Effects of Fluctuating Temperatures on the Latent Period of Lettuce Downy Mildew (*Bremia lactucae*)

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ABSTRACT

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The effects of fluctuating temperatures on the length of the latent period of downy mildew (*Bremia lactucae*) were studied on six lettuce cultivars. Potted plants were inoculated with *B. lactucae*, incubated at 13 C for 12 or 24 h to initiate infection, and subjected to different temperature treatments by moving them to growth chambers operating at diurnally alternating temperatures, moving them outdoors to naturally varying temperatures, or holding them at constant conditions. Subsets of plants incubated at fluctuating temperatures were returned to constant conditions at 24-h intervals to simulate a wide range in thermal exposure during the latent period. Cumulative numbers of sporulating lesions were recorded for each plant daily from 5 to 14 days after inoculation. Minimum and median latent periods (LP_0 and LP_{50}, respectively) were determined for each cultivar and temperature treatment at the end of the experiments. LP_0 and LP_{50} were expressed as days, in degree-hours (accumulated hourly temperatures above a threshold of 0 C), and in units of accumulated hourly developmental rates derived from a nonlinear function fit to values of LP_0 from a previous constant-temperature study. We found that the variability in LP_0 and LP_{50} was greater when latent periods were measured in degree-hours (coefficient of variation [CV] between 6.2 and 30.9%) or developmental rates ($6.4\% \leq CV \leq 48.6\%$) than when they were measured in days ($4.0\% \leq CV \leq 17.5\%$). Thus, there seems to be little advantage in using degree-hours or developmental rates to model latent periods of lettuce downy mildew. Values of LP_0 were shorter at low and longer at high temperatures with fluctuating temperatures compared to constant temperatures with the same mean. Possible reasons for the difference between constant- and fluctuating-temperature data are discussed.

Additional keyword: *Lactuca sativa*.

Temperature is an important driving force in all physiological processes affecting growth and development of plant pathogens. For example, fungal growth rates, when plotted against temperature, generally follow a negatively skewed unimodal curve (2,5,22). Fungal incubation and latent periods usually decrease hyperbolically as temperatures increase toward the optimum; at temperatures above the optimum, the curves ascend again (3,14). There has been continuing interest in the mathematical description of pathogen and disease development in relation to temperature for use in research and management models (2,3,22,27,28).

Models that accumulate thermal time (degree-days or -hours) are widely used to describe the dependence of development on time and temperature (16,30). This method is based on the assumption that the relationship between development and temperature above a species-specific threshold (base temperature) is linear and that the amount of heat needed to complete a given stage in the development of a poikilothermic organism is constant (3,27). Damaging effects of temperatures above the optimum are usually ignored. Degree-day and -hour models have been widely applied to fungal plant pathogens. For example, Blaeser and Weltzien (4) reported that 50 degree-hours were needed for infection of grape leaves by *Plasmopara viticola* after wet inoculations. Schrödter and Ullrich (23) found that 1,543 degree-hours above a base temperature of 7 C passed before symptoms of *Phytophthora infestans* appeared on potato leaves (cv. Erstling).

Nonlinear developmental rate models offer an alternative approach for modeling the dependence of development on time and temperature. Such models are derived from the reciprocals of development times (e.g., latent periods) at different constant temperatures (3,21,22,28). The sum of developmental rates equals 1.0 when development is complete. This method is based on the assumption that the effects of temperature on development are additive and that a constant temperature during some reasonably small interval can be assumed (3). Developmental rate models have been widely applied in insect phenology (27,28) but only rarely in plant pathology (2,22).

Our overall research project is directed toward designing management models for downy mildew (*Bremia lactucae* Regel) on lettuce (*Lactuca sativa* L.) in coastal California. The effects of environmental factors (temperature, humidity, and wetness period) on spore germination, infection, and sporulation of *B. lactucae* are well characterized (15,18,24,26). A quantitative description of the latent period of *B. lactucae*, however, is still lacking. Verhoeff (26) did a series of incubation experiments on lettuce seedlings (cv. Proeftuins Blackpool) at constant and slightly varying temperatures (referred to as constant conditions hereafter). He observed the shortest latent periods (4–7 days) when inoculated plants were incubated at 20–22 C. Latent periods were longest (24–34 days) when plants were kept at 6 C.

Variable temperatures are typical of the environments of most plant pathogens, yet constant-temperature experiments are routinely used to determine epidemiological parameters. Several authors have questioned the usefulness of constant-temperature data for deriving models to predict pest development at fluctuating temperatures in the field (8,9,28). Therefore, epidemiologists should investigate the effects of variable environments on pathogen development, both theoretically and experimentally. The purpose of this study was to quantify latent periods of lettuce downy mildew for a wide range of fluctuating temperatures, to evaluate degree-hour and developmental rate models for describing latent periods under such conditions, and to compare the effects of constant and fluctuating temperatures on latent period.

