

Species of *Fusarium* Isolated from River and Sea Water of Southeastern Spain and Pathogenicity on Four Plant Species

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ABSTRACT

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Species of *Fusarium* were isolated from water samples collected from the Andarax River and coastal sea water of the Mediterranean in Granada and Almería provinces of southeastern Spain. In total, 18 water samples were analyzed from the Andarax River, and 10 species of *Fusarium* were isolated: *Fusarium anthophilum*, *F. acuminatum*, *F. chlamyosporum*, *F. culmorum*, *F. equiseti*, *F. verticillioides*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. sambucinum*. In addition, five species were isolated from 33 sea water samples from the Mediterranean Sea: *F. equiseti*, *F. verticillioides*, *F. oxysporum*, *F. proliferatum*, and *F. solani*. When considering the samples by their origins, 77.8% of the river water samples yielded at least one species of *Fusarium*, with *F. oxysporum* comprising 72.2% of the total isolates. In the case of marine water, 45.5% of the samples yielded at least one species of *Fusarium*, with *F. solani* comprising 36.3% of the total isolates. The pathogenicity of 41 isolates representing nine of the species collected from river and sea water during the study was evaluated on barley, kohlrabi, melon, and tomato. Inoculation with *F. acuminatum*, *F. chlamyosporum*, *F. culmorum*, *F. equiseti*, *F. verticillioides*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. sambucinum* resulted in pre- and post-emergence damping off. Pathogenicity of *Fusarium* isolates did not seem to be related to the origin of the isolates (sea water or fresh water). However, the presence of pathogenic species of *Fusarium* in river water flowing to the sea could indicate long-distance dispersal in natural water environments.

Almería and Granada provinces, in the southeast of the Iberian Peninsula, stretch along 330 km of coastline on the Mediterranean Sea (Fig. 1). Along the shore, intensive agriculture using plastic greenhouses for production of vegetable crops (tomato, watermelon, pepper, bean, eggplant, and squash.) occurs, and subtropical crops (avocado, cherimoya, and sugar cane) are grown in the field. The main water source in this area is the Andarax River. The river's water is used for irrigation and, as a result, the main part of its course is dry most of the year.

Diseases produced by species of *Fusarium* are common in these areas. They were in the past, and are still today, a problem for greenhouse crops in Almería.

The following species have been listed as pathogens on agricultural crops in this region: *Fusarium oxysporum* f. sp. *lycopersici* (race 0 and 1) (54), *F. oxysporum* f. sp. *melonis* (race 0, 1, 2, and 1-2) (50), *F. oxysporum* f. sp. *niveum* (49), *F. ox-*

ysporum f. sp. *melongenae* (49,60), *F. oxysporum* f. sp. *basilici* (21), *F. oxysporum* f. sp. *asparagi* (11,51), *F. oxysporum* f. sp. *dianthi* (54), *F. oxysporum* f. sp. *radicis-lycopersici* (53), *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (30), *F. solani* in broad bean (*Vicia faba*; 52), pea (*Pisum sativum*; 56) and French bean (*Phaseolus vulgaris*; 55), *F. proliferatum* (asparagus) (11,13), *F. culmorum* (asparagus and carnation) (11,51), and *F. equiseti* (broad bean; 52).

Research that has focused on the occurrence of the genus in aquatic habitats is not common, perhaps because *Fusarium* spp. Generally are considered to be soilborne fungi. Backhouse et al. (2) published an extensive study on the biogeography of *Fusarium* spp. in Australia, but the aquatic habitat was not covered. *Fusarium* spp. have been reported in marshy waters (19), and *F. merismoides* was found by Booth (4) in polluted water and mud. Articles on fungi in fluvial and lacustrine water mention the presence of *Fusarium* spp., but as a decomposer of leaves and branches from fallen trees in river channels (8,9,40,62). The spread of *Fusarium* spp. via irrigation water has also been described for some species causing plant diseases in greenhouses (14).

