the absence of leaf wetness. In such situations, an infection model based only on leaf wetness may underestimate infection. Studies on the influence of humidity and interrupted leaf wetness periods on infection could be helpful in refining the original model.

The objectives of this study were 1) to examine the influence of dry period and of initial wet period duration during interrupted wet period on infection, 2) to study the combined effects of humidity and temperature on infection with and without short initial wetness period, 3) to establish infection criteria and to develop mathematical models describing the effect of these factors on infection so that these models (and criteria) can be eventually used to correct the original infection model based on temperature and leaf wetness duration (9).

MATERIALS AND METHODS

Plant production. Carrot plants (cv. Dagger) were seeded in 13-cm-diameter pots with a 3:1 (v/v) mixture of organic soil (27–30% organic matter) and perlite. Fertilizers (200 ppm of 19-52-19 N-P-K) were applied every 2 days. For experiments on interrupted leaf wetness, the plants were placed in a growth chamber maintained at 20 C with 12 h of light per day (200 $\mu E \cdot m^{-2} \cdot s^{-1}$). In experiments on temperature and RH, the carrot plants were grown in a greenhouse adjusted to 22 ± 2 C with 12 h of light per day (200–300 $\mu E \cdot m^{-2} \cdot s^{-1}$). All experiments were conducted with 5-wk-old carrot plants (7).

Inoculum production and inoculation. A single-spore culture of *C. carotae* was maintained on carrot leaf infusion agar at 26 C under 12-h light per day until it was required for inoculation (6–8). Fresh cultures were obtained by successive inoculations of carrot leaves and reisolations from infected leaves (8). Conidia were harvested from 12-day-old cultures using a solution of 0.01% Tween 80. The concentration of the conidial suspensions was

adjusted to 10^4 conidia per milliliter using an hemacytometer. Percent spore germination was estimated for all inoculations by spraying three water agar plates with the conidial suspension used for the inoculations. One agar plate was sprayed at the beginning, one in the middle, and one at the end of each inoculation. The number of germinated spores was counted 3 h after the plates were sprayed.

At the sixth leaf stage, the second and third true leaves from the bottom were tagged. The tagged leaves were inoculated on both surfaces until runoff using an artist air brush (model 350, Badger Air Brush Co., Franklin Park, IL) operated at 100 kPa of air pressure. Immediately after inoculation, the plants were placed in a mist chamber or a humidity-controlled growth chamber (model PGW36 M10, Conviron, Winnipeg, with RH controlled by bypass dehumidification) kept at the required temperature. Because carrot leaves take less than 10 min to dry off in a growth chamber, drying time was not included in the wetness duration.

The effects of interrupted leaf wetness and of temperature and RH on infection were examined in four series of experiments. The first two experiments were conducted to investigate the effects of dry period and duration of initial wet period on infection, respectively. The third and fourth experiments were conducted to examine the effects of temperature and RH on infection, without and with an initial leaf wetness period, respectively. All four experiments were arranged as a completely randomized design, conducted twice, and each treatment included four experimental units (four plants, two leaves per plant). Infection was quantified by counting the number of lesions on each inoculated leaf at 2-day intervals starting 10 days after inoculation and continuing until two similar readings were obtained. The numbers of lesions on each of two inoculated leaves per plant were summed, and the total number of lesions per plant was used in all analyses.

Influence of dry period and duration of initial wet period on infection. After inoculation with the conidial suspension of *C. carotae,* the plants were subjected to either continuous or interrupted wet periods. The interrupted wet period consisted of an initial wet period followed by a dry period and a final wet period. A dry period was defined as percent RH less than 65 ± 5%. Humidity during the dry period was monitored using a data logger (CR-10, Campbell Scientific Canada Corp., Alberta). The interrupted wet period consisted of initial and final wet periods of 24 and 12 h, respectively, separated by a dry period of 3, 6, 12, 18, 24, 30, or 36 h. Durations of the continuous wet periods were 36, 39, 42, 48, 54, 60, 66, and 72 h. Thus, for each interrupted wet period treatment, there was a corresponding continuous wet period treatment with the same total duration. In a second experiment, the effects of various durations of initial leaf wetness period on infection were tested. After inoculation the plants were subjected to an initial wet period of 0, 3, 6, 12, 18, 24, 30, 36, 42, or 48 h, followed by a fixed dry period of 6 h and a final wet period fixed so that the total length of the cycle was equal to 48 h.

