

## Verification of a Model of Spore Germination at Variable, Moderate Temperatures

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### ABSTRACT

A concept of the development of germ tubes as  $f$  steps taken at  $P$  per hour produced the half-time ( $t_{1/2}$ ) and variance ( $s^2$ ) that characterize the sigmoid course of germination of wet *Alternaria solani* spores at steady temp. At steady temp from 4 to 29 C, the number of steps  $f$  was constant, and the isothermal  $P$  increased linearly with warmth. At variable temp from 10 to 29 C, because  $f$  was constant and the spores promptly took on the isothermal  $P$  at whatever temp they

had, the  $t_{1/2}$  of germination could be calculated from an integration of  $P$  or even a simple heat-sum during development. The  $s^2$  could be calculated from the ratio  $f/P^2$  where  $P$  is the isothermal rate at the temp prevailing near  $t_{1/2}$ . Although little development occurred at 4 C, exposure to 4 C on wetting did speed subsequent development somewhat.

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

Although the goal is calculating or predicting the course of development in varying temp outdoors, controlled experiments and observations of development are generally made at steady temp in the laboratory. Choosing spore germination as an example of development, we have devised and tested a method for calculating the course of germination at variable temp from 4 to 29 C, using observations of germination at steady temp.

The course of development is depicted in Fig. 1. One can say that after a lag, germination of a batch of spores proceeds at a rapid rate, and finally slows as the limit of 100% is approached. Alternatively, one can say that there is a characteristic time  $t_{1/2}$  of germination for the spores, and inherent variation (1) makes the curve of Fig. 1 sigmoid with the slope or rate being, in fact, a measure of the variance of the individual germination times around the ideal  $t_{1/2}$ . Germination increases up to 90% or even more with time as the cumulative normal curve (5). When nearly all spores germinate (as *Alternaria solani* spores do

in water at moderate temp) the course of germination is represented by the mean germination time  $t_{1/2}$  and the variance  $s^2$  of the germination times of individuals around the mean.

We must now relate the two parameters  $t_{1/2}$  and  $s^2$  to the environment; i.e., temp. One could, in principle, tabulate  $t_{1/2}$  and  $s^2$  for any temp history. Since this would be neither practical nor elegant, we have instead sought a model that is easier to grasp and use, and that evokes the process of development. In the concept of Fig. 2, the development of spores is a journey of  $f$  steps from a first step, which we have chosen as the moment of wetting of *Alternaria* spores, to a final step reached when a germ tube has appeared (4). The plausible assumption is made that the spores follow a path dictated by the environment and defined by its length  $f$ , and that they progress at a rate  $P$  that is also dictated by the environment. The movement through the boxes or steps is defined by:

$$dC_i/dt = P (C_{i-1} - C_i) \quad , \text{hour}^{-1} \quad (\text{Eq. 1})$$

which causes a fraction  $P$  of the count  $C^i$  in the  $i^{\text{th}}$  of  $f$  boxes to progress per hour and causes a dispersion among the population in their times of germination; i.e., when they leave the  $f^{\text{th}}$  box.

Essentially the model replaces the observed  $t_{1/2}$  and  $s^2$  by two other quantities that evoke the biological process, and it permits some simplification. It can be seen intuitively, and demonstrated mathematically (3), that at steady temp and with several steps, the rate  $P$  is the number of steps divided by the average time  $t_{1/2}$  to complete them:

$$P = f/t_{1/2} \quad , \text{hour}^{-1} \quad (\text{Eq. 2})$$

Put the other way around,  $1/P$  is the average time (h) spent on each step. Parlange (3) showed that when  $f$  is sufficiently great,

$$f = (t_{1/2}/s)^2 \quad , \text{dimensionless} \quad (\text{Eq. 3})$$

Waggoner and Parlange (4) have observed germination of *Alternaria* spores at 25 and 45 C and found that both  $P$  and  $f$  increase markedly from the cooler to the warmer, indicating that development is swifter, but along a longer path at the warmer than at the cooler temp. Through a range of cool and moderate temp in the present study, we shall simply find development along a single path,  $f$

constant, the  $P$  within the range proportional to warming, and  $t_{1/2}^{-1}$  and  $s^{-1}$  proportional to warming.