MATERIALS AND METHODS

Plant culture. Six cultivars were selected as a sample of the range of morphological and physiological forms present in culti-

vated lettuce. Crisphead-type cultivars included Salinas, South Bay, and Holborn Standard, the latter with a high level of field resistance (6). The cultivars Prizehead and Buttercrunch were included as Leaf- and Butterhead-type lettuces, respectively. Proeftuins Blackpool, another Butterhead lettuce, was included to allow comparisons with Verhoeff's reference study (26). Seed samples were supplied by E. J. Ryder (USDA-ARS, Salinas, CA), Northrup King Seed Co. (Minneapolis), Harris Moran Seed Co. (Salinas, CA), and Ferry Morse Seed Co. (Mountain View, CA).

Seedlings were grown in square 150-cm^3 plastic pots (one plant per pot) in vermiculite and kept in a growth chamber at a temperature regime of 22/15 C (day/night) and a 12-h photoperiod with a photon flux density of 282–300 μmol m^{-2} s^{-1} during the light phase. The plants were watered on alternate days with half-strength Hoagland's solution (7) or deionized water.

Inoculation and infection. Two isolates of *B. lactucae* were used in our experiments. The first isolate, California pathotype III (20), had been maintained in the laboratory for about 3 yr. The second isolate, a mixture of pathotypes with virulence to downy mildew resistance genes *Dm1* through *Dm8*, *Dm10*, and *Dm12* through *Dm15*, had been collected in a field near Castroville, CA, during July 1993. Both isolates were maintained and inoculum was produced as described previously (10). Only sporangia from lesions that were at least 2 days but not more than 3 days old were harvested for the preparation of inoculum. Germinability of sporangia was determined before inoculation and was always >80% at densities similar to those used in the experiments. Plants were inoculated at the four-leaf stage (when they were 4–5 wk old) by spraying a sporangial suspension (5×10^4 ml^{-1} in deionized water with 0.02% Tween 80) onto the upper leaf surfaces to runoff. After inoculation, plants were enclosed in wet plastic bags and kept in darkness at 13 C for 12 or 24 h to initiate infection (preincubation period).

Constant-temperature experiment. A preliminary experiment was done with constant temperatures to verify that our conditions for inoculation, incubation, and plant growth gave results that were consistent with Verhoeff's reference study (26). Cvs. Salinas, Proeftuins Blackpool, and Holborn Standard were inoculated either with pathotype III or the field isolate of *B. lactucae*. After inoculation and preincubation (24 h at 13 C in darkness), three to four plants of each cultivar-pathotype combination were moved to growth chambers operating at temperatures of 7.2 ± 0.37 C (mean ± standard deviation [SD]), 12.1 ± 0.88 C, 18.4 ± 1.13 C, and 22.3 ± 1.69 C. The plants were kept in these chambers until the experiment was terminated 24 days after inoculation. The experiment was repeated once with similar temperature treatments. Temperature and relative humidity (RH) were monitored with thermistors and sulfonated polystyrene humidity transducers, respectively (Campbell Scientific Inc., Logan, UT). Sensor signals were sampled every 5 min with data loggers, and 60-min averages were computed from the measurements. Each night, plants were enclosed in wet plastic bags (from about 1900–2030 h until 0700-0830 h local time) to provide conditions conducive to symptom expression and sporulation (15,24,26). No attempts were made to control RH during the light phase. The light/dark sequence in the growth chambers was adjusted to 12/12 h.

Variable-temperature experiments. After inoculation and preincubation, infected plants were subjected to different temperature treatments by moving them to growth chambers operating at diurnally alternating temperatures, moving them outdoors to naturally varying temperatures, or holding them at constant conditions. Subsets of plants incubated at fluctuating temperatures were returned to constant conditions at 24-h intervals to simulate a wide range in the accumulation of degree-hours and developmental rates over time. With the exception of experiment 1, all plants were moved back to constant conditions before the end of the latent period to avoid confounding temperature effects on latent period with effects on sporulation. Temperature and RH in all treatments were monitored as described above. The light/dark sequence in the growth chambers was adjusted to match the sequence prevailing outdoors at the same time.

Four independent experiments were done in this manner, with different temperature treatments and cultivars included in each experiment (Fig. 1). Because the experiments were not identical, they cannot be considered true replicates. However, the experimental design was adequate to insure the reproducibility of the results.

First experiment. Cvs. Salinas, Prizehead, and Buttercrunch were inoculated with pathotype III. After preincubation for 24 h, two plants of each cultivar were exposed to six temperature

