In Vitro Interactions of Fusarium and Verticillium Wilt Fungi with Water, pH, and Temperature

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ABSTRACT

Fusarium oxysporum f. sp. *vasinfectum* and the microsclerotial form of *Verticillium albo-atrum* pathogenic to cotton grew progressively slower in soil as the predominantly matric water potentials in soil were lowered below 0. In contrast, both fungi grew optimally at about −10 to −30 bars at 25 C in either agar media or in soil when water potential was regulated osmotically. The osmotic stimulation of both fungi was independent of the solute used and occurred in agar media when water potentials were adjusted with NaCl, KCl, CaCl$_2$, MgCl$_2$, a salt mixture, or sucrose, and on straw in sterile soil when water potentials were osmotically adjusted. At water potentials below the stimulatory range, growth declined linearly and was zero at near −100 bars for *V. albo-atrum* and at near −115 bars for *F. oxysporum*. A water stress of −40 to −60 bars reduced the growth of both to about half the maximum rate. Both fungi grew well at pH values ranging from 5.2 to 8.6, and no significant interactions of pH and water potential were observed in either fungus. A highly significant temperature × water interaction occurred in both wilt fungi: the magnitude of growth stimulation with slightly reduced osmotic water potential increased with rising temperature. At 35 C and pH 6, for example, *F. oxysporum* grew 2-4 times as fast at about −25 bars as at about −3 bars; at 35 C, *Verticillium* spp. did not grow on cornmeal dextrose agar, but when KCl was added to reduce the water potential to −30 to −40 bars, considerable growth occurred. No new in vitro responses to water, pH, temperature, or to interactions of these were observed that explain the different distributions of these wilt fungi in nature.

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Fusarium wilt of cotton, caused by *F. oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. & Hans., has historically been a major problem in the sandier, relatively acidic soils of the humid cotton belt of southeastern USA. Verticillium wilt, caused by *V. albo-atrum* Reinke & Berth. and *V. dahliae* Kleb., has prevailed in the irrigated Southwest (7, 10, 17, 18). Most students of these diseases believe that Verticillium wilts are favored by lower temperatures than are Fusarium wilts (1, 8, 18). This is undoubtedly true, but Verticillium wilt is common in the Mississippi River "delta" region, with Fusarium wilt of cotton being more common on either side. This particular distribution may be influenced by soil or some factors other than temperature.

Even though Verticillium wilt of cotton was first reported in the southeastern USA, its prevalence in the Southwest could be due to its occurrence in native plants. This argument for the current predominance of Verticillium over Fusarium wilt in the Southwest does not seem adequate. Most Fusarium-wilt fungi have relentlessly followed their hosts, and one wonders why the cotton wilt Fusarium, though present in parts of the Southwest, is not more generally distributed in that region.

Soils of the two regions differ in salt content, pH, clay minerals, and somewhat in texture. The present study (10) of the effect of water potential, pH, and temperature on growth of the two pathogens was undertaken in the hope of discovering something that

might explain the distributions of these fungi in nature.

MATERIALS AND METHODS.—Difco cornmeal dextrose agar (CMDA), 19 g/liter, was used as the basal medium unless otherwise stated. *F. oxysporum* f. sp. *pisi* (Linford) Snyd. & Hans. race 1, two isolates of *F. oxysporum* f. sp. *vasinfectum*, and microsclerotial forms of *V. albo-atrum* from cotton were used as test organisms.

Colony diameter on osmotically adjusted agar media.—The osmotic water potential of the basal medium was adjusted to various levels by adding NaCl, KCl, $CaCl_2$, $MgCl_2$, or sucrose at various molal concentrations by interpolatation of the values from the water activity (a_w) tables given by Robinson & Stokes (12, 13). The a_w values of different molalities of $MgCl_2$ were calculated by the formula of Robinson & Stokes (12). The salt mixture (SM) consisted of NaCl, KCl, and Na_2SO_4 in the molal ratio of 5:3:2; and a_w values for different concentrations are given by Scott (14). The a_w values were then converted into total water potentials (ψ) by the formula

$$\psi = \frac{RT}{V} \log a_w = 10.6 \ T \log_{10} a_w$$

where R = ideal gas constant, T = absolute temperature, and V = volume of a mole of water. The a_w for the basal medium was approximately 0.999 (= −1.6 bars). The salts were first put into sterilized 2-liter Erlenmeyer flasks, and 1 liter of deionized-distilled water was added to the flasks. The mouth of each flask was kept covered with aluminum foil to reduce evaporation and the flask was swirled until the salt dissolved. Then the dehydrated cornmeal dextrose agar was added and the flask was swirled for a few seconds and autoclaved at 121 C for 20 min. To prevent formation of precipitates, preparations of media osmotically adjusted with $CaCl_2$ and $MgCl_2$ were autoclaved before and after adding cornmeal dextrose agar plus 2 g of yeast extract. The pH of the media was adjusted to 7.0 before and again after sterilization.

