Effect of Water Potential on Reproduction and Spore Germination by *Fusarium roseum* 'Graminearum,' 'Culmorum,' and 'Avenaceum'

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ABSTRACT

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Production of macroconidia by isolates of *Fusarium roseum* 'Graminearum' (Group I, sensu Francis and Burgess), 'Culmorum,' and 'Avenaceum' (all isolated from Washington state), on either potato-dextrose agar or water agar containing carnation leaf pieces and adjusted to different water potentials with either KCl or NaCl, was consistently maximal at about −15 bars. Sporulation by these isolates was nil at about −80 bars and below for Culmorum and at −50 to −60 bars and below for Graminearum and Avenaceum. In contrast, production of macroconidia by two isolates of Graminearum from Pennsylvania (Group II, sensu Francis and Burgess) was maximal at −1.4 to −3.0 bars (the basal medium without salts) and progressively less as the water potential was dropped to −40 to −50 bars, the lower limit. Group II (Pennsylvania) isolates of Graminearum produced perithecia in culture but Group I (Washington) isolates did not. Perithecial production was maximal at about −15 bars and nil at about −50 bars and below. Percentage spore germination for all isolates, including conidia, chlamydospores, and ascospores, was uniformly maximal at all water potentials between about −1 (the highest tested) and −20 bars, was progressively less as the water potential was lowered below −20 bars, and was prevented in the range of −60 to −80 bars. The isolates of Graminearum Group I behaved more like those of Culmorum than like those of Graminearum Group II.

Additional key words: soilborne pathogens, wheat, *Triticum aestivum.*

Optimal and minimal water potentials for mycelial growth have been determined for many phytopathogenic fungi (5,6). In contrast, very little is known about the effects of water potential on other phases in the life cycles of plant pathogens; eg, reproduction and spore germination. To date, information is most complete for *Phytophthora* species. Mycelial growth by these species is generally maximal at −5 to −10 bars and occurs down to −30 and −40 bars (17), sporangia form only at water potentials greater than −4 bars (8,9), and zoospores are released only at soil matric potentials greater than −25 bars (12,13). Very little is known of the relative sensitivities or responses of sexual or asexual stages of ascomyceteous fungi to changes in water potential.

Fusarium roseum Lk. emend. Synder & Hansen 'Graminearum' (= *Gibberella zeae* (Schw.) Petch) and 'Culmorum' each cause foot rot of wheat under dryland conditions in the Pacific Northwest (3) and Australia (10). *Fusarium roseum* 'Graminearum,' 'Culmorum,' and 'Avenaceum' (= *G. avenacea* Cook) also cause headblight and scab of wheat and barley, and stalk rot of corn in areas of high summer rainfall; eg, the Corn Belt of the USA, the Yangtze River area of the People's Republic of China, southern Brazil, South Korea, and southern Europe. Francis and Burgess (10) have classified Graminearum isolates into Group I (causes root and foot rot of wheat and produces perithecia infrequently or not at all) and Group II (causes head blight of wheat and corn stalk rot and produces perithecia abundantly). All isolates of these Fusaria tested to date have grown at water potentials down to −90 to −100 bars (4,7,19), but information is not available on the effects of water potential on other stages of their life cycles.

Graminearum Groups I and II, Culmorum, and Avenaceum are suited for studies of the effects of water potential on sexual and asexual sporulation because they produce several spore types (conidia, chlamydospores and ascospores). This study was undertaken to determine the influence of water potential on the production of macroconidia and perithecia and the germination of macroconidia, ascopores, and chlamydospores. Such information may help clarify the epidemiology of *Fusarium* diseases of cereals.

MATERIALS AND METHODS

Isolates of *Fusarium* and their sources. Six isolates of *Fusarium roseum* were used, including one of Culmorum, one of Avenaceum, and two each of Graminearum Groups I and II. The isolates of Culmorum and Avenaceum were from wheat and lentils (11), respectively, from Washington state. The two isolates of Graminearum Group I were from wheat and soybeans, respectively, from Washington state; and the two of Group II were from corn and carnation, respectively, from Pennsylvania. The wheat plant with Graminearum Group I had typical symptoms of foot rot (3). The soybean plant from which Graminearum Group I was isolated was from an experimental plot at Lind, WA, previously planted to wheat; it was morphologically and culturally indistinguishable from the wheat isolate.