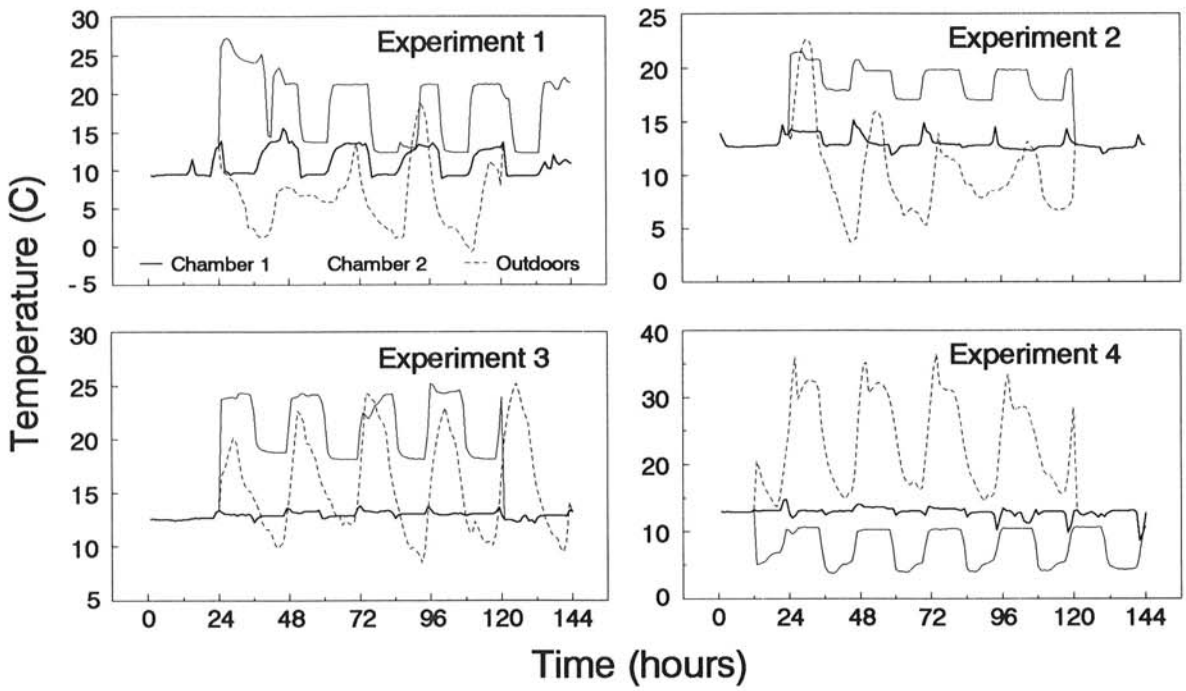

Fig. 1. Temperature treatments used to quantify latent periods of lettuce downy mildew at fluctuating temperatures in four independent incubation experiments. Infected lettuce plants were kept in growth chambers at constant or diurnally alternating temperatures or incubated outdoors at naturally fluctuating temperatures. Subsets of plants incubated at fluctuating temperatures were returned to constant conditions at 24-h intervals to simulate a wide range in thermal exposure during the latent period.

treatments during the latent period. Treatment groups I and II were incubated continuously in growth chambers at alternating temperatures of 15/10 C and 22/12 C (day/night), respectively. Groups III to VI were exposed to naturally varying temperatures outdoors (ranging from −0.7 to +17.9 C) for 24, 48, 72, and 96 h, respectively, and then returned to the 15/10 C chamber. The day length outdoors was about 9.3 h (December, latitude ~38.5 N).

Second experiment. Cvs. Salinas, South Bay, and Buttercrunch were inoculated with pathotype III. After preincubation for 24 h, two plants of each cultivar were exposed to six temperature treatments. Group I was incubated continuously in a reference growth chamber at 13 C. Group II was subjected to an alternating temperature cycle of 20/17 C for 96 h in a second growth chamber and then moved back to the reference chamber. Groups III to VI were exposed to naturally varying temperatures outdoors (ranging from 3.7 to 22.7 C) for 24, 48, 72, and 96 h, respectively, before they were returned to the reference chamber. The day length outdoors was about 10.5 h (February).

Third experiment. Cvs. South Bay, Buttercrunch, Prizehead, and Holborn Standard were inoculated with pathotype III. After preincubation for 24 h, two plants of each cultivar were exposed to six temperature treatments. Group I was incubated continuously in a reference growth chamber at 13 C. Groups II and III were subjected to alternating temperature cycles of 24/18 C in a second growth chamber for 48 and 96 h, respectively, and then moved back to the reference chamber. Groups IV to VI were exposed to naturally varying temperatures outdoors (ranging from 8.5 to 25.2 C) for 72, 96, and 120 h, respectively, and then returned to the reference chamber. The day length outdoors was about 12.8 h (April).