Data analysis. The total number of lesions per plant was transformed to proportion of number of lesions obtained under continuous wet period (PCWP) as follows: PCWP = number of lesions in interrupted wet period/number of lesions in corresponding continuous wet period.

The PCWP for each interrupted wet period treatment was used to evaluate relationships between the continuous wet period and interrupted wet period of the same duration including dry period. The number of lesions per plant was used to compare the lesion production in the interrupted wet period with that in the 36-h continuous wet period. The number of lesions obtained for the seven interrupted wet period treatments were compared with the number of lesions resulting from 36 h of continuous wetness to determine if the infection can be attributed only to the initial and final wet periods. The *F* test was used to determine if pooling of the two experimental trials was allowed. The PCWP for all treatments (separately for each experiment) was subjected to analysis of variance and regression analysis to find equations that best described the PCWP as a function of dry period duration and as a function of initial wet period duration.

Influence of temperature and RH on infection. After inoculation with the conidial suspension of *C. carotae,* the plants were placed in four chambers all adjusted to a specific temperature and to RHs of 84, 88, 92, and 96%, ±1.5%. Humidity in the chamber was continuously monitored using a data logger (CR-10, Campbell Scientific) and the growth chamber sensor. The wet treatment was created by enclosing the plants in plastic bags. After an infection period of 72 h, all of the plants were returned to the greenhouse until symptom development. This procedure was repeated for temperatures in the RH-controlled chambers of 16, 20, 24, 28, and 32 C (in a random order). A second experiment was designed to study the effects of temperature and RH when the plants were exposed to a short leaf wetness period before the exposure to various levels of RH (84, 88, 92, and 96%) and leaf wetness. Immediately after inoculation the plants were enclosed in plastic bags to maintain leaf wetness. After 6 h the plastic bags were removed, except for those of the leaf wetness treatment in which plants were retained in the plastic bags. After a total of 72 h, all of the plants were returned to the greenhouse until symptom development. This procedure was repeated for temperatures in the RH-controlled chambers of 16, 20, 24, 28, and 32 C (in a random order).

Data analysis and model development. The total number of lesions per plant was transformed to proportion of maximum number of lesions (PML) as follows: PML = number of lesions observed/maximum number of lesions observed.

The PML obtained for all humidity treatments was subjected to analysis of variance and linear regression analysis to find equations that described best the PML as a function of the percent RH and temperature, without and with an initial wet period of 6 h.

RESULTS

Influence of duration of dry periods on infection. Interrupted wet periods resulted in significantly ($P = 0.05$) fewer lesions per plant than continuous wet periods (Table 1). An increase in the duration of dry periods, between 24 h of initial and 12 h of final wet periods, significantly reduced infection as compared with those at a corresponding duration of continuous wet period, except for the 3-h interruption that resulted in more lesions than the corresponding continuous wet period treatment (39-h continuous wet period) (Table 1). The number of lesions produced under a 36-h dry period was 29% of the number of lesions obtained at the corresponding continuous wet period treatment (72-h con-

TABLE 1. Influence of length of dry period during interrupted wet period and continuous wet period on infection of carrot leaves by conidia of *Cercospora carotae*

Treatment (h)					
IWP[u]			CWP[v]	Lesions per plant[w]	
Wet	Dry	Wet	Wet	IWP	CWP
24	0	12	36	54.6 c[x]	54.6[y]
24	3	12	39	92.1 a	61.4[z]
24	6	12	42	69.4 b	91.2[z]
24	12	12	48	65.9 bc	101.2[z]
24	18	12	54	71.7 b	122.9[z]
24	24	12	60	72.7 b	137.6[z]
24	30	12	66	53.0 c	144.4[z]
24	36	12	72	55.5 c	188.2[z]

