Effect of Temperature, Dew Period, and Age of Leaves, Spores, and Source Pustules on Germination of Bean Rust Urediospores

Martin W. Imhoff, C. E. Main, and K. J. Leonard

Graduate research assistant, professor, and plant pathologist, respectively, (third author, U.S. Department of Agriculture, SEA, AR) all at the Department of Plant Pathology, North Carolina State University, Raleigh 27650.

Journal Series Paper 6696 of the North Carolina Agricultural Research Service, Raleigh.

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This work was carried out with the continued support of Donna Jarlenski. The assistance of Mein Wann with mathematical and statistical analyses is gratefully acknowledged. We also are indebted to C. Lee Campbell for contributing ideas and for translation of the Analytis temperature growth function paper (1), and to Marvin Williams for continued advice on visual aids.

Accepted for publication 4 November 1980.

ABSTRACT

Imhoff, M. W., Main, C. E., and Leonard, K. J. 1981. Effect of temperature, dew period, and age of leaves, spores, and source pustules on germination of bean rust urediospores. Phytopathology 71:577-583.

Urediospores of *Uromyces phaseoli* were incubated on wetted snap bean leaf disks resting on water agar in seed germination chambers. Minimal germination occurred at 10 and 25 C; none occurred at 4 and 27.5 C. Maximum germination, 93%, occurred within 17.5–22.5 C. At 15–22.5 C, 90% of all germinations occurred within the first 6–8 hr of wetness. Spores stored 14–21 days at 21 C in growth chambers showed little or no reduction in germinability. Spores from old leaves and old pustules germinated only two thirds as well as those from young leaves and young pustules. Germinability after interrupted wetting periods was reduced for well-spaced spores and increased for clumped spores. Spores wetted for at least 2 hr continued germination if relative humidity remained greater than 85%. Spores produced at 24–27 C germinated about half as well as those produced at 16 or 21 C. Equations describing temperature-dew period interactions were developed. Effects of all other treatments could be expressed as fractional responses of the maximum germination at a given temperature and dew period.

Addtional key words: epidemiology, simulation.

As an important link in Gäumann's disease chain (7), spore germination has been studied extensively. The bean rust fungus, *Uromyces phaseoli* var. *typica,* is one of the organisms that has often been used in studies of spore germination. Our interest in the germination of urediospores of *U. phaseoli* var. *typica* came from our efforts to apply Shrum's flexible disease simulator (18) to bean rust. Shrum's simulator differs from others in that its logical structure was designed to be applied to epidemics in general rather than being limited to a specific disease. Defining the effects of environmental factors on the progress of spore germination at hourly intervals is an important step in the use of Shrum's simulator.

Although there have been many studies of the germination of bean rust urediospores, not all of them provide data that is sufficiently extensive or detailed enough to be incorporated into a simulator. Curtis (3) showed that light had little or no effect on urediospore germination. Shands and Schein (17) found that temperatures between 12.5 and 22 C were optimal for urediospore germination, but their data are available only in abstract form. Bell and Daly (2) showed that optimal germination occurred at 20 C and pH 6.0–7.0.

The effect of urediospore age on viability has not been tested thoroughly at temperatures and time intervals important in simulating bean rust epidemics. Davison and Vaughan (5) showed that urediospores remain viable for more than 600 days when stored at −18 C. Schein and Rotem (15) demonstrated that temperatures over a range of 5.5 to 33.2 C and relative humidity over a range of 31 to 95% affected the rate of loss of viability of urediospores exposed in gelatin capsules. Their results are presented as least squares curves without data points, however, which makes it difficult to interpret precisely the effects of urediospore age on germination during the epidemiologically critical first 10–20 days. Furthermore, in the studies of Schein and Rotem (15) and Schein (13), germination was tested at a single wetting period, 18–24 hr, and therefore the data give little epidemiological information for the wetting periods typically found in the field.

Under epidemic conditions, the viability of spores >10–20 days old is unlikely to be critically important. The bean rust fungus is capable of exponential increase with a 3- to 5-day doubling time (10) and 10–20 days constitutes two to three latent periods (12). From the results of Schein's work with inoculum efficiency (14) and

Yarwood's with spore production (20), it can be estimated that 100 spores may lead to the development of five to 10 pustules, each capable of producing as many as 70,000 spores. Therefore, spores that have not germinated within 10–20 days after maturation are not likely to constitute a significant proportion of the total number of spores available.

The objectives of this research were to determine more exactly the effects of time, temperature, length of wetting period, and spore age during the first few latent periods after spore maturity on germination of *U. phaseoli* var. *typica* urediospores, and to evaluate the importance of age of leaves and source pustules, which had not been previously investigated. Estimations of the various effects are described mathematically to facilitate eventual incorporation into Shrum's disease simulator.

MATERIALS AND METHODS

Spores were produced on plants grown in walk-in controlled environment chambers 2.44 × 3.66 × 2.13 m high located at the Southeastern Plant Environment Laboratories in Raleigh (6). A combination of cool-white fluorescent and incandescent lamps in these chambers provided an illuminance ranging 430–480 hlx at canopy level. Day length was based on a light period of 13 hr. Air temperatures were maintained at ±0.25 C of the set point as measured with a #24, type "T" welded-bead copper-constantan thermocouple in a light-shielded, aspirated housing. Day/night temperatures were 16/16, 21/21, and 24/24 C for appropriate experiments. Top-to-bottom airflow was indicated by a Hastings air velocity meter to average 20 m/min. Relative humidity (RH) was measured on a Weather Measure RO 21-10 hygrometer and maintained at 70% or more at all temperatures. Carbon dioxide concentrations were measured with a Beckman IR gas analyzer and controlled at 300–400 ppm by injection of commercial grade CO_2.