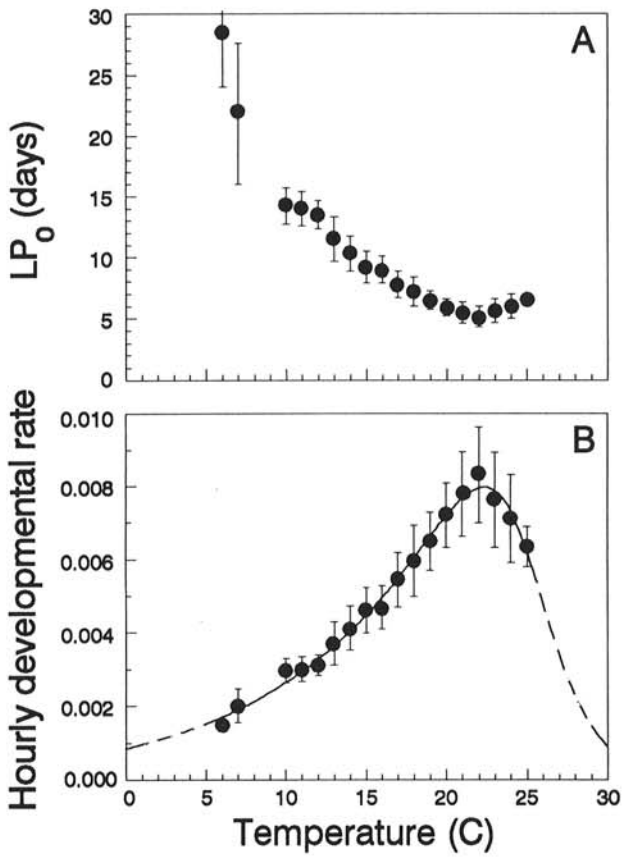

Fig. 2. A, Relationship between temperature and minimum latent period (LP_0) of lettuce downy mildew during incubation at constant temperatures (26). **B,** Relationship between temperature and developmental rate during the latent period, calculated from the data in **A**. The curve was fit to equation 1 (in text), which resulted in the parameter estimates ρ_{25} = 0.0122, ΔH_A = 16,580.3, ΔH_H = 109,960, and $T_{1/2H}$ = 298.20. Vertical bars represent two standard deviations.

Fourth experiment. Cvs. Prizehead, Holborn Standard, and Proeftuins Blackpool were inoculated with the field isolate of *B. lactucae*. After preincubation for 12 h, two plants of each cultivar were exposed to six temperature treatments. Group I was incubated continuously in a reference growth chamber at 13 C. Groups II to IV were subjected to an alternating temperature cycle of 10/5 C in a second growth chamber for 60, 108, and 156 h, respectively, and then moved back to the reference chamber. Groups V and VI were exposed to naturally varying temperatures outdoors (ranging from 13.8 to 36.4 C) for 60 and 108 h, respectively, before they were returned to the reference chamber. The day length outdoors was about 14.5 h (July).

Data collection. Beginning 5 days after inoculation, each plant was examined during midmorning for sporulation on the four oldest leaves. Cumulative numbers of sporulating lesions, pooled over four leaves per plant and two plants per cultivar, were recorded daily until 14 days after inoculation. Minimum and median latent periods (LP_0 and LP_{50}, respectively) were determined for each cultivar and temperature treatment at the end of the experiments.

Model development. Two time-temperature dependent models were compared for their suitability to describe LP_0 and LP_{50}. The first model was based on the summation of degree-hours (hourly temperatures above a threshold of 0 C) until LP_0 or LP_{50} was reached for each cultivar and temperature treatment. The lower threshold of 0 C was adopted because previous work had shown that *B. lactucae* can germinate, grow, and infect at temperatures near freezing (15,24,26). The second model was based on the summation of hourly rates of development until LP_0 or LP_{50} was attained for each cultivar and temperature treatment combination. The underlying developmental rate data were generated by a nonlinear function fit to values of LP_0 from Verhoeff's study (26). Figure 2A shows the relationship between temperature and LP_0 observed in this study (26). When the reciprocals of the data are plotted as rates (expressed in units of days^{-1} or h^{-1}) against temperature, a negatively skewed unimodal curve is obtained (Fig. 2B). We used a nonlinear thermodynamic model (21) to describe the relationship between temperature and developmental rate:

$$r(T) = \frac{\rho_{25}\dfrac{T+273.2}{298}\exp\left[\dfrac{\Delta H_A}{1.987}\left(\dfrac{1}{298}-\dfrac{1}{T+273.2}\right)\right]}{1+\exp\left[\dfrac{\Delta H_H}{1.987}\left(\dfrac{1}{T_{1/2H}}-\dfrac{1}{T+273.2}\right)\right]}, \quad (1)$$

where $r(T)$ = developmental rate (h^{-1}), T = temperature (C), and ρ_{25}, ΔH_A, ΔH_H, $T_{1/2H}$ are parameters. A possible biophysical

TABLE 1. Means and standard deviations of minimum latent period (LP_0)[a] of lettuce downy mildew during incubation at constant temperatures

Cultivar / Pathotype	Temperature (C) LP_0 (days)[b] 7	12	18	22
Salinas				
Pathotype III	21.5 (3.54)	13.2 (1.20)	6.7 (1.63)	7.5 (0.71)
Field isolate	21.0 (2.83)	13.0 (1.41)	7.3 (0.78)	6.8 (0.35)
Holborn Standard				
Pathotype III	22.0 (. . .)[c]	11.3 (. . .)	8.6 (2.97)	7.8 (3.89)
Field isolate	20.5 (2.12)	12.5 (. . .)	9.4 (2.33)	9.8 (2.47)
Proeftuins Blackpool				
Pathotype III	21.0 (2.83)	12.5 (2.12)	7.9 (0.57)	7.0 (0.00)
Field isolate	21.5 (2.12)	12.8 (1.77)	7.0 (0.00)	7.2 (0.00)
Reference study[d]	21.8 (5.19)	13.6 (1.11)	7.2 (1.13)	5.1 (0.76)

[a] Minimum latent period was defined as the time from inoculation to the first appearance of sporulating lesions.
[b] Values are the means and standard deviations of two experiments.
[c] Infection did not occur in one of the experiments.
[d] Data published previously (26) for cv. Proeftuins Blackpool and an uncharacterized isolate of *Bremia lactucae*.

interpretation of the parameters in equation 1 has been proposed (21) based on enzyme reaction rate theory.