The presence of *Fusarium* spp. is not mentioned in marine aquatic research lit-

erature concerning the study of the Fungi Imperfecti (1,17,38). *F. oxysporum*, *F. solani*, and *F. semitectum* have been isolated from the rhizosphere soil of mangrove (47,48). Rebell (39) reported a high recovery of *F. solani* from calcareous beach sands in Florida and the Caribbean islands. *F. solani* commonly occurs in lesions sustained by loggerhead sea turtles (*Caretta caretta*; 7). More recently, several species of *Fusarium* have been described as causing diseases in marine animals (10,15,22,26,41).

Tello and Lacasa (57) studied the presence of species of *Fusarium* in uncultivated land, finding a high proportion of *F. solani* and *F. oxysporum*. These authors questioned the relationship between the isolated species (especially *F. oxysporum*) and those that produced diseases in crops surrounding the sampled uncultivated ground. Tello et al. (57,58) also studied species of *Fusarium* isolated from beach sands in Spain. They reported *F. oxysporum*, *F. solani*, *F. equiseti*, *F. acuminatum*, *F. chlamyosporum* var. *fuscum*, *F. reticulatum* var. *majus*, *F. moniliforme* (sensu lato), and *F. merismoides*. Recently, Nuñez et al. (34) recovered *F. oxysporum* and *F. equiseti* at 7.2, 9.0, and 27.0 m in depth and *F. acuminatum* at 27.0 m in depth in the bay of Almería, supporting the findings of Tello et al. (58) on the occurrence of *Fusarium* spp. in beach sand on the Mediterranean shore. This study, like others, did not address whether or not the *Fusarium* spp. isolated from this habitat were pathogenic to crops in the sampled areas.

The purpose of this study was to collect and identify the species of *Fusarium* present in the Andarax River and Andarax dry riverbeds of southeastern Spain. In addition, the distribution of *Fusarium* spp. at different depths in marine water along the Mediterranean shore of the southeastern Iberian Peninsula was ascertained.

MATERIALS AND METHODS

Andarax River and riverbed sampling. The Andarax River and one of its tributaries, the Isfalada River, which partially run across the mountainous areas of Granada, is the only water source in Almería province (Fig. 1), an arid region characterized by intensive agriculture in plastic greenhouses. Sampling was designed to discover whether the Andarax

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River contained *Fusarium* spp. at its source. In all, 2 samples (Riv1 and Riv2) were collected at the headwaters of the river basin (Fig. 2) and 13 samples (Riv3 to Riv16) from four different locations along 67 km of the river channel (Tabernas, Gador, Pechina, and N340) were obtained after significant rainfall events when the river flowed all the way to the sea. Riv17 and Riv18 samples (Fig. 2) were collected from stagnant water at the mouth of the river that could have originated from rain-induced overflow (hereafter referred to as freshet). However, the water also could have been sea water from the surf. All water samples were manually collected in 200-ml sterile plastic jars (Eurotubo Deltalab, Barcelona, Spain) that were not opened until placement in the water. Sampling times were December 2003 and March, June, and October 2004. After collecting, samples were taken to the laboratory for analysis within 24 to 48 h. Dates and locations of the samples are summarized in Table 1 and Figure 2.

Analysis of the water samples involved gently shaking each jar, transferring 1 ml of the water sample to a 9-cm petri dish containing 10 ml of selective medium cooled to 35°C, and gently agitating the mixture to assure fusion of the sample

water and agar. Thirty-two dishes per sample were set up in this way and randomly divided into four blocks of eight dishes. Plates were incubated for 10 days at laboratory temperature (25°C) under continuous fluorescent light.

In order to determine whether *Fusarium* spp. were present in the sandy riverbed mouth, dry soil samples (Dry1, Dry2, Dry3, Dry4, and Dry5) were collected here in September 2004, 75 days after the last day in which the channel had carried water to the sea. Analysis of soil samples from the Andarax riverbed consisted of drying the soil under aseptic conditions at room temperature (20 to 25°C), crushing, then sifting the sample through a 200- μ m sieve and adding 0.02 g of the sifted soil to a *Fusarium*-selective medium as described by Komada (27) and modified by Tello et al. (59).