Phaseolus vulgaris L. 'Bountiful' plants were grown in 15.2-cm plastic pots containing a gravel Peat-lite (W. R. Grace, Co., Traveler's Rest, SC 29690) mixture (2:1, v/v). Plants were irrigated twice each day with nutrient solution (6) and placed in two rows, 1 m apart, containing 30 plants each on perforated metal platforms supported 80 cm above the chamber floor. First and second trifoliates were inoculated 11 days after emergence (young leaves) by brushing race 34 bean rust urediospores (8) (derived from a single-pustule isolate through three generations) onto selected leaves. A fine mist of deionized water at a rate of about 10.6 L/hr from each of four sprayers was then applied to the foliage overnight to simulate dew formation; 3–4 min of mist each hour was sufficient to keep leaves wet with little runoff. Previously uninoculated first and second trifoliates were similarly inoculated approximately 25 days after emergence (old leaves). In this paper, spores obtained from leaves that were inoculated 11 days after emergence are referred to as spores from young leaves. Similarly, spores obtained from leaves that were inoculated 25 days after emergence are referred to as spores from old leaves.

All germination experiments were carried out in darkened, temperature-controlled (±0.5 C), saturated-humidity, seed-germination chambers (6). All germinations (except those in experiments designed to test this technique) were performed on adaxial surfaces of 2-cm-diameter bean leaf disks (from plants grown in growth chambers) placed on 2% water agar in 60 × 15-mm plastic petri plates. Unless otherwise noted, spores were collected from 1- to 2-day-old pustules on young leaves and immediately used in germination tests. Except in experiments testing effects of temperature, all spores were produced at 21 C air temperature and germinated at 22.5 C. Leaf disks were chosen at random from leaves of different ages.

Approximately 24 hr before germination tests were initiated, leaves from which spores were to be collected were tapped to remove spores that might have matured a few days earlier. Thus, most of the spores collected the following day had matured during the previous 24 hr. Pustules erupted between 7 and 8.5 days after inoculation, so that the ages of pustules on leaves from which spores were collected seldom varied by more than 1 day. Germination tests always began between 0600–0800 hours when spores were collected by tapping the source leaves over a sheet of waxed paper. Leaf disks used for germination testing were cut from uninfected leaves with care so that their surfaces were never touched. The disks were quickly arranged into circular groups of six and inoculated as follows: approximately 3–5 mg of spores were dropped onto a combination of 7-cm-diameter 30 and 38-μm (500- and 400-mesh) nested sieves above the leaf disks. Spores were brushed through the 30-μm sieve with a camel's-hair brush and allowed to settle through the 38-μm sieve and onto the leaf disks for about 10 sec. The upper sieve was then removed, and the lower one was brushed and allowed to remain over the leaf disks for 5 sec. This procedure separated clumped groups of spores into single spores, distributed them uniformly and reproducibly onto all leaf disks (six at a time), and kept spore concentration at about 100/cm^2, which is well below that expected to show germination inhibition effects due to crowding (19). This method also prevented any carrying agent from being deposited onto the leaf surfaces and allowed control of the timing of initial spore wetting.

After all leaf disks were inoculated, one or two disks were placed on each of the water agar plates that had been preconditioned to the desired temperature overnight. As soon as all the leaf disks were arranged in plates, all of the plates were misted with distilled water (pH 6.5–7.0) from a hand-pumped sprayer until all the disks were visibly covered with water. Lids were replaced and all petri dishes were placed in germination chambers regulated at the desired temperatures. The entire inoculation procedure never took longer than 15 min, and was designed to simulate natural spore deposition and dew formation.

At designated time intervals after initial wetting, one or two plates were removed from each treatment, sprayed with 0.1% safranin in 95% ethanol, and stored at 4 C until the leaf disks could be examined microscopically. Examination of the agar surrounding the leaf disks showed that very few spores had been washed from the disks during misting, incubation, and staining. Safranin stained the spore cell wall and germ tube contents darkly, while cell walls of empty germ tubes stained lightly. Determinations of percent germination usually were based on microscopic examination of 200 spores. Germination counts always were made within 3 wk of the experiment. Light was easily transmitted through the leaves for these determinations. After 5–6 hr of incubation, many spores had germinated and penetration of the leaf had already occurred, leaving behind a detached, degenerated, empty, very lightly stained germ tube and an empty spore, usually with no visible connection between the two. At this point, therefore, counts had to be made of empty vs ungerminated spores. Since the ethanol in the staining solution acted as a dehydrating agent, ungerminated spores appeared characteristically doughnut shaped, while germinated spores appeared crushed and empty. Some spores required a judgment between the two classes, thus creating a possible source for a random or systematic error.

Effect of germination substrate. A comparison of germination on wetted leaf disks, on leaf disks kept in a saturated atmosphere, and on surface-wetted agar (Difco-Bacto) was performed simultaneously. Spores from 4-wk-old pustules were used to test germination on young vs old leaf disks. Spores from 6- to 7-day-old pustules were used to test germination on the surfaces of abaxial vs adaxial and growth chamber- vs outdoor-grown leaf disks.

Effect of temperature. To determine the effects of a range of incubation temperatures, spores were germinated at 4.0, 10.0, 15.0, 17.5, 20.0, 22.5, 25.0, and 27.5 C. All temperatures were tested with the same spore lot simultaneously. A repeat temperature experiment using spores from 1- to 2-day-old pustules on old leaves at 15.0 and 22.5 C was performed in order to reexamine a suspected anomaly at 15.0 C.

Effects of spore age and storage conditions. Germination of spores stored at 21 C and RH greater than 70% was determined both with spores stored dispersed on plastic petri plates by the sieve technique described above, and with spores stored aggregated at the base of a 3-ml glass vial. After 0, 7, 14, or 21 days of storage, leaf disks were appressed to the plastic plates with dispersed spores in order to deposit spores on them before wetting. With the aggregated storage treatment, approximately 2 mg of spores was

removed from the vial and applied to leaf disks by using the previously described sieve technique. Germination tests were then carried out simultaneously for each treatment.