Approximately 25 ml of medium were poured into each petri dish, which was then inoculated near the center with mycelium (grown on CMDA) measuring about 1.5-2.0 mm in diam obtained by a gentle scraping of the periphery of the parent cultures. Each culture and treatment were replicated five times. After inoculation, the dishes were sealed in new polyethylene bags and incubated at 25 C. The diameter of the colonies was measured at 3 or 4 and again at 6 or 7 days for *F. oxysporum*, and at 7 or 8 and 14 or 15 days for *V. albo-atrum* after inoculation.

Linear mycelial growth on straw in soil at water potentials adjusted matrically and osmotically.—Ritzville silt loam (RSL) and Palouse silt loam (PSL) from eastern Washington were used following the methods and techniques used by Cook et al. (6). The soil was sieved through a 40-mesh screen and autoclaved in large beakers at 121 C for 1 hr on each of 2 consecutive days, oven-dried at 105 C

for 48 hr, and stored in new polyethylene bags (size 4 X 2 X 12 inches), 100 g/bag. All manipulation of the sterilized soil was done in a closed transfer chamber.

Matric potentials of the soil were adjusted by aseptically adding different amounts of sterile distilled water. Osmotic potentials were adjusted by adding different molalities of NaCl and KCl solutions (previously sterilized in an autoclave at 121 C for 15 min). The bags were then sealed with rubber bands. The soil and water or salt solutions were mixed well by kneading the soil within the bags, then stored at 5 C for 36 hr for equilibration. The soil was then transferred to 140-ml sterile glass jars, the mouths of which were covered with Parafilm.

Inoculum plugs 2-mm in diam from the edges of the parental cultures were placed near the middle of the concavity of 4-cm pieces of unweathered wheat straws, longitudinally bisected and previously autoclaved at 121 C for 1 hour. Five inoculated and one check straw were buried horizontally at least 1 cm below the soil surface in each jar, and the soil was tamped with a glass rod. Only one test fungus was used in a jar, and no straws were in contact with each other. The jars were kept in plastic boxes at 25 C.

F. oxysporum f. sp. *vasinfectum* isolate 141-inoculated straws were removed from the glass jars 2 days after inoculation; *V. albo-atrum* (Texas cotton isolate 131)-inoculated straws were removed after 6 days. The straws were washed gently with tap water, dipped into 0.1% cotton blue-lactophenol for 1 min, and examined under a dissecting microscope. Total soil water potential for each soil and treatment was determined with a Peltier thermocouple psychrometer (Wescor Model C51), and water retention curves for RSL and PSL were determined as described by Cook & Papendick (5).

pH X water potential.—KCl was dissolved in distilled water, CMDA was added, the media were adjusted to the desired pH with HCl or KOH, buffer was added, and then media were autoclaved at 121 C for 15 min. Phosphate buffer was used in one experiment; and Tris [tris(hydroxymethyl)amino methane] maleate-NaOH buffer at 0.2 M concentration in a second experiment. The pH values of the buffered media, after autoclaving and cooling, were: phosphate buffer 5.6, 6.2, 7.0, and 7.8; Tris maleate-NaOH series, 5.2, 6.0, 7.0, and 8.6. Total water potential of each treatment was determined by thermocouple psychrometry after the media had solidified. Two cotton isolates of *F. oxysporum* and of *V. albo-atrum* were incubated 4 and 16 days, respectively, at 25 C, and then colony diameters were measured.

Temperature X water potential.—Water potentials in CMDA were adjusted with KCl, and the pH was adjusted to 6.0 with HCl or KOH. Incubation of the same fungal isolates used in the previous section was at 15, 20, 25, 30, and 35 C for 4 days for *Fusarium* isolates and 16 days for *Verticillium* isolates.