Model evaluation. Means, SD, and coefficients of variation (CV) of LP_0 and LP_{50} (expressed as days or by the degree-hour or developmental rate models) were computed based on the observed data. We used CV to summarize the variation in latent period (over the different temperature treatments) not accounted for by the models.

Linear regression analyses were done to compare the effects of constant and variable temperatures on LP_0 (in days). For the constant-temperature case, values of LP_0 from Verhoeff's study were regressed against temperature, ranging from 9 to 20 C (linear range). For the variable-temperature case, values of LP_0 from our experiments were regressed against mean incubation temperature (calculated over the entire latent period) separately for each cultivar. Effects of temperature on LP_0 were interpreted in terms of the magnitude and significance levels of the slope coefficients.

RESULTS

Constant-temperature experiment. Latent period under constant conditions depended strongly on temperature (Table 1). The first sporulating lesions appeared in the 18- and 22-C treatments 5 days after inoculation. At 7 and 12 C, sporulation did not occur before days 18 and 11, respectively. Latent period was not affected by cultivar at low temperatures (7 and 12 C). At higher temperatures (18 and 22 C), cv. Holborn Standard had the longest latent periods. Values of LP_0 on cv. Proeftuins Blackpool were similar to values published previously (26) for this cultivar (Table 1). At 22 C, we observed longer latent periods than reported previously.

Variable-temperature experiments. The variable-temperature treatments resulted in temperature extremes ranging from −0.7 C to 36.4 C (Fig. 1), with means of 9.5–18.2 C over the entire latent period. This range is representative of the environmental conditions in coastal California during the growing season (data for

TABLE 2. Means, standard deviations (SD), and coefficients of variation (CV) of minimum latent period (LP_0)[a] of lettuce downy mildew during incubation at fluctuating temperatures

Cultivar	LP_0 in days			LP_0 in degree-hours[b]			LP_0 in developmental rates[c]		
	Mean[d]	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
Experiment 1									
Salinas	8.7	0.75	8.6	2,418	432.9	17.9	0.60	0.202	33.7
Prizehead	9.0	0.84	9.3	2,535	580.8	22.9	0.64	0.252	39.7
Buttercrunch	9.8	1.03	10.5	2,776	466.0	16.8	0.69	0.222	32.2
Experiment 2									
South Bay	8.3	0.52	6.2	2,581	241.5	9.4	0.72	0.131	18.2
Salinas	8.7	0.82	9.4	2,683	243.6	9.1	0.75	0.123	16.4
Buttercrunch	10.8	0.50	4.6	3,188	198.9	6.2	0.85	0.069	8.1
Experiment 3									
Prizehead	7.5	0.55	7.3	2,653	266.8	10.1	0.80	0.100	12.5
South Bay	8.0	0.89	11.1	2,808	468.4	16.7	0.84	0.160	19.0
Holborn Standard	10.2	0.41	4.0	3,481	313.5	9.0	1.03	0.110	10.7
Buttercrunch	10.5	0.84	8.0	3,586	316.8	8.8	1.06	0.110	10.4
Experiment 4									
Prizehead	9.0	1.41	15.7	2,838	857.8	30.2	0.73	0.135	18.5
Proeftuins Blackpool	9.5	0.55	5.8	3,005	685.9	22.8	0.76	0.091	11.9
Holborn Standard	11.7	1.03	8.8	3,652	794.8	21.8	0.94	0.102	10.9

[a] Minimum latent period was defined as the time from inoculation to the first appearance of sporulating lesions.
[b] Accumulated hourly temperatures above a threshold of 0 C.
[c] Developmental rates were calculated with equation 1 (in text) and accumulated over time. Values are dimensionless.
[d] Means, SD, and CV were calculated from data pooled over all temperature treatments in the respective experiments. Figure 1 explains the temperature treatments.