Sporulation media and methods of adjusting of water potential and control of temperature. All experiments were carried out with four different medium-solute combinations: homemade potato-dextrose agar (PDA) or water agar containing sterile pieces of carnation leaves (18); osmotic potentials of both media were adjusted with either KCl or NaCl (7,19). The carnation leaves were from actively growing plants that had not been sprayed with fungicides. The green blades were cut into pieces 1 × 1.2 cm, placed

in open paper bags in layers no deeper than 6 mm, dried in an oven at 75–85 C for about 2 hr, and then cooled overnight. The crisp dry leaf pieces were placed in a layer 3 cm deep in a 10-L desiccator and sterilized with propylene oxide. Five pieces of carnation leaf tissue were used in each dish of the water agar medium. The required amount of salt for a given osmotic potential (range, −1.4 to −114 bars) was dissolved in 1 L of medium in an Erlenmeyer flask. The medium with dissolved salt was then autoclaved at 121 C for 20 min. After being autoclaved, the molten media were dispensed into plastic petri dishes, 15 ml per dish. PDA without the added salt had a water potential of about −3 bars and water agar with carnation leaf pieces about −1.4 bars.

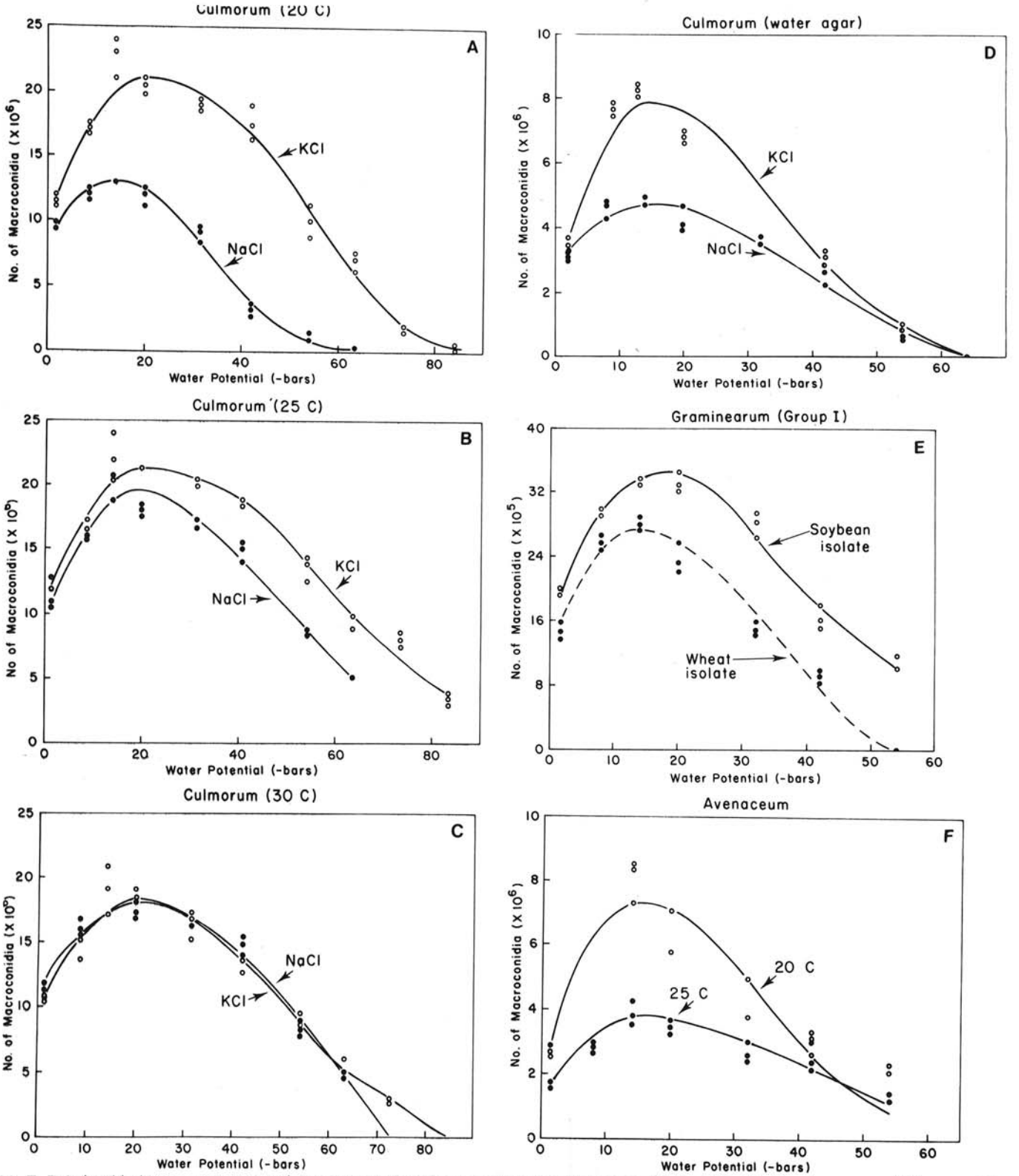

Fig. 1A–F. Relationship between water potential and production of macroconidia by Washington isolates of **A–D,** *Fusarium roseum* 'Culmorum,' **E,** 'Graminearum' Group I, and **F,** 'Avenaceum.' Culmorum was grown on PDA adjusted to different water potentials with KCl or NaCl and incubated at **A,** 20 C **B,** 25 C or **C,** 30 C and on water agar containing sterile pieces of carnation leaves, adjusted with KCl or NaCl, and incubated at **D,** 20 C. The isolates of Graminearum Group I were grown on water agar with carnation leaf pieces adjusted to different water potentials with KCl and incubated at 20 C (E). Avenaceum was grown at 20 and 25 C on PDA adjusted to different water potentials with KCl. Each curve is drawn according to the cubic equation, $\hat{Y} = b_0 + b_1X + b_2X^2 + b_3X^3$ in which X = water potential and $\hat{Y}$ = predicted number of macroconidia.

All substrate water potentials were confirmed by direct measurement with a dew-point hygrometer (14). A disk was cut from representative plates of the respective media and transferred directly to the sample chamber of the Wescor C-52 unit. Whatman No. 1 filter paper disks that had been soaked in KCl solutions of known molalities were used to prepare a standard curve for the instrument.

In a typical study, each isolate was grown first on PDA. Disks (3 mm in diameter) were cut with a cork borer from the advancing margin of 7-day-old cultures and placed in the center of each petri dish of osmotically adjusted or basal (no KCl or NaCl) medium. Each culture and treatment were replicated three times. After being seeded, the dishes were enclosed in new polyethylene bags and incubated at either 20, 25, or 30 C. Light was provided for 12 hr each day during incubation with a combination of fluorescent and incandescent fixtures.

