Germination of Alternaria solani Spores in Changing Osmotic Pressures

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The authors thank Barbara Wooding for her help.
Accepted for publication 15 January 1976.

ABSTRACT

WAGGONER, P. E., and J-Y. PARLANGE. 1976. Germination of Alternaria solani spores in changing osmotic pressures. Phytopathology 66:786-789

Alternaria solani spores germinate more rapidly in 0.3 M sucrose than in either more or less dilute sucrose solutions, but the ratio of mean and standard deviation of germination times is about $\sqrt{14}$ over a wide range. Frequent changes in sucrose concentration and thus osmotic pressure retard germination in proportion to the number of changes.

Additional key words: mathematical model, water.

The development of an organism in a changing environment must be understood and then expressed as a function of the environment if its progress is to be anticipated. An important case is the germination of a pathogenic fungal spore, which must be calculated in a simulator of a plant disease (9).

The effect of temperature on germination of *Alternaria solani* spores has now been studied at length (10, 11, 12). Development was summarized by the mean and variance of germination times of individual spores, and these parameters in turn were conceived as the outcomes of development rates through several equally spaced stages. Over a wide range of temperature, the observed mean and variance indicated 14 of the conceptual stages. For many stages (e.g., 14 as in the observed germination) the increase in the portion germinated resembles a cumulative normal curve, whereas for a few stages it resembles a cumulative Poisson curve (2, 5). From 4 to 30 C, the number of stages was constant, and the rate in a variable environment promptly attained the rate at the same temperature in a steady environment. Thus, the mean germination time in a variable environment can be calculated simply by integrating the rates. When spores were warmed several times to 30 to 40 C, each warming appeared to increase the number of stages, thus lengthening the path to germination, with a consequent delay in germination. The next step is to ask whether germination rates at varied hydration can be simply integrated, like rates at moderate temperatures, or whether changing hydration, like warming above 30 C, delays germination.

The minimum relative humidity for germination of *A. solani* is 87% or a water potential of −190 bars and it increases sharply with increasing humidity, reaching 100% at about 90% humidity or −140 bars potential (4).

The water potential of the environment can be changed conveniently and quickly by an osmotic solute; e.g., salt, sugar, or alcohol. The effect equals that of a corresponding change in humidity only if the spore is an ideal osmometer that completely excludes the solute. In fact, many solutes diffuse into plants; e.g., cations are taken in by ascospores with half-times of about 15 minutes (8); and the apparent osmotic pressure of sucrose is only 0.7 to 0.8 of its potential because it is not perfectly excluded by cell membranes.

The effects of solutes differ because some are taken in more readily, some are toxic in addition to their osmotic effect, and some are nutrients. Thus, in several tissues sucrose moves into cells somewhat less than mannitol (6), and polyethylene glycol is more toxic to roots than is mannitol (3). Further, salts and sucrose affect fungal growth differently, possibly because of nutrition (7) and membrane structure (1).

This review shows that a solute can affect germination somewhat like a change in humidity, but with an interesting difference. At first the solute dehydrates the cell, but as the solute diffuses in, the internal osmotic pressure and the turgidity increase and growth resumes (6). Thus, changing solute concentration provides an alteration among environmental states where fluctuations may logically differ from the sum of the states. We shall show that the germination of *A. solani* conidia is slowed by some steady osmotic pressures produced by sucrose within the range of 0 to 50 bars and, further, that *changing* pressures within this range especially delays germination.

MATERIALS AND METHODS

We have previously described (10) the *A. solani* isolated from potato, the production of spores on paper, and the storage of the spores in a dry, cool environment. At the beginning of an experiment, spores were brushed onto moistened Millipore filters (HAWP02500, 0.45 μm pore). The filters rested on a porous plastic base manufactured for the filter, and solution was slowly pipetted onto the filter and sucked through. This procedure was used because when the hemispheric cap of the filter holder was installed and solution forced through, ungerminated spores were washed onto the cap and remained there, which decreased the percentage of ungerminated spores on the filter. The osmotic pressure

was maintained steady by passing 5 to 10 ml of the same solution through the filters every 10 to 30 minutes, or it was changed by substituting different concentrations. After various times, germination was stopped by killing the spores by lowering a cover glass smeared with Trypan blue in lactophenol onto a filter. From 100 to 200 spores on each filter were observed to determine germination.

