The Interactive Effects of Temperature and Osmotic Potential on the Growth of Aquatic Isolates of *Fusarium culmorum*

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The mycelial growth of 10 *Fusarium culmorum* strains isolated from water of the Andarax riverbed in the provinces of Granada and Almeria in southeastern Spain was tested on potato-dextrose-agar adjusted to different osmotic potentials with either KCl or NaCl (−1.50 to −144.54 bars) at 10°C intervals ranging from 15° to 35°C. Fungal growth was determined by measuring colony diameter after 4 d of incubation. Mycelial growth was maximal at 25°C. The quantity and capacity of mycelial growth of *F. culmorum* were similar at 15 and 25°C, with maximal growth occurring at −13.79 bars water potential and a lack of growth at 35°C. The effect of water potential was independent of salt composition. The general growth pattern of *Fusarium culmorum* growth declined at potentials below −13.79 bars. Fungal growth at 25°C was always greater than growth at 15°C, at all of the water potentials tested. Significant differences were observed in the response of mycelia to water potential and temperature as main and interactive effects. The number of isolates that showed growth was increasingly inhibited as the water potential dropped, but some growth was still observable at −99.56 bars. These findings could indicate that *F. culmorum* strains isolated from water have a physiological mechanism that permits survival in environments with low water potential. Propagules of *Fusarium culmorum* are transported long distances by river water, which could explain the severity of diseases caused by *F.culmorum* on cereal plants irrigated with river water and its interaction under hydric stress or moderate soil salinity. The observed differences in growth magnitude and capacity could indicate that the biological factors governing potential and actual growth are affected by osmotic potential in different ways.

INTRODUCTION

Little research has been done on *Fusarium* in aquatic habitats. The presence of 10 *Fusarium* species has been reported in marshy waters (Gordon 1960). *Fusarium merismoides* was mentioned by Booth (1971) in polluted water and mud. Articles on fungi in fluvial and lacustrine water mention the presence of *Fusarium* as a decomposer of leaves and branches from trees fallen into the water (Willoghby and Archer 1973; Bärlocher and Kendrix 1974; Chamier et al. 1984; Revay and Gözczol 1990). *F. culmorum* and *F. aquaeductum* (Roldan et al. 1989; Roldan and Honrubia 1990) and *Fusarium* sp. (Casas and Descals 1997), which were considered saprophytes, have been sporadically isolated from river channels in Southeast Spain. Palmero et al. (2008a) reported the presence of the *Fusarium* genus in water from the Andarax riverbed and coastal water samples of the Mediterranean Sea. Ten species of *Fusarium*, including *F. culmorum*, have been isolated from river water, but although five different species of *Fusarium* were isolated from the Mediterranean Sea at the mouth of the river, no *F. culmorum* was found.

The fungus reproduces asexually by means of conidia, which form the main mode of dispersal. The dispersal of spores of *Fusarium culmorum* was investigated in aquatic systems as biological control agent for the aquatic weed *Hydrilla verticillata* (Smither-Kopperl 1999). Spores in the water were dispersed by the water current (Palmero et al. 2008a). The study of the pathogenicity of nine isolates of *F. culmorum* obtained from river water showed that most of the isolates (eight out of nine) caused seed coat decay and root rot on barley prior to emergence (Palmero 2008b).

On the other hand, seedling blight, foot rot, and head blight (*Fusarium* head blight (FHB)) or scab caused by *F. culmorum* are associated with dry soils. Cook (1981), refers to this ability to grow and cause disease at low water potential and proposes that organisms unable to develop in substrates possessing a water-potential of less than −40 bars would be restricted to areas (or years) with high rainfall or irrigated crops; those able to grow at less than −60 bars would be the only pathogens able to cause disease in dry climates.

Plant pathogens such as *F. culmorum* are exposed to environments with low water potential (−28 to −40 bars). Different

studies using isolates obtained from diseased plants focussed on the effects of salt concentration on the growth of *Fusarium culmorum* (Cook and Christen 1976). However we were unable to locate any study with *F. culmorum* isolated directly from soil nor from aquatic habitats where the effects of osmotic potential on the growth were evaluated. Studies on the mycelial growth of 18 *Fusarium solani* strains isolated from sea beds of the southeastern coast of Spain indicated that some *Fusaria* have the capacity to adapt metabolically to environments with low water potential (Palmero et al. 2008d).