Measurement of asexual sporulation and perithecial formation. The amount of asexual sporulation by each isolate was estimated after 15 days in culture for Culmorum and Avenaceum and after 30 days in culture for the isolates of Graminearum. Spores were collected by pouring 20 ml of distilled water containing Tween-20 into each dish; the agar surface was then rubbed gently with a camel's hair brush, and spores per milliliter in the resulting suspensions were counted with a haemacytometer.

Perithecia were counted under a dissecting microscope 30 days after the cultures were started. The counts were made within 50-mm^2 areas that were marked at random on the carnation leaves or on the PDA surface with an 8-mm-diameter cork borer. Only perithecia that appeared to be mature were counted.

Measurement of macroconidial, ascospore, and chlamydospore germination. The influence of osmotic potential on germination of macroconidia, ascospores, and chlamydospores was determined on water agar adjusted osmotically with KCl. Macroconidia were obtained from 15-day-old PDA cultures of the respective fungi. For ascospores, the corn isolate (Graminearum Group II only) was grown on water agar containing carnation leaves; the perithecia produced were then crushed on glass microscope slides to extrude the ascospores. For chlamydospores (Culmorum only), macroconidia on 13-mm-diameter Millipore filters were sandwiched (15) between pairs of 25-mm-diameter Nuclepore filters (Nuclepore Corporation, Pleasanton, CA 94566) that were sealed at the edges with stopcock grease (15) and these "sandwiches" were buried in moist ($\cong -1/3$ bar) nonsterile field soil for 15 days at 20 C. During incubation in soil, the macroconidia either were converted into chlamydospores or were lysed. The macroconidia, ascopores, and chlamydospores were suspended separately in distilled water (0 bars) or distilled water containing KCl of the same molalities (osmotic potentials) as the respective osmotically adjusted water agar on which they were subsequently germinated. About 10 ml of water or KCl solution containing the test spores was poured on the surface of 10 ml of solidified water agar in a petri dish. The spores were allowed to settle on the agar for a few minutes and then the surplus liquid was drained away. The plates were then incubated for the duration of the experiment at 25 C and at a slight angle to permit the surface liquid to collect and remain on one side. The concentration of spores in the salt solution and the time allowed for settling on the agar were such that each ×10 microscope field of the agar surface had 1–10 spores. The percentage of spore germination was determined after 4, 8, 12, and 24 hr. Each experiment was conducted three times with macroconidia of Graminearum Group I, macroconidia and ascopsores of Graminearum Group II, macroconidia and chlamydospores of Culmorum, and macroconidia of Avenaceum.

