Influence of Temperature and Leaf Wetness Duration on Infection of Strawberry Leaves by *Mycosphaerella fragariae*

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ABSTRACT

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In controlled environment studies, the influence of temperature and wetness duration on infection of strawberry leaves by *Mycosphaerella fragariae* was quantified by inoculating plants with a conidial suspension and incubating them at various combinations of temperature (5 to 35°C) and leaf wetness duration (0 to 96 h). Infection was expressed as the number of lesions per square centimeter of leaf surface and relative infection was used to develop an infection model. Younger leaves were more susceptible to infection. Regardless of temperature and duration of leaf wetness, only few lesions developed on the oldest (19 to 21 days old) and intermediate leaves (12 to 15 days old), respectively (maximum of 1.7 and 2.3 lesions per cm^2) as compared to the youngest leaves (5 to 7 days old; maximum of 12.6 lesions per cm^2). On the youngest leaves, lesions developed at all temperatures except at 35°C, and the number of lesions, for all leaf wetness durations, increased gradually from 5 to 25°C and decreased sharply from 25 to 30°C. For temperatures of 15 and 20°C, the number of lesions increased gradually when leaf wetness duration increased from 12 to 96 h. At 25°C, the number of lesions increased with increasing leaf wetness from 12 to 48 h and then at a higher rate from 48 to 96 h. The optimal temperature for infection was 25°C. For most temperatures, a minimum of 12 h of leaf wetness was necessary for infection (more than 1 lesion per cm^2). Relative infection was modeled as a function of both temperature and wetness duration using a modified version of the Weibull equation (R^2 = 0.98). The resulting equations provided a precise description of the response of *M. fragariae* to temperature. The model was sufficiently flexible to account for most characteristics of the response of *M. fragariae* to wetness duration. The model was used to construct a risk chart that can be used to estimate the potential risk for infection based on observed or forecasted temperature and leaf wetness duration.

Additional keywords: common leaf spot, disease management, disease modeling.

Leaf spot, caused by *Mycosphaerella fragariae* (Tul.) Lindau (= *Ramularia tulasnei* Sacc.), is injurious to numerous cultivars of strawberry (*Fragariae* X *ananassa* Duschesne) in Canada (4,6,10) and in the United States (5,11,14,24,25). Initial symptoms are small purple spots that first appear on young leaves. As the spots enlarge, the centers become necrotic with distinct red borders. Lesions produced by *M. fragariae* differ depending on host cultivars and environmental conditions during infection (10,16,20). The pathogen may also attack the petioles and fruit calices, causing symptoms similar to those found on the leaves, although lesions on the petioles are more elongated than those found on leaves (22). Fruit infection occurs under severe epidemics, in which case some or all seeds and underlying areas become discolored, rendering the fruit unmarketable (11). In eastern Canada, epidemics of leaf spot occur from June to October and can reduce fruit market value and affect vegetative growth in the next season (17).

Several reports suggest that better management of leaf spot is possible through exploitation of host resistance (14,16,19). However, several cultivars recommended for Canada are susceptible (6,15). For new plantings, it is recommended to apply the first fungicide 3 to 4 weeks after planting and two more times at 2-week intervals (3,21). During fruit bearing years, recommendations vary. In Ontario (21) it is suggested to apply the first spray when flower buds are visible in the crown and again after renovation (mowing of strawberry plants). The Quebec protection guide (3) recommends to apply the first spray in early spring and subsequent sprays when first flowers appear, additional sprays being applied when conditions are favorable to disease development. In practice, growers tend to apply fungicides on a calendar basis, regardless of the level of risk of infection, because consumers are reluctant to pick strawberries in fields infected by leaf spot. Furthermore, the Quebec guide mentions that leaf spot develops under cold and moist conditions, which is not supported by scientific data. Satisfactory control of leaf spot is currently obtained with several applications of protectant fungicides, however, it is not clear whether these sprays are needed.

Studies on some aspects of the ecology of *M. fragariae*, mainly conidia germination and in vitro growth requirements, were reported by Fall (10). Fall observed no lesions on young leaves, numerous lesions on middle-age leaves, and a few lesions on old leaves 12 days after inoculation. Fall also noted that host penetration took place at 10, 15, and 20°C, but that symptoms were more severe at 20 and 25°C. Plakidas (24) reported that the pathogen penetration into leaves occurred only through stomata. Elliott (9) reported the effects of temperature and moisture on conidiospore germination and germ tube elongation of *M. fragariae* on glass slides.