Studies dealing with conidial germination and viability of *Fusarium culmorum* showed that conidia always germinated in distilled water. The pattern of conidial germination observed in *F. culmorum* when osmotic potential was increased was similar of that observed in *F. verticilloides, F. oxysporum, F. proliferatum* and *F. chlamydosporum*. A great diminution of spore germination was found in −13.79 bars solutions (Palmero et al. 2008c).

We studied the influence of osmotic potential on hyphal growth of aquatic isolates of *F. culmorum* in order to explain the effect of hydric stress or moderate salinity on this fungus, its absence in the seabed at the mouth of the Albuñol River (Spain) in which other Fusaria were found, and the severity of the disease caused by this fungus in cereal plants irrigated with water from the river.

MATERIAL AND METHODS

To determine the interactive effects of temperature and osmotic potential on the growth of *Fusarium culmorum* we used an 10×2×3×6×5 factorial experimental design, wherein strains 1 to 10 were the first factor, salt type was the second factor (NaCl and KCl), temperature (15−25−35°C) was the third factor, osmotic potential (−1.50; −13.79; −41.79; −70.37; −99.56 and −144.54 bars) was the fourth factor and each combination of isolate, temperature, and water potential was replicated 5 times.

Isolates used in salinity and temperature tests.

The origin and code of the isolates of *Fusarium culmorum* tested can be seen on Table 1. All strains used in this study are stored at the University of Almería (Plant Prod. Dept.) and at the Polytechnic University of Madrid (E.U.I.T. Agrícola) culture collections.

TABLE 1
Origin of *Fusarium culmorum* isolates used in tests

Sample code	Location	Coordinates (X,Y)	
13R, 14R	Gador	545687	4090006
15R, 16R, 17R	Gador	545098	4090435
18R	Rambla of Tabernas	574058	4090020
21R, 22R, 23R, 24R	Pechina	549372	4086274

TABLE 2
Relations between the osmotic potential of the medium (ψ) and the concentrations of KCl and NaCl

	Product amount (g / l of PDA)$_{(3)}$	
ψ (bars)	NaCl$_{(2)}$	KCl$_{(2)}$
−1.50$_{(1)}$	0.0	0.0
−13.79	17.6	22.2
−41.79	52.0	68.8
−70.37	84.8	112.0
−99.56	115.2	152.8
−144.54	156.6	212.5

(1) Cook (1981).
(2) Jakobsen et al. (1972).
(3) Potato Dextrose Agar (PDA).

Growth Media

The culture medium used was Potato Dextrose Agar (PDA) (Difco). To achieve targeted osmotic pressures, different batches of the medium were amended with various amounts of NaCl and KCl according to the schedule in Table 2.

Study of Mycelial Growth. Each of the isolates originally grown on selective medium of Komada (1971) was subcultured on PDA. To examine mycelial growth at various temperatures and osmotic pressures, 1 cm diam. agar discs of were excised from the margins of two-week-old PDA cultures and aseptically transferred to the surface of fresh PDA medium.

These cultures were incubated in complete darkness at either 15°C, 25°C, or 35°C. Cultures were examined after 4 d under a dissecting microscope and colony margins were marked with permanent ink on the reverse side of the Petri dishes. For each colony, the mean radial mycelial growth was calculated by measuring two different colony radii in each of five plates per combination of isolate, osmotic pressure and temperature. The growth was corrected by subtracting the 1 cm diameter of the original plug of inoculum.

Statistical Analysis of Data. Statistical analyses were performed using SPSS software, version 11.5.1, of Leadtools Company. For the amount of growth (measure of growth after 4 days), parametric and non-parametric analysis of variance were carried out. χ^2 tests were made to check whether the differences were significant.

To assess the influence of temperature and osmotic pressure on growth, several comparative analyses of averages were performed; these analyses were parametric (one-way ANOVA) when Levene's test indicated no significant heterogeneity of variance, or non–parametric (Kruskal–Wallis test, Mann–Whitney post hoc test) when the heterogeneity of variance was significant.