RESULTS

Influence of water potential on formation of macroconidia. Formation of macroconidia by isolates of Culmorum, Avenaceum, and Graminearum Group I was maximal at about −15 bars (Fig. 1). Culmorum produced macroconidia on PDA at water potentials down to −80 bars (Fig. 1A–C) and on water agar with carnation leaf pieces down to −60 bars (Fig. 1D). The two isolates of Graminearum Group I on water agar with carnation leaf pieces (Fig. 1E) likewise produced macroconidia at water potentials only down to −60 bars. Production of macroconidia by all isolates of Graminearum on PDA was so sparse that comparisons at different water potentials were not meaningful; consequently these treatments were abandoned. Production of macroconidia by Avenaceum (Fig. 1F) was prevented on PDA at about −60 bars and lower. At water potentials between about −60 bars (−80 bars in the case of Culmorum on PDA) and from −90 to −100 bars, the respective fusaria grew as mycelium on the various media but produced essentially no macroconidia within the time allowed (15 days for Culmorum and Avenaceum and 30 days for Graminearum).

In contrast to the isolates of Graminearum Group I, the two isolates of Graminearum Group II from Pennsylvania formed the greatest number of macroconidia (Fig. 2A) on the basal water agar plus carnation leaves (−1.5 bars). These isolates formed progressively fewer macroconidia with each incremental drop in water potential and none below −40 bars or slightly lower (Fig. 2A). Mycelial growth, on the other hand, was similar to that of the isolates of Group I, being maximal at −10 to −20 bars, and prevented only when the water potential was about −90 bars or slightly lower.