TABLE 3. Means, standard deviations (SD), and coefficients of variation (CV) of median latent period (LP_{50})[a] of lettuce downy mildew during incubation at fluctuating temperatures

Cultivar	LP_{50} in days			LP_{50} in degree-hours[b]			LP_{50} in developmental rates[c]		
	Mean[d]	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
Experiment 1									
Salinas	9.8	1.03	10.5	2,797	674.7	24.1	0.71	0.286	40.3
Prizehead	10.5	0.89	8.5	3,028	936.9	30.9	0.78	0.379	48.6
Buttercrunch	11.5	1.09	9.5	3,284	416.3	12.7	0.84	0.222	26.4
Experiment 2									
South Bay	9.5	0.55	5.8	2,938	249.9	8.5	0.82	0.131	16.0
Salinas	9.5	0.55	5.8	2,938	240.9	8.2	0.83	0.128	15.4
Buttercrunch	10.8	0.50	4.6	3,188	198.9	6.2	0.85	0.069	8.1
Experiment 3									
Prizehead	9.0	0.89	9.9	3,119	384.7	12.3	0.93	0.120	12.9
South Bay	9.2	0.98	10.7	3,170	485.4	15.3	0.94	0.160	17.0
Holborn Standard	10.8	0.98	9.1	3,691	245.7	6.7	1.09	0.070	6.4
Buttercrunch	11.0	0.63	5.7	3,745	375.1	10.0	1.11	0.130	11.7
Experiment 4									
Prizehead	9.3	1.63	17.5	2,941	799.2	27.2	0.76	0.127	16.7
Proeftuins Blackpool	10.0	1.09	10.9	3,157	591.6	18.7	0.82	0.080	9.8
Holborn Standard	12.2	1.17	9.6	3,797	773.2	20.4	0.98	0.106	10.8

[a] Median latent period was defined as the time from inoculation to the appearance of 50% of all sporulating lesions.
[b] Accumulated hourly temperatures above a threshold of 0 C.
[c] Developmental rates were calculated with equation 1 (in text) and accumulated over time. Values are dimensionless.
[d] Means, SD, and CV were calculated from data pooled over all temperature treatments in the respective experiments. Figure 1 explains the temperature treatments.

the years 1961–1990 from the National Oceanic and Atmospheric Association climatic stations in Salinas and Santa Maria, CA).

Sporulating lesions appeared first on older leaves of cvs. Salinas, South Bay, Prizehead, and Proeftuins Blackpool 7–9 days after inoculation, followed by the development of the remainder of the lesions during the subsequent two or three nights. Sporulation on cvs. Buttercrunch and Holborn Standard did not occur before day 10. The cumulative number of lesions on these cultivars was always lower than on cv. Salinas or South Bay at the end of the experiments. Mean values of LP_0 (Table 2) ranged from 7.5 days on cv. Prizehead (experiment 3) to 11.7 days on cv. Holborn Standard (experiment 4). Mean LP_{50} (Table 3) varied between 9.0 days on cv. Prizehead (experiment 3) and 12.2 days on cv. Holborn Standard (experiment 4).

When latent periods were measured in days, they showed little variation with temperature (Fig. 3A and B). For example, values of LP_0 on cv. Salinas in experiment 1 ranged from 7.5 to 9.5 days, with a mean of 8.7 ± 0.75 days. Values of LP_{50} on the same cultivar varied between 8.5 and 10.5 days (mean 9.8 ± 1.03 days). There was a small trend toward longer latent periods at lower temperatures, however (groups V and VI, Fig. 3A and B). When latent periods were modeled as degree-hours or developmental rates, they showed greater variability with temperature (Fig. 3C–F) due mainly to longer latent periods at high temperatures (group II in both experiments). Based on data from all four experiments, LP_0 and LP_{50} showed the least variation with temperature when they were measured in days, with CV between 4.0 and 17.5% (Tables 2 and 3). Variability in LP_0 and LP_{50} was always greater when they were modeled as degree-hours ($6.2\% \leq CV \leq 30.9\%$) or developmental rates ($6.4\% \leq CV \leq 48.6\%$).

A comparison of latent periods observed at constant temperatures (26) with those observed at fluctuating temperatures showed that the relationship between LP_0 and temperature was not as strong at fluctuating temperatures as at constant temperatures (Fig. 4). Linear regression of the values of LP_0 obtained in variable-temperature treatments against mean incubation temperatures resulted in a slope coefficient of -0.13 days C^{-1} ($P \leq 0.0515$), indicating only a small effect of temperature on latent period within the range of temperatures tested (Table 4). When similar regression analyses were done for each cultivar separately (Table 4), there was no statistically ($P > 0.10$) significant effect of temperature on LP_0, except for cv. Salinas (slope $= -0.21$ days C^{-1}; $P \leq 0.0164$). In contrast, for the constant-temperature data, a