In total, 18 water samples from the river and five soil samples from the dry riverbed were analyzed. Sixteen petri dishes per sample were used and divided into four blocks of four dishes. Plates were incubated for 10 days at laboratory temperature under continuous fluorescent light. The mean number of CFU per petri dish and the standard deviation of the mean was calculated for all *Fusarium* colonies and used as the basis for comparisons.

Coastal sea water sampling in the provinces of Almeria and Granada. In Almeria, 20 seawater samples were collected from the Andarax River delta (Table 1; Fig. 2), distributed along the intertidal zone on the beach to about 100 m away from the mean high tide line (mhtl) at depths of 0 m (Sea4 to Sea8), 2 m (Sea9 to Sea13), 4 m (Sea14 to Sea18), and 6 m (Sea19 to Sea23) (Fig. 2). Other samples (Sea1, Sea2, and Sea3) already had been collected 1 month earlier at different depths (Table 1) in the same location. Along the coast of Granada, three seawater samples (Sea24 to Sea26) were collected at different depths at the mouth of the Albuñol River. In this area, there is intense agricultural activity in greenhouses located adjacent to the beach. Soil samples were collected at distances of 50, 150, and 300 m from the mhtl (Table 1; Fig. 3). In the Cabo de Gata-Nijar Natural Parck where there is no fluvial channel mouth or agricultural activity, seven sea bed samples (Sea27 to Sea33) were collected at depths of approximately 4.5 m (Table 1; Fig. 2). Three of the samples (Sea27 to Sea29) were separated from one another by 150 m and also separated from the other four samples (Sea30 to Sea33) by about 1,500 m (Table 1; Fig. 2).



Fig. 1. Geographical location of Almeria and Granada provinces (southeastern Spain).

Sea water samples were analyzed following the same methodology as indicated for river water samples.

Maintenance and identification of *Fusarium* spp. The whole collection was maintained on potato dextrose agar (PDA) and Komada's media and stored at 4°C in the fungus collection of the Plant Production Department of the University of Almeria and in the Polytechnic University of Madrid. The identification procedures and taxonomic criteria of Nelson et al. (32) and Leslie and Summerell (28) were followed to assign isolates to the *Fusarium* spp. level.

Pathogenicity tests. In total, 41 isolates of *Fusarium* (Table 2) obtained during both river and marine water sampling were inoculated onto barley (*Hordeum vulgare* L.) cv. CCE6, melon (*Cucumis melo* L.) cv. Canary yellow, kohlrabi (*Brassica oleracea* L. var. *gongyloides*) cv. Nabilcol, and tomato (*Lycopersicon esculentum* Mill.) cv. Marmande. These species were chosen because they are the main herbaceous crops in Almeria and Granada provinces. Seed were first disinfected with sodium hypochlorite (active Cl₂ at 40 to 50 mg/liter) for 15 min, washed with sterilized water, then germinated in paper toweling over 3 to 7 days at room temperature (20 to 25°C).

Inoculation tests followed a modification of the technique proposed by Messiaen et al. (29). Inoculum for each isolate was prepared by growing the culture for 2 weeks in complete darkness on PDA plates kept at 25°C until the colony reached the edge of the dish. Plastic 350-ml greenhouse pots were filled to two-thirds capacity with disinfected (30 min at 120°C) vermiculite substrate (Agroalse S.L. Poligono Virgen de los Dolores, 220, no. 15; Valencia, Spain). A fungal colony was then scraped off a PDA plate and added to the surface of a pot; three pots were used per *Fusarium* isolate. Then, 10 pregerminated seeds (with a root length of 1 to 2 cm at the time of sowing) were placed into each pot and covered with a surface layer of 1 cm of disinfected vermiculite. Once the pot was full, it was watered until saturation, and then with 250 ml of water every 2 days. Care was taken to keep pot leachates from contaminating other pots by keeping inoculated pots with different isolates in different trays and removing excess water from the trays daily. A sterile agar control was included in the inoculation test for each species of *Fusarium* tested. Inoculated and control plants were kept in a growth chamber set at 25 to 28°C under a photoperiod of 16 h at 12,000 lux.