At variable temp, the relations between the parameters are (3):

$$f \approx \int_0^{t_{1/2}} P(t) dt \quad , \text{dimensionless} \quad (\text{Eq. 4})$$

and equation 6 becomes,

$$s^2 \approx f/P^2(t_{1/2}) \quad , \text{hour}^2 \quad (\text{Eq. 5})$$

where  $P(t)$  and  $P(t_{1/2})$  are the rates at times  $t$  and  $t_{1/2}$ . In the isothermal case, equations 4 and 5 reduce to equations 2 and 3, respectively. Equations 4 and 5 assume that temp varies slowly around germination time; more complicated expressions were also derived for the general case although they are not necessary here. Waggoner and Parlange (4) showed that when the temp of developing spores was changed from 45 to 25 C, they promptly took on the isothermal rate  $P$  characteristic of spores that had been steadily at 25 C.

If the spores developing at variable temp within the moderate range of 4 to 29 C promptly take the isothermal rate for any temp they are at, and if they also follow an unvarying path with constant  $f$ , a considerable simplification is possible. Employing equations 2 and 3,  $f$

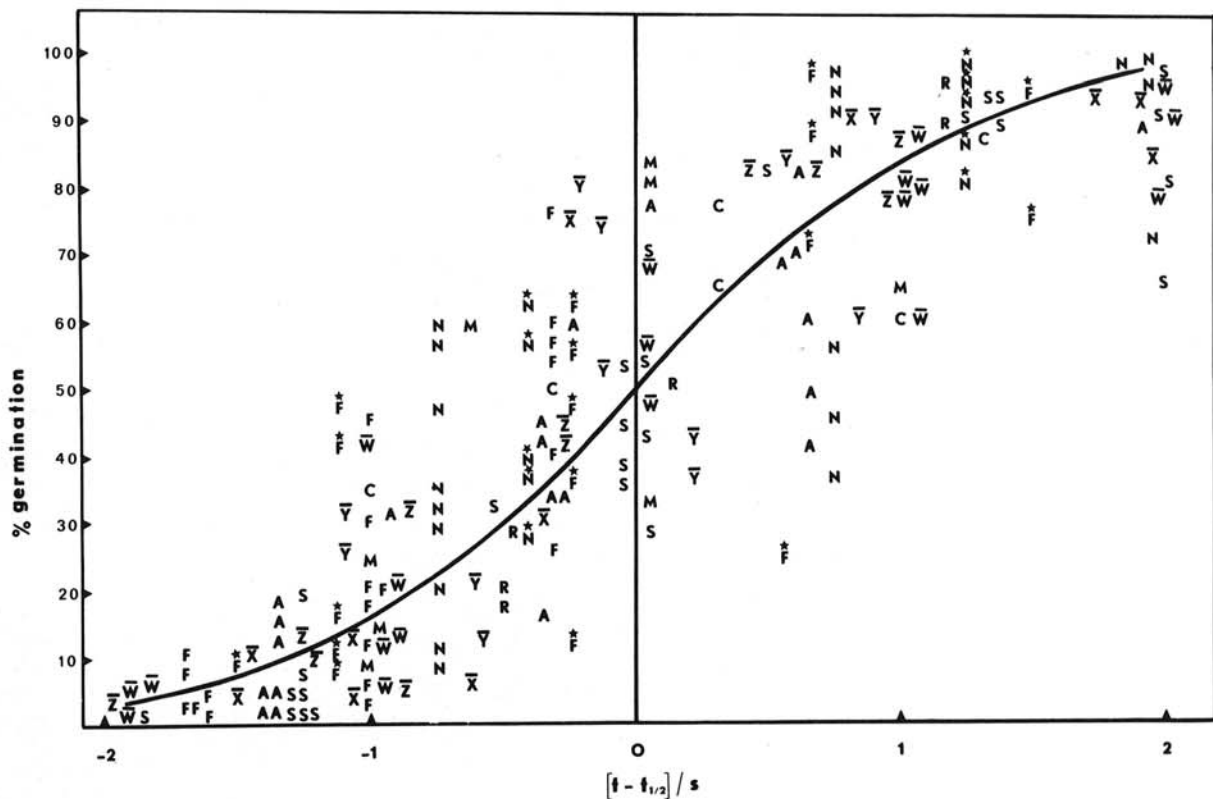


Fig. 1. The time course of germination of *Alternaria solani* under different regimes. Time has been normalized by subtracting  $t_{1/2}$  and dividing by  $s$  for each regime. The curve is the normal ogive with zero  $t_{1/2}$  and unit  $s$ . Regimes identified by C, F, N, S, R, M, A correspond to the isothermal treatments of Table 1. Letters with a bar,  $\bar{V}$ ,  $\bar{X}$ ,  $\bar{Y}$ ,  $\bar{Z}$ , correspond to the varying temp of Table 2. Letters with the star,  $\bar{F}$ ,  $\bar{N}$ , correspond to 0.5 h at 4 C, followed by 10 C and 16 C, respectively.



Fig. 2. The box model which was developed to describe the time-rate of germination of *Alternaria solani* spores. The spores pass through  $f$  steps on their way to germination. The count  $C_i$  of spores in each step is represented by the level of shading and changes as the fraction  $P$  per hour progresses.

and  $P$  can be eliminated from equation 4 to obtain

$$\int_0^{t_{1/2}} dt/\bar{t}_{1/2} = 1, \text{ dimensionless} \quad (\text{Eq. 6})$$