RESULTS.—*Psychrometer reading versus thermodynamic calculations.*—Total water potentials for CMDA media adjusted with increasing molal concentrations of NaCl and KCl were determined by

thermocouple psychrometry (5, 6), and the results compared with calculations based in water activity values given by Robinson & Stokes (12, 13). Experimentally determined water potentials agreed so closely with the calculated value that results obtained from osmotic or matric forces can be accepted with reliance (10), provided none of the solutes used in osmotic tests is toxic to the test organism.

Colony diameter on osmotically adjusted agar media.—Both fungi tolerated all solutes reasonably well (Fig. 1), with KCl and the salt mixture being most favorable for both. The stimulation at -10 to -20 bars was greater for *V. albo-atrum* than for *F. oxysporum* f. sp. *vasinfectum*, but the latter fungus tended to grow at lower water potentials.

Influence of osmotic and matric water potentials on linear growth on straw in soil.—Linear growth of both fungi was greatest at -10 bars with NaCl, and at -15 to -20 bars with KCl (primarily osmotic water potential, Fig. 2). When different amounts of water alone were added to soil, neither fungus was stimulated with reduced water potential (primarily matric water potential, Fig. 3). These data also indicate that extinction of growth by low water potential occurs at a slightly higher water potential in one soil than in another.

pH X water potential.—The fungi responded so nearly the same in media adjusted with phosphate buffer or with Tris maleate-NaOH buffer that only the data of the latter system are presented (Fig. 4).
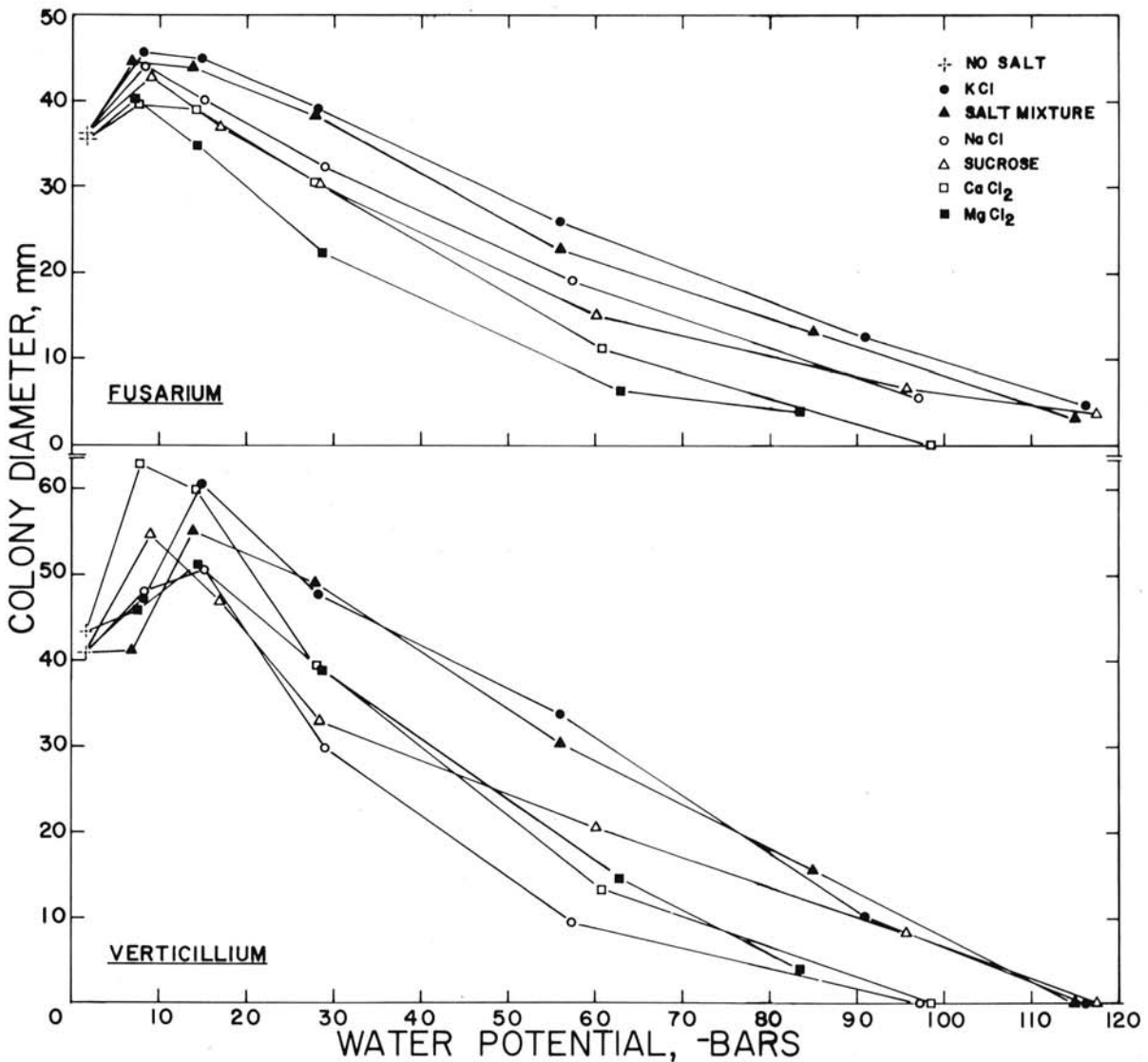

