Influence of Water Activity, Temperature, and Their Interaction on Germination of *Verticillium albo-atrum* Conidia

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ABSTRACT

Conidia were germinated at three temperatures in media in which available water, expressed as water activity, was controlled at three levels. Rate of germination in the basal medium (0.9964 water activity) was most rapid at 25 C and was inhibited at 15 and 30 C. Lowering the water activity at a particular temperature decreased rates of germination. However, at a low water activity (0.9778) germination rate was greater at 38 C than at 25 C. Thus, the effect of temperature on germination appears to be dependent on the water activity of the medium.

It is commonly recognized that water is one of the most important factors in spore germination of microorganisms (3, 4, 12, 16-18). In many of these studies available water is usually expressed as the water content of the substrate. However, Scott (11-13) concluded that a,,, is a more useful measure of the water available for microbial growth. Water activity expresses the chemical potential of water in solutions; for ideal solutions it is equal to the mole fraction of water present and for water in air it is equal to the corresponding relative humidity expressed as a fraction; RH/100.

Spores of plant pathogenic fungi generally germinate under conditions of high available water (1, 10). In general, departures from the optimal a,, will affect the latent period, rate, and percentage germination of fungus spores. Scott (12) summarized these observations by noting that when the temperature remains constant, an a,,, less than the optimum reduces the rate and increases the latent period of germination. Also, changes in temperature reduce the rate and narrow the range of a,, in which spores can germinate. Scott (12) concluded that bacterial and fungal spores can best tolerate water stress at their optimal germination temperature.

In this paper we wish to report on the effect of a,, on the germination of *Verticillium albo-atrum* conidia and show that at low a,, (0.9778) the optimal temperature for germination was higher than at higher levels of a,..

MATERIALS AND METHODS

Use of osmometry to determine directly the a,, status of solutions in water relations studies gives accurate values and overcomes many difficulties encountered in media preparation when calculation of a,,, is attempted with Raoult's law. A standard vapor pressure osmometer (Mechrolab Model 301A, F&M Scientific Division of Hewlett-Packard) for aqueous operation at 25 C and fitted with a self-draining solvent cup and a high range decade ΔR scale was used to measure a,, values. This instrument relates concentration to a change in resistance reading in ohms (ΔR) required to balance a Wheatstone bridge circuit in comparison to pure solvent. Based on the results of Burge (2), who showed that osmotic coefficient values of sodium chloride solutions obtained by use of this instrument are the same as those reported by Robinson and Stokes (9), a calibration curve of experimentally determined ΔR values versus molal (w/w) NaCl solutions was constructed and used for determining the a,, of unknown solutions. All ΔR values were obtained after standardizing the instrument with 0.01 M NaCl and allowing 4 min for stabilization.

Spore inocula used for cultures to produce spores for germination studies were obtained from a subculture made from a single spore isolate of *V. albo-atrum* Reinke & Berth isolated from a diseased Norway maple tree by N. E. Caroselli. Spores were harvested from mycelium grown in modified Czapek's liquid medium for 9 days at 25 C in still culture. With differential centrifugation a population of spores of uniform size (6-8μ) was obtained which had less than 1% germination. Random observations showed that the spores were unincullate and the nuclear number in relation to spore size was similar to that of Hansen (6).

Spore germination media were prepared by mixing sterilized buffer and nutrient stock solutions. When an a,, below the basal medium (0.9964) was desired, KCl was added to the buffer component. Water activities of the media were determined with the calibration curve previously described. An aliquot of spore suspension containing 10⁶ spores in sterile, distilled-deionized water was added last. Inoculated cultures (25 ml in 125-ml Erlenmeyer flasks) were incubated in the dark at 25 C for up to 24 hr. During incubation samples were removed, killed, and fixed, and percentage of germination was determined by counting aliquots of the samples in a hemacytometer counting chamber. Germination counts represent the averages obtained from two separate flasks with not more than a 5% difference between individual counts. Variations of these experiments were repeated five times with similar results (5).

