

## COMPARATIVE STUDY OF THE PATHOGENICITY OF SEABED ISOLATES OF *FUSARIUM EQUISETI* AND THE EFFECT OF THE COMPOSITION OF THE MINERAL SALT MEDIUM AND TEMPERATURE ON MYCELIAL GROWTH

D. Palmero <sup>\*1</sup>, M. de Cara <sup>2</sup>, C. Iglesias<sup>1</sup>, L. Gálvez<sup>1</sup>, J.C. Tello<sup>2</sup>

<sup>1</sup> Universidad Politécnica de Madrid (UPM). EUIT Agrícola. Ciudad Universitaria s/n. 28040 Madrid, Spain; <sup>2</sup> Universidad de Almería (UA), Dpto. Producción Vegetal. Cañada de San Urbano s/n. 04120 Almería, Spain.

Submitted: November 11, 2010; Returned to authors for corrections: November 26, 2010; Approved: May 16, 2011.

### ABSTRACT

The pathogenicity of seven strains of *Fusarium equiseti* isolated from seabed soil was evaluated on different host plants showing pre and post emergence damage. Radial growth of 27 strains was measured on culture media previously adjusted to different osmotic potentials with either KCl or NaCl (-1.50 to -144.54 bars) at 15°, 25° and 35° C. Significant differences and interactive effects were observed in the response of mycelia to osmotic potential and temperature.

**Key words:** Salinity, sodium chloride, potassium chloride, osmotic pressure, temperature.

### INTRODUCTION

*Fusarium equiseti* is cosmopolitan (18, 13). Messiaen and Casini (15) considered it a typically soil-borne species, common in warm temperate and subtropical areas. Joffe and Palti (11) found this fungus to be pathogenic to cucurbits and avocado, and stated that its pathogenicity had been underestimated. Since then, it has been reported to cause stem rot in maize and root rot in winter wheat (3), and has been associated to Fusarium head blight disease in wheat and barley (27) (8). Bloomberg (2) reported *F. equiseti* to be pathogenic to pine species in forest nurseries. Sanders and Cole (25) also isolated it from bluegrass crowns that exhibited symptoms of Fusarium blight disease. A previous study on *F. equiseti* isolates obtained from saline seabed soil samples (24) showed

that *F. equiseti* was pathogenic mainly during seedling preemergence.

Tello *et al.* (30, 31) isolated *Fusarium* species from high salinity sand soils from beaches on the Mediterranean shore in Spain. More recently, Nuñez *et al.* (19) recovered *F. oxysporum* and *F. equiseti* at 7.2, 9 and 27 m depths and *F. acuminatum* at a 27 m depth in the bay of Almeria, supporting the findings of Tello *et al.* (31). Palmero *et al.* (20, 24) reported the presence of the *Fusarium* genus in coastal saline water carried by winds to the Mediterranean Sea and stated that these species came from water flowing from the river. In a sampling carried out 78 days after the last freshet from the river to the sea, *F. equiseti* was found at 2 m, 4 m and 6m depths. In fact, *F. solani* and *F. equiseti* were isolated from water gathered 19 days after the last freshet and were also present 78 days later.

\*Corresponding Author. Mailing address: Universidad Politécnica de Madrid (UPM). EUIT Agrícola. Ciudad Universitaria s/n. 28040 Madrid, Spain.; Tel.: +34 3363713.; E-mail: [daniel.palmero@upm.es](mailto:daniel.palmero@upm.es)

Such results suggest that these species might be able to survive in a saline aquatic medium for at least two months.