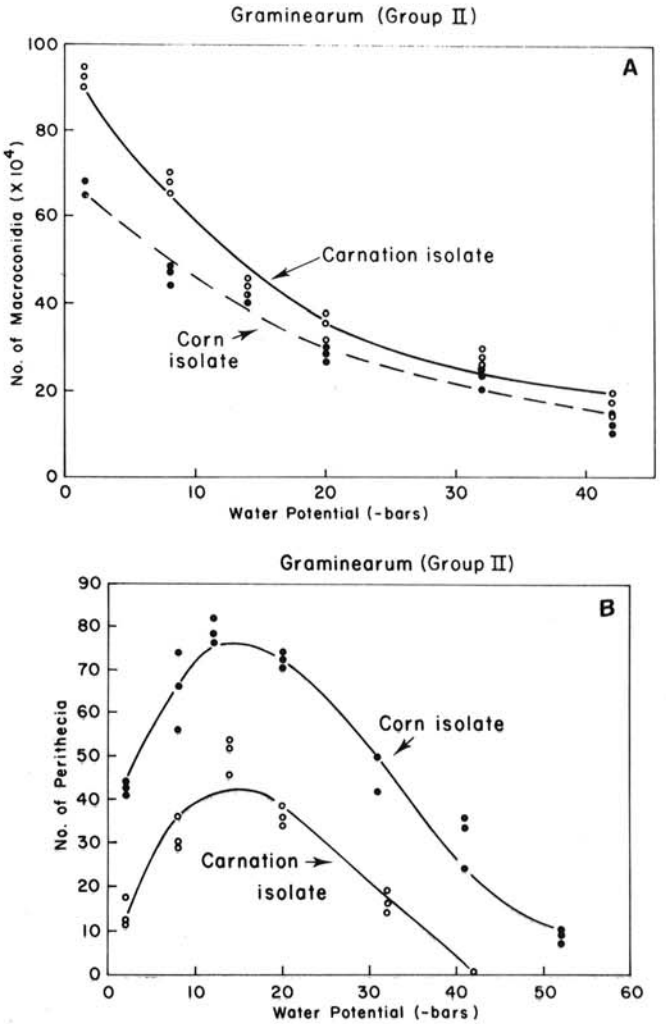

Fig. 2A,B. Relationship between water potential and production of **A,** macroconidia and **B,** perithecia by corn and carnation isolates of *Fusarium roseum* 'Graminearum' Group II grown at 20 C on water agar containing pieces of carnation leaves and adjusted with KCl to different water potentials. The curve for macroconidia (A) is drawn according to the equation, $\hat{Y} = b_0 + b_1X + b_2X^2$, in which X = water potential and $\hat{Y}$ = predicted number of macroconidia. The curve for perithecia (B) is drawn according to the cubic equation, $\hat{Y} = b_0 + b_1X + b_2X^2 + b_3X^3$, in which X = water potential and $\hat{Y}$ = predicted number of perithecia.

At 20 C, and to some extent at 25 C, Culmorum produced more macroconidia with KCl than with NaCl as the solute, whether on PDA (Fig. 1A and B) or water agar with carnation leaf pieces (Fig. 1D). The optimal water potential for sporulation by Culmorum was the same for both solutes (about −15 bars) and did not change with temperature or medium solute combination in spite of differences in total sporulation. On the other hand, minimal water potential for sporulation did change with the medium-solute combination; it tended to be lowest with media or temperatures that favored the greatest total sporulation (eg, Fig. 1A).

The number of macroconidia produced by Culmorum, Avenaceum, and Graminearum Group I on the media adjusted to