[u] The interrupted wet period (IWP) consisted of initial and final wet periods of 24 and 12 h, respectively, separated by a dry period of various lengths.
[v] The continuous wet period (CWP) consisted of an uninterrupted leaf wetness period equal to the total duration of the corresponding IWP treatment.
[w] Means of two trials with four plants per trial, two leaves per plant.
[x] Values followed by the same letter are not significantly different according to the Waller-Duncan *K*-ratio *t* test (*K*-ratio = 100).
[y] The same data were used for both 24-0-12 IWP and 36-h CWP treatments.
[z] Mean values between IWP and CWP columns differ significantly ($P = 0.01$) according to the least significant difference test.

tinuous wet period) for both trials. Mean number of lesions per plant from interrupted wet period treatments with dry periods ranging from 3 to 24 h were significantly higher than the mean number of lesions obtained from 36 h of continuous wetness, except for the treatment with a 12-h dry period. However, for dry periods of 30 and 36 h, the number of lesions was not significantly different from 36 h of continuous wetness and from the treatment with a 12-h dry period. Dry periods of 6–36 h have a significant effect on lesion production ($P = 0.0001$), and a first-order polynomial model explained the relationships between dry period durations and PCWP ($R^2 = 0.77$) (Fig. 1).

Influence of initial leaf wetness duration on infection. Plants exposed to an initial wet period of 0–24 h had fewer lesions than the control (48-h continuous wet period) (Fig. 2). The PCWP increased linearly with increase in initial wet period (Fig. 2). The interrupted wet period treatments with no initial wet period produced 8 and 18% of the number of lesions obtained at the 48-h continuous wet period for the first and second trials, respectively. The initial wet period duration has a significant effect on infection ($P = 0.0001$), and a first-order polynomial model explained the relationship between dry period durations and PCWP ($R^2 = 0.74$) (Fig. 2). For these two experiments the F test indicated no significant difference ($P > 0.05$) between the two experimental trials.

Influence of RH and temperature on infection. In general, the number of lesions per plant increased with an increase in humidity level for all temperatures (Fig. 3). The relationship between RH and PML was not linear for the temperatures studied. Maximum number of lesions was reached under leaf wetness for all temperatures. The number of lesions increased with increase in temperatures ranging from 16 to 28 C and decreased at 32 C. For the plants exposed to an initial wet period of 6 h (Fig. 3B), the number of lesions increased rapidly between 84 and 96% RH, but the increase was rather slow at 96 and 100% RH (leaf wetness). No lesions were observed at 84% RH (Fig. 3) and only a few at 88% RH (Fig. 3A) when plants were not exposed to an initial wet period. However, the number of lesions increased rapidly between 88 and 100% RH. In general, the number of lesions observed on the plants exposed to an initial wet period (Fig. 3B) was higher for all RH levels and for all temperatures than those of plants exposed to humidity only (Fig. 3A). Percentage of spore germination of the inoculum used in these experiments varied from 92 to 97%, and the F test indicated no significant difference ($P > 0.05$) between inocula. Therefore, the effect of inoculum associated with the temperature treatment (different inoculum was used for each temperature) was considered

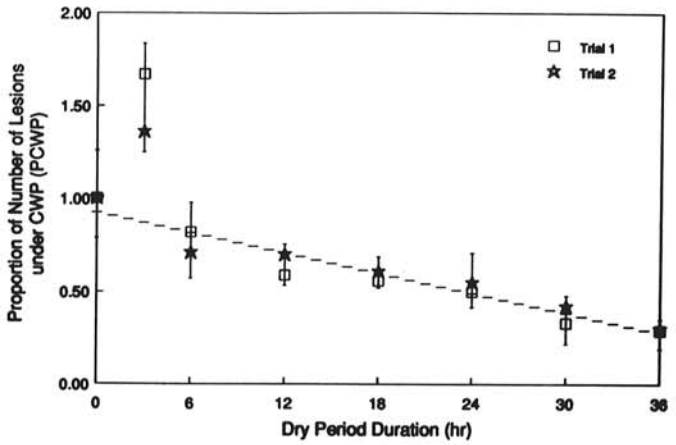

Fig. 1. Influence of duration of dry periods on infection of carrot leaves by *Cercospora carotae*. The regression equation is $Y = 0.9243 - 0.018X$, $R^2 = 0.77$, where Y is proportion of number of lesions obtained under continuous wetness and X is the dry period duration between the 24-h pre- and 12-h postdry wet periods. R^2 is the coefficient of determination. The dashed line represents the regression line and the error bars represent the range of observed values. Each point is an average of observations made on four plants, two leaves per plant. The 3-h dry period treatment was not included in the regression analysis.