Fig. 1. Growth of cotton isolate *Fusarium oxysporum* f. sp. *vasinfectum* isolate 141 in 3 days and of *Verticillium albo-atrum* from cotton in 14 days on cornmeal dextrose agar adjusted osmotically with various salts and sucrose. Grown at 25 C and pH 7.0.
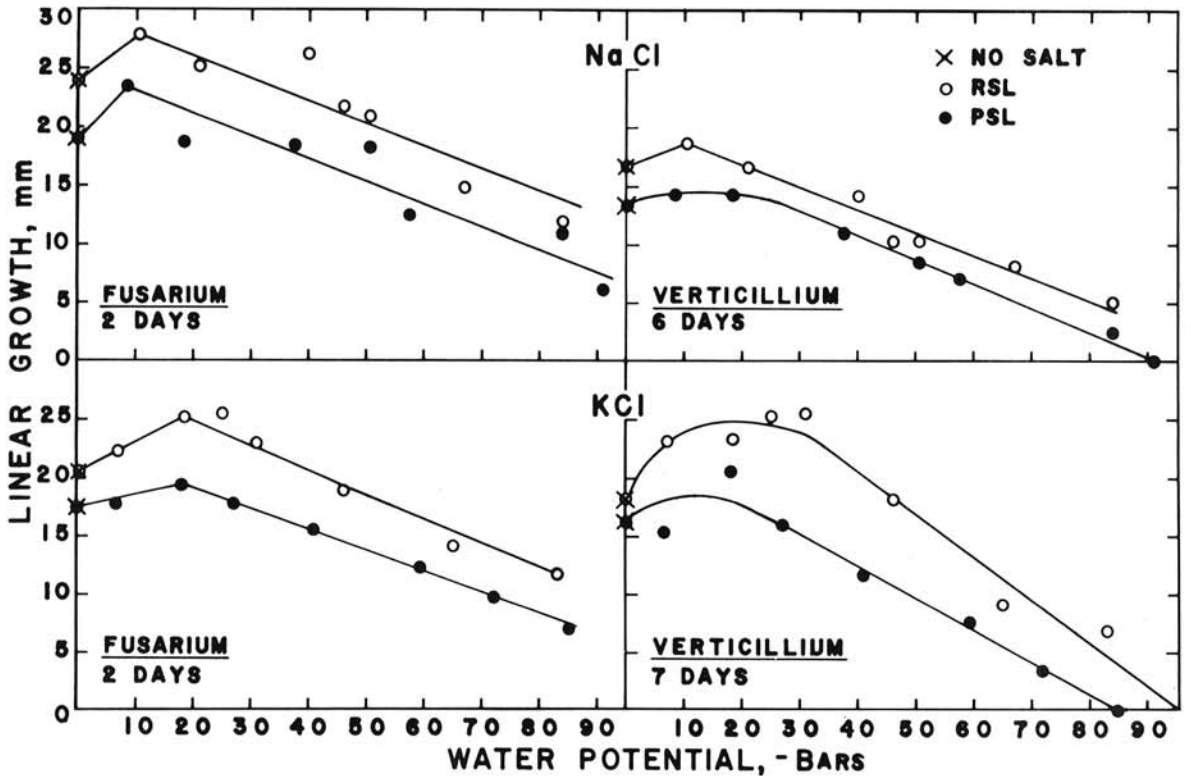
Fig. 2. Linear growth of *Fusarium oxysporum* f. sp. *vasinfectum* isolate 141 in 48-60 hr and of *Verticillium albo-atrum* Texas cotton isolate 131 in 6-7 days on split wheat straws buried in sterile Ritzville silt loam (RSL) and in Palouse silt loam (PSL) adjusted osmotically to different water potentials with NaCl and KCl. Incubated at 25 C.
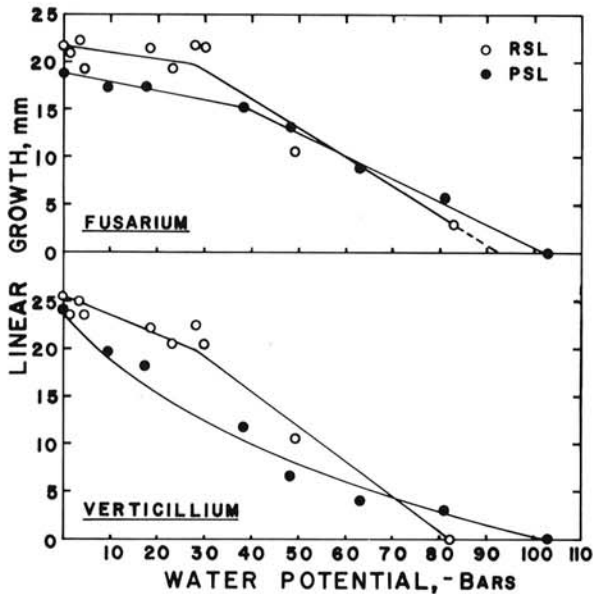