Preliminary experiments showed that within a pH range of 3.75 to 8.50, 5.00 was optimum. At 25 C pH values of 3.75, 5.00, and 8.80 resulted in percentage germination values of 2.5, 42.0, and 8.5%, respectively, for a 10-hr incubation period. Exploratory experiments on the effect of temperature on spore germination indicated that temperatures above 30 C severely inhibited germination while at 25 C germination was rapid; at 15 C germination was markedly inhibited.

RESULTS AND DISCUSSION

Germination curves showing percentage germination of *V. albo-atrum* conidia during 24 hr at different a,, levels with optimal
Latent Period and Time to 50\% Germination from End of Latent Period for Conidia of V. albo-atrum at Different Temperatures and Levels of $a_w$

<table>
<thead>
<tr>
<th>$a_w$</th>
<th>Latent Period</th>
<th>Time to 50% Germination From End of Latent Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 C</td>
<td>25 C</td>
</tr>
<tr>
<td>0.9964</td>
<td>4.0</td>
<td>2.1</td>
</tr>
<tr>
<td>0.9882</td>
<td>6.0</td>
<td>3.0</td>
</tr>
<tr>
<td>0.9778</td>
<td>10.0</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Germination temperature (25 C) and optimal pH (5.0) are given in Figure 1. Maximal percentage of germination and rate were obtained at the highest $a_w$ level (0.9964). Total percentage of germination and rate decreased with successively lower levels of $a_w$. The time required to reach the maximal total percentage germination at each $a_w$ level increased with decreasing $a_w$ (8 hr for 0.9964; 11 hr for 0.9882; 16 hr for 0.9778; 20 hr for 0.9674). The latent period also increased with decreasing $a_w$ levels.

The latent period was taken at the 5\% level of germination since at this level it was apparent that the spore population was very close to or just entering a steady state of germination, i.e., at the end of the latent phase. Examination of the latent period times (Table I) indicates that at optimal temperatures (25 C) the latent period was shortest for all $a_w$ levels tested, and, as the temperature was lowered (15 C) or raised (30 C), the latent period increased. The time required to reach 50\% germination once germination had entered the steady state phase, i.e., time to 50\% germination from end of latent period, increased with increasing $a_w$ at each temperature (Table I). However, it took less time at 30 C than at 25 C, indicating a faster rate of germination at 30 C than at 25 C. This feature of germination is better illustrated when germination rate (\%/hr) was determined at the 50\% level of germination (Fig. 2). Thus, at 0.9778 $a_w$ germination rate at 30 C was greater than at 25 C. However, at 0.9964 $a_w$ germination rates at 25 and 30 C were very similar, and at 0.9882 $a_w$ 25 C had the highest rate. At all $a_w$ values tested, 15 C gave the lowest germination rate, but the 25 C value was close to the 15 C value at 0.9778 $a_w$.

The shapes of the germination curves (Fig. 1) are very similar to curves obtained by Skujins and McLaren (14) describing urease activity at selected relative atmospheric humidities. If germination is considered as a morphological expression of metabolic activities mediated by enzymes, then decreased rates of enzyme activities would be reflected in decreased amounts and rates of germination. Thus, as a working hypothesis, we propose that the effect of $a_w$ on decreased spore germination is caused by the effects of decreased water availability on rates of enzyme reactions.

The result whereby the combination of 30 C and 0.9778 $a_w$ was more favorable to germination than was 25 C at the same $a_w$ (Fig. 2) suggests that temperature optimum for germination is related to the $a_w$ at which germination occurs. In relationship to enzyme activities this result may be interpreted to indicate that at certain temperatures enzymatic rates are greater at lower than at higher levels of $a_w$. Such an effect may be related to enzyme activities influenced through interactions of available water and allosteric regulation (15), or reversible association-dissociation of isozymes into subunits (8, 19), or perhaps an expression of an influence of hydration directly on the properties and behavior of protein molecules (7, 14).
LITERATURE CITED