where  $t_{1/2}$  is the  $t_{1/2}$  for isothermal germination at the temp existing at time  $t$ . If in addition to a constant  $f$ , the spores have a rate  $P$  that increases linearly with temp  $T$  above a threshold  $T_0$ , then  $1/t_{1/2}$  is proportional to  $(T - T_0)$  with a proportionality constant  $\beta$ , and

$$1/\bar{t}_{1/2} \approx \beta (T - T_0), \text{ hour}^{-1} \quad (\text{Eq. 7})$$

and equation (6) becomes,

$$\int_0^{t_{1/2}} (T - T_0) dt = 1/\beta, \text{ degree-hours} \quad (\text{Eq. 8})$$

Since equation 8 is the familiar heat-sum or "degree-day" equation first surmised by Reaumur (2), the heat-sum equation can be derived exactly when development is mimicked by the box model as long as: (i) the temp fluctuates within the range of constant  $f$  or a constant path, and (ii) the spores promptly assume the isothermal rate proportional to the warming.

Equation 6 gives  $t_{1/2}$  for a known temp history, and equation 5 yields  $s$ . Hence the assumptions of constant path and prompt assumption of new rate permit us to predict easily the response of development to an arbitrary and variable temp history. Note that the standard deviation  $s$  is inversely proportional to the rate prevalent at time  $t_{1/2}$ , and not proportional to an average rate.

Figure 2 is a physically attractive, if greatly simplified, model of germination. We shall now test whether the further simplifications derived from the model and represented by equations 5 through 8, allow the

TABLE 1. The isothermal germination of wet *Alternaria solani* spores. Germination is summarized by the  $t_{1/2}$  hours for 50% germination of the spores and the  $s$  hours standard deviation. The corresponding  $f$  and  $P$  are calculated from equations 2 and 3 (see text). The uncertainty in the data is such that the  $t_{1/2}$  and  $s$  could be adjusted to yield  $f = 14$  for all treatments.

Treatment	Temp (C)	Germination		Box model		Repetitions
		$t_{1/2}$ (h)	$s$ (h)	$f$ ( $h^{-1}$ )	$P$ (no.)	
C	4	22	5.9	14	0.6	3
F	10	5.5	1.48	14	2.5	8
N	16	2.5	0.67	14	5.6	9
S	25	1.5	0.40	14	9.3	11
R	29	1.2	0.30	14	12.3	3
M	32	1.5	0.40	14	9.3	4
A	35	2.6	0.70	14	5.3	7

<sup>a</sup>Treatments were described in detail by Waggoner and Parlange (4).

calculation of germination during any temp fluctuations within 4 to 29 C.

