Mathematical Model for Spore Germination at Changing Temperature

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ABSTRACT

In the field, organisms develop in temp, radiation, and moisture conditions that are variable rather than steady, and that are often detrimental rather than temperate. A mathematical model is presented for predicting one aspect of development, the germination of spores, under variations of temp. The model predicts the failures in germination of *Alternaria solani* spores and the average and variability in the germination time among individual spores when temp varied from 25 to 45 C. In the model, it is conceived that developing spores either progress through, or die in, a series of stages between dormancy and the appearance of germ tubes; and that temp affects the number of stages successfully completed and the rates of progress or death. The rates and number of stages could be calculated at any instant from the current environment and currently attained development stage, and the calculation did not require information about the often complex changes in temp that the spore had encountered previously. In principle, the model can be generalized to other development criteria, to other organisms, and to other environmental variations in the field.

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Flowering, hatching, or germination may be hastened, or the organism may be killed, by changing temp. These developments from stage to stage may be summarized by the fraction (H) of the population that does not perish and eventually flowers, hatches, or germinates; the time $t_{1/2}$ for half to arrive at that stage; and the variance (s^2) of the individual arrival times around $t_{1/2}$. Practically, H for a germinating spore could determine need for a fungicide, $t_{1/2}$ could guide the time of spraying, and s^2 could aid in choosing a fungicide with the appropriate persistence. Because the environment around developing organisms is normally variable while behavior is customarily observed in constant environments, a dual problem arises (i) in choosing the constant environment, and then (ii) relating the observations to behavior in the variable outdoor environment.

In developing a system of experimentation, summary, and model of development in fluctuating environments we have varied only the temp and employed the germination of the spores of *Alternaria solani*, the pathogen of potato and tomato early blight, *Alternaria solani*, which is important in simulating epidemics from weather observations (7). Spores of *Alternaria solani* germinate well from 26 to 28 C with limits of 1 and 45 C, and the thermal death point is 50 C maintained for 25 min (1). Because predictions for the hatching of insect eggs failed most when temp fluctuated most violently (4), we developed and tested our model under the extreme conditions of nearly instantaneous changes from 25 to 45 C.

MATERIALS AND METHODS.—The fungus was grown for 7 days in casamino acid media with constant shaking (2). Then the 75 ml of medium was decanted, the mycelium suspended in water, ground for 1 min, and washed twice by centrifugation and suspension in water. The mycelium was suspended in 0.02 M phosphate buffer (pH 6.3), and a portion was pipetted onto filter paper in glass trays. After about 30 hours at 25 C in the fluorescent illumination of a laboratory and then about 12 hours in the dark at 20 C, the paper, laden with spores, was dried in the air for 1 day and afterwards stored over silica gel at about 5 C. Spores from different sheets of paper prepared at one time and stored were less variable than spores grown for separate experiments.

TABLE 1. The germination of wet *Alternaria solani* spores at 25 C after various treatments at 45 C. The treatments are described by the delay of t_d hours before heating to 45 C, the t_{45} hours at 45 C and the t_b hours between split exposures to 45 C. Germination is summarized as the eventual or maximum germination percentage H, the $t_{1/2}$ hours for germination of H/2 percent of the spores, and the s hours standard deviation times of individual spores around $t_{1/2}$

	Treatment			Germination			
Code	t_d	t_{45}	t_b	H	$t_{1/2}$	s	Repetitions
S		0		100	1.2	0.32	7
F	0.08	0.25		95	5.1	1.14	6
B	0.08	0.50		90	9.0	1.58	4
M	0.08	1.00		80	16.8	2.18	5
L	0.08	1.50		70	24.6	2.68	3
G	0.50	0.25		89	6.0	1.55	3
K	1.00	0.25		81	7.0	1.92	3
T	0.50	0.50	0.67	78	11.4	2.18	2
X	0.08	0.17		97	3.9	0.95	1
Y	0.50	0.17		92	4.4	1.30	1
Z	0.50	0.33	0.50	85	8.0	1.79	1

Rapid and precise temp changes were obtained in aluminum bread pans with 0.7-mm-thick bottoms lowered into baths until the outside of the floor and walls were exposed only to the thermostatic water. The pans were closed with polyvinyl chloride film backed by 1 cm of styrofoam. When a cold microscope slide was laid upon the warm floor of the pan, the upper surface of the slide warmed half the difference between its previous and final temp in 17 sec.