Fig. 3. Linear growth of *Fusarium oxysporum* f. sp. *vasinfectum* (Isolate-141) in 2 days and of *Verticillium albo-atrum* Texas cotton (Isolate-131) in 6 days on split wheat straws in sterile Ritzville silt loam (RSL) and in Palouse silt loam (PSL) at 25 C, adjusted matrically to different water potentials by addition of water alone.

Both fungi tolerated wide pH ranges over the range of water potentials provided.

Temperature X water potential.—All four isolates responded strongly with interactions between temperature and water potential (Fig. 5). As the temperature increased, the fungi grew relatively better in drier media. The most striking response was that of *V. albo-atrum*; neither isolate grew at 35 C on ordinary CMDA, but the addition of salt enabled both to grow at 35 C, especially in the range of −30 to −50 bars. *Fusarium* sp. isolates responded similarly but to a lesser degree.

DISCUSSION.—The similarity of in vitro responses of Fusarium and Verticillium wilt fungi to pH, salts, and water potential make it unlikely that these factors acting directly in soil influence their distribution in nature. It is more likely that the influence of these physical and chemical factors (2, 4, 7, 9, 17, 19) is indirect. Smith & Snyder (16) found that chlamydospores of a wilt *Fusarium* germinated much more poorly in one soil than in another. They attributed the difference between the soils to biological factors. Possibly the cotton wilt *Fusarium* of India, prevalent on heavy, alkaline soils (2), has adapted to these soils in contrast to the prevalent situation in the USA, or the necessary biological factor(s) is lacking from the particular Indian soils.