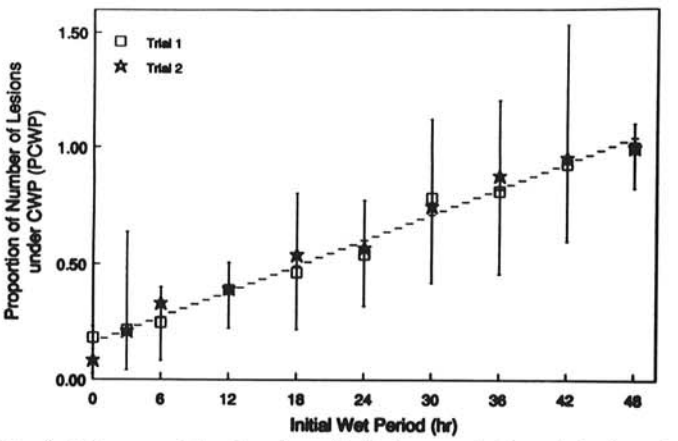

Fig. 2. Influence of the duration of initial wet periods on infection of carrot leaves by *Cercospora carotae*. The regression equation is $Y = 0.1593 + 0.0184X$, $R^2 = 0.74$, where Y is proportion of number of lesions obtained under 48-h continuous wetness and X is the duration of the initial wet period. R^2 is the coefficient of determination. All initial wet periods were followed by a 6-h dry period and then by a wet period to make up the total of 48 h. The dashed line represents the regression line and the error bars represent the range of observed values. Each point was an average of observations made on four plants, two leaves per plant.

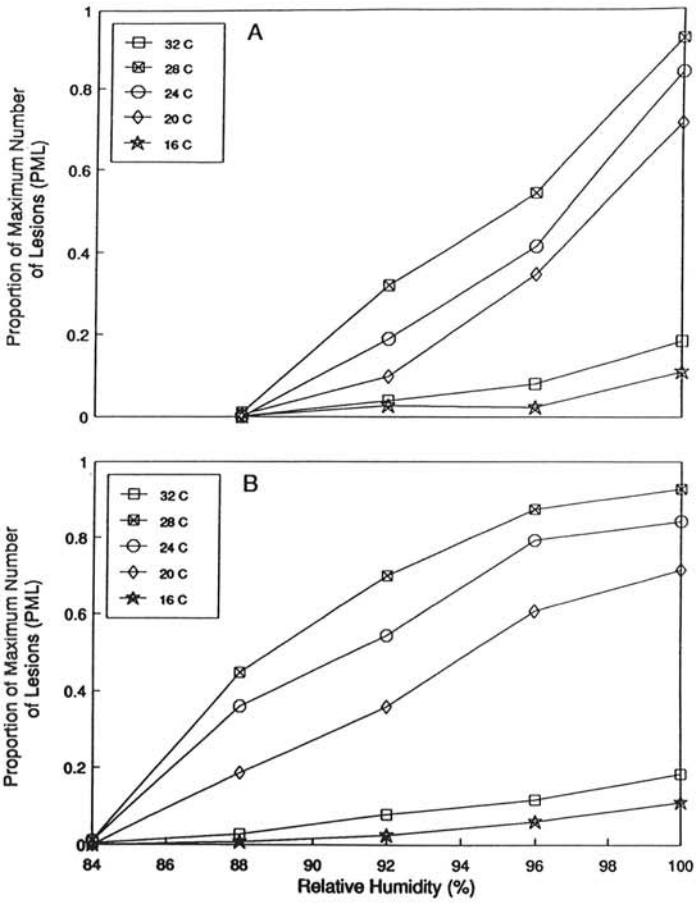

Fig. 3. Observed proportion of the maximum number of lesions of *Cercospora carotae* observed on carrot leaves at various temperatures and relative humidities. **A,** Plants exposed to continuous humidity. **B,** Plants exposed to 6 h of leaf wetness before being subjected to various levels of relative humidity. The maximum number of lesions observed at 28 C under leaf wetness was 550 and 492 for the first and second trials, respectively. Each point was an average of observations made on 10 plants (two experimental replications, five plants per replication, two leaves per plant).