Shortly before an experiment, spores were brushed from a paper onto slides. At time 0, the slides and dried spores were sprayed with water containing 0.5% orange juice (8). At time 0, the slides were in moist chambers at a nominal 25 C, and subsequently they were suddenly heated by placing them in a pan at a temp steady within 0.1 of 45 C. After various times, development was stopped by killing the spores with a drop of Trypan blue in lactophenol. Germination was estimated in a sample of 100 as the number that had grown germ tubes half as long as the spore diam. Since the statistical interaction of treatment by repetition in following weeks was significantly greater than the variance between samples on one slide, only one sample was generally examined for each time and treatment.

In Table 1 the treatments are described in terms of the time t_d of delay between time 0 and the first maltreatment by exposure to 45 C, the maltreatment time t_{45} at 45 C, and the time t_b between two maltreatments. For example, in the standard treatment S the spores remained at 25 C, while in experiment F they were heated to 45 C after 0.08 hours and kept there 0.25 hours before their return to 25 C. In experiments T and Z, the 0.50- and 0.33-hour

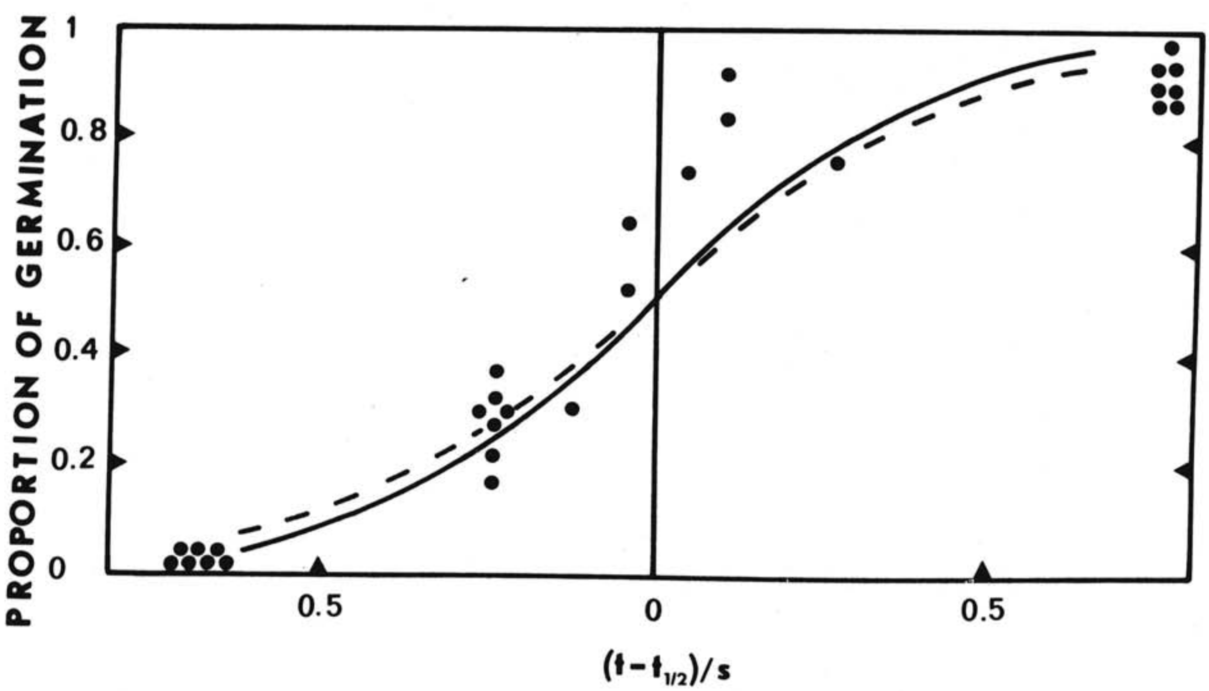

Fig. 1. The time course of germination of *Alternaria solani* spores at steady 25 C. Time is measured in hours from a time $t_{1/2}$ of 1.2 hours when half the spores have germinated, and germination of the populations rises from 0 to 1 at completion. The dashed curve is a normal ogive with a $t_{1/2}$ of 1.22 and standard deviation s of 0.39 hours, and the solid curve has parameters of 1.2 and 0.32 hours.

maltreatments were divided into two halves by 0.67 or 0.50 hours at 25 C. Experiments were performed weekly for nine wk, and in each weekly experiment about five treatments were each examined with five slides incubated for various times to reveal the $t_{1/2}$, s^2 and H for each treatment. The number of weekly repetitions of the treatments range from seven of the standard S to one of X, Y, and Z.