Plants in plots were rated every 5 days for percent emergence. After 20 days, plants were evaluated for the percent damping off (44) and pregerminated seed that did not emerge were uncovered and symptoms evaluated for root rot and seed rot (hereafter referred to as preemergence damping off). Reisolation and identification of *Fusarium* spp. were done on PDA medium for all plants and isolates. The experiment was repeated.

Statistical analysis of data. Analysis of variance on the isolates of *Fusarium* used for the inoculation test were carried out using STATGRAPHICS Plus 5.1 statistical package software (StatPoint, Inc., Herndon, VA). Data on percent damping off in test 1 and test 2 were not significantly different ($P = 0.05$), and were combined for analysis.

RESULTS

Analysis of water samples from the Andarax River. No species of *Fusarium* were recovered from the headwaters of the Andarax River. However, 18 water samples from the Andarax River yielded a total of 1,059 isolates of *Fusarium* (Fig. 2; Table 3). Of these, 351 isolates (33.1%) were identified as *F. oxysporum*, 292 (27.6%) as *F. solani*, 229 (21.6%) as *F. equiseti*, 78 (7.4%) as *F. culmorum*, 52 (4.9%) as *F.*

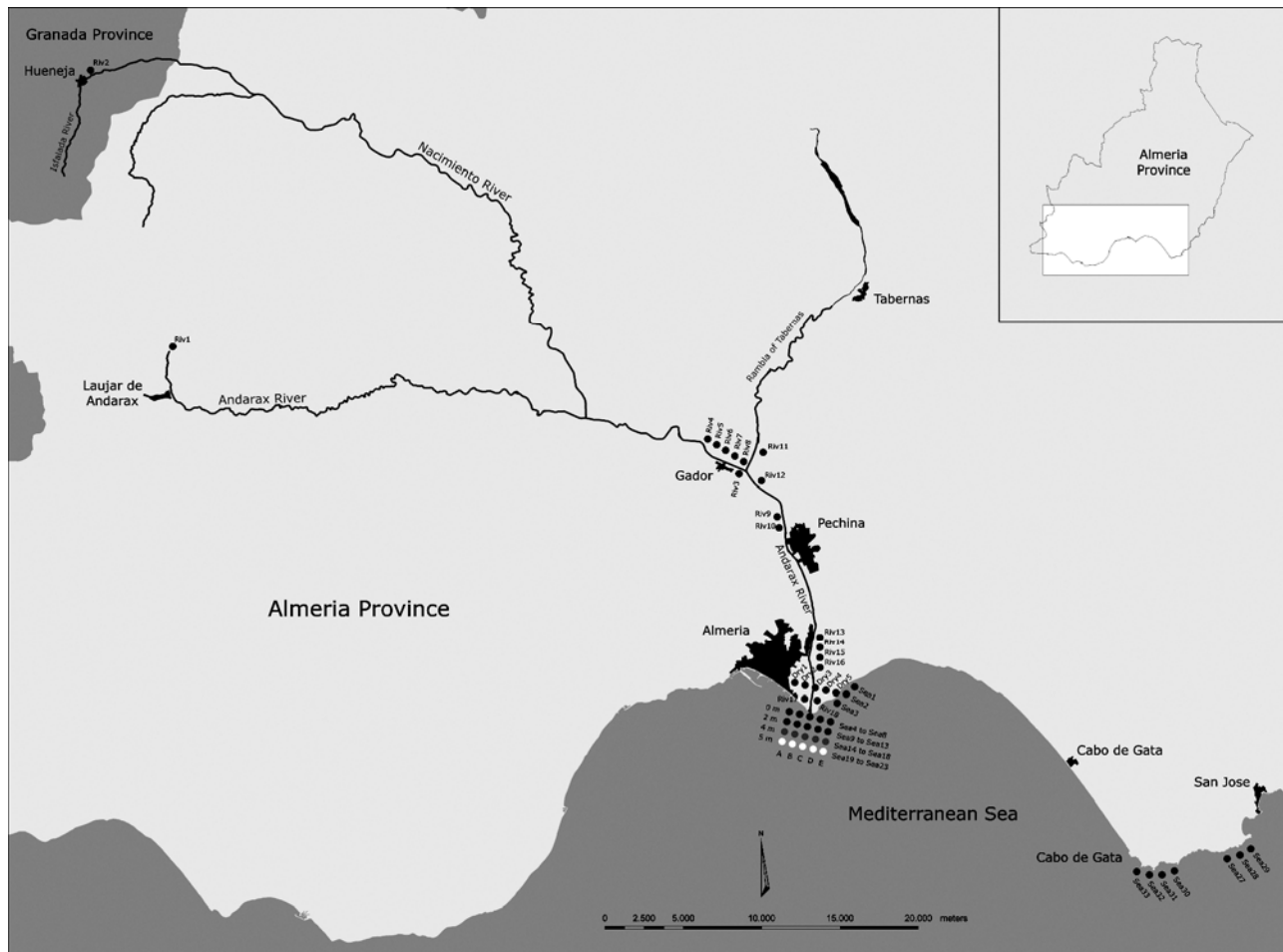


Fig. 2. Location of water samples collected from the Andarax and Isfalada Rivers and marine waters from the coast of Almería in southeastern Spain.