Studies on the mycelial growth of *F. solani* and *F. culmorum* strains isolated from water or seabeds of the south-eastern coast of Spain indicate that some Fusaria have the capacity to adapt metabolically to environments with low osmotic potential (21, 23). Further studies on *Fusarium* spp. showed that conidial germination in *F. solani* and *F. equiseti* was positively affected by the osmotic potential of the aqueous medium (22). *F. equiseti* showed the highest germination percentage (12.36%) in solutions with osmotic potentials of -144.54 bars. The first saline concentration tested ( $c_e = 24.8$  mS/ml) tolerates the viability of *F. equiseti*, which reaches 132.000 CFU/ml at 48 h of incubation. The purpose of this work was to study the pathogenicity of seabed isolates of *F. equiseti* on four different plant species and assess the interactive effects of temperature and osmotic potential on the mycelial growth of the tested isolates.

The isolates used in this study were collected from the shoreline (0.10 m depth) in the mouth of the Andarax River (Almeria, Spain) (X: 550856, Y: 4074317 coordinates, European Datum 1950, UTM zone 30 N). The salinity of sea water measured from 0.5m to 1m depths for 3min varied between 50.06 and 54.40 mSxcm<sup>-1</sup> (37.95 - 40.8 g NaClx1-1).

Sea water samples were analysed following Palmero *et al.* (24). Isolates were classified to the *Fusarium* species level following the identification procedures and the taxonomic criteria of Nelson *et al.* (18) and Leslie and Summerell (13).

All the strains used in this study were stored at the University of Almería (Plant Production Department), Spain and at the Polytechnic University of Madrid (E.U.I.T. Agrícola), Spain (depository numbers Fm0A-1 to Fm0A-27). Seven of these isolates were randomly selected for pathogenicity tests.

The selected *Fusarium equiseti* isolates were inoculated onto barley (*Hordeum vulgare* L.) cv. CCE6, melon (*Cucumis melo* L.) cv. Canary yellow, kohlrabi (*Brassica oleracea* L. cv.

*gongylodes*) cv. Nabilcol, and tomato (*Lycopersicon esculentum* Mill.) cv. Marmande. Seeds were first disinfected with sodium hypochlorite (40-50 mg/L of active Cl<sub>2</sub>) for 15 min, washed with sterilized water and then germinated in paper toweling for 3 to 7 days at room temperature (20 to 25°). Inoculation tests were carried out using a modification of the technique proposed by Messiaen *et al.* (16). A steril agar control was also included in the inoculation test. Inoculated and control plants were kept in a growth chamber at 25-28° C under a photoperiod of 16 hours at 12,000 lux.

Plants in plots were rated every 5 days for percent emergence. After 20 days plants were evaluated for percent damping off (26), and non-emerged pregerminated seeds were uncovered and evaluated for symptoms of root and seed rot (referred to as pre-emergence damping off, hereafter). The experiment was then repeated.

To determine the interactive effects of temperature and osmotic potential on the growth of *F. equiseti*, we used an 10x2x3x6x5 factorial experimental design, where strains 1 to 10 were the first factor, salt type (NaCl and KCl) was the second factor, temperature (15-25-35°C) was the third factor, and osmotic potential (-1.50; -13.79; -41.79; -70.37; -99.56 and -144.54 bars) was the fourth factor. Each isolate, temperature, and osmotic potential combination was replicated 5 times.

Each of the isolates originally grown on Komada's selective medium (12) was subcultured on PDA (Difco). The media were amended with varying amounts of NaCl and KCl following Besri (1). A 27x2x3x6x5 factorial experimental design was used, where strains 1 to 27 were the first factor, salt type (NaCl and KCl) was the second factor, temperature (15, 25 and 35°C) was the third factor and osmotic pressure (-1.50,-13.79,-41.79, -70.37,-99.56 and -144.54 bar) was the fourth factor. Each isolate, temperature, and osmotic pressure combination was replicated 5 times.

Agar discs of a 1 cm diameter were excised from the margins of two-week-old PDA cultures and aseptically transferred to the surface of fresh PDA medium. Cultures were

examined after 4 days under a dissecting microscope and colony margins were marked with permanent ink on the reverse side of the Petri dishes. Mean radial mycelial growth was calculated for each colony by measuring two different colony radii in five plates of each isolate, osmotic potential and temperature combination. Growth was corrected by subtracting the 1 cm diameter of the original plug of inoculum.