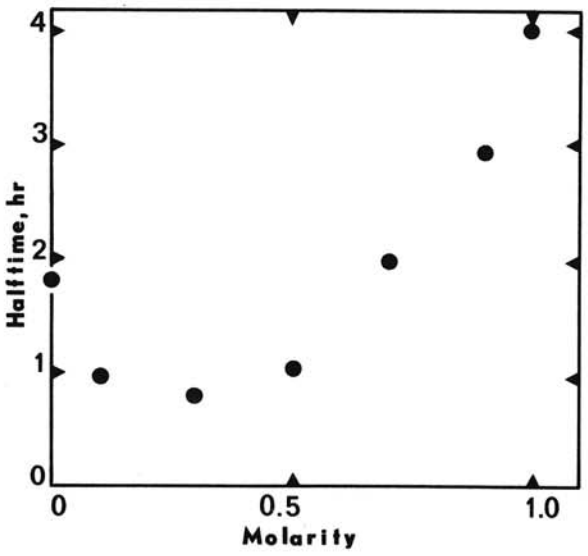

Fig. 1. The half time of germination of *Alternaria solani* spores in 0 to 1 M sucrose. The half time of 16 hours in 2 M sucrose is not shown in the figure.

RESULTS AND DISCUSSION

Alternaria solani spores were first incubated at steady osmotic pressures from 0 to 25 bars by periodically renewing the sucrose solution. Since there was little difference in the germination curves caused by renewing the solution every 10, 20, or 30 minutes, washing itself did not affect the spores greatly. Figure 1 summarizes the half-times ($t_{1/2}$) observed with half-hourly washings of 0 to 1 M sucrose at 20 C. We observed a minimum for $t_{1/2}$ at 0.3 M, which was the optimal concentration of sugar for germination. At 2 M sucrose, $t_{1/2}$ was about 16 hours. The curve shown is for 20 C; different curves would be obtained for different temperatures (12).

The entire courses of germination at all steady molarities or osmotic pressures were depicted on a single graph (Fig. 2) by employing a normalized time $T = t/t_{1/2}$ with $t_{1/2}$ taken from Fig. 1. The theoretical germination curve (Fig. 2) corresponds to a cumulative normal distribution with a standard deviation of 0.27 T. In terms of our model this corresponds to 14 stages (11). We note the usual asymmetry around $t_{1/2}$ (13, 14). In view of the scatter of the experimental observations, however, little would be gained by replacing the symmetrical normal distribution by the asymmetric Poisson distribution.

Changing, rather than steady, osmotic pressure of sugar delayed germination. The change between 0.9 and 0.1 M was the largest employed. If a change itself made no