RESULTS.—At 25 C in seven repetitions of S, germination increased from none at 0.50 hours to about half at 1.20 hours and on to nearly 100 percent at 2.0 hours (Fig. 1). Assuming that a normal cumulative curve or ogive would represent germination reasonably well as it does the hatching of insect eggs (6), we converted the percentages to probits and fitted a straight line to them (3). The dashed curve corresponding to the estimated $t_{1/2}$ of 1.22 and s of 0.39 hours is drawn on Fig. 1 (and although a chi-square shows that the deviations of the observations from the curve are statistically significant) the normal curve remains a practical representation of development in seven experiments repeated during 7 wk.

Next, the results of all 11 treatments (Table 1) were summarized by:

$$H = H_o - t_{45}\,(0.15 + 0.6\ t_d) \qquad (1)$$

states that H was less than the H_o of 1 where spores were not exposed to 45 C, and it was less according to the maltreatment time t_{45} and also the delay t_d.

The results were also examined for $t_{1/2}$ and s^2 by dividing germination by the H for the treatment and plotting these as functions of time on arithmetic-probability paper where a normal ogive is straight. Zero germination and germination near H were neglected. The following summarize the $t_{1/2}$ and s^2 obtained by inspection:

$$t_{1/2} = t_o + t_b + t_{45}\,(15 + 8\ t_d) \qquad (2)$$

$$s^2 = t_{1/2}\ /\ 12 + t_{45}\,(2.5 + 10\ t_d) \qquad (3)$$

where t_o is the $t_{1/2}$ of 1.2 hours in the standard S experiment at 25 C. For a given delay t_d, increasing maltreatment t_{45} lengthens $t_{1/2}$. Thus in treatments F, B, M, and L increasing t_{45} with the same t_d of 0.08 lengthened $t_{1/2}$. The deleterious effect of t_d is shown by comparing treatments F, G, and K, which had increasing delay t_d with constant maltreatment t_{45}: increasing t_d from 0.08 to 0.50 and finally 1.00 decreased germination after 6 hours from a range of 48-85%, to 16-69%, and finally 20-35%. On the other hand, equation 2 says that t_b merely delays $t_{1/2}$ by exactly t_b. This rule for t_b applies only when t_{45} is not too small since $t_{1/2}$ must approach t_o when t_{45} approaches zero whatever t_b is. In all our experiments t_{45} is sufficiently long so that equation 2 is an adequate summary although somewhat illogical since it cannot be applied when t_{45} is nearly zero.

The rule for s^2 shows that germination is more variable at longer $t_{1/2}$ and even more after maltreatment and when maltreatment is delayed.

Finally we must show how well the summarizing rule for H, $t_{1/2}$ and s^2 fit their variation among treatments, how well the normal ogive fits the course of germination, and how reproducible the observations are. In the standard treatment S, equation 3 makes s equal to 0.32, while an estimate of 0.39 was obtained above by a statistical method (3). Since the shorter s from equation 3 simply steepens the relation to the solid curve of Fig. 1, making it fit the observations around $t_{1/2}$ more closely, there is no reason not to accept equations 2 and 3. Figure 2 is a single, graphical test for all treatments. First, the H, $t_{1/2}$ and s were calculated from the three summary rules. Then the percentages from all treatments were transformed to a scale of 0 to 100 by dividing by H. (After any long t_d before maltreatment as in treatment K, the germination after t_d was subtracted). Lastly the observation times were normalized by subtracting $t_{1/2}$ and dividing by s.

If in equations 1, 2, and 3, the assumption of the normal ogive, and reproducibility were all perfect, all observations from the 11 treatments over the nine weekly experiments would fit the curve of Fig. 2. Beginning with eventual germination H, we see its effect in the observations at normalized times near 2.0. Ideally, these percentages should be very near 100; they range from 75-100 with no evidence that they do not tend toward 100. Not shown on the graph are forty-one observations at normalized times far longer than 2 that range from 51 to 114% with no evidence that they do not tend toward 100. The clustering of the points along the curve, and their fairly even division above and below it, suggest that the rules for H, $t_{1/2}$ and s^2 summarize the effect of hot temp upon the viability of spores and their rapidity and similarity of germination times.

