Slowing of Spore Germination with Changes Between Moderately Warm and Cool Temperatures

Paul E. Waggoner and Jean-Yves Parlange

The Connecticut Agricultural Experiment Station, New Haven 06504.
The authors thank Barbara Wooding for her assistance.
Accepted for publication 4 December 1974.

ABSTRACT

Although *Alternaria solani* spores germinated with equal rapidity at 16 or 35 C, frequent changes from 16 to 35 C more than doubled the germination time. We infer that germination follows different paths at 16 and 35 C and that changing paths slows germination.

Phytopathology 65:551-553

Additional key words: mathematical model, *Alternaria solani*.

A method for calculating development of an organism in a changing environment is being tested by comparison with the germination of *Alternaria solani* spores in steady and changing temperatures. When the spores are cooler than 38 C, they germinate abundantly, and their course of development at steady temperature can be described by the mean germination time $t_{1/2}$ and the variance s^2 of times around the mean. The $t_{1/2}$ and s^2 are used to calculate two parameters that are mathematically equivalent, but evoke the biological process. One is the number f of steps in the germination path, and the other is the rate P of progress from one step to the next. Under steady or isothermal conditions ($t_{1/2}$, s^2) and (f, P) are related by

$$f = (t_{1/2}/s)^2;\ P = t_{1/2}/s^2 \qquad \text{(I)}$$

where f and P are functions of temperature that characterize the development of the spores under isothermal conditions (1, 2).

For spore germination from 4 C to about 30 C, the number f of steps was a constant 14, and rate P increased linearly with temperature (Fig. 1). This regime below 30 C was dubbed the "linear" range (3).

Even when spores germinated under varying temperatures in the linear region, simple rules predicted the outcome (2): The half time is

$$f = \int_0^{t_{1/2}} P\,(t)\,dt \qquad \text{(II)}$$

where f remains 14, and P (t) is the isothermal rate from Fig. 1 for the temperature at time t. The standard deviation is

$$s^2 = f/P_{1/2}^2 \qquad \text{(III)}$$

When spores were warmed to 45 C and then cooled to the linear range, the total germination decreased rapidly with the duration of exposure to 45 C (2). More important for the present investigation, equations II and III still applied, but the rate P for 45 C in equation II was very fast. An empirical relation was also derived for the change of f in the changing environment. It is intuitive that the rate P should increase with temperature, as in a chemical reaction, and f must then increase to accommodate the harm during exposure to 45 C.

To complete the study of temperature interactions and our method of calculation, we now examine the interactions between the linear regime of 4 to 30 C and the intermediate regime where temperature is warmer than 30 C, but not so hot that germination ceases under isothermal conditions. Since equations II and III applied in warmer and cooler regimes, we expect them to hold here as well. We already know that germination and P are slower at steady 35 C than at steady 30 C (3). The task here is to reconcile this obervation of a slowing P on warming from 30 to 35 C withthe observation that warming to 45 C speeds up P.

MATERIALS AND METHODS.—Most of our materials and methods have been described elsewhere (2, 3) and will not be repeated here.

After being moistened, the *A. solani* spores were exposed to 16 C in the linear range and 35 C in the intermediate range; 35 C is about at the midpoint of the intermediate range, and P is about the same at 16 and 35 C (Fig. 1). If the rules of the linear range applied to changes between 16 and 35 C as well, alternations between these temperatures would not affect germination speed, and the outcome could not be distinguished from the germination at either steady 16 or steady 35 C. Hence, any deviation from the simple rules that apply in the linear range should be easily detected with this particular pair of temperatures.