Parametric and non-parametric analyses of variance were carried out to test the effects of salt concentration and temperature on growth (measure of growth after 4 days). Chi-square ( $\chi^2$ ) tests were made to compare whether differences in the number of isolates that exhibited growth at different salt compositions were significant. The influence of temperature and osmotic potential on growth was assessed using parametric analyses (one-way ANOVA) when Levene's test indicated no significant heterogeneity of variance, and non-parametric

analyses (Kruskal-Wallis test, Mann-Whitney post hoc test) when the heterogeneity of variance was significant. Analysis of variance was carried out using STATGRAPHICS Plus 5.1 statistical software package (StatPoint, Inc. 2325 Dulles Corner Boulevard, Suite 500 Herndon, Virginia 20171). Non parametric statistical analyses of salinity response were performed using SPSS software (version 11.5.1).

Pathogenicity results for the 7 isolates are shown in Table 1. Barley and kohlrabi were the most susceptible hosts. All the tested *F. equiseti* isolates affected seedlings, causing damping off during pre-emergence, although no symptoms were observed once seedlings emerged. Only isolate *Feq4* caused significant pre-emergence damping off on melon, while isolates *Feq 2*, *Feq3*, *Feq4* and *Feq7* were pathogenic after seedling emergence. Isolates *Feq1*, *Feq3* and *Feq5* caused a significant decrease in seedling emergence on tomato.

**Table 1.** Incidence of damping off on four plant species inoculated with isolates of *Fusarium equiseti* (values with the same lower case letter did not differ significantly).

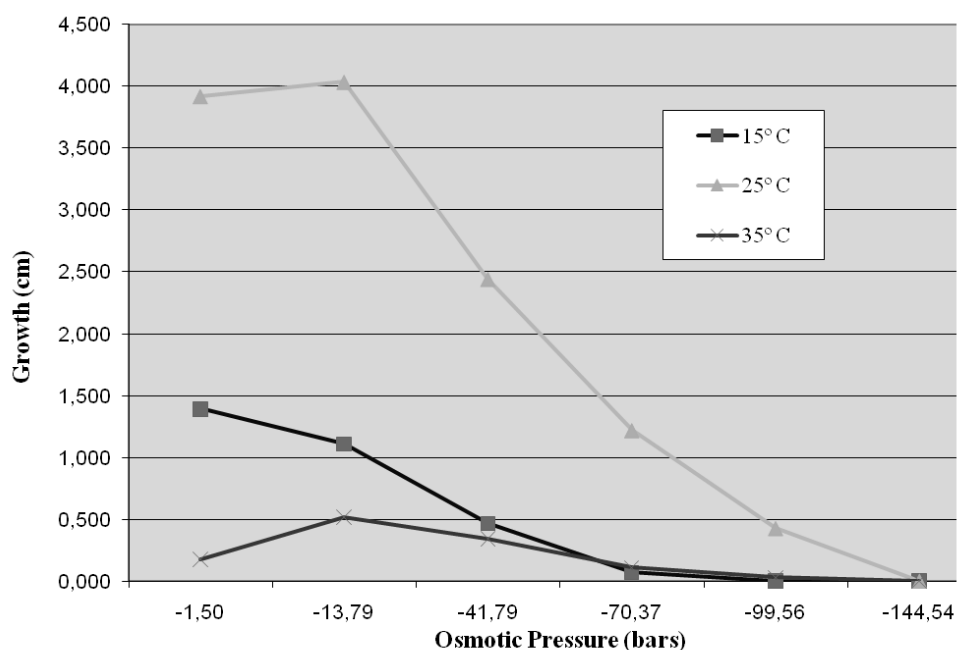
Isolate number	Barley*		Kohlrabi*		Melon*		Tomato*	
	%emergence	% survival	%emergence	% survival	%emergence	% survival	%emergence	% survival
<i>F. eq 1</i>	27** ± 12.5b	27 ± 12.5 a	62 ± 6.2 cd	62 ± 6.2 cd	90 ± 14.1 ab	77 ± 4.7 bc	57 ± 14.3 ab	55 ± 14.7 ab
<i>F. eq 2</i>	7 ± 4.7 a	7 ± 4.7 a	50 ± 7.1 bc	50 ± 7.1 bc	90 ± 8.2 ab	37 ± 17.0 a	77 ± 18.9 bc	68 ± 18.4 abc
<i>F. eq3</i>	17 ± 9.4 ab	17 ± 9.4 a	0 ± 0.0 a	0 ± 0.0 a	90 ± 8.2 ab	50 ± 16.3ab	48 ± 6.2 a	48 ± 6.2 ab
<i>F. eq 4</i>	27 ± 4.7 b	23 ± 9.4 a	65 ± 4.1 cd	65 ± 4.1 cd	80 ± 8.2 a	53 ± 12.5 ab	67 ± 12.5abc	65 ± 12.2 abc
<i>F. eq 5</i>	50 ± 8.2 c	50 ± 8.2 b	25 ± 25.5 ab	25 ± 25.5 ab	83 ± 12.5 ab	67 ± 24.9 abc	48 ± 6.2 a	47 ± 8.5 a
<i>F. eq 6</i>	20 ± 0.0 ab	20 ± 0.0 a	38 ± 23.6 bc	38 ± 23.6 bc	90 ± 8.2 ab	70 ± 21.6 abc	72 ± 8.5 bc	72 ± 8.5 bc
<i>F. eq 7</i>	20 ± 16.3 ab	20 ± 16.3 a	38 ± 22.5 bc	38 ± 22.5 bc	100 ± 0.0 b	43 ± 17.0 ab	72 ± 10.3 bc	70 ± 12.2 abc
<i>Control</i>	70 ± 8.2 d	70 ± 8.2 b	87 ± 4.7 d	87 ± 4.7 d	100 ± 0.0 b	100 ± 0.0 c	82 ± 2.4 c	82 ± 2.4 c