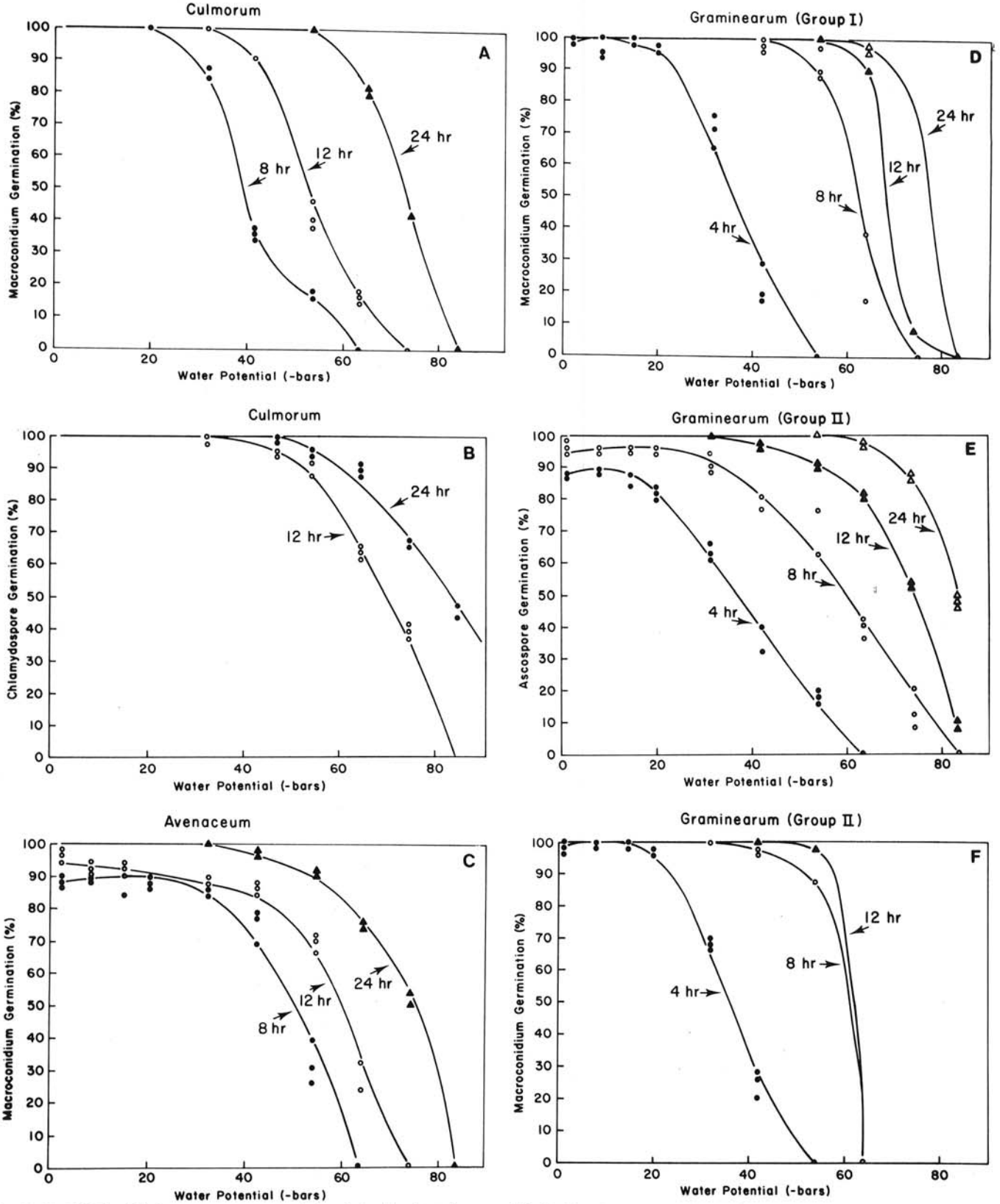

Fig. 3A–F. Relationship between water potential and germination of spores of **A,B,** *Fusarium roseum* 'Culmorum,' **C,** 'Avenaceum,' **D,** 'Graminearum' Group I, and **E,F,** Graminearum Group II at different times during incubation at 25 C on water agar adjusted to different water potentials with KCl. The water used to suspend the spores and transfer them from their respective sources to the water agar contained KCl sufficient to provide the same water potential as the water agar on which they were subsequently incubated.

different water potentials could be predicted by the cubic function $\hat{Y} = b_0 + b_1X + b_2X^2 + b_3X^3$ in which X = water potential and $\hat{Y}$ = predicted number of macroconidia (1). Correlation coefficients between the predicted and actual number of spores at different water potentials by the cubic function was 0.95 for Culmorum, 0.85 for Avenaceum, 0.97 for the wheat isolate of Graminearum, and 0.93 for the soybean isolate of Graminearum. The relationship between water potential and sporulation for the Graminearum Group II fit the equation $\hat{Y} = b_0 + b_1X + b_2X^2$ in which X = water potential and $\hat{Y}$ = predicted number of macroconidia (Fig. 2A). The correlation coefficient between the predicted and actual number of macroconidia at different water potentials was 0.97 for both the carnation and corn isolates.

Influence of water potential and temperature on formation of perithecia. The production of perithecia by the two isolates of Graminearum Group II was maximal at about −15 bars on either PDA or water agar with carnation leaf pieces and with KCl as the solute (Fig. 2B). The corn isolate formed more perithecia and also exhibited a lower minimal water potential for perithecial formation (about −55 or −60 bars) compared with the carnation isolate (−40 bars). Perithecial formation was generally greater on water agar containing carnation leaf pieces (Fig. 2B) than on PDA, and with KCl (Fig. 2B) as the solute compared with NaCl; but for the production of macroconidia, the optimal water potential was the same regardless of the medium-solute combination. Conditions that favored high total numbers of perithecia also favored perithecial formation at the lowest water potentials.

The relationship between water potential and production of perithecia of Graminearum Group II followed the equation $\hat{Y} = b_0 + b_1X + b_2X^2 + b_3X^3$ in which X = water potential and $\hat{Y}$ = the predicted number of perithecia. Correlation coefficients between the predicted and actual number of perithecia produced at different water potentials on water agar with carnation leaf pieces incubated at 20 C were 0.93 and 0.90 for the corn and carnation isolates, respectively.

Influence of water potential on germination of macroconidia, chlamydospores, and ascospores. Ascospores of the corn isolate of Graminearum Group II (Fig. 3E), macroconidia and chlamydospores of Culmorum (Fig. 3A and B), and macroconidia of Avenaceum (Fig. 3C) and the wheat isolate of Graminearum Group I (Fig. 3D) all germinated maximally at all water potentials between −1.4 and about −20 bars, progressively slower with each incremental drop in water potential below −20 bars, and not at all below −80 bars in the time allowed. Macroconidia of the corn isolate of Graminearum Group II also germinated maximally down to −20 bars, but did not germinate at water potentials below about −65 bars (Fig. 3F).