The effect of pustule age on spore germination was studied by using spores collected from pustules aged 1–2, 7, 14, 21, and 28 days that had developed on leaves inoculated when young. Therefore, the tests were conducted weekly and not simultaneously. The effect of source leaf age on germination was studied by using spores from 1- to 2-day-old pustules that had developed on old vs young leaves. The effect of old leaves was studied approximately 25 days after the young-leaf test.

The effect of prewetting spores was tested on well dispersed spores and on spores aggregated as though still resting in a pustule. Spores produced on old leaves were either applied to the disks with the sieve technique (dispersed) or approximately 1 mg of spores was placed in one small area of the leaf (aggregated). The dispersed treatment disks were then placed on water agar and wetted with the hand-pump mister. The aggregated treatment disks were placed on water agar and received one or two drops of water directly over and entirely encompassing the spore aggregate. After 0, 1, 2, 3, and 4 hr of prewetting (dispersed) or 0, 4, 9, 12, and 20 hr of prewetting (aggregated), the petri plate lids were removed and the respective treatments were left in circulating air at approximately 70% RH. Leaf disk surfaces had visibily dried within 15–20 min. After 30–60 min, the plates were moved to a less windy area of the same corridor and exposed to the air for approximately 20 hr. Final wettings for germination of spores in the dispersed treatment were carried out as usual. For the aggregated treatment, however, the spore aggregates were first brushed over the leaf with a camel's-hair brush. Final spore concentrations remained below 300 spores per leaf disk.

One experiment was designed to test how much germination might continue if spores were allowed to dry at >85% RH after a wetting period. This experiment was performed in a greenhouse using a mixture of spore collections. Spores were applied with a quantitative inoculator (14) onto leaf disks placed on water agar and kept wet for 2, 4, 6, 8, 10, or 12 hr followed by 0, 2, or 4 hr at high RH with the petri dishes open. Visible wetness disappeared from the leaf disks within 15–20 min after the dishes were opened. After completion of a treatment, disks were sprayed with safranin as in other experiments. These germination tests were conducted at 22.5 ± 1.5 C underneath greenhouse benches at night.

A final experiment tested germination of spores produced on plants grown at 16, 21, and 24 C air temperature. Due to differing plant growth rates, these treatments were not carried out simultaneously. These germination tests were conducted at 20 C.

Mathematical descriptions. Since all curves of percent germination vs time at all temperatures were expected to be asymmetrically S-shaped, the equation of Richards (11),

$$G = A(1 \pm Be^{-kt})^{1/(1-m)} \quad (1)$$

was used to fit these curves. In this equation G represents percent germination; A is the maximum possible germination at a given temperature (equal to the germination at 16 or 24 hr of wetting), B is a scale-position parameter that is positive if $m > 1$ and negative if $m < 1$, e is the base of the natural logarithms, k expresses the rate of change of the function, t is time in hours, and m determines the position of the inflection point.

Richards' model was fitted to the data by using an IBM 370 computer equipped with Statistical Analysis System (SAS) nonlinear modeling procedures (9). An iterative procedure employing partial derivatives of G with respect to B, m, and k was used. The nonlinear least squares approach was chosen since irregularities in the data prevented accurate estimation of m, which is necessary for determining B and k by simple linear regression. After curves had been fit for all temperatures, simple expressions for m, k, and B as functions of temperature were found, which fit the data well. An expression for the parameter A as a function of temperature was found by using the method of Analytis (1), again processed according to the SAS nonlinear procedures.

Effects of all factors other than temperature during wetting period were expressed as fractional reductions in germination abilities from the maximum obtained; ie, that of fresh spores from a 1- to 2-day-old pustule on a young leaf at a given temperature and incubation period.

RESULTS

Wet leaf disks promoted a more rapid germination rate than did water agar or unwetted leaf disks (Fig. 1A). A second experiment with a different spore lot and Noble agar gave nearly identical results except that spores germinated at an even lower rate on Noble agar than on plain agar. The age of the leaf disks contributed neither to germinability nor to variation in the data (Fig. 1B). Disks from outdoor-grown leaves provided a better germination substrate than did those from the growth chamber-grown leaves

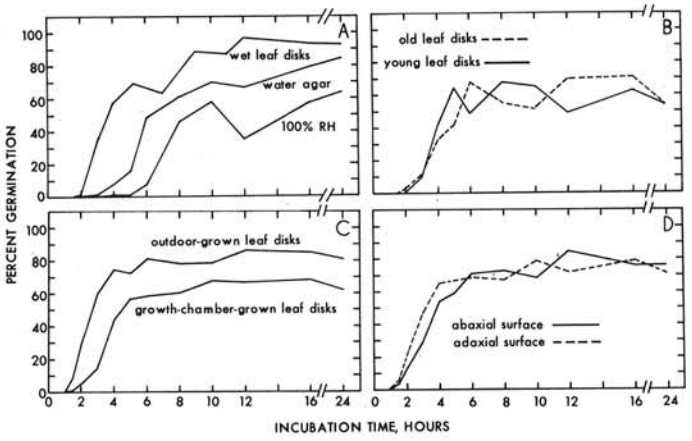

Fig. 1. Percent germination of bean rust urediospores vs time for different germination substrates: **A,** wetted bean leaf disks, water agar, bean leaf disks in saturated humidity; **B,** wetted bean leaf disks from old vs young leaves; **C,** wetted bean leaf disks from growth chamber- vs outdoor-grown leaves; **D,** wetted bean leaf disks, abaxial vs adaxial surfaces. C and D each represent averages of two determinations.