The objectives of this study were (i) to complement Elliot's data through infection experiments done under controlled conditions, under a set of temperatures and leaf wetness durations and according to the age of the leaves; (ii) to derive models describing the response of spore germination to temperature and relative infection to leaf wetness and temperature; (iii) to integrate these models into a global surface response model describing the response of infection to these weather factors; and (iv) to determine risk periods from this surface response model.

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MATERIALS AND METHODS

Inoculum production. A single conidium isolate of *M. fragariae* obtained from naturally infected strawberry leaves collected in 1993 at the Agriculture and Agri-Food Canada experimental farm in l'Acadie, Quebec, was used for inoculation. The stock cultures were stored on potato dextrose agar (PDA) (Difco Laboratories, Detroit) slants maintained at 3°C under mineral oil. The pathogen was subjected to host passage in strawberry leaves (cv. Kent) and subsequently maintained at room temperature on strawberry leaf agar (SLA) (6). To produce sufficient amount of conidia for inoculations, a mycelial slurry of *M. fragariae* was prepared with homogenizing fungal cultures in a laboratory blender (Stomaker; Seward Medical, London) in distilled sterile water at a ratio of 60 ml of water for each petri dish. The mycelial slurry was inoculated on SLA (1 ml per petri dish) and incubated at room temperature for 1 week. For inoculation of host plants, a conidial suspension was prepared in distilled water containing 0.01% Tween 80 and adjusted to 1×10^5 conidia per ml with a hemacytometer.

Plant production. Dormant strawberry plants (cv. Kent) were transplanted in 13-cm diameter pots filled with a mixture of mineral soil and peat moss (1:1, vol/vol) and were placed in a greenhouse maintained at 23 ± 4°C with a 16-h photoperiod. Fertilizer was applied at the time of transplanting at a rate of 150 ml of 10-52-10 (N-P_2O_5-K_2O) per plant and 1 week later with 200 ml per plant of a nutrient solution prepared by diluting 3 g of 20-20-20 (N-P_2O_5-K_2O) in 1 liter of water. The plants were kept in the greenhouse for 4 weeks. For each inoculation, the 25 most uniform plants were selected. On the day of inoculation, the three youngest expanded leaves of each plant that were 5 to 7, 12 to 15, and 19 to 21 days old , respectively, were tagged and leaf position was identified.

Inoculation and treatments. A conidial suspension of *M. fragariae* was applied to both surfaces of the tagged leaves as a uniform layer of fine droplets with an air brush (pump Model 0211-V45N-G8CX, brush Model H; Paaschi Inc., New York) operated at 100 kPa. Inoculated plants, except those exposed to 0 h of wetness, were placed immediately in a growth chamber (Model E15, Conviron Co., Manitoba, Canada) where the atmosphere was kept saturated by a humidifier (Sovereign, Irvington, NJ) and at a constant temperature of 5, 10, 15, 20, 25, 30, or 35°C. After a postinoculation wetness of 12, 24, 48, 72, or 96 h, five plants were dried for 10 min with an electric fan, transferred to a growth chamber maintained at 15°C for 2 weeks, and transferred to a greenhouse adjusted to 15 ± 3°C for the rest of the incubation period (≈1 week). Postinoculation wetness periods were selected based on preliminary experiments and previous work (9). This procedure was repeated for all selected temperatures. To minimize variation, the order in which plants were inoculated, temperature selected, and the distribution of plants in the mist chamber were randomized for the five leaf wetness durations. The experimental design was a split plot with main units represented by temperature and subunits by leaf wetness duration. The entire experiment was conducted three times.

Because a different batch of conidial suspensions was used for each inoculation, germination of the inoculum was estimated for each batch by spraying conidia onto two water agar plates at the beginning, middle, and end of each inoculation procedure for a total of six plates per batch. The plates were incubated at room temperature for 24 h. Germination on each plate was estimated by observing 50 randomly selected conidia and counting the number of conidia with germ tubes at least one-half the length of the conidium.

To evaluate conidia germination on leaves as a function of temperature, five additional plants were inoculated (for the first and second trials only) and removed from the mist chamber after 24 h. Disks (12-mm diameter) were cut from the youngest tagged leaf (nine disks per leaf) and were fixed and cleared by soaking in glacial acetic acid and ethanol (1:5, vol/vol) for 48 to 72 h. Differential staining between conidia and leaf tissues was achieved by transferring the leaf disks into a solution of lactophenol and ethanol (1:3, vol/vol) with a drop of aniline blue. After soaking for at least 48 h (27), 100 conidia, randomly selected from nine leaf disks originating from the same plant, were observed. Conidia were considered germinated when the germ tube was at least half the length of the conidia. The data were expressed as percent germinated conidium.