\* Greenhouse inoculation studies were repeated.

\*\* Percent of germination and survival (Value ± SD).

There were no significant differences in the number of isolates that exhibited growth at different salt compositions ( $\chi^2 = 0.172$ ;  $p = 0.679$ ). Of all the tested isolates, 52.4% grew in the medium amended with NaCl, while 50.3% grew in the KCl medium. Salt composition did not have a significant effect on growth [ $F(1,970) = 0.539$ ;  $p = 0.626$ ], as an average growth of 0.911 and 0.896 was obtained for the NaCl and KCl media, respectively.

*F. equiseti* growth at different osmotic potentials showed the same pattern at 25° and 35°C (Fig.1). Maximal growth was observed at -13.79 bars of osmotic potential with a steady sharp decrease beginning at -41.79 bars. These results are in agreement with those obtained with species of the genus *Fusarium* (5, 6, 7, 21, 23) where low osmotic potentials were advantageous to fungal growth.



**Figure 1.** Growth of *Fusarium equiseti* versus temperature and osmotic pressure (variance does not exceed 5% of the means)

The greatest growth occurred at 25°C at all osmotic potentials. Osmotic potential had a significant effect on growth (Fig. 1).

The  $\chi^2$  test showed significant differences in viability versus osmotic potential ( $\chi^2 = 377.235$ ;  $p < 0.001$ ). At 15°C, low osmotic potential (-1.50 and -13.79 bars) had a minimal or no effect on cultural viability, while an acute decrease in growth capacity was observed (>50%) at -70.37 bars. At 25°C, 27.78% of isolate cultures were still viable at -99.56 bars. There are clear differences in the growth response of *F. equiseti*. At 15°C, growth was maximal at the highest osmotic potential, while at 25° and 35°C, growth was maximal at -13.79 bars (Fig.1).