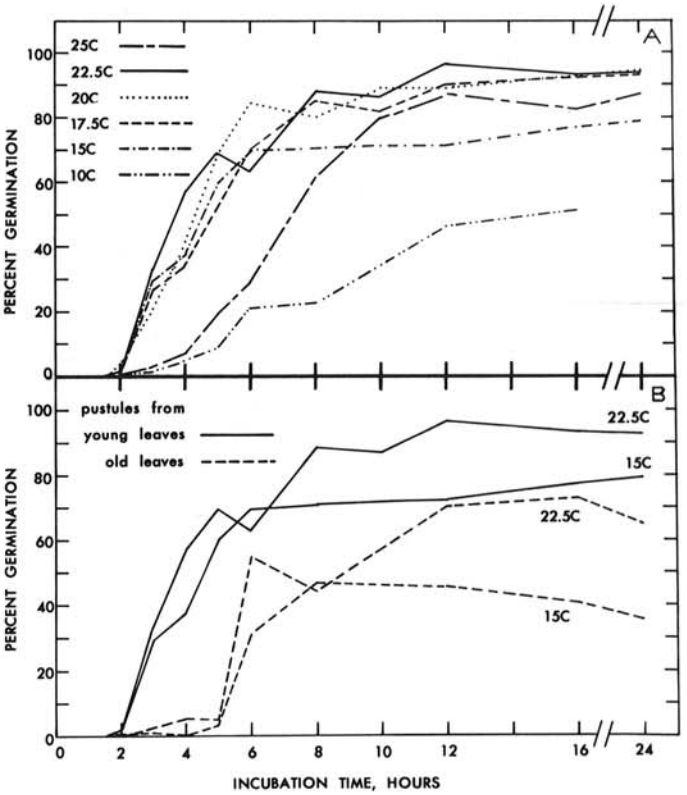

Fig. 2. A, Percent germination of bean rust urediospores vs time at six temperatures. There was no germination at 4 and 27.5 C. **B,** Comparison of germination rates and asymptotic values for two bean rust urediospore lots at 22.5 and 15.0 C.

(Fig. 1C), but in both indoor and outdoor grown leaves there was little difference between spore germination on the abaxial and adaxial surfaces (Fig. 1D).

Figure 2A presents data that show the effect of temperature and wetting period. Germination did not occur at 4 or 27.5 C. Thus, these temperatures served as minimum and maximum temperatures used in the Analytis model. Germination at temperatures between 15.0 and 22.5 C was similar except that at 15.0 C it had a high initial rate but consistently low asymptotic value. This effect occurred again when the experiment was repeated (Fig. 2B). Above and below this range germination rates were reduced.

Parameter values giving the best fit of Richards' model at each temperature are given in Table 1. It was hoped that the m, B, and k parameters could be expressed as simple functions of temperature, but because the best-fitting parameter values do not follow such a pattern, the process described below was used to obtain the step functions given in Table 2 for the parameters as functions of temperature. The m parameter was approximately linear below 22.5 C, and 23.75 C was chosen as the end point of the step function because it is halfway between 22.5 and 25 C, a temperature for which no data existed. After m was fit as such, k parameter values were chosen by trial and error until, as a compromise between accuracy (as measured by error sum of squares) and simplicity, the values in Table 2 were chosen. B was similarly chosen, but after having fit m and k, choices of B values were limited if the error sum of squares was to be minimized. Thus, the values in Table 2 reflect compromises of simplicity and accuracy for all the parameters and may not actually represent the best possible fit to the data of Fig. 2A.

The original data of Fig. 2A are shown in three dimensions in Fig. 3 for the purpose of comparison to the germination predicted by the step functions chosen in Table 2. Figure 4 only approximates these results, however, since the smoothness of the curves parallel to the temperature axis is not really known. Figure 4 was derived by first plotting S-shaped curves at each temperature tested, using the parameter values for Eq. 1 found in Table 2. Then curves parallel to the temperature axis were drawn as smoothly as possible. The general shapes of the surfaces are similar, although the fit given by the step functions is not as good as the fit given by the parameters in Table 1 for each individual curve of percent germination vs time. The rapid rate of germination within the first 8 hr, the broad range of temperature optima, the slow increase to the optimum from the minimum temperature, and quick drop-off to the maximum temperature are pronounced. Notice in both Figs. 3 and 4 that at the 24-hr incubation period, the graph of percent germination vs temperature is an asymmetric inverted U-shaped curve. The Analytis model describes this curve, the parameter A at a given temperature being found as follows:

$$Q = aT^b(1-T)^c \quad (2)$$

$$A = 93(Q), \quad (3)$$

TABLE 1. Parameter values of Richards' model giving best fits to curves of rate of bean rust urediospore germination at eight temperatures, as determined by Statistical Analysis System[a] (SAS) iterative nonlinear procedures

Temperature (C)	Parameter[a]				
	m	k	B	A[b]	A[c]
4.0	...	....	...	0	0
10.0	1.126	0.370	1.452	50	46.5
15.0	0.793	0.769	−2.150	72	76.3
17.5	0.512	0.414	−1.803	93	88.3
20.0	1.077	0.798	1.414	93	94.5
22.5	0.434	0.482	−2.059	93	94.5
25.0	1.707	0.687	70.360	88	83.7
27.5	...	...	...	0	8.3

[a] Parameters used in Eq. 1; see text for details and SAS procedures (9).
[b] Estimated graphically from Fig. 2.
[c] Predicted by Analytis' (1) method.