negligible, and data from all temperatures were pooled. The influence of RH and temperature on infection was described by the following equations for experiments without and with initial wetness, respectively:

$$\text{Arcsin}\sqrt{\text{PML}} = 16.334 - 0.6844H + 0.6569 \times 10^{-4}H^2 + 2.378T - 0.3853T^2 + 0.0142T^3 - 0.1527 \times 10^{-4}T^4 + 0.0542HT - 0.1111 \times 10^{-3}HT^2 \quad (1)$$

$$\text{Arcsin}\sqrt{\text{PML}} = -26.862 + 0.0575H - 0.2443 \times 10^{-3}H^2 + 3.9208T - 0.4144T^2 + 0.0141T^3 - 0.1537 \times 10^{-4}T^4 + 0.0384HT - 0.7872 \times 10^{-4}HT^2 \quad (2)$$

where H is percent RH and T is temperature (C) (Fig. 4). The polynomial model accounted for 97 and 96% of the variation in PML for experiments without and with initial wetness, respectively. Both models indicated a quadratic relationship between H and PML and a quartic relationship between T and PML. The interaction between H and T and between H and T^2 were found to be significant. Although the coefficient of determination was high for both models, the two models overestimated the PML at 20, 24, and 28 C and 96 and 100% RH (Fig. 4). The lack of a definite pattern of distribution of residuals indicated that the models are appropriate.

DISCUSSION

The interrupted wet period significantly reduced infection. This warrants incorporation of the effect of interrupted wet period into the infection model based on duration of continuous leaf wetness (9). Plants given a 24-h initial wet period followed by 3- to 24-h dry periods have more lesions per plant than those exposed to a 36-h continuous wet period, except for the 12-h dry period for which the number of lesions was not significantly different. However, for dry interruptions of 30 and 36 h the number of lesions per plant was not significantly different than that for the 36-h continuous wet period. Germinated spores can survive dry periods and resume growth when wetted again. These observations were similar to those reported for *Cercospora* species on other plants (14). Good and Zathureczky (12) demonstrated that spores of *C. musae* have a considerable ability to tolerate drying.

Increased infection observed under a 24-3-12 wet-dry-wet period compared with a 36-h continuous wet period remains unexplained, and histopathological studies are needed to fully understand the mechanism of spore germination and penetration by *C. carotae*. This phenomenon can be partially explained by the presence of large droplets of water on the leaf surface, which may have reduced germ tube contact with the leaf surface. Either the 3-h dry period stimulated the infection process and the effect gradually reduced with increase in dry period up to 24 h, or the continuous wet period is not the optimum.

The 6-h dry period then was used to examine the effects of initial wet period durations on infection. Dry interruptions occurring after an initial wet period of 24 h or less resulted in fewer lesions than those after 24 h. These results supported conclusions of a previous experiment on infection of carrot leaves by *C. carotae* (9), which indicated that a minimum of 24 h of leaf wetness was required to induce infection in growth chamber experiments.

Infection under interrupted wetness by a fungal pathogen is due to either rapid germination and penetration or capacity of the germinating spores to survive intermittent drying. Although no detailed studies of germination, penetration, and survival of spores were performed, our results suggest that it is probably the ability of spores of *C. carotae* to survive drying rather than rapid germination and penetration that is responsible for successful infection under interrupted wet period. In practice, it means that two wet periods separated by a dry period of $\leq$12 h should be considered as one infection period. Also, the cumulative effect must be calculated for wet-dry-wet periods if the temperature is favorable.