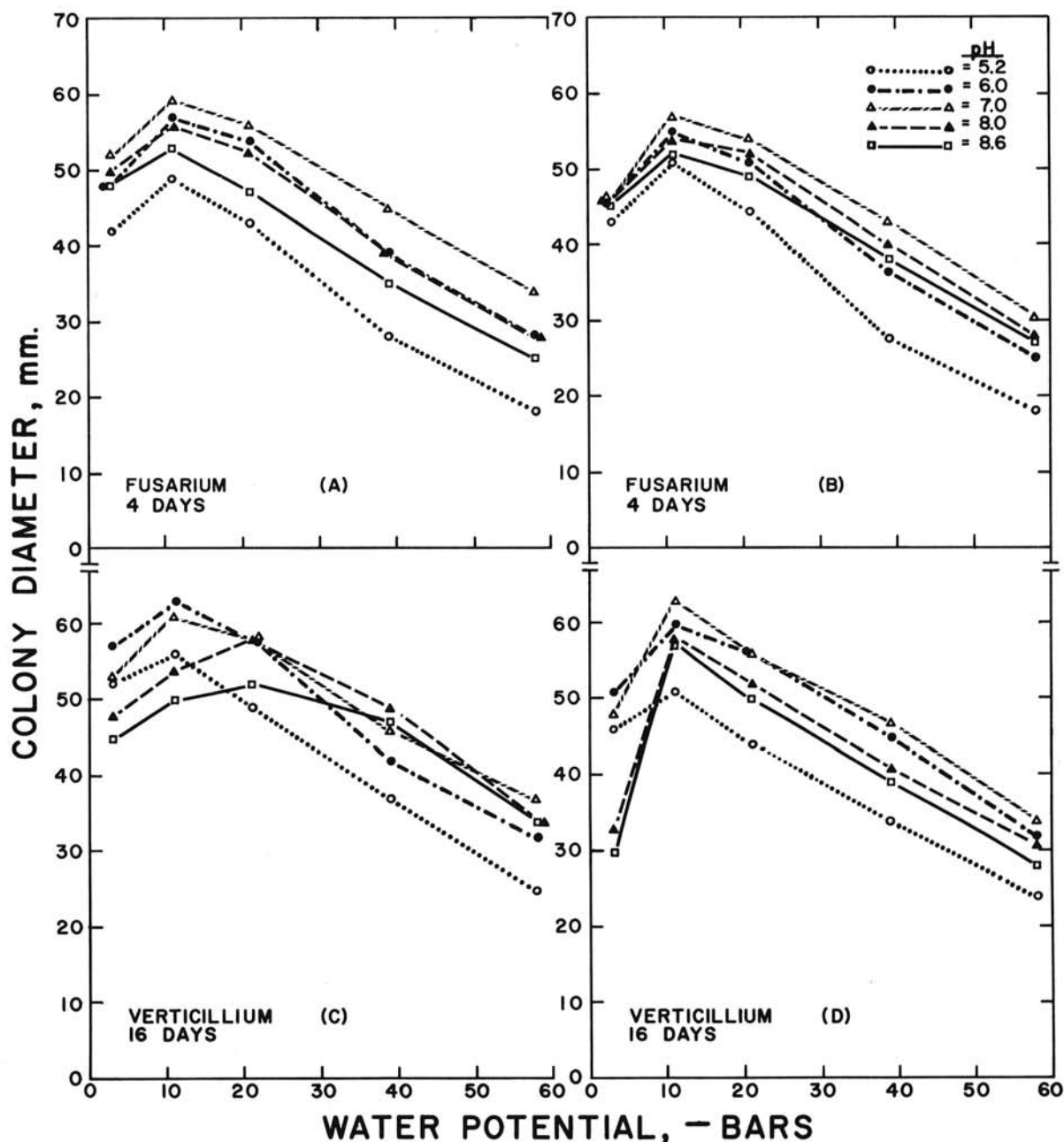*Verticillium* grows relatively faster at lower

Fig. 4. Influence of pH and water potential upon the growth of *Fusarium oxysporum* f. sp. *vasinfectum* isolate 141 (A) and of isolate 144 (B), and upon *Verticillium albo-atrum* from cotton (C, D) at 25 C. Water potentials adjusted with KCl, pH adjusted with Tris [tris(hydroxymethyl)amino methane] maleate-NaOH. Basal medium cornmeal dextrose agar.

temperatures, and Verticillium wilt of cotton is favored by lower temperatures than Fusarium wilt (1, 8, 18). The agreement between the temperature growth optima in water-rich media and in vivo (1, 8) of these two wilt fungi is remarkable. In many fungal diseases, the temperature optima for mycelial development in vitro and for disease development differ, often widely. The host-parasite interaction of these wilts in susceptible plants must be relatively

simple.

