

Water Relations of Respiration of *Verticillium albo-atrum* Conidia

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Water is one of the most important factors influencing respiration of microorganisms (3, 4). Although various respiratory substrates are either inhibitory or stimulatory to the respiration of conidia of *Verticillium albo-atrum* Reinke & Berth. (2), little is known of the physiology of respiration as affected by available water requirements. Scott (10) has stated that the available water requirements of microorganisms are best considered in terms of water activity (a_w); a_w as a fundamental property of aqueous solutions is equal to P/P_o , where P is the vapor pressure of the solution and P_o is the solvent vapor pressure (9), and a_w is numerically the same as relative humidity, and can be expressed as relative humidity (per cent) = $a_w \times 100$.

Determination of a_w and preparation of media are as follows (6): The a_w of different media was determined by first ascertaining the solute concentrations as reflected in solution resistance readings (ΔR) by using a vapor pressure osmometer ("Mechrolab" Model 301A). The (ΔR) values of the media were then converted to a_w by using a calibration curve. The curve was prepared by plotting the (ΔR) values of different molal concentrations of sodium chloride against the a_w value of similar solutions, as given by Robinson & Stokes (7).

The basal medium used as the respiratory substrate and for spore germination had a pH of 5 and consisted of 10 ml of a sucrose nitrate solution (30 g sucrose, 3.5 g NaNO_3 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.025 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 1.0 g KCl in 1 liter deionized distilled water), 12.5 ml of citrate phosphate buffer (0.1 M), and 2.5 ml of spore suspension with 6.5×10^8 spores/ml.

Media with a_w levels lower than that of the basal medium were obtained by the addition of KCl in approximate amounts calculated from the a_w table of Robinson & Stokes (7). To prevent change of pH due to addition of KCl, different proportions of both the citrate and phosphate solutions were used to make a total volume of 12.5 ml. This caused no change in the desired a_w levels. All components of the media were separately sterilized by milipore filter (0.45 μ).

Since the use of KCl to obtain a_w values below that of the basal medium would slightly increase the volume above 25 ml, the use of equal aliquots of solutions of different a_w values for respiratory studies will result in a difference in the amount of nutrients and number of spores. To maintain constant substrate nutrients and spore number, the following procedure was undertaken. Specific gravity of solutions having different a_w were determined. By the use of these specific gravities, the aliquot volumes of the respiratory substrates to be dispensed in the Warburg flasks were calculated as 3.0108 ml, 3.0930 ml, and 3.1695 ml for a_w 0.9964, 0.9830, and 0.9674, respectively. Experiments were conducted using duplicate Warburg flasks after storing the original solution in a refrigerator at 4 C overnight (10 hr) to allow the spores to reach a_w equilibrium. Oxygen consumption was determined at 25 C in darkness by standard manometric technique (12). In most cases the replicate flasks exhibited a difference in oxygen consumption of less than 10%.