DISCUSSION.—With all our observations summarized in equations 1, 2, and 3, we can now discuss them in terms of a box model, Fig. 3, which appeals to our intuition about biology and implies relations among the parameters. Successful spores will pass through the series, while unsuccessful ones will die in one of the stages. The stages might be different concns of an essential compound, or they might be unrecognized physical developments after the initial wetting of the dormant desiccated spore and before the concluding appearance of the germ tube.

In the conception that is the model, the spores begin in the first of f stages when they are wetted and have progressed to the final (f + 1) stage when germinated. The progress of the population can be described by the net change dC_n/dt spores per hour in the number or census C_n of spores in the n^{th} stage. Spores reach the n^{th} stage from the preceding $(n-1)^{th}$ in proportion to the number C_{n-1} in the preceding stage, some pass on to the $(n + 1)^{th}$ in proportion to C_n, and some die in the n^{th} stage in proportion to C_n. If P per hour is the relative rate of passage between stages, and B per hour the relative rate of death in a stage,

$$dC_n/dt = P(C_{n-1}\text{-}C_n)\text{-}BC_n \qquad (4)$$

At the favorable 25 C B_{25} is zero. By defining the stages as stages reached at equal intervals of time, e.g. every 5 min, we make P the same P_{25} in all stages.

Since no spores germinate at 45 C, P_{45} and B_{45} cannot be learned from experiments at a steady 45 C. It is reasonable to assume, however, that so long as the path to germination is not entirely different, these rates will be faster at warmer temp as chemical reaction rates would be faster. It is also reasonable that (i) P_{45} and B_{45} will vary with the stage of development when the exposure to 45 C begins, and (ii) P_{45} and B_{45} will be constant in all stages during the exposure to 45 C as P_{25} was constant during exposure to 25 C. Since t_b in our experiments is always much briefer than $t_{1/2}$, the stage of development could scarcely change during t_b, and we do not expect P_{45} varies significantly with t_b.

While heating may accelerate P, it may also destroy essential material in the survivors and thus delay their germination when they return to 25 C. In the model, therefore, maltreatment may increase the number of stages f and delay germination despite any speeding of P during t_{45}. The system for making the essential material may also be injured, the spores may "remember" their maltreatment in the form of a damaged physiology that is not described solely by an increase in f, and P may not return to the former P_{25} but instead pass through the increased f stages at a new P'_{25} although the temp is again 25 C. Alternatively, the spores may have a short memory, and the rate may quickly return to the rate P_{25} in spores that have never been maltreated. Obviously a system with long memory that is difficult to analyze for specification at every instant must include history, while a system with short memory is easily specified by the stage and environment at that instant. Length of memory will determine the difficulty of calculating development in fluctuating environments, and we shall test memory as well as the model.

The mathematics of the box model, which have been examined elsewhere (Parlange, *unpublished*), can be