After an incubation period of 18 to 21 days, the number of lesions was counted on each inoculated leaf, but because lesions appeared over a period of a few days, counting was done twice (18 and 21 days after inoculation) to make sure that all potential lesions were counted. The area of each of the different leaves was measured with a leaf area meter (Model LI-3000; Li-Cor, Lincoln, NE) immediately following the second lesion assessment. The number of lesions per square centimeter of leaf was transformed to relative infection (RINF) by dividing the number of lesions per square centimeter of each leaf by the maximum obtained for the same leaf age from any of the temperature–leaf wetness duration combinations in one experimental run.

Data analysis. Homogeneity of variance between the experiments was tested to determine whether the percent conidia germination of inoculum varied significantly among inoculations (28). This test was necessary because the temperatures were tested over time and with different inoculum suspensions (temperature and inoculum effects were confounded). Analysis of variance by general linear models (GLM) was used to test the significance of leaf age on the number of lesions per square centimeter and of temperature on conidia germination. To test the effects of temperature and leaf wetness duration on the number of lesions per square centimeter, means of lesions per square centimeter were taken over the five inoculated plants for each experiment, and experimental runs were treated as blocks. Effects of temperature and leaf wetness duration on mean lesions per square centimeter were compared by analysis of variance using GLM. Statistical analyses including modeling procedures were done with the statistical analysis system (SAS Institute Inc., Cary, NC).

Effect of temperature on conidia germination. Percent conidia germination on strawberry leaves was calculated separately for each temperature and each experiment. The relationship of percent conidia germination to temperature (degrees celcius) was described by the following equation proposed by Duthie (8)

$$PCG = E' \{\exp[(T - F)G/(H + 1)]\}/\{1 + \exp[(T - F)G]\} \quad (1)$$

where PCG is percent conidia germination at a given temperature (T), E' is equal to $E[(H + 1)/H]H^{1/(H+1)}$, E is the maximum response, F is a location parameter proportional to the optimum temperature, G is the intrinsic rate of decline from the maximum as the temperature deviate from the optimum, and H is the degree of asymmetry of the curve. To estimate the value of parameters E, F, G, and H, partial derivatives equations were obtained and the Marquardt iterative method of the nonlinear regression (NLIN) procedure in SAS was used.

Model development. To describe the effects of temperature and duration of leaf wetness on relative infection, a surface response model was fitted to the data following several distinct steps. The equations for response of foliar parasites to combined effects of temperature and duration of wetness proposed by Duthie (8) were used to develop the surface response model. Equation 2 was used to represent the effect of duration of leaf wetness on relative infection

$$Y_w = A \times (1 - \exp\{-[B \times (w - C)]^D\}) \quad (2)$$

where Y_w is the response to duration of wetness, A is the upper limit of the response (upper asymptote), B is the intrinsic rate of

increase in the response, C is the length of delay in the response, D is the portion of the period of wetness in which the response decelerate, and w is the wetness duration in hours.

First, the upper asymptote (A) was established by expressing the maximum RINF as a function of temperature (T) using equation 3

$$A = E' \{\exp[(T - F)G/(H + 1)]\}/\{1 + \exp[(T - F)G]\} \quad (3)$$

where A is the upper asymptote at a given temperature (T), E' is equal to $E[(H + 1)/H]H^{1/(H+1)}$, E is the maximum response, F is a location parameter proportional to the optimum temperature, G is the intrinsic rate of decline from the maximum as the temperature deviates from the optimum, and H is the degree of asymmetry of the curve. To estimate the value of parameters E, F, G, and H, partial derivatives equations were obtained and the Marquardt iterative method of the NLIN procedure in SAS was used.

To avoid overparameterization, the parameter C in equation 2 was fixed to 0 assuming that RINF responded immediately to increase in leaf wetness duration after a minimum of 12 h. To estimate the value of parameters B and D, partial derivatives equations were obtained and the Marquardt iterative method of the NLIN procedure in SAS was used.

The final step consisted in integrating the asymptote (A) equation, the estimated value of B, the fixed value for C ($C = 0$), and the estimated value of D in equation 2 to obtain the predicted RINF as a function of both temperature (T) and duration of wetness (w). Several criteria were used to evaluate the models: (i) randomness and normality of residuals; (ii) goodness of fit between predicted and observed values (R^2); and (iii) standard deviation around the regression lines. All nonlinear regressions were evaluated by performing a simple regression analysis between the predicted values and the actual observations as paired observations. Analysis of these regressions were done by testing whether the intercept was significantly different from 0, the slope from 1 and if the appropriate F value was significant (7).