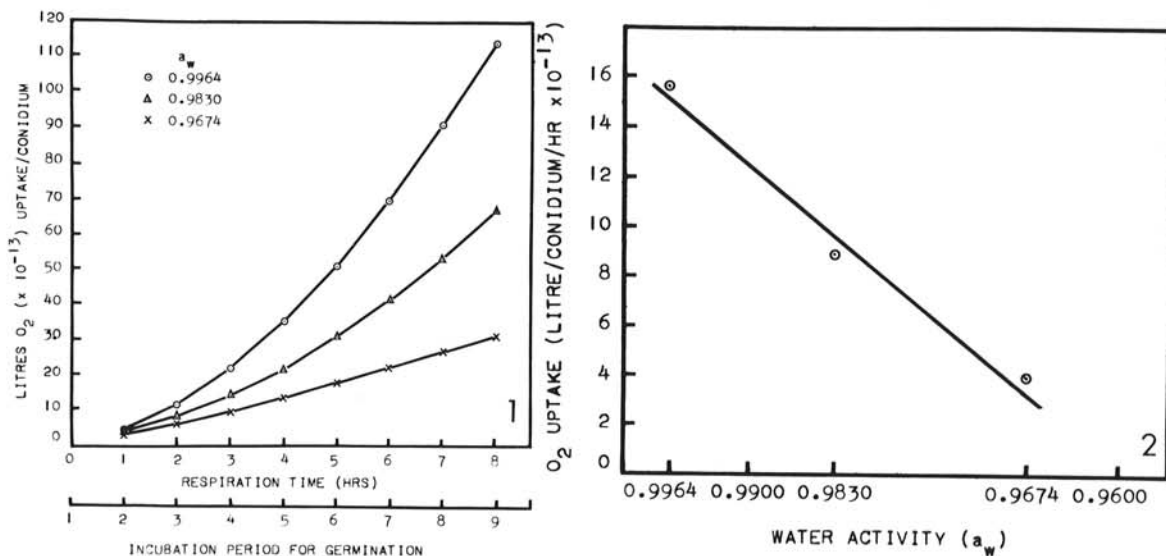


Fig. 1-2. 1) Total oxygen uptake by conidia of *Verticillium albo-atrum* in liquid germination media of differing water activities (a_w). Liters $\text{O}_2 (\times 10^{-13})$ /conidium in 8 hr respiratory period at 25 C in relation to a_w level. 2) Effect of three different water activity (a_w) levels on rate of respiration after 8-hr respiratory period of *V. albo-atrum* conidia at 25 C.

Concurrently, a study of the germination of conidia in the same a_w levels, temperature, and pH was undertaken to compare the respiratory response with that of germination (protrusion of the germ tube).

Total respiration and rate of respiration are greater in higher levels of a_w (Fig. 1, 2). In the lower a_w (0.9674), the total respiration exhibits a steady rate and shows essentially a linear relationship. But with increase of a_w of the respiratory substrate (0.9964 and 0.9830), the rate and total uptake of oxygen increased. Germination occurs (Fig. 3) during this period of respiration. Since substrate water plays an important role in germination of *V. albo-atrum* conidium (8), a comparison between germination and respiration was made (Fig. 3). At high a_w level (0.9964), there is a gradual increase of respiratory rate and total oxygen uptake with increased germination. However, in low a_w (0.9674), the respiratory rate and total oxygen uptake follows a steady rate and is not affected by the germination of conidia. There is no evidence of a sudden burst of respiration due to initiation of the germ tube either in high or low levels of water. Perhaps the lack of respiratory response to germination in a water-stressed condition indicates that the processes influencing the morphological aspects (germ tube protrusion) of germination differ from the processes controlling physiological activities. However, both are affected by the

a_w of the substrate. Allen (1) has noted that the conditions which permit germination also favor a rise in respiratory rate. He states that "the rise during the course of germination is gradual and is not correlated with the emergence of the germ tube". Allen's observation (1) supports the data presented in this paper of the relationship between emergence of germ tube and respiratory rate, which remains unaffected by the difference in the available water level of the substrate.

Although there is a definite relationship between the a_w level of the substrate and respiration and germination of the conidium, the mechanism responsible is not known, partly because of a lack of knowledge of the nature of the permeability of conidial membrane. It may be speculated that certain metabolic activities involving enzymatic reactions associated with the germination and respiration of fungal conidia can be influenced by a_w . In vitro studies have shown that available water regulates enzyme reaction by enzyme hydration (5, 11).

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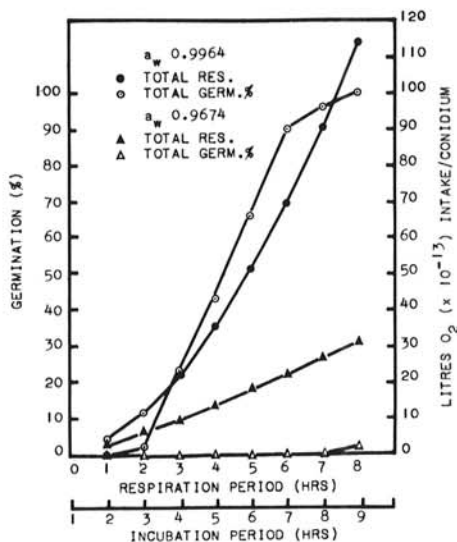


Fig. 3. Comparison between total percentage of *Verticillium albo-atrum* conidia germinated and accumulative respiration per conidium at two different water activity (a_w) levels at 25 C.