TABLE 2. Richards' (11) m, k, and B parameters used in Eq. 1, as step functions of temperature, T. See text for A as a function of temperature

Parameter	Temperature range, T (C)
m = −0.0543 (T) + 1.616	T < 23.75
m = 1.7	T > 23.75
k = 0.37	T < 10.95
k = 0.54	T > 10.95
B = 1.45	T < 10.95
B = 1.0	10.95 < T < 15.95
B = 2.0	15.95 < T < 23.75
B = 70.0	T < 23.75

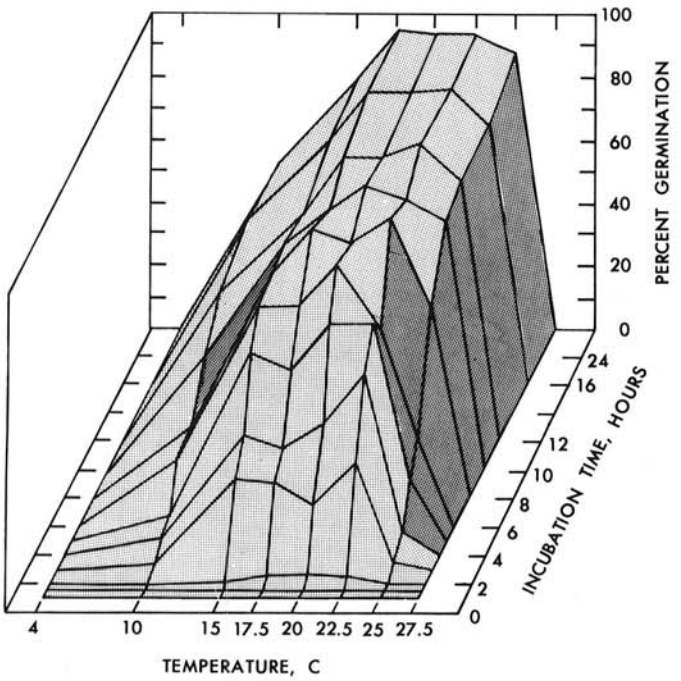

Fig. 3. Percent germination of bean rust urediospores vs temperature and time. Data of Fig. 2 represented in three dimensions.

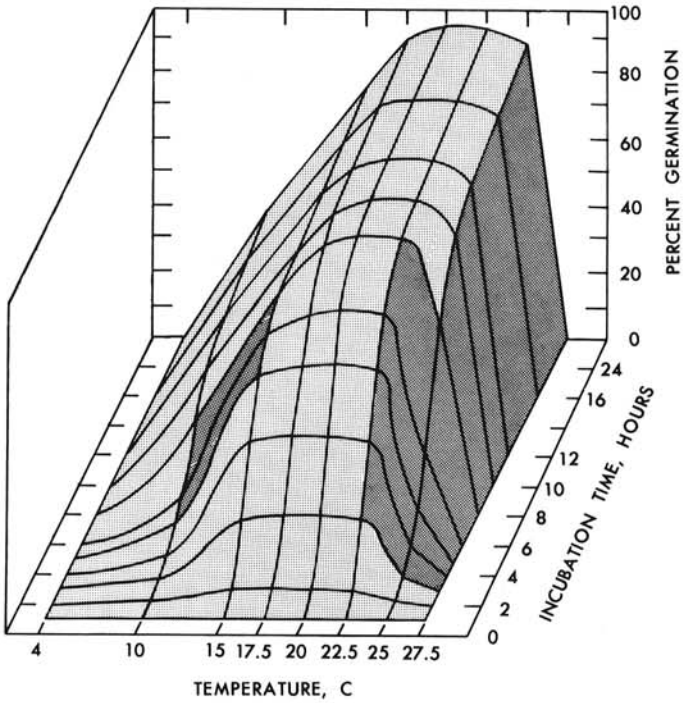

Fig. 4. Approximate percent germination of bean rust urediospores predicted by functions in Table 2 at a given temperature and incubation period for fresh spores from young pustules on young snap bean leaves.

in which A is germination of fresh spores from 1- to 2-day-old pustules on young leaves at a given temperature after 24 hr of wetness, T = (degrees C −4)/23, a = 0.03866, b = 1.06829, c = 2.40398, and 93 is a constant representing the maximum germination under optimal conditions. The predicted values of A for the eight temperatures studied are given in Table 1.

Figure 5A displays the data on aging of spores dispersed in plastic petri dishes. After 21 days of aging, the rate of germination was reduced, but the percent germination after 24 hr of wetness remained constant over the range of aging times tested. Differences between the germination curves for spores stored 0, 7, and 14 days of aging appear indistinguishable, given the variability of the data. Figure 5B represents data for spores treated as in Fig. 5A, but stored aggregated. Again, after 21 days of aging, rate of germination seems slowed, but for shorter periods of aging the curves are indistinguishable. In this experiment, the final percent germination after 24 hr was lower for spores aged 21 days than for the other treatments.

Spores from pustules ≥1 wk old usually germinated as rapidly as those from 1- to 2-day-old pustules, but the final percent germination was lower (Fig. 6). Spores from 3-wk-old pustules appeared to germinate more slowly than those from younger or older pustules.

Spores from newly formed pustules on young leaves had higher germinability than did those from newly formed pustules on old leaves (Fig. 7). In fact, 4-wk-old pustules on leaves infected when they were young produced spores that germinated as well as those produced in newly formed pustules on leaves infected when they were old.

Prewetting of dispersed spores for 1, 2, or 3 hr reduced total germination by similar amounts but did not affect the initial rates of germination (Fig. 8A). Prewetting dispersed spores for 4 hr reduced total germination, but increased initial germination rates so that the asymptotic value was approached after only 4 hr of the second wetting period. Little or no germination occurred during prewetting periods of up to 4 hr because the spores were from pustules on old leaves and were slow in germinating, as shown in Fig. 7. Figure 8B shows that prewetting aggregated spores may increase, rather than decrease, germinability. Spores prewetted for 4 and 9 hr showed the same germinability (as opposed to viability). Spores used in these experiments were fresh spores from young pustules on old leaves. Without 12 or 20 hr of prewetting these spores germinated at lower percentages than did fresh spores from young pustules on young leaves.

