

**Effect of Temperature, Relative Humidity, and Rehydration Rate on Germination of Dried Sporangia of *Phytophthora infestans***

K. P. Minogue and W. E. Fry

Department of Plant Pathology, Cornell University, Ithaca, NY 14853.  
Supported in part by USDA-CSRS grant 701-15-54.  
Accepted for publication 4 March 1981.

**ABSTRACT**

Minogue, K. P., and Fry, W. E. 1981. Effect of temperature, relative humidity, and rehydration rate on germination of dried sporangia of *Phytophthora infestans*. *Phytopathology* 71:1181-1184.

The ability of sporangia of *Phytophthora infestans* to germinate after exposure to an unsaturated atmosphere was studied in relation to the conditions of exposure (temperature, relative humidity, and time) and to the rate of rehydration of the sporangia before germination. About 30% of dried sporangia germinated when rehydrated over a period of 2 min in a dew chamber; however, when rehydrated rapidly by immediate transfer from a dry atmosphere to the germination medium, fewer than 1% germinated. Differences in the rate of rehydration appear to account for

some of the disagreement among previous researchers over the resistance of sporangia to drying. The sporangial death rate was similar at 15 and 20 C and was not significantly affected by relative humidity (RH) between 40 and 88% at these temperatures. The average half-life of the sporangial population under these conditions was 5.5 hr. The death rate was significantly faster at 30 C; the half-life at this temperature was 1.4 hr at 40% RH and 3.8 hr at 88% RH.

The ability of windborne sporangia of *Phytophthora infestans* (Mont.) de Bary to establish new foci of potato late blight and to contribute to infection within foci depends in part on the ability of the sporangia to withstand drying after release from the sporangiophores. Since the early work of Crosier (2), the conventional opinion has been that the sporangia can survive only a short time in a dry atmosphere. This view was challenged by de Weille (3), who found little change in percentage germination after

as long as 10 hr at 31% relative humidity (RH). He attributed this disagreement to the fact that he used detached sporangia, whereas Crosier had worked with sporangia still attached to the sporangiophores. At about the same time, however, Glendinning and co-workers (5), also using detached sporangia, obtained results not much different from Crosier's and, more recently, Warren and Colhoun (9) found that sporangia died within a few minutes after detachment, even when the humidity was as high as 95%. Rotem and Cohen (7), using infectivity rather than germination as their measure of sporangial viability, observed much lower rates of decline; in addition, these rates depended on RH at 30 C but not at 10 C.

Clearly, evaluating the epidemiologic role of windborne sporangia is difficult if their ability to withstand drying is unknown.

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0031-949X/81/11118104/\$03.00/0  
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While variation among fungal isolates may account for some of the disagreement in the literature, the wide discrepancies encountered suggest that methodological differences may be responsible for the conflicting results. Work on the basidiospores of some *Bolbitaceae* (10) has shown that the rate of rehydration of a dry spore is sometimes a critical determinant of its subsequent ability to germinate; the spore may be killed if it is rehydrated too quickly. Because previous workers used different rehydration techniques, we attempted to evaluate the effect of rehydration rate on sporangia of *P. infestans*. In addition, the influence of temperature and relative humidity on the sporangial death rate was determined under carefully controlled conditions.

## MATERIALS AND METHODS

**Production and harvest of sporangia.** Sporangia were harvested from late-blight lesions collected from an unsprayed field plot of the potato cultivar Katahdin, which had been inoculated with an isolate of *P. infestans*, race 0 (4). Leaves bearing lesions were collected at 0900 hours, and sporangia were harvested within 1 hr; during this time, leaves were kept in a sealed paper bag to protect them from desiccation. Sporangia were detached from the sporangiophores by touching the surface of a lesion to a glass coverslip. Generally, the sporangia from about 10 lesions were used for each experiment, and the harvesting procedure was arranged so that each coverslip received some sporangia from each lesion. Only lesions free of surface moisture were used, because preliminary experiments showed that sporangia from moist lesions died much more rapidly upon drying than those from dry lesions.

**Rehydration of sporangia.** Either immediately after harvest, or following exposure to a dry atmosphere in a controlled-environment chamber, sporangia were transferred from the coverslip to an agar germination medium (see below) by pressing the coverslip briefly against the agar surface. For rapid rehydration, coverslips were placed directly on the medium. For slow rehydration, they were put first in a small dew chamber, made from a storage jar (10 cm in diameter by 8 cm high) lined with a moist paper towel. The chamber was placed on a cold plate set at 10 C. Dew became visible on the coverslips after about 1 min and formed a continuous film within 2 min. The coverslips were then removed, and the sporangia were transferred to the germination medium.