TABLE 3
The effects of salt composition on the growth of aquatic isolates of *Fusarium culmorum* (mean± SD)

Type of salt	n	Growth (cm)	Maximum (cm)
NaCl	180	1.289 ± 2.092	7.5
KCl	180	1.445 ± 2.309	7.5
Total	360	1.367 ± 2.201	7.5

RESULTS AND DISCUSSION

Growth in the Presence of Different Salt Compositions

There were no significant differences in the number of isolates that exhibited growth at different salt composition ($\chi^2 = 0.181$; $p = 0.671$). Of all isolates tested, 44.4% grew in media amended with NaCl and 42.2% grew when KCl was used as a solute. No significant difference [F (1.358) = 0.448; $p = 0.504$] in the amount of growth was observed with the different salt compositions with a mean of 1.367 cm (Table 3). These results are consistent with those obtained by Sung and Cook (1981).

The fact that growth amount and capacity were not significantly affected by salt composition indicates that ions formed by the salts do not have different chemical effects on the physiology of the fungi. Any differences in other parameters should therefore be due to physical effects (osmotic pressure), which only depend on the quantity of ions present.

The extent of growth of *Fusarium culmorum* at different osmotic pressures varied between 15 and 35°C. Maximal growth was observed at −13.79 bars of osmotic potential. No growth occurred at 35°C. More growth occurred at 25 than at 15°C at all osmotic potentials. The differences in extent of growth at different osmotic potentials were statistically significant (Fig. 1).

It is worth noting the difference in the response of the number of isolates that exhibited growth and the extent of growth. This was particularly striking at 25°C, where growth capacity only diminished at very low osmotic pressures, while the average growth initially increased with decreasing osmotic pressure to −13.79 bars, and declined gradually with increasing salinity. A similar pattern occurred at 15°C. These results indicate that the physiological determinants of capacity and extent of growth are affected quite differently by the presence of dissolved salts in the culture medium.

TABLE 4
The effect of osmotic pressure on the growth capacity of aquatic isolates of *F. culmorum*

Osmotic pressure (bars)	Growth capacity (%)[a]	Lack of growth capacity (%)[a]	Total (%)
−1.50	667	33.3	100.0
−13.79	71.7	28.3	100.0
−41.79	66.7	33.3	100.0
−70.37	43.3	56.7	100.0
−99.56	11.7	88.3	100.0
−144.54	0.0	100.0	100.0

[a]Percentage of isolates that grew.

Growth vs. Osmotic Pressure and Temperature

The χ^2 test showed that there were significant differences in the growth capacity versus osmotic pressure ($\chi^2 = 116.606$; $p < 0.001$) (Table 4). Optimal pressure for growth of isolates of *Fusarium culmorum* was −13.79 bars. Experimental results obtained by others (Cook and Papendick 1972; Cook et al. 1972; Cook and Christen 1976) with species of the genus corroborated these findings showing low osmotic pressure being advantageous to fungal growth.

Table 5 shows that significant differences existed between ability to grow and osmotic pressure. There was minimal or no effect on growth capacity at the first three pressures tested, with an sharp decrease in cultural (>50%) at −70.37 bars. Growth

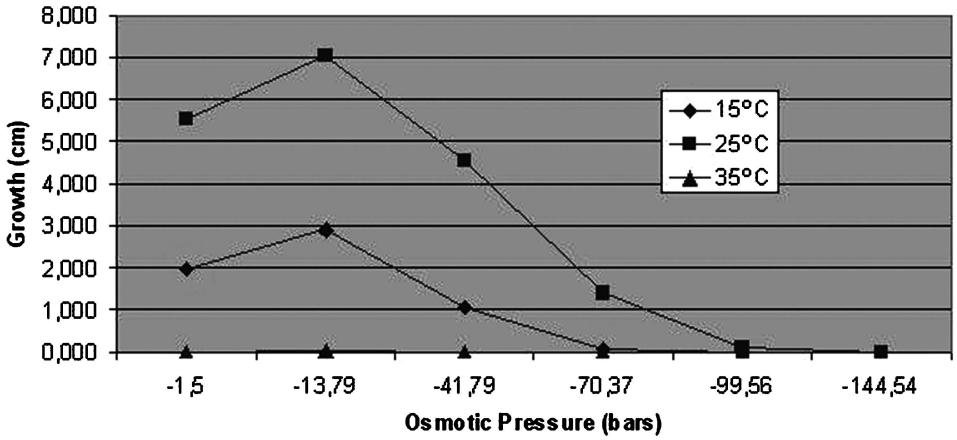

FIG. 1. Growth of aquatic isolates of *Fusarium culmorum* versus temperature and osmotic potential (variance does not exceed 5% of the means).