It is worth noting the difference in the response of the number of isolates that grow and the extent of this growth. This was particularly notable at 25° C, where viability only diminished at very low osmotic potentials, while average growth initially increased with decreasing osmotic potential to -13.79 bars, and gradually declined with increasing salinity. A similar pattern occurred at 35° C. These results indicate that the

physiological determinants of the frequency of isolates that grow and among these, the extent of growth, are affected quite differently by the presence of dissolved salts in the culture medium.

The two-way ANOVA (temperature x osmotic potential) showed statistically significant differences in the response of *F. equiseti* to temperature [ $F(2.971) = 428.870$ ;  $p < 0.001$ ;  $\eta^2 = 0.471$ ], osmotic potential [ $F(5.971) = 155.760$ ;  $p < 0.001$ ;  $\eta^2 = 0.449$ ], and the interaction between temperature and osmotic potential [ $F(10.971) = 51.924$ ;  $p < 0.001$ ;  $\eta^2 = 0.352$ ]. The significant interaction between temperature and osmotic potential explains the previously noted change in the growth response of *F. equiseti* at lower osmotic potentials.

This work on 27 different isolates of *F. equiseti* shows that hyphal growth may be stimulated at low osmotic potentials (-13.79 bars) over 25°C. This agrees with the results of Cook et al (7) for *F. culmorum*. Sung and Cook (28) studied the effect of osmotic potential on sporulation in one isolate of *F. culmorum*, which was consistently maximal at about -15 bars. These results are consistent with those obtained by Cook and

Christen (5) for *F. culmorum* and *F. graminearum* and by Palmero *et al.* (22, 24) for *F. solani* and *F. culmorum*.

*F. equiseti* can be found worldwide (13). According to Gerlach and Nirenberg (9) *F. equiseti* is only mildly parasitic and of minor economic importance. Conversely, other authors have found that *F. equiseti* causes stem rot in maize and root rot in broad bean (29), winter wheat and other small grains in the field and in storage (2, 10).

We found that *F. equiseti* isolates were pathogenic on melon and tomato prior to seedling emergence and on melon after emergence. These results agree with those of Joffe and Palti (11) who reported that *Fusarium equiseti* is pathogenic on cucurbits.

Results from the growth tests are consistent with those obtained by Sung and Cook (28) for *F. roseum* and by Palmero *et al.* (21, 23) for *F. culmorum* and *F. solani*. These authors found that mycelial growth and the ability to grow were not significantly affected by salt composition. This indicates that ions formed by the salts do not have different chemical effects on the physiology of the fungi. Thus, differences in other parameters may be due to physical effects (osmotic potential), which only depend on the number of ions present.

Cook (4) stated that low water potential or irrigation with saline water may free a soilborne pathogen from the usual dampening influence of background soil microbiota by limiting growth of other organisms more than that of the pathogen itself. Experimental results provide clear information on the pathogenicity of *F. equiseti*. This work also confirms that *F. equiseti* isolates are well adapted to moderate saline habitats and that the positive effect of salinity on mycelial growth is greater at higher temperatures. Effects on antagonist-pathogen interactions must be studied in relation to salinity.

We do not know if the effect of high salinity on growth in our experiments was due to the effect of temperature on osmotic potential or vice versa. However, the ability of the microorganism to grow in saline media or dry soils at high temperatures seems to confer an advantage over other microorganisms in warm saline soils or dry environments.

Cook (4), Besri (1) and Triky Dotan *et al.* (32) reported that some species of *Fusarium* were able to escape antagonism by virtue of ability to grow at very low potentials. This study would therefore include pathogenic isolates of *F. equiseti* in this group of species.

Previous studies have found that winds can provide considerable allocthonous elements from North Africa to the circum-Mediterranean area (14, 17). On the other hand, the presence of *Fusarium* spp. propagules carried out by winds has been reported. Experimental results show that *Fusarium equiseti* is pathogenic and has the capacity to grow at low osmotic potentials, which could explain the prevalence of the genus *Fusarium* in the wind and soils in semiarid environments. The pathogenicity of *F. equiseti* on seedling emergence is now demonstrated, but its economic significance remains to be elucidated. Further work is necessary to understand the physiological mechanisms of salt tolerance in *Fusarium equiseti* and how the pathogenicity of isolates might be affected in moderate salinity soils or water stress situations.