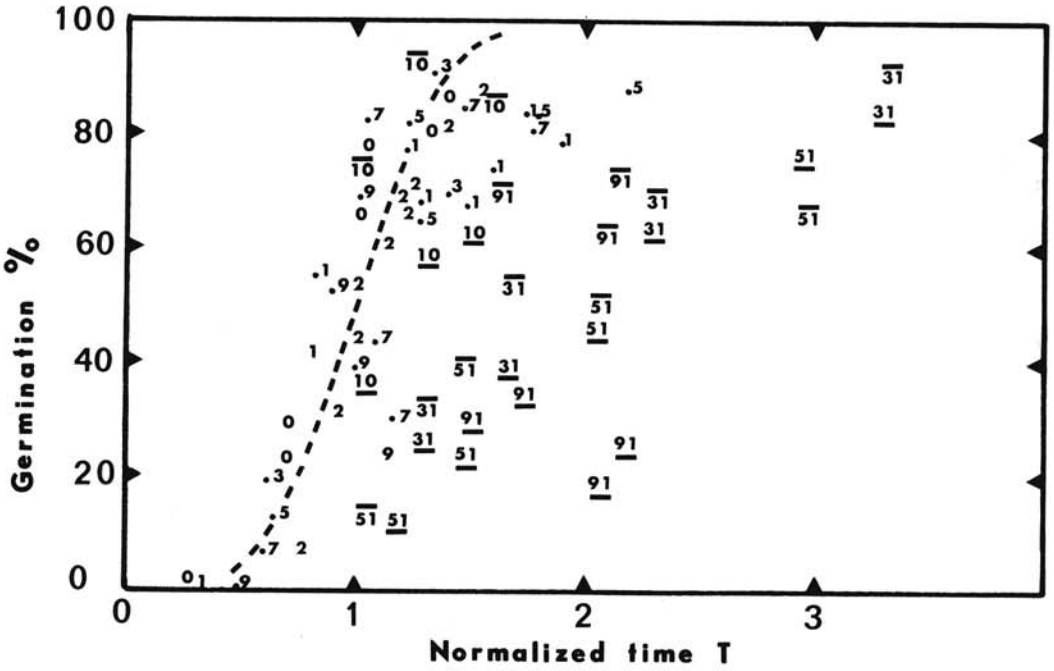

Fig. 2. Courses of germination of *Alternaria solani* spores in steady osmotic pressures indicated by the sucrose molarity, 0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0 M. Courses of germination in changing osmotic pressures caused by changing sucrose molarity between 0.1 and 0, 0.3 and 0.1, 0.5 and 0.1, and 0.9 and 1 are indicated by 10, 31, 51, and 91, respectively. Overlined numbers indicate changes every half and underlined every quarter hour. The normalized time $T = t/t_{1/2}$ is obtained by dividing the actual time by a half time deduced from Fig. 1. The dashed line corresponds to the model of germination through 14 states.

difference, then germination would proceed one-half the time at 0.9 M as Fig. 1 indicates for steady 0.9 M and one-half the time at 0.1 as Fig. 1 indicates for steady 0.1 M. A $t_{1/2}$ was calculated from this rule of simple addition of progress and used to normalize the times for 0.9 and 0.1 M in Fig. 2. In fact, germination in the changing environment proceeded more slowly than this simple addition of progress in steady environments, and the symbols 91 in Fig. 2 rise more slowly than the theoretical curve. For other molarities, one sees that the smaller the change in molarity, as between 0.1 and 0 M, the less was the delay in germination.

Changing osmotic pressure at 15-minute intervals delayed germination more than changing it at 30-minute intervals. This can be seen in Fig. 2 by comparing change from 0.9 to 0.1 M at 15-minute intervals shown by underlined 91 with the same change at 30-minute intervals shown by overlined 91.

The observations presented in Fig. 1 and 2 establish that (i) a steady osmotic pressure speeds or slows germination as it is near or far from the optimum of about 0.3 M solute, and (ii) changing pressures delay germination, especially when the change is great or frequent. These results resemble changes in temperature above and below 30 C where change delays germination (12). In the present case of osmotic pressure, the mechanism of delay seems clear: a changed external osmotic pressure causes a changed turgor inside the cell; after a time suggested by the 0.25 hour required for diffusion of cations into ascospores (8), the osmotic solute equilibrates and turgor is restored to one suitable for development.

Although the qualitative effect of change is clearly exhibited in Fig. 2, these results do not lend themselves readily to quantitative analysis within the framework of our model because differences among the changes are of the order of the scatter in the data.

For quantitative analysis we now discuss only the results of germination under extreme conditions; i.e., for changes between 0 and 2 M. For such conditions we expect the effects to be so large that no ambiguity in interpretation is caused by the relatively smaller scatter in the data. The results of four experiments each repeated once are shown in Fig. 3, depicting the germination curves for 0, 1, 2, and 4, half-hour time intervals at 2 M. Note that in Fig. 3 the $t_{1/2}$ at steady 0 M is about 1 hour instead of the 1.8 hour indicated in Fig. 1 because for the case of Fig. 3 temperature was 30 C instead of 20 C, significantly shortening the $t_{1/2}$ (11).

By analogy with the case of changing temperature (11) we expect the model to apply to the present case of changing osmotic pressure with

$$f_o + n \Delta f = P_o t_{1/2} + n \Delta t \Delta P$$

$$s^2 = [f_o + n \Delta f] / P_o^2,$$

where $f_o \simeq 14$ is the number of stages in the steady case, $P_o \simeq 14$ hour^{-1} is the approximate rate in water to give a $t_{1/2}$ of 1 hour, n is the number of time intervals of duration Δt spent at 2 M, and Δf and ΔP are the constant increases in f and P per interval at 2 M. The advantage of the model, if it is applicable, is that only the two parameters Δf and ΔP have to be measured, and the two equations can then be