DISCUSSION

Production of macroconidia by the isolates of *F. roseum* Culmorum, Avenaceum, and Graminearum Group I was best at about −15 bars, a water potential very similar to the optimum for mycelial growth by these fungi (4,7). The optimal for sporulation was similar with either PDA or water agar containing carnation leaf pieces as the basal medium, and with either KCl or NaCl as the solute used to adjust water potential. Production of macroconidia by these isolates was prevented at water potentials of about −50 to −60 bars (−80 bars for Culmorum on PDA), which are higher than those limiting to their mycelial growth (4,7). Fungi commonly require a narrower range of environmental conditions for sporulation than for mycelial growth (2).

Even though the water potentials of water agar and PDA without added salts (−1.4 and −3 bars, respectively) are considerably higher than optimal for sporulation by these fungi, these and similar water potentials of other common media have been used for decades to cultivate the Fusaria under pure culture conditions and to induce them to produce spores. In contrast to laboratory media, many kinds of living plant tissues, and especially dryland wheat, have water potentials between −10 and −50 bars (6,16) and thus are within the range ideal for sporulation by these fungi. Appropriately, agar media used to cultivate these pathogens probably should be adjusted to about −15 bars with a solute such as KCl to provide optimal conditions for sporulation.

In contrast to the isolates of Culmorum, Avenaceum, and Graminearum Group I, the two isolates of Graminearum Group II both sporulated best at the highest water potential tested (−1.4 bars) and progressively less with each incremental drop in water potential below that of the basal medium. Again, the optimal was the same with either KCl or NaCl. These isolates cause stalk rot of corn, headblight of wheat, and stem and stub rot of carnation, and function mainly aboveground in humid climates where plant surfaces are commonly wet (water potential about 0 bar). Thus, these isolates appear to be well-suited to sporulate maximally in the environment in which they operate.

The response of perithecial production to water potential by the isolates of Graminearum Group II was similar to that of macroconidial production by the isolates of Graminearum Group I, being maximal at about −15 bars and nil at about −50 to −60 bars or below. Thus, as the water potential of the medium was lowered, the isolates of Graminearum Group II produced fewer macroconidia and more perithecia. This raises the possibility that in nature this *Fusarium* makes macroconidia during periods of free moisture and perithecia when plant surfaces are drying. The shift by isolates of Graminearum Group II from macroconidial to perithecial formation as water potential decreased suggests that some aspect of morphogenesis might be responsible for the difference in responses between Groups I and II. All four isolates of Graminearum produced about 10^6 macroconidia per milliliter on the water agar containing carnation leaf pieces, and Group I isolates produced more and Group II fewer macroconidia as the water potential decreased. Perhaps the pattern for macroconidial formation would be similar for Groups I and II were it not for the shift by Group II to perithecial formation.

Germination of macroconidia, ascospores, and chlamydospores was maximal and uniformly high over the entire range of water potentials between ~0 and −20 bars. This is different from mycelial growth which is progressively better (stimulated) as the osmotic potential is lowered over this same range (4,7,19). This stimulation in mycelial growth by osmotic potentials down to −20 bars has been attributed (5) to ion uptake by the hyphal cells, and the resultant development of cell osmotic potentials more ideal for cell functions and maintenance of turgor. Without exogenous ions such as K^+ or Na^+, cell osmoregulation depends entirely on endogenous osmotica, which may be limiting. As an example, hyphal growth became progressively slower (was not stimulated) when water potential was lowered from 0 to −20 bars by matric control (7); ie, where salts were not added. Since spores are rich in solutes (food reserves), perhaps they contain sufficient amounts of endogenous osmotica for osmoregulation down to 0 to −20 bars without an external supply of ions. This could explain the uniformly rapid spore germination over the entire range of 0 to −20 bars. The greater total and the lower minimal sporulation with KCl compared to NaCl at 20 and 25, but not at 30 C, might be explained by a greater uptake of K^+ than of Na^+ at 20 and 25 C. Extra uptake of solute would, in turn, facilitate osmoregulation and maintenance of the turgor required for sporulation at the lower substrate water potentials.

Our results support the conclusion of Francis and Burgess (10) that Graminearum exists as two different groups. Indeed, our results suggest that the isolates of Graminearum Group I behave more like those of Culmorum than like those of Graminearum Group II.

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