## REFERENCES

1. Besri, M. (1980). Influence du potentiel osmotique de l'eau sur la croissance de *Fusarium oxysporum* f. sp. *lycopersici* et de *Verticillium dahliae*. *Phytopathology*. 99, 1-8.
2. Bloomberg, W.J. (1981). Diseases caused by *Fusarium* in forest nurseries. In: *Fusarium: Diseases, biology, and taxonomy*. Nelson, P.E., Toussoun, T.A., Cook, R. (eds). The Pennsylvania State University Press, University Park, USA, p. 178-187.
3. Booth, C. (1971). The genus *Fusarium*. Ed. Commonwealth Mycol. Inst. Kew, England.
4. Cook, R.J. (1973). Influence of low plant and soil water potentials on diseases caused by soilborne fungi. *Phytopathology*. 63, 451-458.
5. Cook, R.J.; Christen, A.A. (1976). Growth of cereal root rot fungi as affected by temperature and water potential interactions. *Phytopathology*. 66, 193-197.
6. Cook, R.J.; Papendick, R.I. (1972). Influence of water potential of soils and plants on root disease. *Annu. Rev. Phytopathol.* 10, 349-374.
7. Cook, R. J.; Papendick, R. I.; Griffin, D.M., (1972). Growth of two root rot fungi as affected by osmotic and matrix water potentials. *Soil Sci. Soc. Am. Proc.* 36, 78-82.
8. Gale, L. R. (2003). Population Biology of *Fusarium* species causing head