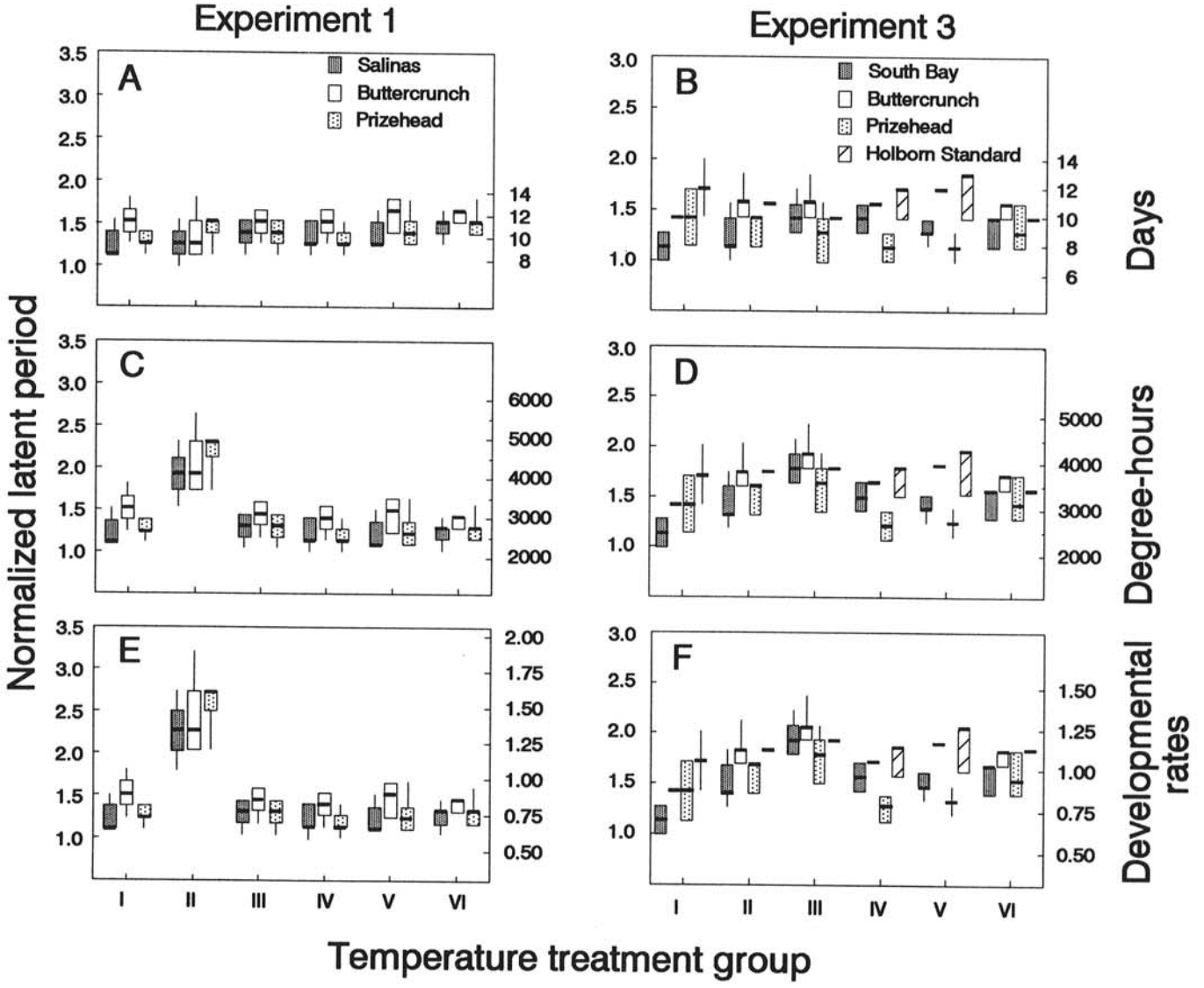

Fig. 3. Latent periods of lettuce downy mildew at fluctuating temperatures in relation to time expressed as **A and B,** days, **C and D,** degree-hours, and **E and F,** developmental rates. Data are from experiments 1 and 3 and are presented as box-plots. The rectangles show the times at which 25% (lower end of the box), 50% (heavy line), and 75% (upper end of the box) of all sporulating lesions appeared. The vertical lines extending from the boxes indicate the times at which the first and last sporulating lesions appeared. LP_0 (minimum latent period) and LP_{50} (median latent period) correspond to the 0 and 50% points, respectively. Data are plotted on a normalized scale, with the shortest observed latent periods in each experiment set to 1.0. (The text explains the temperature treatments.)

highly significant linear relationship was obtained between LP_0 and mean temperatures over the range of 9 to 20 C (slope = −0.90 days C^{-1}; $P \leq 0.0001$) (Table 4; Fig. 4). Slope coefficients of the variable- and constant-temperature data sets were significantly different ($P \leq 0.0001$).

DISCUSSION

Temperature had only a small effect on the length of the latent period of lettuce downy mildew for a wide range of naturally and artificially fluctuating temperatures. This was unexpected in view of previous work done at constant conditions, in which a strong dependence of latent period on temperature had been observed (26). Degree-hour and developmental rate models derived from constant-temperature data did not adequately describe latent period under variable conditions. Based on these results, the usefulness of constant-temperature data for deriving models to predict disease development at variable temperatures in the field seems questionable.

The use of fluctuating temperatures in laboratory experiments for determining epidemiological parameters of plant pathogens has received little attention to date. We are aware of no other study in which latent period has been quantified for a wide range of artificially and naturally varying temperatures. However, our results can be discussed in relation to similar work on the life histories of other poikilothermic organisms (particularly insects) at fluctuating temperatures. Hagstrum and Hagstrum (8), Ratte (17), and Worner (28) reviewed the literature for studies in which insect development times at variable temperatures were compared with expected development predicted by linear or nonlinear models derived from constant-temperature data. They reported that observed development differed from predicted development in most cases. Most commonly, acceleration of development at low and retardation at high temperatures was observed at fluctuating temperatures compared to constant temperatures with the same mean. We observed a similar effect in our study. According to the developmental rate model (equation 1), the first sporulating lesions should have appeared when the sum of developmental rates equaled 1.0, irrespective of the temperature regime. (Some variation due to cultivar would be expected.) However, accumulated developmental rates were usually <1.0 when the first sporulating lesions were observed (Table 2; Fig. 3E and F), confirming accelerated development at fluctuating temperatures.