TABLE 5
The interactive effects of temperature and osmotic pressure on the growth capacity of aquatic isolates of *Fusarium culmorum* (percentages of tested isolates that presented growth)

	Temperature (°C)		
Osmotic pressure (bars)	15	25	35
−1,5	100	100	0
−13,79	100	100	15
−41,79	100	100	0
−70,37	30	100	0
−99,56	0	35	0
−144,54	0	0	0

still occurred at −99.56 bars, at which 35% of isolate cultures grew at 25°C. No growth occurred at −144.54 bars.

Study of the Interaction between Temperature and Osmotic Pressure

The two-way ANOVA (temperature × osmotic pressure) showed statistically significant differences in of the response to temperature [F (2.359) = 1063.093; $p < 0.001$; $\eta^2 = 0.861$], in the response to osmotic pressure [F (5.359) = 416.199; $p < 0.001$; η^2 =0.859, and in the interaction between temperature and osmotic pressure [F (10.359) = 163.776; $p < 0.001$; $\eta^2 = 0.827$]. Sorted according to η^2, these data are as follows (Table 6). A significant interaction between temperature and osmotic pressure causes the previously noted change in the growth response at lower osmotic pressures.

This work on 10 different isolates of *F. culmorum* confirms that hyphal growth may be stimulated at low osmotic potentials (−13.79 bars). This agrees with the results of Cook et al. (1972), who studied the influence of osmotic potentials on a single fungal strain. They found that their strain was progressively stimulated with a decrease in osmotic pressure from −1.5 to −8.2 bars. They noted no stimulation of fungal growth at different matrix potentials. Sung and Cook (1981) studied the effect of osmotic pressure on sporulation by one isolate of *F. culmorum*, which was consistently maximal at about −15 bars. These results are consistent with those obtained by Cook and Christen (1976) for *F. culmorum* and *F. graminearum*.

TABLE 6
Analysis of variance for the main and interactive effects of temperature and osmotic pressure on the growth of aquatic isolates of *Fusarium culmorum*

Variable	F	p	Partial η^2
Osmotic pressure (O. P.)	416.199	0.001	0.859
Temperature	1063.093	0.001	0.861
Temperature × O. P.	163.776	0.001	0.827

The presence of *Fusarium* in samples collected from the river channel as well as from the mouth of the river likely resulted after rainfall events, when flowing water carried particles of soil and organic matter from the riverbanks where cereal crops were being grown. *Fusarium culmorum* isolates were obtained from the river water but not from the seabed. Our experimental results show that the growth response of isolates of *F. culmorum* at 15 and 25°C was very similar, and was clearly affected by high osmotic pressures of the medium (less than −41.79 bars). These results are consistent with those obtained by (Palmero et al. 2008c) for conidial germination where it was uniformly maximal in distilled water and drastically lower in aqueous solutions with high osmotic potentials. There were no algae or vegetation in the sampled area. Our results suggest that the acquired isolates could not survive in high salinity aquatic habitats as saprophytes. These findings may explain the absence of *F. culmorum* in the sea beds of the Mediterranean Sea.

This work also confirms that there can be a stimulation of the hyphal growth at low osmotic potentials (−13.79 bars). The isolates of *F. culmorum* are well adapted to exist in moderate saline habitats. We do not know if the effect of high salinity on growth in our experiments was due to the effect of temperature on osmotic pressure or vice versa. But the ability of a microorganism to grow in saline media or dry soils at high temperatures seems to confer an advantage over other microorganisms in warm saline soils and dry environments both common in cereal crops in the area. The increased use of saline water or water coming from desalination plants for crops irrigation in the studied areas underlines the importance of the research.

Further work is necessary to understand the physiological mechanisms of salt tolerance in *Fusarium culmorum*. From a practical standpoint, the effect of saline waters, used in the irrigation of crops, or hydric stress of cereals on the pathogenicity of *Fusarium culmorum* needs to be investigated in order to enable suitable land irrigation policies in the area.

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