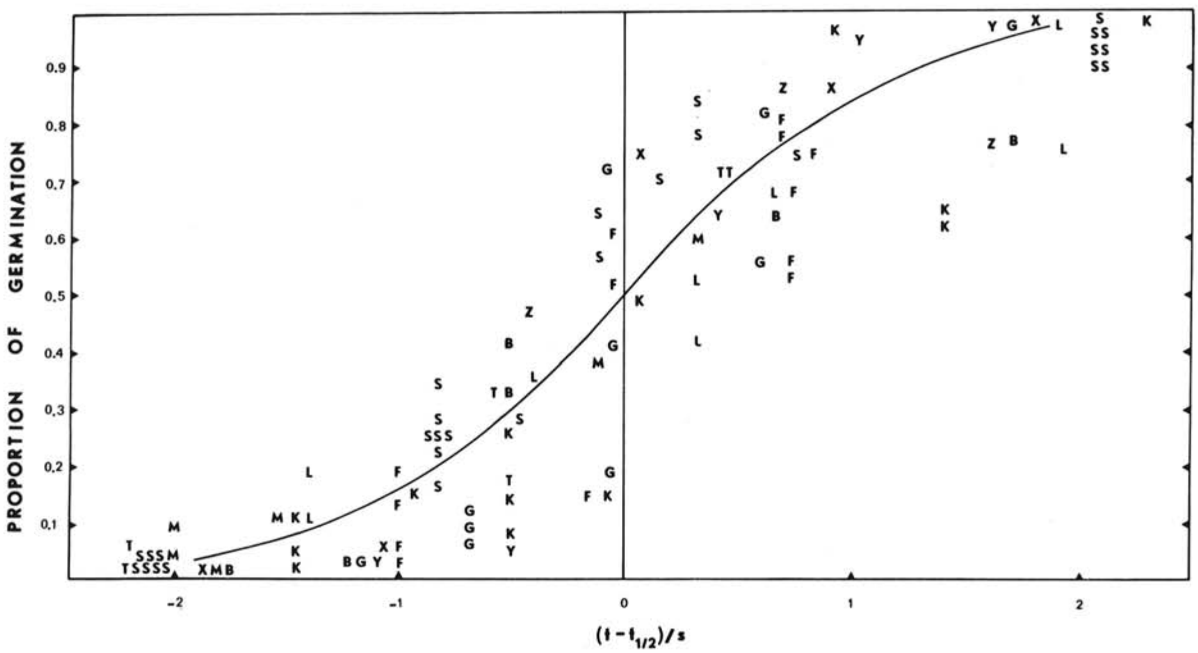

Fig. 2. The three courses of germination of *Alternaria solani* under different regimes of 25 and 45 C, which are identified by the letters of Table 1. Time has been normalized for the different regimes by subtracting the $t_{1/2}$ and dividing by the s of the different regimes, and the $(t - t_{1/2})/s$ is measured along the abscissa. The curve is the normal ogive with zero $t_{1/2}$ and unit s. Forty-one observations at normalized times much longer than 2.0-hour range from 54-114% and are not shown.

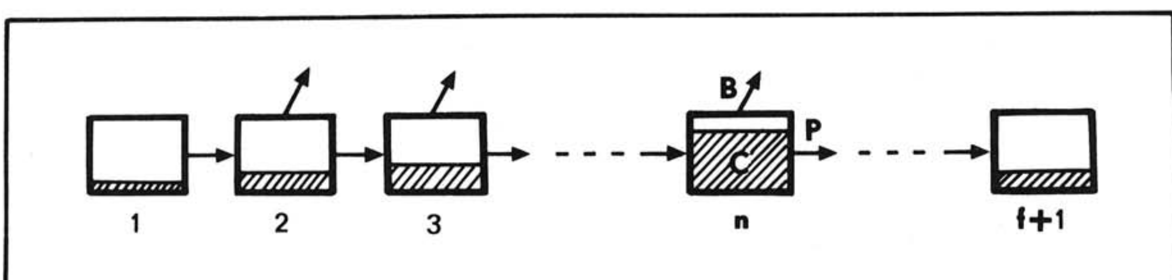

Fig. 3. The box model. The spores pass through f stages on their way to germination. The number C (represented by the level of shading in each box) changes as the fraction P per hour moves forward, while the fraction B per hour dies. At a given time P and B are the same in all boxes.

summarized by three equations:

$$P_{25}\, t_d + P_{45}\, t_{45} + P'_{25}\, (t_{1/2} - t_d - t_{45}) = f \qquad (5)$$

$$s^2 = f / P'^2_{25} \qquad (6)$$

$$H = H_0 \exp(-t_{45}\, B_{45}) \qquad (7)$$

Equation 5 says that the number of boxes is a sum of products of rates and times at those rates. If memory is short, the rates P_{25} before and P'_{25} after maltreatment will be equal, and 5 becomes:

$$P_{45}\, t_{45} + P_{25}\, (t_{1/2} - t_{45}) = f \qquad (8)$$

If equation 8 does not fit the data, the model may still apply, but equation 5 would have to be used. Simple equation 8 says that with no maltreatment the rate is simply the number of stages divided by P_{25}, and when maltreatment does occur and t_{45} is not zero, the maltreated spores must pass more stages f. Equation 6 says that the variance of germination times increases with f, which may have been increased by maltreatment for t_{45} hours, and the variance is less when P'_{25} is rapid. The rate P'_{25} at germination determines s^2 whether or not P'_{25} equals the earlier P_{25}. In equation 7 the final germination fraction H decreases exponentially with the time of maltreatment as has generally been observed in heat sterilization (5).

The test of the model with short memory lies in a comparison of equations 6, 7, and 8 derived from the model and equations 1, 2, and 3, which summarize the observations. When H is not much smaller than H_o, as in our experiments, equation 7 from the model is approximately:

$$H \cong H_o\, (1 - t_{45}\, B_{45}) \qquad (9)$$

Since the corresponding equation from the observation is equation 1,

$$H \cong H_o\, (1 - t_{45}\, B_{45}) \cong H_o\, [1 - t_{45}\, (0.15 + 0.6\, t_d)] \qquad (10)$$

where the observed proportionality between destruction and t_{45} shows the compatibility of model and observation. Equation 10 provides an estimate of B_{45}, which can then be employed in equations 7 or 9 to calculate eventual germination H.