**Germination.** The germination medium contained, per liter of water, the filtered extract of 10 g of dried, crushed lima beans (autoclaved 30 min in 800 ml of water); 2 g of Difco yeast extract; 10 g of dextrose; 10 mg of gentamicin sulfate; 10 g of agar; and 2 ml of each of vitamin and trace element stock solutions (1). Germination

was assessed after a 24-hr incubation in the dark at 18 C. Under these conditions, all germination was direct. Three to five hundred sporangia were counted in each sample.

**Effects of temperature and RH.** An apparatus was constructed for the precise control of temperature and relative humidity (Fig. 1), to determine the effects of these factors on the sporangial death rate. Relative humidity was controlled via a three-stage process. Air from a compressed-air line was filtered through cotton wool and activated charcoal and brought near saturation by passing through a 1-L flask, within which deionized water was continuously atomized. When high RH was required, the flask was heated to promote evaporation of the mist. The air then entered a 1.5-m coil of 6.4-mm (0.25-in.) OD copper tubing immersed in a water bath. The dew point of the air (which varied in different experiments from 1.5 C to 28 C) was set at the desired level by adjusting the temperature of this bath. The air was then brought to its final temperature (15, 20, or 30 C) in a second coil immersed in a warm water bath and was conducted into an opaque chamber 11 cm in diameter and 10 cm deep. Airflow through the apparatus was adjusted to 10 L/min.

The temperature of the warm water bath was monitored with a mercury thermometer. The RH setting of the apparatus was displayed by an instrument receiving inputs from thermistors immersed in the two water baths. The calibration of this instrument was checked periodically with a psychrometer. The apparatus allowed control of temperature within 0.2 C and RH within 2%.

The effects of temperature and relative humidity on the survival of sporangia were determined with this apparatus. Coverslips bearing sporangia were placed in the chamber of this apparatus immediately after the sporangia were removed from the sporangiophores. At intervals, two coverslips were removed from the chamber, and the sporangia were slowly rehydrated in the dew chamber. Germination was then assessed as described above.

**Analysis of data.** Time-course data were fitted to a first-order exponential decay model,

$$g(t) = g(0)e^{-kt}, \quad (1)$$

where  $g(t)$  is percentage germination at time  $t$ ,  $e$  is the base of natural logarithms, and  $k$  is a rate constant estimated as the slope of the linear regression of  $\ln g(t)$  on  $t$ . Because the initial levels of viability differed somewhat, regressions were calculated separately for each experiment; a pooled estimate of the slope for each temperature-RH combination was then calculated as described by Snedecor and Cochran (8, Sec. 14.6). Differences in  $k$  among temperature-RH combinations were tested for significance at  $P = 0.95$  (8). Half-lives were computed from equation (1) by solving for  $t$  when  $g(t) = 0.5g(0)$ .

## RESULTS

**Effect of rehydration rate.** Rapid rehydration of sporangia by transferring them directly to the agar surface was lethal to all but a small proportion. This was true even when they were dried only long enough to lose turgidity (as indicated by the collapse of the wall of the sporangium), which required no more than 5 sec after removal from the sporangiophore. In contrast, sporangia that were rehydrated slowly in the dew chamber germinated well. In five experiments of two replicates each, the mean germination of such sporangia after slow rehydration was 29.6%; after rapid rehydration, the mean was 0.5%. After drying for 1 hr at 20 C and 70% RH, the means were 32.5 and 0.8%, respectively. The differences between rehydration treatments were significant at  $P = 0.995$ .

Attempts were made to optimize the slow rehydration procedure by varying the rate of dew formation (that is, by varying the temperature of the cold plate) or by varying the length of time the coverslips were left in the dew chamber. Within the ranges of 0 to 20 C and 2 to 10 min, however, the conditions of dew formation had no detectable effect on the viability of the sporangia.

**Effect of temperature and RH on viability.** The decline in the viability of sporangia over time seemed to fit the exponential model

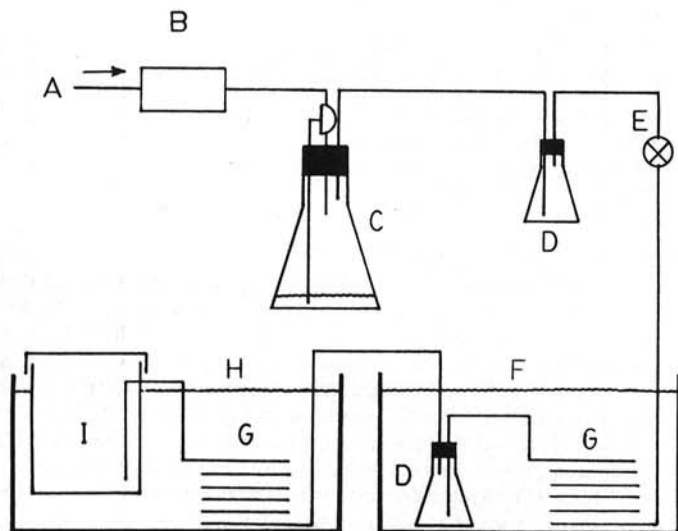


Fig. 1. Apparatus for controlling temperature and relative humidity. A, Compressed-air line; B, filter (cotton wool and activated charcoal); C, saturator (atomizer enclosed in a 1-L flask); D, water traps; E, flow control valve; F, cold water bath; G, copper coils; H, warm water bath; I, chamber.