MATERIALS AND METHODS.—*Alternaria solani* isolated from potato was caused to sporulate on sheets of moist filter paper, and the spore-laden papers were stored over silica gel in a refrigerator. When an experiment was performed, spores were dusted from about 8 cm<sup>2</sup> of the paper onto each of several slides. At 0 hours the slides were sprayed with a 0.05% suspension of orange juice, and later at four or five times chosen to reveal the course of germination, development was stopped by dropping Trypan blue onto a slide. The developing spores were kept moist by enclosing the slides in staining jars held horizontally in a water bath or refrigerator or on the laboratory bench. The half-time of a temp change varied from 8 min on the central slide within a jar on the bench, to 2 min on the outer slide within a jar in the water bath. Germination was estimated by noting the number of spores out of 100 that had germ tubes at least half as long as the spore diam. The  $t_{1/2}$  and  $s^2$  were estimated for each experiment of four or five slides and times of development by plotting the observed probit of germination versus time, and then drawing a straight line through the points. Except for enclosing the slides in the staining jars, these are the same methods and materials described at greater length by Waggoner and Parlange (4).

RESULTS AND DISCUSSION.—*Steady temp.*—In 45 experiments during 6 mo, the  $t_{1/2}$  for germination at 4, 10, 16, 25, 29, 32, 35 C ranged from fully 22 h to only 1 h, and the  $s$  for the same temp ranged from 5.9 to 0.3 h, Table 1. The corresponding  $f$  and  $P$  estimated by equations 2 and 3 are also indicated in the table. Figure 1 shows the observed germination as a function of the transformed time  $(t - t_{1/2})/s$ , where  $t_{1/2}$  and  $s$  were taken from Table 1. The scatter between experiments performed at the same temp but at different dates during 6 mo can be seen. If germination always followed the normal distribution, all points would fall on the sigmoid curve. Although  $t_{1/2}$  and  $s$  were chosen to give the least bias (i.e., the experimental points fall more or less equally on both sides of the normal ogive) there is, of course, some uncertainty in the tabulated values of  $t_{1/2}$  and  $s$ .

Several conclusions, nevertheless, stand out. Spores warmer than 29 C behave differently from cooler ones. At cooler temp,  $t_{1/2}$  and  $s$  decrease with increasing temp. Since  $t_{1/2}^{-1}$  and  $s^{-1}$  vary essentially linearly with temp cooler than 29 C, the number  $f$  of boxes in the range 4 C to 29 C is nearly constant at  $14 \pm 1$ . At higher temp, 32 and 35 C, both  $t_{1/2}$  and  $s$  increase with temp, so that  $f$  is still about 14.

The rate  $P$  also increases with temp almost linearly in the range 4 C to 29 C, which is therefore denoted as the

"linear range" in the following. At warmer temp the rate  $P$  decreases with temp, the rate at 32 C being the same as at 25 C and the rate at 35 C about the same as at 16 C. Waggoner and Parlange (4) dealt with a higher temp, but in the following paragraphs we are mainly concerned with the linear range of 4 C to 29 C.

Although the correspondence between  $(t_{1/2}, s)$  and  $(f, P)$  is trivial at steady temp, the virtue of the model is already apparent. While  $t_{1/2}$  and  $s$  vary with temp, showing they depend on the environment,  $f$  is a constant for the spores, showing it is an intrinsic property unaffected by temp. In other words, the observation that  $f$  is a property of the spores unaffected by temp indicates that the variability in the population, as measured by  $s$ , is not caused by the experiment, but is inherent in the population (1). The advantage of the model will become more apparent when variable temp conditions are considered.

*Variable temp.*—Spores were exposed to variable temp in 17 experiments during three months (Table 2). Since  $f$  is essentially constant, the  $t_{1/2}$  for each experiment could be calculated by equation 6 and the  $s^2$  by equation 5. The correspondence of the observations to these predictions is shown in Fig. 1. Since the data points fall more or less at random around the normal ogive, the correspondence is adequate. The first experiment, V, was performed under continuously varying temp, increasing

TABLE 2. The germination of wet *Alternaria solani* spores under varying temp. Values of  $t_{1/2}$  and  $s$  are predicted from equations 5 and 6 (see text) with an  $f$  of 14 and the isothermal rates. Room temp varied from experiment to experiment, but remained close to 25 C.