RESULTS

Germination of the conidia used as inoculum varied from 89 to 95%, and a F test indicated that it did not differ significantly among batches of inoculum ($P = 0.40$). Analysis of variance indicated that temperature significantly affected conidia germination on leaf ($P \leq 0.0001$). When the number of lesions per square centimeter for each leaf age was averaged over temperatures and leaf wetness durations, there were significantly ($P \leq 0.0001$) more lesions per square centimeter on 5 to 7 day old leaves than on 12 to 15 and 19 to 21 day old leaves with 2.29, 0.49, and 0.24 lesions per cm^2, respectively. Moreover, for most temperatures and leaf wetness durations, more lesions developed on young leaves (5 to 7 days old at the time of inoculation) than on leaves that were more than 12 days old (Fig. 1). In general, infection on intermediate (12 to 15 days old) and on older leaves (19 to 21 days old) was low with a maximum of 2.3 and 1.7 lesions per cm^2, respectively, at 25°C and 96 h of leaf wetness as compared to 12.6 for the 5 to 7 day old leaves. Overall, mean number of lesions per square centimeter was similar (no significant block effect) for the three experimental runs (Table 1). However, temperature, leaf wetness duration, and the interaction between temperature and leaf wetness duration significantly affected the number of lesions per square centimeter produced on 5 to 7 day old leaves (Table 1).

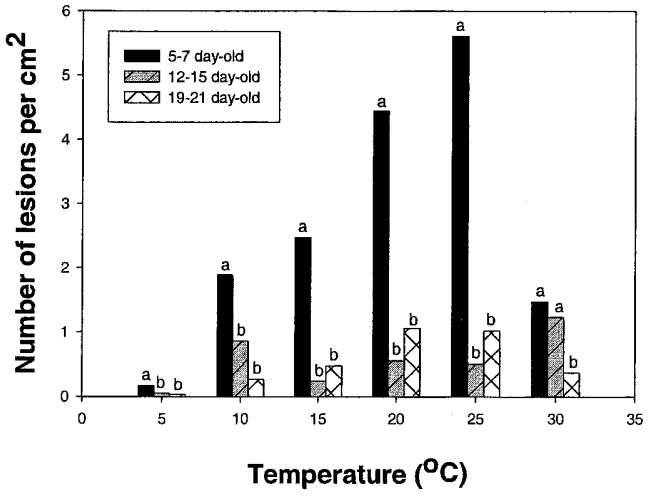

Fig. 1. Infection of strawberry leaves of different ages by *Mycosphaerella fragariae* as a function of temperature. Each point is an average of observations made on 75 leaves (three experimental repetitions with five plants per replication averaged over five durations of leaf wetness). In each temperature, values followed by the same letter within columns are not significantly different according to LSD ($P = 0.05$).

Effect of temperature on conidia germination. Results of the two experimental repetitions were similar with a maximum spore germination of 92% observed at 25°C. Germination was slightly higher for the first run (Fig. 2), but the overall response to temperature was similar. The difference between the two experimental runs varied from 0.34 to 9.18% at temperatures of 35 and 15°C, respectively. Percent conidia germination varied from 2% at 35°C to 92% at 25°C. Percent conidia germination increased with increasing temperature, reached the maximum at 25°C, and sharply declined with increasing temperature to attain the minimum at 35°C (Fig. 2). The response of conidia germination to temperature (T) was best described as

$$PCG = E' \{\exp[(T - 27.1529) \times 0.6344/(4.9670 + 1)]\}/ \{1 + \exp[(T - 27.1529) \times 0.6344]\} \quad (4)$$
$$\text{where } E' = 94.1189[(4.9670 + 1) / 4.9670]\, 4.9670^{1/(4.9670+1)}$$

Parameter estimates and their associated errors are presented in Table 2. The intercept of the regression of the predicted values against observed germination percentage was not significantly different from 0 ($P > 0.05$), and the slope was not significantly different from 1 (Table 3). The F value for both intercept equal to 0 and slope equal to 1 was 0.400, and the coefficient of determination was 0.98. The model (equation 4) adequately predicted percent germination with an error of 5 and 6% for experimental run one and two, respectively. The optimum temperature for conidia germination, as predicted by the model, was 24.6°C.

Effect of temperature and leaf wetness duration on infection. Because variation in numbers of lesion per square centimeter of leaf on intermediate and older leaves for the different temperatures and durations of leaf wetness tested was very small, data other than young leaves were not used in model development. Infection of susceptible strawberry leaves (5 to 7 days old) by *M. fragariae* occurred at duration of leaf wetness of 12 to 96 h.