Mild drying periods after initial wetting periods did not abruptly halt the germination process (Fig. 9). Treatments with 0 hr dry periods showed the germination expected from previous experiments. Germination percentage increased during the mild drying periods in all cases, but never exceeded the germination received from treatments of identical total treatment time and 0 hr of drying time. For example, the treatment of 4 hr of visible wetness followed by 4 hr of mild drying did not produce as much germination as that from 8 hr of visible wetness followed by immediate safranin staining (0 hr drying time). A 4- to 5-hr wetting time appeared to trigger the spore's germination mechanism.

Spores produced on plants at 21 and 16 C germinated equally well (Fig. 10), but spores produced on plants at 24 C had dramatically reduced germination capacity. Light-shielded copper-constantan thermocouples used to measure leaf temperature indicated that in any chamber, temperatures of leaves in direct light were 1–3 C higher than air temperatures, depending on leaf height and shading. Temperatures of leaves closer to the light source differed from the air more than did temperatures of lower leaves. Reduced germination of spores from plants grown at 24 C may have resulted from 25–27 C leaf temperatures. It is of further interest that spores produced in 7-day-old pustules on plants at 24 C germinated better than those produced in young pustules. This

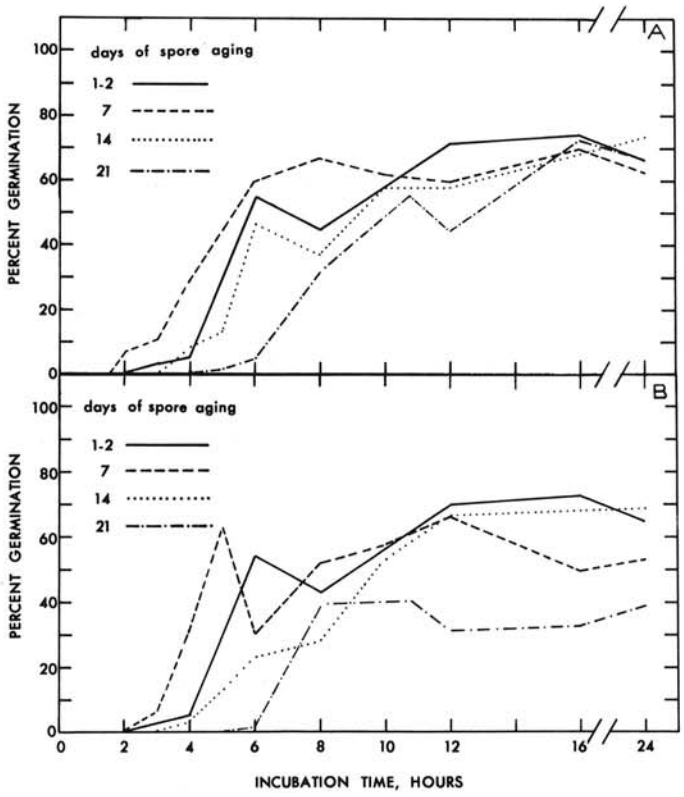

Fig. 5. A, Effect of bean rust urediospore aging on percent germination vs time. Spores were stored dispersed (<100 spores per square centimeter) on plastic petri dishes. **B,** Effect of bean urediospore aging on percent germination vs time. Spores were stored aggregated in a glass vial.

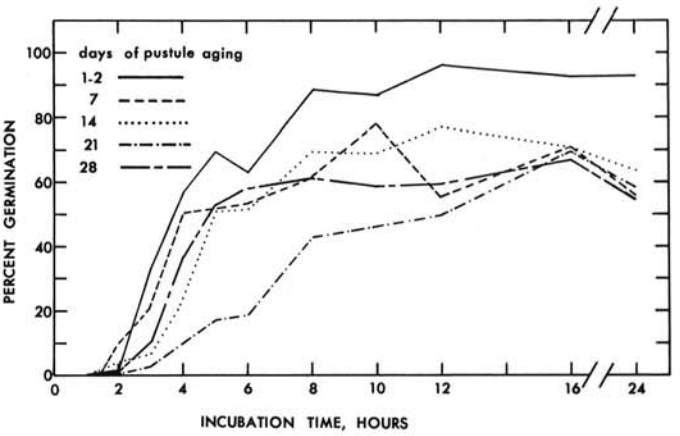

Fig. 6. Effect of source pustule aging on percent germination of bean rust urediospores vs time.

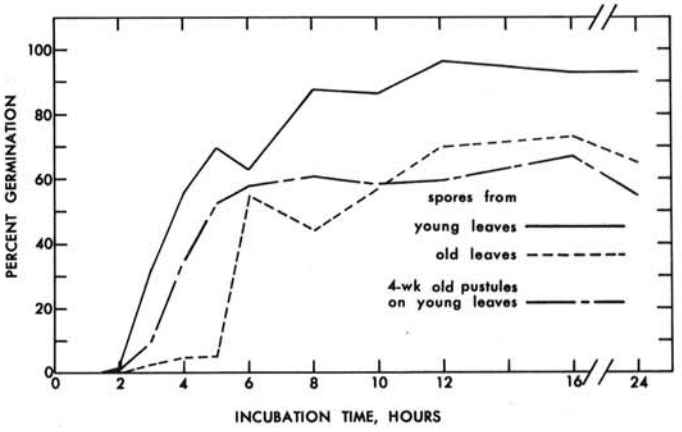

Fig. 7. Effect of two snap bean leaf ages on percent germination of bean rust urediospores vs time. For this comparison, spores were from freshly opened pustules on old vs young leaves. Dashed curve is for comparison of young pustules on old leaves to old pusutles on initially young leaves.

phenomenon had not been observed in any other treatments, and it may reflect the effect of higher leaf temperatures of leaves near the tops of the plants as opposed to lower temperatures of older leaves that were shaded by newly formed leaves.