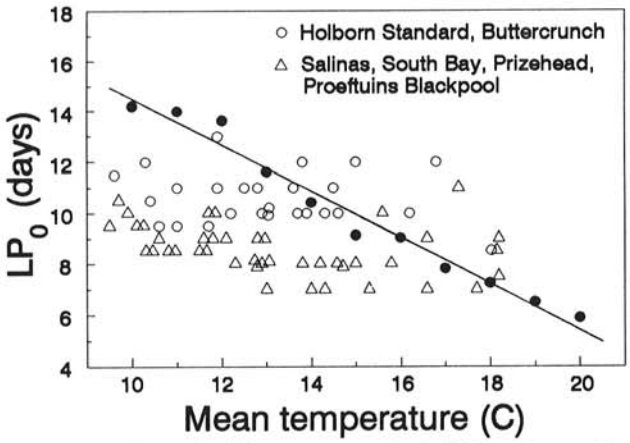

Fig. 4. Comparison of minimum latent periods (LP_0) of lettuce downy mildew at constant (●) and fluctuating (○ and △) temperatures with the same mean. Constant-temperature data are from Figure 2A. Variable-temperature data are pooled over the four experiments, with the six cultivars classified into two groups with similar latent periods. The regression line for the constant-temperature data is shown.

TABLE 4. Slope coefficients of linear models relating minimum latent period (LP_0)[a] of lettuce downy mildew to mean temperature during incubation at fluctuating temperatures

Cultivar	df	Slope coefficient (days C^{-1})	$P > \|t\|$[b]
Buttercrunch	15	−0.14	0.1889
Holborn Standard	11	−0.11	0.5394
Prizehead	17	−0.17	0.1026
Proeftuins Blackpool	5	−0.04	0.6288
Salinas	11	−0.21	0.0164
South Bay	11	−0.06	0.6388
All cultivars	75	−0.13	0.0515

[a] Minimum latent period was defined as the time from inoculation to the first appearance of sporulating lesions.
[b] H_0: Slope coefficient = 0.

Messenger and Flitters (13) and Worner (28) discussed several factors that could explain the difference between observed development in vivo and predictions based on constant-temperature development models. The first factor is related to a simple but important mathematical property of nonlinear functions. Because development curves are nonlinear, there will always be a difference between the growth rate at the mean temperature (as obtained from constant-temperature studies) and the "average" rate obtained by summation of these constant-temperature rates at fluctuating temperatures (1,12). This effect, first described by Kaufmann (11), is known as "rate summation effect" in the entomological literature (28). Likewise, if the temperature is too low for development at constant conditions, development will still occur at temperatures fluctuating around the same mean, provided that subthreshold temperatures have no permanently damaging effect (12,13,17). The second factor is related to a possible physiological mechanism. It has been suggested that low-temperature fluctuations have a "stimulative" or "synergistic" effect on development (28). Ratte (17) summarized some theories for this phenomenon in insects. A hypothesis that could be extended to other poikilothermic organisms suggests that a diurnal periodicity of activity and rest, as conditioned by fluctuating temperatures, could result in an energetic advantage in terms of oxygen efficiency and nutrient uptake. However, whether such physiological mechanisms occur in plant-pathogenic fungi is unknown.

Other environmental, host, and/or pathogen factors may interact with temperature in their effects on latent period. Downy mildews are known for their dependence on the daily rhythm of alternating light and dark for sporulation (14,25,26,29). Yarwood (29) showed that the ability of several downy mildews to sporulate was "inherent in the host-parasite complex" and conditioned by the daily light-dark cycle, rather than being directly dependent on external factors. Thus, it is conceivable that an approximately constant number of day-night cycles is needed to initiate sporulation. Moreover, host colonization and sporulation in downy mildews also depend on diurnal cycles in internal (leaf) and external (atmospheric) water potential (14,25). In our study, moisture was not limiting for the onset of sporulation because plants were always enclosed in wet plastic bags overnight. During the light phase, however, humidity in the different temperature treatments was not controlled. Factors such as humidity or light (which were similar but not constant among the treatments) may have had a stronger effect on colonization and latent period than temperature. However, we cannot make assertions regarding these hypotheses without further data.

From a practical viewpoint, the information provided in this paper could assist in understanding and managing downy mildew epidemics on lettuce. For example, the first sporulating lesions on cv. Salinas appeared on average 8.7 ± 0.75 days after inoculation, with only a small effect of the temperature regime. It should be feasible to use this information to identify days on which infection likely occurred based on field observations of the appearance of new lesions (19).

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