TABLE 1. Analysis of variance for the effects of temperature (T) and leaf wetness duration (w) on infection of strawberry leaves by *Mycosphaerella fragariae*

Source[y]	df	Sum of square	$P > F$[z]
Block	2	2.31	NS
T	6	400.89	*
Error a	12	1.30	NS
w	4	206.84	*
Tw	24	285.77	*
Error b	56	0.19	NS

[y] Analysis of variance was performed on means of lesions per square centimeter over the five inoculated plants for each experiment, and experimental runs were treated as block. Only the data for the 5- to 7-day old leaves were used in the analysis.

[z] * = Significant at $P \leq 0.01$, and NS = not significant at $P > 0.05$.

Variation among the results of the three experimental repetitions was small and varied for the different temperatures tested. At 5, 20, 25, and 35°C, the variation was small and trends were similar. The highest discrepancy in the experimental repetitions was observed at 15 and 30°C with RINF for the third run approximately twice the values observed for the first and second runs (Fig. 3). The highest number of lesions was observed at 25°C and 96 h with 12.3, 11.6, and 14.1 lesion per cm^2. At 20°C, the number of lesions increased gradually from 12 to 96 h of postinoculation wetness. At 25°C, the number of lesions increased linearly from 12 to 48 h and then more rapidly from 48 to 96 h. The highest infection was obtained at 25°C and 96 h of leaf wetness (average of 12.6 lesions per cm^2). An average of 2.53 and 1.58 lesions per cm^2 of leaf surface was observed on plants exposed to 12 h of leaf wetness at 10 and 30°C, respectively.

The upper asymptote (parameter *A*) was highest at 25.2°C with a value of 1.0 and lowest at 5 and 35°C with values of 0.074 and 0.001, respectively. The response of the upper asymptote to temperature was best described by the following equation (Fig. 4)

$$A = E' \{\exp[(T - 27.0460) \times 1.0826/(7.0047 + 1)]\}/ \{1 + \exp[(T - 27.0460) \times 1.0826]\} \quad (5)$$

where $E' = 1.0013 \times [(7.0047 + 1) / 7.0047] \times 7.0047^{1/(7.0047+1)}$

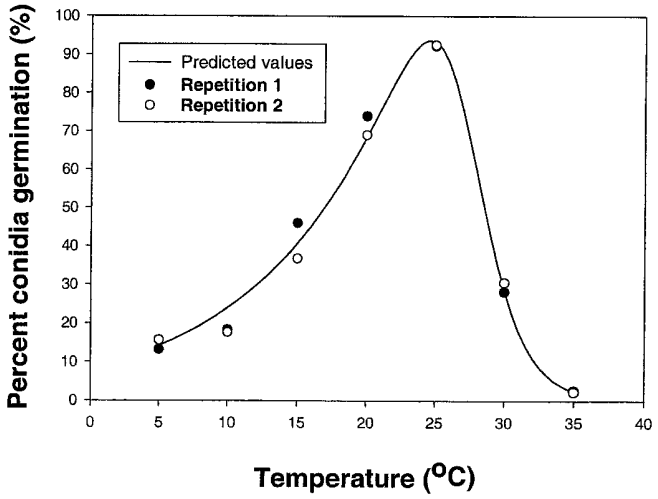

Fig. 2. Conidia germination as a function of temperature after an infection period of 24 h. The line represents the predicted value calculated with equation 4, and the symbols represent the mean of five observations for each of the two experimental repetitions.

TABLE 2. Parameter estimates for the model, $PCG = E' \{\exp[(T - F)G/(H + 1)]\}/\{1 + \exp[(T - F)G]\}$ where *PCG* is percent conidia germination at a given temperature (*T*), E' is equal to $E[(H + 1)/H]H^{1/(H+1)}$, that described the response of conidia germination to temperature

Parameter[z]	Estimate	Asymptotic standard error	Asymptotic 95% lower	Confidence interval upper
E	94.1189	2.5486	88.4302	99.7876
F	27.1529	2.8817	20.7320	33.5737
G	0.6344	0.0632	0.4936	0.7753
H	4.9670	0.0287	4.9032	5.0309

[z] Equation 4.

Parameter estimates and their associated errors are presented in Table 4. The intercept of the regression of the calculated values of *A* against the predicted values was not significantly different from 0 ($P > 0.05$), and the slope was not significantly different from 1 (Table 3). The *F* value for both intercept equal to 0 and slope equal to 1 was 0.056 and the coefficient of determination was 0.98.