DISCUSSION

Water agar traditionally has been used as a spore germination substrate. Results presented here indicate that bean rust urediospores germinate more rapidly on leaves, so water agar probably should not be used in epidemiological studies of effects of variable dew period conditions. One might expect that indoor and outdoor grown leaves would provide a different surface microflora. There would be many physical, chemical, and biological factors involved that might lead to differences in spore germinability on the two substrates. It is biologically reassuring to note that germination was better on the more natural substrate encountered by the spore.

The variability of the data is obvious. Spores incubated for 8 hr should not show less germination than those incubated for 6 hr. The age of leaf disks does not contribute to the observed variability, nor does differences between the upper or lower surfaces of the leaf. The errors from a smooth curve could therefore stem from the sample size, judgmental errors in determining empty vs ungerminated spores, and inherent variability due to each hourly reading having been drawn from a separate leaf disk and petri plate. These sources of error could lead to the observed variability in the data.

The data represented in Fig. 8B indicate that spores remaining in pustules do not lose germinability when exposed to a wetting period. In fact, the wetting period can increase germinability. Data from previous experiments indicated that fresh spores produced on old leaf tissue had lower germinability than those produced on young tissue. However, from Fig. 8B it appears that if such spores are exposed to water for 12–20 hr while aggregated, they regain lost germinability. This suggests the possibility of more germination inhibitor in pustules on old leaves than in those on young tissue, rather than an actual loss of viability. This could be a survival mechanism, such that as young succulent leaf material becomes scarce, greater proportions of the spores become resistant to germination stimuli. This might increase spore dormancy and extend overwintering capabilities or survival during long-distance disperal as increasingly greater proportions of the bean leaf tissue mature.

Spore lots appearing to be only about 70% viable were actually greater than 95% germinable, given proper stimuli (Fig. 8B). Schipper et al (16) developed a method for synchronous germination of rust urediospores and summarize other workers' attempts to stimulate presumably viable yet dormant spores to germinate. Results presented in Fig. 8B further illustrate that viability studies should carefully choose conditions for germinability and thus viability.

The major differences between this study and previous similar studies are that leaf and pustule age are viewed as important variables, germination was tested at 12 incubation periods by a technique similar to natural infection processes, and spore aging effects were studied during epidemiologically crucial time periods. The data for temperature effects (Fig. 2A and B) are similar but more detailed than the data of Shands and Schein (17) and Bell and Daly (2). The choice of 4 and 27.5 C as lower and upper temperature thresholds, respectively, also is supported by their studies. The 15.0 C anomaly found in this study was repeatable and may represent a common but very interesting biological phenomenon. That is, the presence of biochemical anomalies at 15, 30, and 45 C as studied by

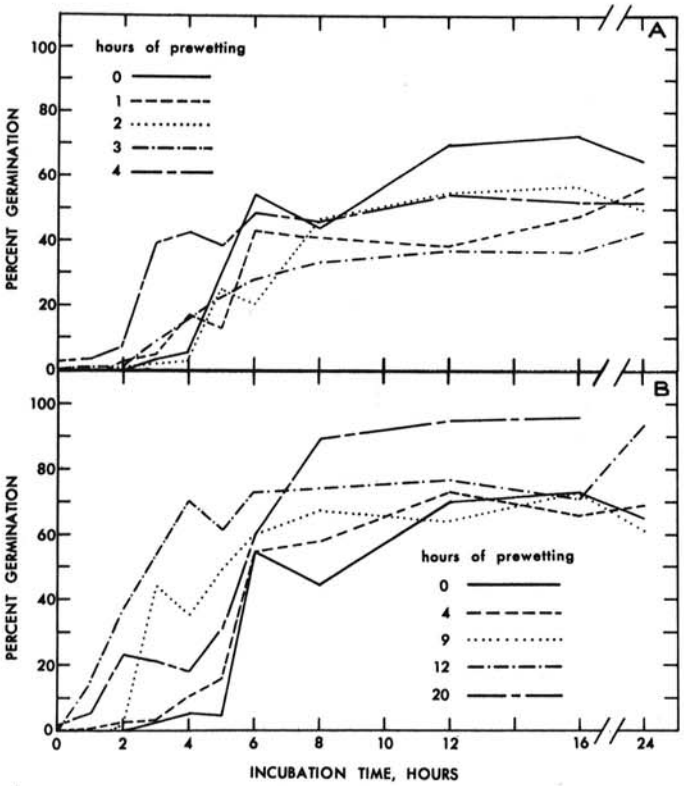

Fig. 8. A, Effect of prewetting periods followed by 24-hr drying periods on percent germination of bean rust urediospores vs time. Spores were prewetted while dispersed on bean leaf disks. **B,** Effect of prewetting periods followed by 24-hr drying periods on percent germination of bean rust urediospores vs time. Spores were prewetted while aggregated on bean leaf disks.

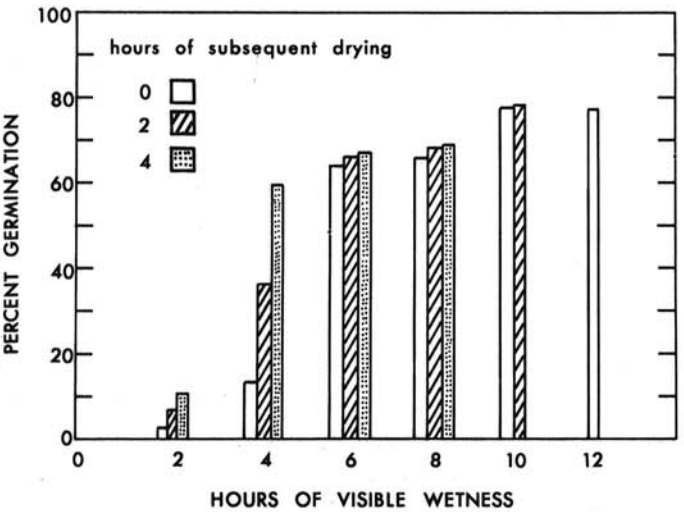

Fig. 9. Effect of mild (relative humidity >85%) drying periods on increased bean rust urediospore germination after initial wetting periods.