adequately, with no apparent curvature in the logarithmic plots (Figs. 2-4). The sporangial death rates were similar at 15 and 20 C and were not significantly affected by RH between 40 and 88% at these temperatures (Table 1, Figs. 2 and 3). The half-lives of the sporangial populations in these experiments averaged 5.5 hr. At 30 C and 88% RH, however, the half-life was 3.8 hr, while at 40% RH, it was 1.4 hr (Fig. 4). The effect of RH at this temperature was significant at  $P = 0.95$ .

### DISCUSSION

The marked effect of rehydration rate on sporangial viability appears to explain some of the disagreement among previous researchers over the ability of sporangia to survive drying. When de Weille (3) rehydrated sporangia before assessing germination, his method (breathing onto a cooled slide) resembled the slow rehydration method used here. In contrast, Warren and Colhoun (9), who found sporangia to be very sensitive to drying, used a fast rehydration method (washing dry sporangia from slides). Because loss of viability coincided in their experiments with loss of turgidity, it seems likely, in view of the results reported here, that

TABLE 1. Effect of temperature and relative humidity on the death rate and half-life of sporangia of *Phytophthora infestans*

Temperature (C)	Relative humidity (%)	Death rate constant, $k^a$ ( $hr^{-1}$ )	Half-life (hr)
15	40	0.132 a	5.3
15	88	0.151 a	4.6
20	40	0.112 a	6.2
20	70	0.114 a	6.1
20	88	0.124 a	5.6
30	40	0.513 b	1.4
30	88	0.181 c	3.8

<sup>a</sup>Slope of the linear regression of the natural logarithm of percentage germination on time. Values of  $k$  followed by the same letter are not significantly different at  $P = 0.95$  by the  $F$ -test (8, Sec. 14.6).

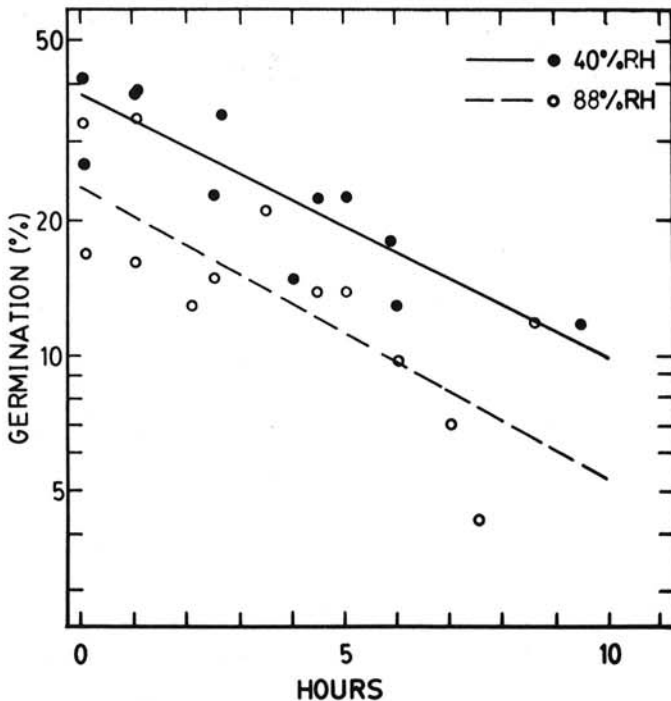


Fig. 2. Percentage germination of sporangia of *Phytophthora infestans* after exposure for the indicated times to 40 and 88% relative humidity (RH) at 15 C. Each point is the average of two replicates of 300-500 sporangia each. Germination is plotted on a logarithmic scale. Lines are calculated from equation (1), with  $k$  and  $g(0)$  estimated by the linear regression of the natural logarithm of percentage germination on time.

the different rehydration methods explain the difference in results. Other methodological differences—such as the mode of germination (direct or indirect) and the techniques for producing and harvesting sporangia—may also be responsible in part for the conflicting results; however, the pronounced effect of rehydration rate implicates it as a major contributing factor.

The rates of sporangial rehydration are not stated in the work of Glendinning et al (5) or Rotem and Cohen (7); without this information, their results must be interpreted with caution.