The surface response of RINF to combined effects of temperature and leaf wetness duration was obtained by integrating equation 5, describing the response of the asymptote (parameter *A*) to temperature, in equation 2 with $C = 0$. Estimates of parameters *B* and *D* and their associated errors are presented in Table 4. The two- and three-dimensional representations of the response of equation 6 are given in Figures 3 and 5, respectively, and the final equation was

$$Y_w = A \times (1 - \exp\{-[0.0168 \times (w - 0)]^{3.1223}\}) \quad (6)$$

where *A* is calculated from equation 4. Inspection of residual plots indicated a random pattern of distribution for all temperatures and leaf wetness durations. The model provided a good prediction of RINF for all durations of leaf wetness (Fig. 6). The intercept of the regression of the predicted values against the observed RINF equal to –0.020 was significantly different from 0 ($P \leq 0.01$) with a slope of 1.012, which was not significantly different from 1 (Table 3). The *F* value for both intercept equal to 0 and slope equal to 1 was 5.270 and was significant ($P \leq 0.01$), and the coefficient of determination was 0.98 (Table 3).

Based on the final model (equation 6), the optimum temperature for infection of strawberry leaves by *M. fragariae* was 25.2°C. The minimum duration of leaf wetness required to induce infection was 12 h. Overall, the model tended to overestimate infection at 5°C and at leaf wetness of 24 h.

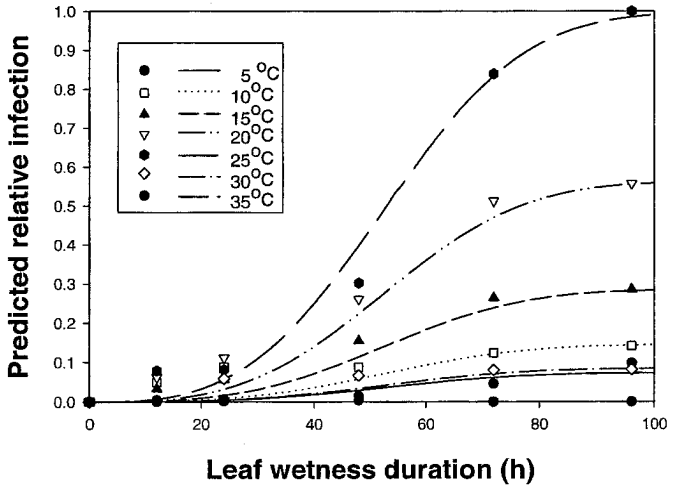

Fig. 3. Relative infection by *Mycosphaerella fragariae* on strawberry leaves of 5 to 7 days old at various temperatures and leaf wetness durations. The lines represent the predicted value calculated from equation 6, and the symbols represent the observed values for each experimental repetition (average of observations made on 15 plants; three experimental repetitions with five plants per repetition).

TABLE 3. Linear regression of the predicted against observed values for conidia germination (equation 4), upper asymptote (*A*) expressing the maximum relative infection (equation 5) and relative infection (equation 6)[z]

Equation	*n*	Intercept B_0	*T* for $B_0 = 0$	Slope B_1	*T* for $B_1 = 1$	*F* value for $B_0 = 0$, $B_1 = 0$	R^2
4	14	0.014	0.934 NS	0.971	–0.936 NS	0.400 NS	0.98
5	21	–0.001	–0.116 NS	0.996	–0.163 NS	0.056 NS	0.98
6	35	–0.020	–2.976 *	1.012	–0.517 NS	5.270 *	0.98

[z] * = Significant at $P \leq 0.01$, and NS = not significant at $P > 0.05$.

DISCUSSION

In this study, infection of strawberry leaves by *M. fragariae* was more severe on young leaves than on older leaves. The low numbers of lesions observed on leaves older than 12 days could have resulted from resistance of the leaf tissues to infection by the pathogen (ontogenic resistance) or to lesion development in leaf tissues. These results are contradictory with those reported by Fall (10), who studied the effect of leaf age on infection under greenhouse conditions and reported no symptoms on young leaves, severe symptoms on middle-aged leaves, and sporadic presence of spots on older leaves. The observations of Fall (10) should be interpreted with care because there was no mention of the concentration of the inoculum or the exact age of leaves. Zheng and Sutton (29) observed a different effect of leaf age for strawberry leaf scorch caused by *Diplocarpon earlianum* and reported higher infection on older leaves than on newly expanded ones. This may partially explain why under field conditions the two leaf diseases can coexist.