Treatment	Germination Repetition		
	$t_{1/2}$ (h)	$s$ (h)	(no.)
V 4, 10, 17.5, 19, 21, 22, 24.5 C, at 0, 1, 2.5, 3, 3.5, 4, 5 h	3.5	0.5	5
X 2 h at 10 C; then at room temp	3.0	0.4	3
Y 0.5 h at 4 C; 0.75 h at room temp; then at 10 C.	4.0	1.5	5
Z 0.5 h at 4 C; 0.75 h at room temp; then at 16 C.	2.45	0.7	4

slowly from 4 C to room temp, and its outcome followed the rule. Continuous temp variations, however, provide a less critical test of the two rules (Eq. 5 and 6) than sudden changes, because a system that is affected by only the immediate past is more affected by a larger change in a shorter time. To provide a more crucial test, three

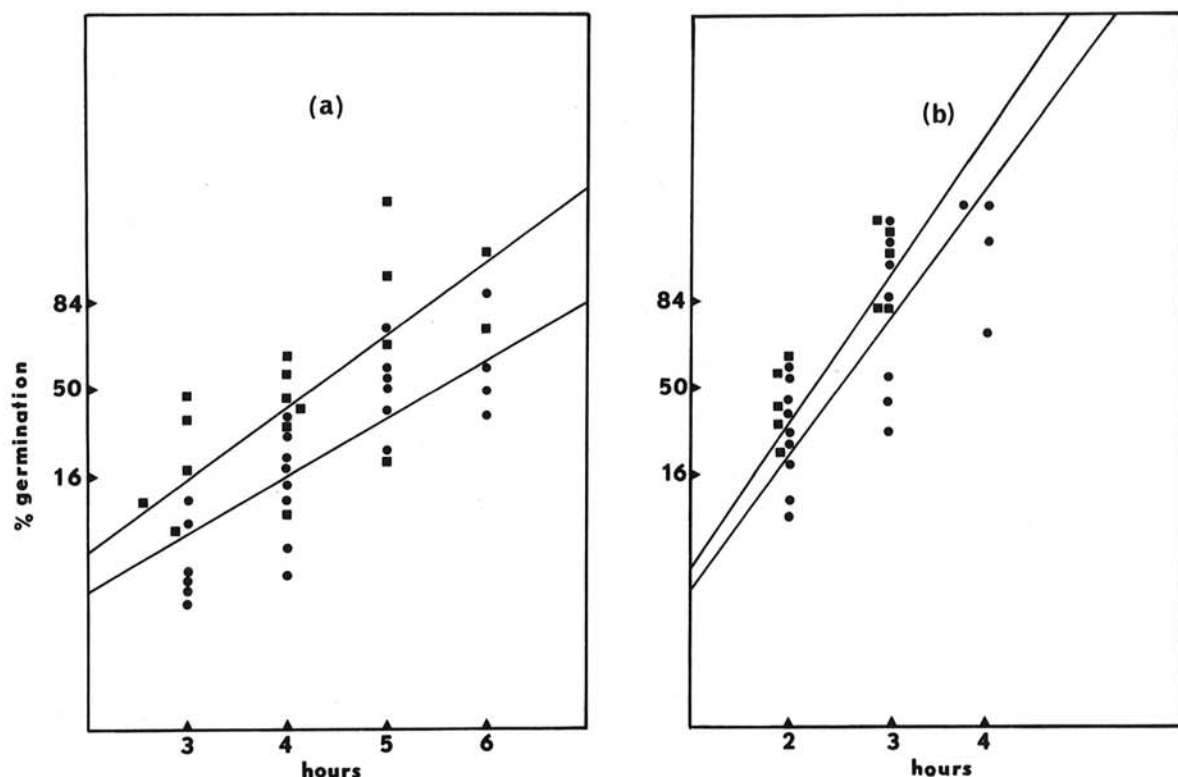


Fig. 3-(A, B). Time course of germination after 0.5 h of chilling at 4 C. The course has been made linear by transformation of percentages to probits. Time is measured from the end of chilling. A) Chilling followed by 10 C (regime F) is represented by squares, and the straight line corresponds to  $f=14$  and  $t_{1/2}=4.25$ . The isothermal exposure to 10 C (regime F) is represented by dots, and the straight line corresponds to  $f=14$  and  $t_{1/2}=5.5$ . B) Chilling followed by 16 C (regime N) is represented by squares, and the straight line corresponds to  $f=14$  and  $t_{1/2}=2.25$ . Isothermal exposure to 16 C (regime N) is represented by dots, and the straight line corresponds to  $f=14$  and  $t_{1/2}=2.5$ .

experiments, X, Y, Z, with sudden temp variations, were also performed. The times of exposure to given temp were chosen to influence the outcome significantly according to the model. Transition from cold (10 C) to warm (27 C) was studied in X and subsequent transitions from warm to 10 or 16 C in Y and Z, respectively. Although these extreme changes are critical for the model, Fig. 1 shows that the simple rules work well. In both Y and Z, 0.5-h exposures at 4 C did not effect the final outcome at warm temp since the rate is so slow at 4 C. Note, however, that in both Y and Z, room temp followed the cold 4 C.

*Temperature interactions.*—Finally, cooler temp were caused to succeed 4 C to learn whether the exposure to 4 C would affect the outcome when development was slowed by 10 or 16 C. Figure 3, which summarizes 27 experiments performed during a 3-mo period, exhibits the effect.