chlamyosporum, 19 (1.8%) as *F. verticillioides*, 16 (1.5%) as *F. acuminatum*, 14 (1.4%) as *F. sambucinum*, 6 (0.6%) as *F. proliferatum*, and 2 (0.2%) as *F. anthophilum* (sensu Nelson et al. [32] and Gerlach and Nirenberg [18]). No *Fusarium* spp. were isolated at the headwaters of the river basin (Riv1 and Riv2).

Samples Riv17 and Riv18 were collected after 75 days of drought, during which time the channel remained dry (Ta-

ble 3). From these samples, four isolates were assigned to *F. equiseti*, two isolates to *F. oxysporum*, and one to *F. verticillioides*. No *Fusarium* spp. were isolated from the dry riverbed samples (Dry1 to Dry5).

Coastal water sampling. Analysis of water samples from Mediterranean coastal areas along the mouth of the Andarax River (Fig. 2) (Sea1 to Sea3) showed the presence of *F. solani*. When samples were collected at depths greater than 1.5 m, *F.*

oxysporum, *F. equiseti*, and *F. verticillioides* were also recovered (Table 3). In a larger sampling made 78 days after the last freshet from the river to the sea (Sea4 to Sea23), 15 isolates in total were identified as *F. equiseti*. Results showed the absence of *F. oxysporum* and presence of *F. solani* and *F. equiseti* in 6 and 9 samples, respectively, out of a total of 20 analyzed samples.

In the analysis of water samples from the Mediterranean Sea taken at the mouth

Table 1. Date, code, location, and coordinates (European Datum 1950 UTM zone 30°N) of water and dry riverbed samples from the Andarax River and marine water samples from the coast of Almeria and Granada