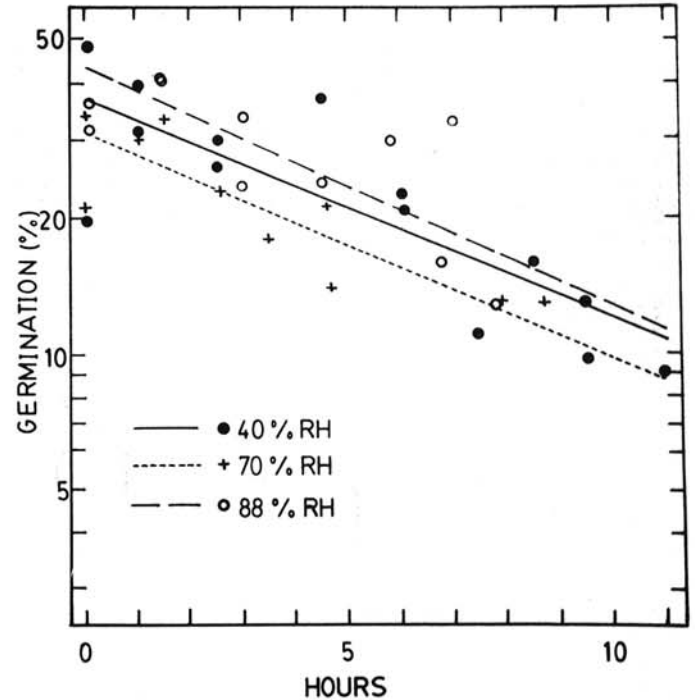


Fig. 3. Percentage germination of sporangia of *Phytophthora infestans* after exposure for the indicated times to 40, 70, or 88% relative humidity (RH) at 20 C.

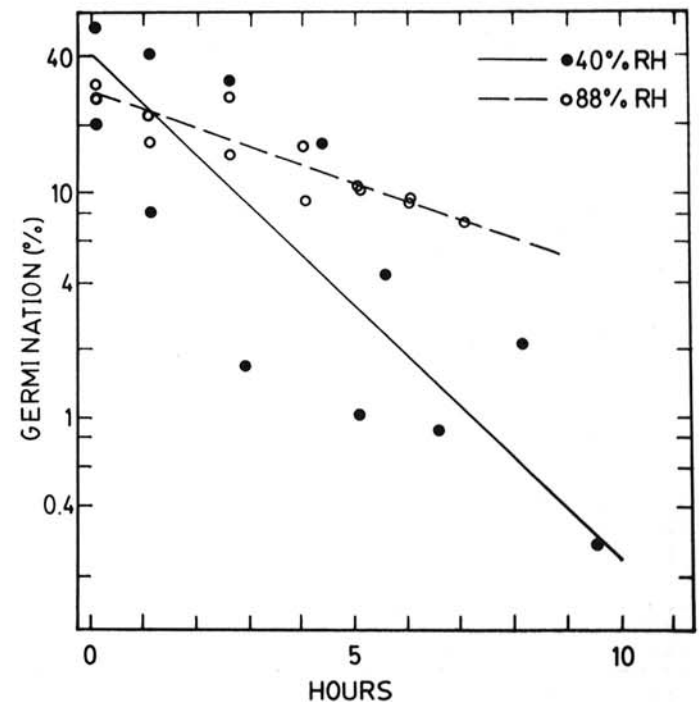


Fig. 4. Percentage germination of sporangia of *Phytophthora infestans* after exposure for the indicated times to 40 or 88% relative humidity (RH) at 30 C.

Qualitatively, the sporangial death rates we found are not inconsistent with those found by Rotem and Cohen: their observation that the rates are affected by relative humidity only at higher temperatures, for example, is confirmed. Because the sporangia in their experiments were lying on leaf surfaces, the conditions to which the sporangia were exposed probably differed from the measured air temperature and relative humidity; consequently, a precise quantitative comparison of their results with ours is not possible.

Whether the rehydration effect has any direct relevance to epidemiologic processes in the field is unclear, although it most likely is a phenomenon of only minor importance. Under the dry conditions most conducive to wind dispersal (6), most sporangia probably deposit onto dry leaves and are rehydrated slowly as dew forms at night. Rapid rehydration, while it may happen in some circumstances, is probably less common.

The sporangial death rates reported here suggest that in cool weather, regardless of humidity, many sporangia should survive through the day until conditions suitable for infection occur at night. Even on warm days, a small but epidemiologically significant proportion may survive until nightfall. Windborne sporangia are important in maintaining an epidemic through periods of rainless weather (7) and in establishing new foci far from sources of infection. For both roles, the ability of sporangia to survive dry conditions is clearly vital.

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