The final test of the correspondence between model and data is a comparison of equations 2 and 3 with 6 and 8. When f is eliminated from equations 6 and 8,

$$t_{45}\, P_{45} = P_{25}\, (P_{25}\, s^2 - t_{1/2} + t_{45}) \qquad (11)$$

P_{25} is estimated from the case of zero t_{45} and the observed $t_{1/2}$ and s^2 of the standard S experiment, in units of hours (hr):

$$P_{25} = 1.2 \text{ hr} / (0.32)^2 \text{ hr}^2 = 12/\text{hr} \qquad (12)$$

Then equation 11 from the model is solved for P_{45}, and $t_{1/2}$ and s^2 are replaced by equations 2 and 3 from the observations. If model and observations are compatible, then the expression for P_{45} must be independent of t_{45} because, as the reader will remember, P_{45} in the model was supposed to be constant during the t_{45} of maltreatment.

$$\begin{aligned} P_{45} &= P_{25}\, (P_{25}\, s^2 - t_{1/2} + t_{45}) / t_{45} \\ &= 12\, (12\, t_{1/2} / 12 + 12\, t_{45}\, (2.5 + 10\, t_d) - t_{1/2} + t_{45}) / t_{45} \\ &= (12\, (31 + 120\, t_d)) \end{aligned} \qquad (13)$$

Indeed P_{45} is independent of t_{45}, and hence the model with short memory evidently fits the data. If instead equations 2 and 3 had not been independent of t_{45} then equation 11 could not have been derived from simple equation 8 but would have required the complex equation 5, indicating a different P_{25} before and after maltreatment and a long memory.

Our goal is a model for calculating development in a fluctuating environment, and we are now ready for the simple (but extreme) cases of 25 and 45 C, using the model of Fig. 3 where the spores have a short memory. First the parameters must be estimated. P_{25} has already been set at 12 hr^{-1}. The number f of boxes is calculated from equation 6 and the observations summarized in equations 2 and 3:

$$f = 12\, t_o + 12\, t_b + 12\, (45 + 128 t_d)\, t_{45} \qquad (14)$$

Finally, B_{45} is $(0.15 + 0.6\, t_d)$ according to equation 10, and P_{45} is $12\, (31 + 120\, t_d)$ according to equation 13. One can now employ the conception of the boxes, these estimates of the parameters, and equation 6, 7, and 8 to calculate the development during fluctuations between 25 and 45 C.

The original advantage of the model over the equations 1, 2, and 3 that merely summarized the observations was the plausibility of germinating spores passing through stages and the logical relation between $t_{1/2}$ and variance. Now, however, we see that the model is a tool for interpreting the physical significance of the data, as in testing the concept of short vs. long memory. Also the model permits P_{45} to be faster than P_{25} as in a chemical reaction with the delayed germination caused by more stages f.

Outdoor application of the model will likely require calculating the increase in the number of germinated spores C_{f+1} while more spores are entering the first box and increasing C_1. If spores begin germination in batches and if an entire batch begins in a time short relative to s, the development of each batch can simply be calculated by the foregoing equations and their contributions to the number of germinated spores simply added to obtain C_{f+1}.

An alternative to this following the development of each batch would be watching the change in C in each stage and neglecting the time when the individual arrived in each stage. Watching the change in C would be accomplished by integrating equation 4. Batches are more practical if there are few batches, many stages, or fast rates. A short memory in the spores is, of course, convenient if one is following the batches, but it is essential if he is watching the stages.

Since the model fits observations of germination and the spores have a short memory even in a violently changing environment, the development of spores or other organisms should be calculable from (i) the temp and (ii) the stage of development at any moment. The goal of calculating development in a variety of fluctuating environments, rather than the single fluctuation of 25 to 45 C, may therefore be attainable. In our experiments, the stage was simply specified unambiguously by t_d, and the task ahead is defining the stage at every instant in various fluctuating environments. One possibility is defining the stage of development at every instant by the number of stages that would be present should the temp suddenly return to a standard temp, e.g. 25 C. Again the value of the tested system of boxes is apparent as a means of progressing from a long table of H, $t_{1/2}$ and s^2 for all conceivable fluctuations on to a concise physical model.

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