- blight of grain crops. *In: Fusarium head blight of wheat and barley.* Leonard, K.J., Bushnell, W.R. (eds). *Am. Phytopathol. Soc.*, St. Paul, MN. p. 120-143
9. Gerlach, W.L.; Nirenberg, H. (1982). The genus *Fusarium*. A pictorial atlas. Mitt. Biol Bundesanst. Land-Forstwirtschaft. Berlin-Dahlen.
  10. Hashem, M.; Moharam, A.M.; Zaied, A.A.; Saleh, F.E.M. (2010). Efficacy of essential oils in the control of cumin root rot disease caused by *Fusarium* spp. *Crop Protection*, 29: 1111-1117.
  11. Joffe, A. Z.; Palti, J. (1967). *Fusarium equiseti* (Cda) Sacc. *Isr J Bot Basic Appl Plant Sci.* 16: 1-18
  12. Komada, H. (1975). Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8, 114-125.
  13. Leslie, J.F.; Summerell, B.A. (2006). The *Fusarium* Laboratory Manual. Blackwell Publishing. Ames, Iowa, USA p. 388.
  14. Menéndez, I.; Díaz Hernandez, J.L.; Mangas, J.; Alonso, I.; Sanchez-Soto, P. J. (2007). Airborne dust accumulation and soil development in the North-East sector of Gran Canaria (Canary Islands, Spain) *J. Arid Environ.* 71, 57-81
  15. Messiaen, C.M.; Casini, R. (1968). Recherches sur les fusarioses IV. La systématique des *Fusarium*. *Ann. Epiphyte* 19, 387-454.
  16. Messiaen, C.M.; Belliard-Alonzol, L.; Barriere, Y.; De La Tullaye, B. (1976). Étude qualitative des *Fusarium roseum* dans les sols des environs de Versailles, sous diverses rotations ou associations végétales. *Ann. Rev. Phytopathol.* 8, 269-282.
  17. Moreno, A.; Cacho, I.; Canals, M.; Prins, M.A.; Sánchez-Goñi, M.F.; Grimalt, J.O.; Weltje, G.J. (2002). Saharan Dust Transport and High-Latitude Glacial Climatic Variability: The Alboran Sea Record. *Quaternary Res.* 58, 318–328.
  18. Nelson, P.E.; Toussoun, T.A.; Marasas, W.F.O. (1983). *Fusarium* species. A manual for identification. Ed. The Pennsylvania State University Press. USA.
  19. Nuñez, F.J.; Palmero, D.; Iglesias, C.; De Cara, M.; Sinobas, J.; Tello, J.C. (2006). Biogeografía de especies de *Fusarium* en el litoral mediterráneo de España. *Bol. San. Veg. Plagas.* 32, 137-149.
  20. Palmero, D.; de Cara, M.; Iglesias, C.; Tello, J.C. (2008a). *Fusarium* species isolated from water from fluvial channels and sea beds of the south eastern coast of Spain. *J. Plant Pathol.* 90 (3), 48-49.
  21. Palmero, D.; de Cara, M.; Iglesias, C.; Ruíz, G.; Tello, J.C. (2008b). The interactive effects of temperature and osmotic potential on the growth of marine isolates of *Fusarium solani*. *J. Ind. Microbiol. Biot.* 35, 1405-1409.
  22. Palmero, D.; de Cara, M.; Iglesias, C.; Ruíz, G.; Tello, J.C. (2008c). Effects of water potential on spore germination and viability of *Fusarium* species. *J. Ind. Microbiol. Biot.* 35, 1411-1418
  23. Palmero, D.; de Cara, M.; Iglesias, C.; Tello, J.C. (2009a). The interactive effects of temperature and osmotic potential on the growth of aquatic isolates of *Fusarium culmorum*. *Geomicrobiol. J.* 26, 321-325
  24. Palmero, D.; Iglesias, C.; de Cara, M.; Lomas, T.; Santos, M.; Tello, J.C. (2009b). Species of *Fusarium* Isolated from River and Sea Water of Southeastern Spain and Pathogenicity on Four Plant Species. *Plant Dis.* 93 (4), 377-385
  25. Sanders, P.; Cole, H. (1981). The *Fusarium* diseases of turfgrass. *In: Fusarium: Diseases, biology, and taxonomy.* Nelson, P.E., Toussoun, T. A., Cook, R. (eds). The Pennsylvania State University Press, University Park. p. 195-209.
  26. Schumann, G.L.; D'Arcy, C.J. (2006). Essential plant pathology. *Phytopathology*, St. Paul, MN, USA.
  27. Shaner, G.E. (2003). Epidemiology of Fusarium Head Blight of small grain cereals in North America. *In: Fusarium Head Blight of Weath and Barley.* Leonard, K.J., Bushnell, W.R. (eds). APS Press, *Phytopathology*, St. Paul, MN, USA. p. 84-119.
  28. Sung, J.; Cook, R.J. (1981). Effect of water potential on reproduction and spore germination of *Fusarium roseum* "Graminearum", "Culmorum" and "Avenaceum". *Phytopathology.* 71 (5), 499-504.
  29. Tello, J.C.; Lacasa, A. (1985). Micosis de las habas (*Vicia faba* L.). La podredumbre negra del pie. Cuadernos de Fitopatología. 4, 143-160.
  30. Tello, J. C.; Lacasa, A.; Rodríguez, M.C. (1990). Presence of some *Fusarium* species on Spanish beaches. Proc 8th Congress of the Mediterranean Phytopathological Union. Agadir (Morocco), 137-138.
  31. Tello, J. C.; Rodríguez, M. C.; Lacasa, A. (1992). Importancia de *Fusarium* en las arenas de playas de España. ITEA 88, 77-94.
  32. Triky-Dotan, S.; Yermiyahu, U.; Katan, J.; Gamliel, A. (2005). Development of crown and root rot disease of tomato under irrigation with saline water. *Phytopatology.* 95 (12), 1438-1444.



All the content of the journal, except where otherwise noted, is licensed under a [Creative Commons License](https://creativecommons.org/licenses/by-nc/4.0/)