M. fragariae produced lesions on expanding strawberry leaves at temperatures from 10 to 30°C provided that leaf wetness was at least 12 h, but at 5 and 35°C only a few lesions developed. The low number of lesions observed at 5°C can be explained by the inability of conidia to germinate (10) or by the requirement for longer leaf wetness at this temperature than in other *Mycosphaerella* spp. (12,13). In the present study, at 5°C we observed only 14% conidia germination on leaves exposed to 24 h of wetness. Fall (10) also reported that the content of conidia was destroyed (coagulated) by exposure at 35°C for 24 h, which explains the absence of lesions at 35°C. These observations were

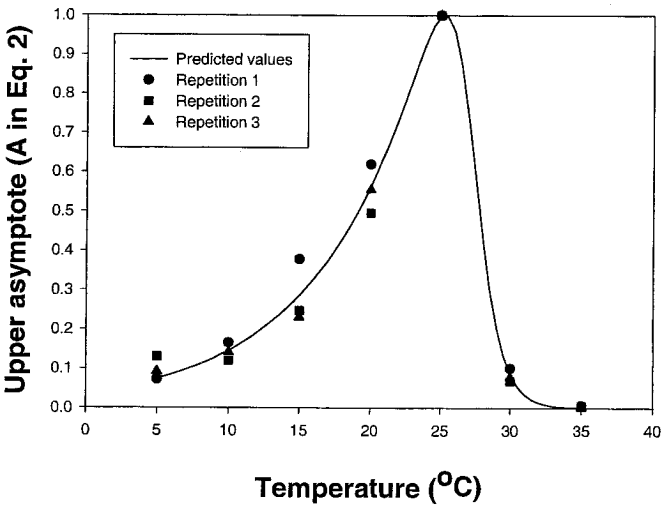

Fig. 4. Response of the upper asymptote (parameter *A* in equation 2) expressing the maximum relative infection to temperature. The line represents the predicted value calculated from equation 5, and the symbols represent the observed values for each experimental repetition.

TABLE 4. Parameter estimates for the model $A = E' \{\exp[(T - F)G/(H + 1)]\}/\{1 + \exp[(T - F)G]\}$ for $Y_w = A \times \{1 - \exp[-(B \times (w - C))^D]\}$ that described the response of relative infection to combined effects of temperature and leaf wetness duration

Parameter[z]	Estimate	Asymptotic standard error	Asymptotic 95% lower	Confidence interval upper
E	1.0013	0.0314	0.9351	1.0674
F	27.0460	4.2489	18.0817	36.0103
G	1.0826	0.1564	0.7526	1.4126
H	7.0047	0.0268	6.9481	7.0613
B	0.0168	0.0004	0.0160	0.0176
D	3.1223	0.3089	2.4937	3.7509

[z] Equation 5 was used for parameters *E*, *F*, *G*, and *H* and equation 6 was used for parameters *B* and *D*.

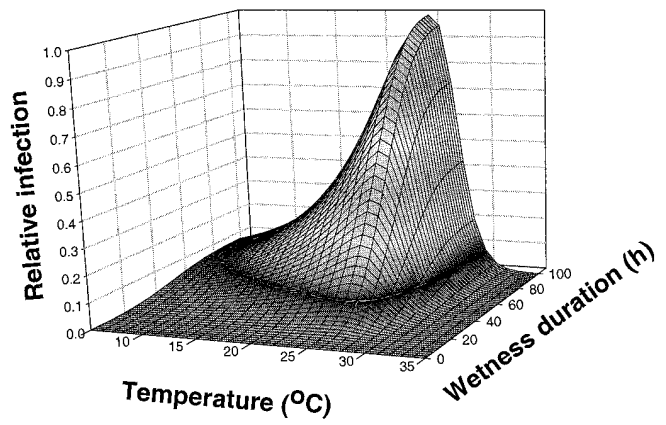

Fig. 5. Three-dimensional response of relative infection by *Mycosphaerella fragariae* to the combined effects of temperature and leaf wetness duration. The predicted values were calculated using equation 6 (described in text).

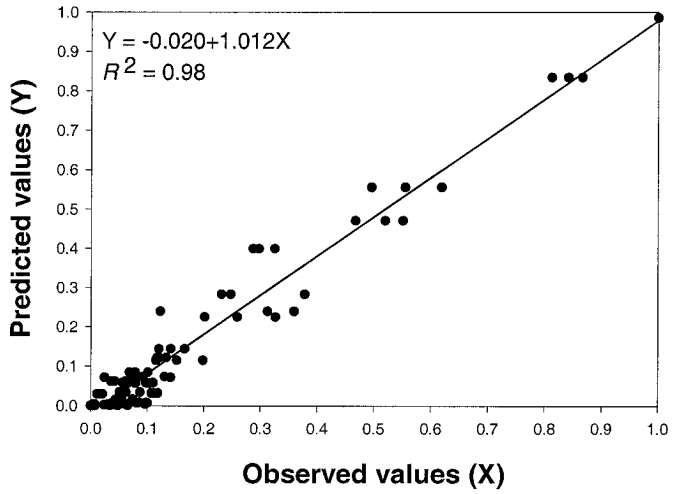

Fig. 6. Regression of the predicted values from equation 6 against observed relative infection of strawberry leaves by *Mycosphaerella fragariae*.