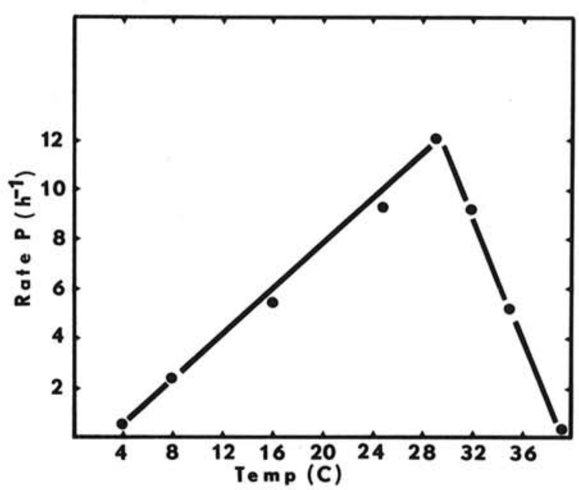

Fig. 1. The isothermal rate P of progress in steps per hour estimated from the course of germination of *Alternaria solani* spores at steady temperatures.

The spores were subjected to two temperature regimes: (i) starting at 16 C and switching to 35 C after various intervals, or (ii) starting at 35 C and switching to 16 C after various intervals. Germination was observed periodically and $t_{1/2}$ and s estimated by regressions of probits of germination upon time.

RESULTS AND DISCUSSION.—In the first experiments (Table 1), the temperature was changed once after 90 minutes, and germination was observed after 2 and 3 hours. Germination was also observed at 4 hours, but it was essentially complete and in a range where the course of germination does not exactly follow the cumulative normal curve (4). The experiment was repeated twice with both spore populations I and II, which were grown at different times.

Germination is clearly more rapid when spores were changed from 35 to 16 C rather than from 16 to 35 C. The rate with change from 35 to 16 C is practically identical to isothermal germination at either 35 or 16 C. That is, no harm in the sense of inhibited germination is caused by the change, perhaps because the first temperature is close to the temperature where germination ceases altogether, but the second temperature is more favorable in the sense of being within the linear range. On the other hand, the estimates of $t_{1/2}$ and s for the regime of 16 to 35 C together with equations II and III suggest that in that case f should be about 24 and P at 35 C about 10. Because of experimental variation, of course, these numbers should not be trusted beyond the qualitative fact that switching from 16 C to 35 C is clearly harmful to the spores. The changes in $t_{1/2}$, s, f, and P are less pronounced here than following fluctuations between 25 and 45 C since 35 C is far less harmful than 45 C (2). Other experiments were performed with different pairs of temperatures and changes of temperature at different times, but the basic conclusions remained unchanged:

—1)—One temperature change from the intermediate to the linear range caused no harm, and equations II and III applied with the isothermal rates and with f about 14.

—2)—One temperature change from the linear to the intermediate range was harmful, and equations II and III applied, but the rate at the intermediate temperature was faster than the rate under isothermal conditions and f was larger than 14. However, the changes in f and P were slight.

These results were rationalized by imagining two paths to germination with a change from 16 to 35 C forcing the spores to change paths. It is consistent with this that more changes between 16 and 35 C should cause more harm. At each change the spores must use a different path and the additional travel between the paths could cause a large f and hence large $t_{1/2}$ and s, which could not possibly be mistaken for variation in the data. Since one change from the linear to intermediate range increased the path length by 10 steps (from 14 to 24) and increased the rate P to 10 h^{-1}, it is plausible that additional changes from 16 to 35 C would each increase f by an additional 10 steps with rates P about 10 at 35 C and about 6 at 16 C. The validity of this assumption was tested.

Table 2 summarizes the outcome of changes from 35 to 16 C and 16 to 35 C at various frequencies. Standard deviations and half times were estimated by regression on time of germination as probits. The correlation coefficients ranged from .89 to .98.

In the first regime of Table 2, the temperature was changed once after 90 minutes. The result is essentially the isothermal one as explained earlier. With one change from 16 to 35 C we increase f by 10 and P at 35 C to 10 h^{-1} and obtain the values of $t_{1/2}$ and s in parentheses, computed according to equations II and III.

With changes every 45 minutes, there are two or three harmful warmings from 16 to 35 C before $t_{1/2}$ is reached. The numbers in parentheses are those predicted by equations II and III when f increases by 10 for each warming from 16 to 35 C.