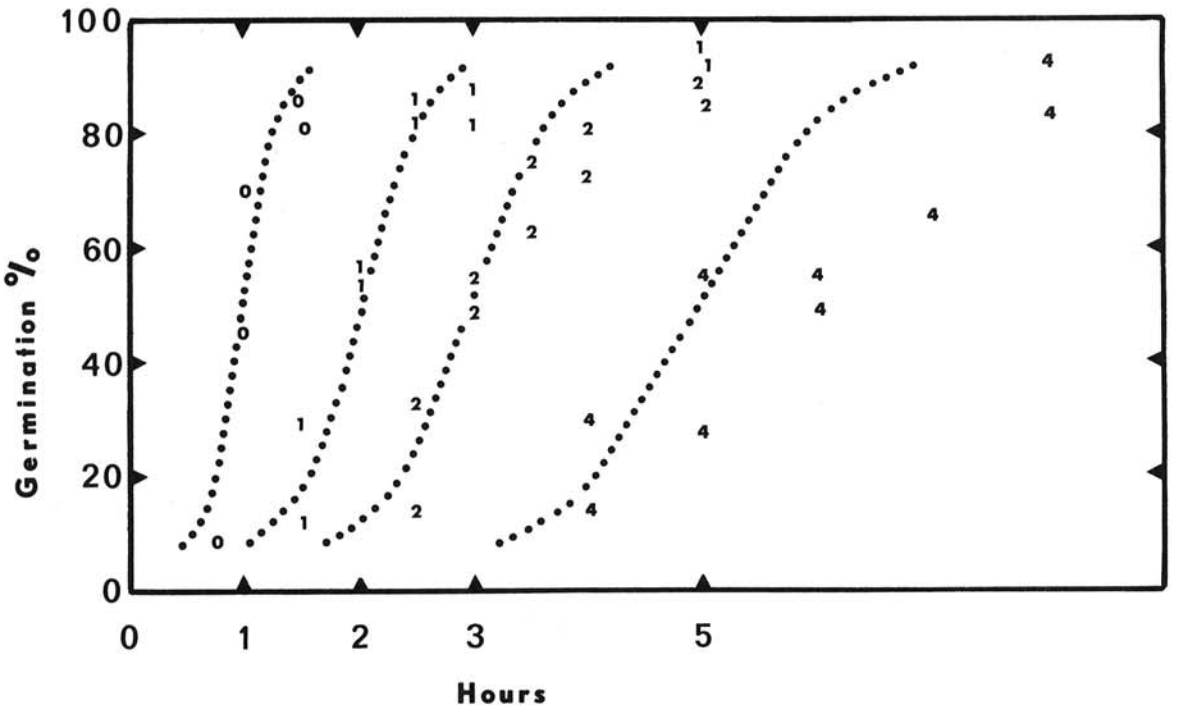

Fig. 3. Courses of germination of *Alternaria solani* spores with large changes in osmotic pressure. Observations are shown by the number of times (0, 1, 2, and 4) that sucrose was changed every half hour between 0 and 2 M. Dotted lines correspond to the model with $\Delta f = 56$ and $\Delta P = 84^{-1}$.

used for any n. In principle one experiment, i.e., for one value of n, yields Δf and ΔP from the measurement of $t_{1/2}$ and of the standard deviations. For instance, taking $t_{1/2} = 3$ hour and $s = 0.8$ hour when $n = 2$ yields $\Delta f = 56$ and $\Delta P = 84$ hour^{-1}. These values in turn can be used to predict the germination course with one and four changes, yielding the curves shown in Fig. 3. The predictions are clearly adequate.

In the case of four changes, however, the actual germination lags behind the predicted course, particularly for times longer than $t_{1/2}$, indicating that many changes damage the spores. This further was exemplified by eight changes. These observations are not plotted on Fig. 3. At times less than the predicted $t_{1/2}$ of 9 hours the germination course lagged slightly behind the prediction, but even more important, the germination never reached 100%. Even after 24 hours, germination only reached 60%, which indicated that some irreversible damage destroyed some of the spores after many changes.

In conclusion, we have shown that frequent changes in sucrose concentration retard germination, i.e., the halftime is longer than would be observed if the germination rate under fluctuating conditions were equal to the rate under steady conditions for the appropriate concentration. A mathematical model of germination describes the process adequately and suggests that the main effect of frequent changes is to increase the number of conceptual stages in the germination path appreciably. Finally under extreme osmotic potential, if the changes are too frequent, the germination rate is below 100% even after a long time.

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