Leaf wetness is more favorable for infection than RH greater than 84%. High humidity reduced infection but, as for other pathogens, high RH (84% < RH < 100%) is sufficient to allow infection (13–16). A detailed study of the infection process of *C. zeae-maydis* in corn leaves revealed that high RH may be more favorable to spore penetration than free water, which reduces tropistic response toward stomata, appressorium formation, and subsequent penetration (5). A similar trend was not observed for *C. carotae*. Decrease in RH level caused rapid reduction in infection, even though the reduction was less rapid when the plants were exposed to a short initial wetness period (6 h).

Under field conditions in Quebec, periods of leaf wetness are usually preceded and followed by periods of high RH (>90%). A short period of leaf wetness (<6 h) may be sufficient to trigger infection (spore germination and penetration), and subsequent high humidity probably supports the completion of remaining phases of the infection process (colonization).

An understanding of the influence of interrupted wet periods and RH should improve prediction of infection of carrots by *C. carotae* and enable preventive treatment. Two or more wet

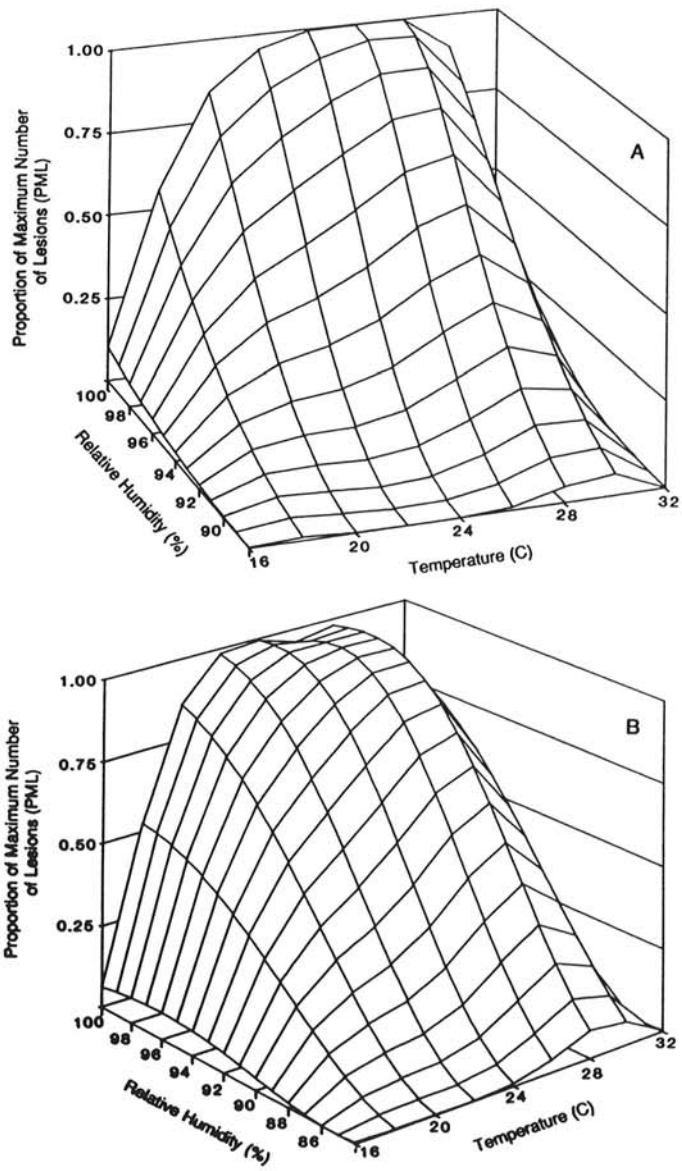

Fig. 4. Proportion of the maximum number of lesions of *Cercospora carotae* predicted by the polynomial model as a function of temperature and percent relative humidity. **A,** Predicted values calculated using equation 1 (see text). **B,** Predicted values calculated using equation 2 (see text).

periods (≤24 h), for each of which no infection is predicted, can be added, and the predicted infection can be corrected to account for the duration of the dry period and of the initial wet period. The wet period also can be extended when the RH is high (>90%).

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