Changing temperature more frequently established that the path to germination was not increased indefinitely. For example, if a harmful change from 16 to 35 C increased f by 10 and P at 35 C were 10 h^{-1}, changing the temperature every 30 minutes would lengthen the path as fast as progress was made. In fact, Table 2 shows that changes every 30 minutes increase $t_{1/2}$ considerably, but that germination did finally occur. Further, changes every 15 minutes did not increase $t_{1/2}$ further.

The final conclusions were: (i) Germination occurs isothermally in two regimes. One, below 30 C, is called the linear range, and there the rate P of germination increases with temperature. Another regime, from 30 to 40 C is called the intermediate range and there the isothermal rate decreases with temperature. (ii) Although

TABLE 1. The percentage, $t_{1/2}$, and s of *Alternaria solani* spores germinated at 2 and 3 hours when two populations of spores were incubated for 90 minutes at 16 or 35 C and then changed to 35 or 16 C

Spore population	Temperature regime (C)	Germination (%) 2 (hours)	3 (hours)	$t_{1/2}$ (hours)	s (hours)
I	35 to 16	38	85		
		43	77		
II		23	86		
		16	78	2.4	0.7
I	16 to 35	6	37		
		3	48		
II		1	47		
		3	55	3.1	0.6

TABLE 2. The mean, $t_{1/2}$, and standard deviation s of germination times when *Alternaria solani* spores were changed between 16 and 35 C at intervals of 90, 45, 30, and 15 minutes. The spores were first exposed to the first temperature specified in the regime. The numbers in parentheses are predictions from equations II and III and based upon assumptions that P is 10 h^{-1} at 35 C and that f increases on each warming from 16 to 35 C

Temperature regime (C)	Interval (minutes)	$t_{1/2}$ (hours)	s (hours)
35 to 16	90	2.4	0.7
16 to 35		3.1 (3.0)	0.6 (0.5)
35 to 16	45	4.1 (4.0)	1.2 (1.1)
16 to 35		4.9 (4.8)	1.1 (1.2)
35 to 16	30	7.8	2.2
16 to 35		7.2	2.1
35 to 16	15	7.2	2.7
16 to 35		8.2	3.0

germination occurs when temperatures change from one range to the other; e.g., between 16 and 35 C, every change from the linear to the intermediate range is harmful in the sense that the germination path is lengthened while the rate at the hot temperature on that new path is faster. (iii) Frequent temperature changes increase the harm until maximum harm is done. At that stage, the germination time was fully three times that at either steady 16 or 35 C.

This concludes a series of papers on the analysis and prediction of development in a variable environment of an organism; i.e., a germinating spore. We found that in all cases our observations could be summarized within a convenient mathematical framework, which describes germination as a development through f mathematically defined steps at a rate P steps per hour. These parameters give the mean and variance of the germination time (1). Three regimes can be found in fluctuating environments: At moderate temperatures, the course of germination can be calculated simply by adding, as degree-days are added, the products of times by rates already observed at steady temperatures (3). In the present paper, we investigated the lengthening path of germination when temperatures warm to an intermediate temperature, 35 C. Finally, when temperatures warm lethally, the model still provides a frame for anticipating mortality as well as altered germination (2).

LITERATURE CITED

1. WAGGONER, P. E., and J. -Y. PARLANGE. 1974. Modeling seasonality, with appendix "Analytic solution to model passages through phenophase. Pages 301-327. In: H. Lieth, ed. Phenology and seasonality modeling. Springer Verlag, Berlin. 444 p.
2. WAGGONER, P. E., and J. -Y. PARLANGE. 1974. Mathematical model for spore germination at changing temperature. Phytopathology 64:605-610.
3. WAGGONER, P. E., and J. -Y. PARLANGE. 1974. Verification of a model of spore germination at variable, moderate temperatures. Phytopathology 64:1192-1196.
4. WELLMAN, R. H., and S. E. A. MC CALLAN. 1942. Analysis of factors causing variation in spore germination tests of fungicides. IV. Time and temperature. Contrib. Boyce Thompson Inst. 12:431-450.