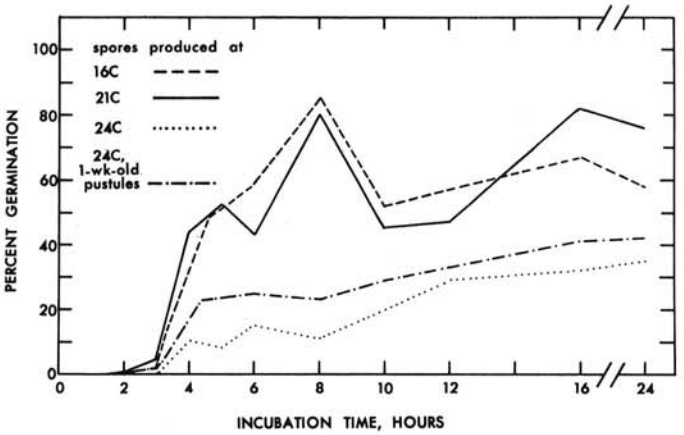

Fig. 10. Percent germination of bean rust urediospores vs time for spores produced in three different air temperatures.

Davey et al (4). The shape of the skewed curve of maximum germination vs temperature (Figs. 3 and 4) is typical of that found for fungal growth (1). For mathematical description purposes, the data appear reasonable, sound, and sufficiently quantitatively detailed.

Richards' equation for fitting S-shaped data and Analytis' model are simple, flexible, and easily applied. Their simplicity serves two purposes: first, with error in data, one does not want a mathematical description that will reproduce those specific errors, but rather wants one that describes expected behavior; second, if necessary, further changes can easily be made in such a model even in computer language form.

The relatively small effect of age of spores up to 21 days on germinability is in contrast with the data of Schein and Rotem (15) who found drastic reductions in germinability of spores stored at 21.2 C and >74% RH. Shrum (*personal communication*), however, obtained results similar to ours. He stored fresh bean rust urediospores on living leaves, and withdrew daily samples to test germinability. He found that the spores retained almost complete germinability for 20 days when incubated 18 hr. Age of spores up to a time equivalent to two or three latent periods has little effect on germinability whether the urediospores are within a pustule or dispersed as long as they are not exposed to wetting cycles too short to allow germination. Under these conditions spore age need not be a factor in the bean rust simulator.

The apparent increased germinability of spores from 4-wk-old pustules compared with those from 3-wk-old pustules (Fig. 6) may be explained by the fact that at 3 wk, most of the pustule areas were nearly necrotic, whereas by 4 wk, new satellite pustules had erupted and were producing spores with germinability similar to those produced by new pustules on old leaves (Fig. 7). The effect of pustule age could be simply handled in a simulator by a statement reducing the output on Eq. 1, the germination percentage expected, to two thirds of its value if the pustule is greater than 4 days old. Day 4 was chosen as a halfway point between 1 and 7 days since the reduced germination is noticed after only 1 wk. Similarly, as in Fig. 7, if a new pustule opens on an old leaf, it is as though the pustule itself is old (on a young leaf) and can be treated as such in a simulator.

Prewetting periods of up to 4 hr reduced subsequent germinability by approximately one third (Fig. 8A). This also can easily be accounted for in a simulator by a fractional reduction from the maximum possible value given by Eq. 1. It also should be noted that a 4-hr prewetting period seems on the threshold for triggering germination with this spore lot. For modeling purposes, a 4- to 5-hr prewetting period will trigger every spore's germination mechanism and a period of 1–3 hr will reduce the spore lot's germinability by approximately one third. Spores not germinated after a 4- to 5-hr wetting period will be assumed to die after drying occurs.

From Fig. 9 it can be seen that if leaves are not dried quickly, germination continues after leaf wetness is no longer apparent. The wetting period should be measured as hours of leaf wetness plus some proportion of hours of subsequent high humidity when entering environmental inputs into a simulator.

The effect of temperature on pustule development is difficult to model because only three temperatures were tested (Fig. 10). However, the fungus is known to germinate well at temperatures at least as high as 22.5 C, and germination is reduced at 25 C (Fig. 2A). A first estimate, therefore, could be that spores produced above 23.75 C, halfway between 22.5 and 25 C, have germination reduced to approximately one half of their maximum possible value. Whether this effect is also removed by lengthy preliminary wetting periods was not investigated.

In summary, whereas many previous studies used only a single incubation time to evaluate germinability, we found that differences in rates of germination within the first 4–6 hr could be detected even when final germination percentages at 12–24 hr did not differ. In field epidemics, the effects of frequent leaf wetness periods of less than 12 hr are likely to be important in determining rates of disease increase.

Different spore lots vary considerably, and the specification of age of spores, pustules, and leaves accounts for a great deal of this variation. Lack of such specification may explain discrepancies in data of similar experiments. Both the Richards and Analytis models were readily applicable to the problems presented in this paper. Simplification of parameter functions due to data variability and focusing interest on the first two to three latent periods of spore maturity also allowed for easy mathematical description. The data reported in this study will be combined with other data on effects of environmental factors on sporulation by the bean rust fungus in order to test the general applicability of Shrum's flexible disease simulator.

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