The *Verticillium* isolates from cotton did not grow at 35 C on the usual laboratory media, but both grew when salt sufficient to attain -30 to -40 bars was added to the media. At high temperature, some hosts recover from Verticillium wilt (18, 20). Living tissues that recover probably have a relatively high water potential. If living tissues were sufficiently dry (-30 to -40 bars), the in vitro interaction of water X
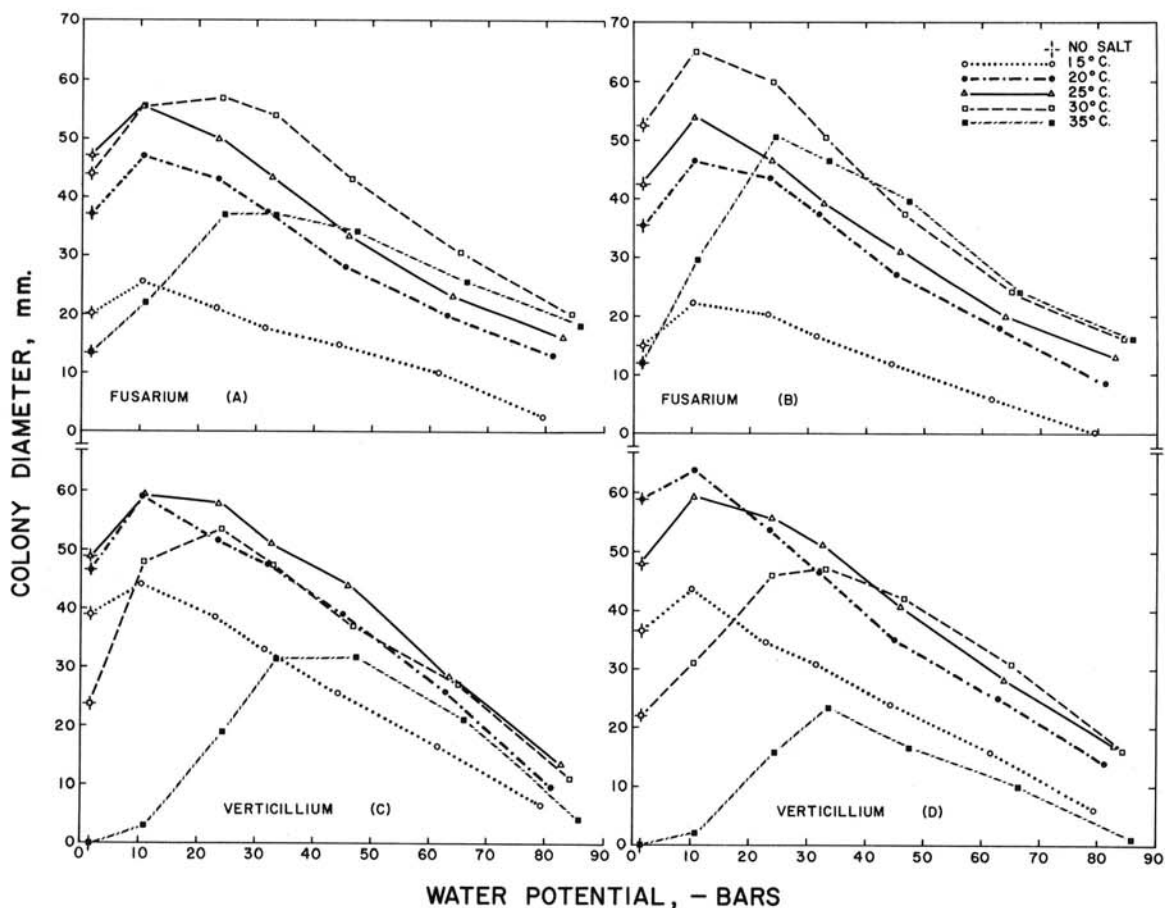
Fig. 5. Influence of water potential and temperature upon the growth of *Fusarium oxysporum* f. sp. *vasinfectum* isolate 141 (A) and isolate 144 (B) in 4 days, and upon *Verticillium albo-atrum* (C, D) from cotton in 16 days. Cornmeal dextrose agar, pH 6, adjusted osmotically with KCl.

temperature would suggest that *Verticillium* should develop in the host even at 30-35 C.

Mozunder et al. (11) found that conidia of *V. albo-atrum* germinated quickly at 25 C in wet media, but at 30 C, germination was quicker in drier media (-31 bars). Thus, germination and hyphal growth apparently respond similarly in temperature × water interactions.

Reducing water potential osmotically (-6 to -12 bars) increases the growth of many microorganisms. Scott (14, 15) postulated that very dilute media increase the energy required by the cell to retain solutes. Others (3) suggest that ion deficits within the cell may restrict the functioning of some enzymes. Whatever the "protective" mechanism of solutes in the medium is, the growth of both organisms was stimulated by osmotic force at high temperature. Because reduction of water potential by matric forces (water added in different amounts to soil) did not stimulate these fungi or those studied by Cook et al. (6), it is unlikely that reduction of water potential itself increases growth.

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