Figure 3-A shows that chilling to 4 C for 0.5 h reduces  $t_{1/2}$  at 10 C from 5.5 to 4.2 h, even though the simple rules of the previous section predicted no effect. An analysis of variance showed that there is less than 5% chance that the difference caused by chilling at 4 C was due to chance. For the same  $f$  of 14, the  $P$  at 10 C is about 3.0 after exposure instead of 2.5, indicating that spore germination progressed faster after chilling. This invigoration could only be discerned by analysis in terms of the model.

Figure 3-B shows a similar, but less striking, decrease in  $t_{1/2}$  when development was at 16 C instead of 10 C; and finally, as already mentioned, no interaction was observable when 4 C was succeeded by room temp. If the degree-day relation (Eq. 8) was assumed, it would fail to predict the right  $t_{1/2}$  when the spores are exposed to 4 C and then to 10 C in succession. At these cold temp, we must keep track of the temp history and use the appropriate rate  $P$  in equation 4, or the appropriate  $t_{1/2}$  in

equation 6, which quantities cannot be obtained by isothermal experiments alone. In principle, at these cool temp, we must take into account any temp interactions, and measure rates as a function of temp before and after the transition. Then, with the correct  $t_{1/2}$  or  $P$ , the model can predict  $t_{1/2}$  and  $s$ .

In the foregoing we have emphasized use of the model as a tool and a framework for the study of germination and most likely other phenological processes. It would be tempting to claim at this stage that our data prove the physical validity of the model. Rather, the good agreement between experimental data and predictions of the model show it is flexible enough to describe a variety of situations, and frequently it enables great simplifications. Actually, the only virtues we claim for the model, and these are not insignificant, are that it is self-consistent, simple, and (more important) useful.

#### LITERATURE CITED

1. JINKS, J. L. 1957. Selection for cytoplasmic differences. *Proc. Roy. Soc. B.* 146:527-540.
2. REAUMUR, R. A. F. de. 1740. Observations du thermometre faites en 1740 a Paris et dans d'autres endroits, soit du Royaume soit des Pays etrangers. *Hist. Acad. Roy. Sci. (Paris)*. 1740:539-566.
3. WAGGONER, P. E. 1973. Modeling seasonality. (With appendix by J.-Y. PARLANGE Pages - in H. Lieth, ed. *Phenology and seasonality modeling*. Springer-verlag, New York. (In press).
4. WAGGONER, P. E., and J.-Y. PARLANGE. 1974. Mathematical model for spore germination at changing temperature. *Phytopathology* 64:605-610.
5. 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.