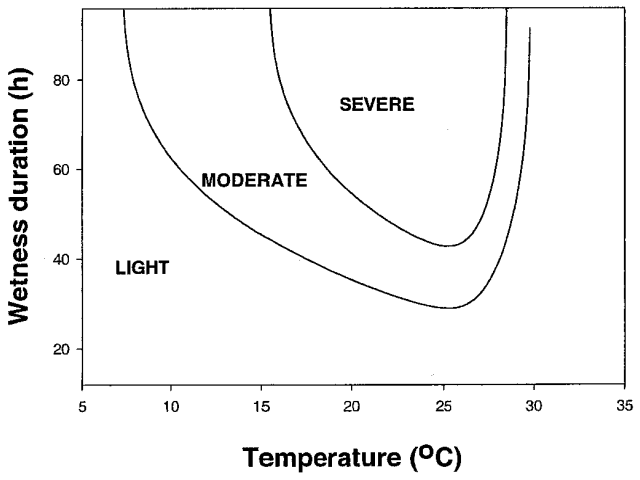

Fig. 7. Chart for risk prediction for infection of strawberry leaves by *M. fragariae* assuming the presence of inoculum. Categories are based on the response surface (equation 6) and isopaths separating the categories correspond to L = light (relative infection [RINF] = 0.00 to 0.10) which corresponds to less than 1.2 lesions per cm^2 of leaf; M = moderate (RINF = 0.11 to 0.30) which corresponds to 1.2 to 3.7 lesions per cm^2 of leaf; H = high (RINF = 0.31 to 1.00) which corresponds to more than 3.7 lesions per cm^2 of leaf.

confirmed by our results, as we observed only 2% conidia germination at 35°C. In this experiment, similar temperature effects were observed for spore germination and infection. Both responses were adequately described by the same model (equations 4 and 5) and the predicted optimum temperatures are similar at 24.6 and 25.2°C for spore germination and infection, respectively.

For all durations of leaf wetness tested, temperatures from 15 to 25°C were more favorable than 5, 10, or 30°C. For temperatures of 15 to 25°C, infection increased slowly with increasing wetness from 12 to 24 h. Increasing wetness duration from 24 to 72 h resulted in rapid increase in infection and then stabilized. This observation is similar to those of Elliott (9), who observed at 18°C a logistic increase in conidia germination on glass slides as a function of time between 12 and 60 h. Under free moisture, he observed ≈20% germination after 12 to 24 h and ≈90% after 36 h. In our experiment, at 20°C, RINF varied from 0.11 to 0.51 at leaf wetness duration from 24 to 72 h and increased to 0.56 after 96 h of postinoculation wetness. Furthermore, the response of *M. fragariae* to temperature and wetness duration observed in this study was similar to what was previously reported for *M. pinodes* on pea seedlings (26).

The modified version of the Weibull equations proposed by Duthie (8) to describe the response of foliar pathogens to both temperature and wetness duration provided a good description of the response of *M. fragariae* under the controlled conditions of this study. The main advantage of these models over other nonlinear models previously reported (1,18,23) is that there is no temperature response of the parameters, such as the asymptote or the rate that are described by polynomial models. In addition, all parameters used to describe the responses to temperature and wetness duration have some biological significance.

This study demonstrates the importance of temperature and duration of leaf wetness on infection of strawberry leaves by *M. fragariae*. The optimum temperature for infection of leaf spot was ≈25°C. The model developed could be used in conjunction with sporulation model (2) to detect periods of high risk for leaf spot development. The pathogen rarely affects the fruit, hence, this allows producers some latitude in terms of controlling leaf spot. Based on our results, it is expected that leaf spot will reach the economic threshold only under high temperature and prolonged leaf wetness periods provided that young leaves are present. In practical terms, this means that fungicide treatments should be applied in early spring and after renovation. A chart for predicting risk of infection was developed for leaf spot (Fig. 7). This chart divides risk into three levels and could be used for scheduling fungicide applications to control leaf spot of strawberry, and the inclusion of host susceptibility, interrupted leaf wetness, and inoculum potential would increase the predictive capacity of the model.

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