Origin, date	Analysis code	Geographic location	Depth (m) ^a	Coordinates	
				X	Y
River samples					
07/03/2004	Riv1	Source of Andarax River (Laujar)	NA	510115	4097286
07/03/2004	Riv2	Isfalada River, tributary of Andarax	NA	504864	4114756
12/22/2003	Riv3	Gador	NA	545687	4090006
	Riv4	Gador	NA	545098	4090435
3/25/2004	Riv5	Gador	NA	545284	4090359
	Riv6	Gador	NA	545472	4090282
6/22/2004	Riv7	Gador	NA	545661	4090201
	Riv8	Gador	NA	545849	4090122
	Riv9	Pechina	NA	549372	4086274
	Riv10	Pechina	NA	549437	4085880
10/09/2004	Riv11	Rambla of Tabernas	NA	574058	4090020
	Riv12	Rambla of Tabernas	NA	546940	4089634
	Riv13	Highway N340	NA	551059	4074998
	Riv14	Highway N340	NA	551047	4074797
12/22/2003	Riv15	Highway N340	NA	551041	4074596
	Riv16	Highway N340	NA	551024	4074396
	Riv17	Stagnant water at the mouth of the Andarax river	0.10	550876	4074383
	Riv18	Stagnant water at the mouth of the Andarax river	0.10	550927	4074371
09/05/2004	Dry1	Dry riverbed at the mouth of the Andarax river	NA	550876	4077383
	Dry2	Dry riverbed at the mouth of the Andarax river	NA	550927	4074371
	Dry3	Dry riverbed at the mouth of the Andarax river	NA	550981	4074359
	Dry4	Dry riverbed at the mouth of the Andarax river	NA	551039	4074345
	Dry5	Dry riverbed at the mouth of the Andarax river	NA	551092	4074334
Sea samples					
07/11/2004	Sea1	Mouth of Andarax Riverbed	0.10	551169	4074231
	Sea2	Mouth of Andarax Riverbed	1.50	551238	4074229
	Sea3	Mouth of Andarax Riverbed	4	551310	4074247
08/09/2004	Sea4	Border of the sea	0.10	550856	4074317
	Sea5	Border of the sea	0.10	550908	4074291
	Sea6	Border of the sea	0.10	550961	4074271
	Sea7	Border of the sea	0.10	551025	4074264
	Sea8	Border of the sea	0.10	551079	4074239
	Sea9	Sea bed	2	550815	4074176
	Sea10	Sea bed	2	550868	4074145
	Sea11	Sea bed	2	550925	4074126
	Sea12	Sea bed	2	550987	4074116
	Sea13	Sea bed	2	551047	4074088
	Sea14	Sea bed	4	550771	4074032
	Sea15	Sea bed	4	550826	4074000
	Sea16	Sea bed	4	550886	4073982
	Sea17	Sea bed	4	550951	4073965
	Sea18	Sea bed	4	551012	4073937
	Sea19	Sea bed	6	550729	4073888
	Sea20	Sea bed	6	550786	4073856
Sea21	Sea bed	6	550844	4073833	
Sea22	Sea bed	6	550913	4073823	
Sea23	Sea bed	6	550976	4073798	
07/11/2004	Sea24	Mouth of Albuñol Riverbed	4.50	485881	4066493
	Sea25	Mouth of Albuñol Riverbed	1.50	485772	4066598
	Sea26	Mouth of Albuñol Riverbed	0.50	485663	4066703
11/17/2004	Sea27	Under the "Cabo de Gata" lighthouse	4.50	577695	4065379
	Sea28	Under the "Cabo de Gata" lighthouse	4.50	578141	4065526
	Sea29	Under the "Cabo de Gata" lighthouse	4.50	578619	4065573
	Sea30	"Cabo de gata"	4.50	572024	4064268
	Sea31	"Cabo de gata"	4.50	572322	4064225
	Sea32	"Cabo de gata"	4.50	572643	4064257
	Sea33	"Cabo de gata"	4.50	573207	4064287

^a NA = not applicable.

of the Albuñol River (Sea24 to Sea26), five isolates were identified as *F. solani*, four isolates as *F. oxysporum*, and two isolates as *F. equiseti*. When samples were collected at depths greater than 1.5 m, five isolates were identified as *F. verticillioides* and two as *F. proliferatum*. *F. oxysporum* and *F. solani* were also isolated from a depth of 4.5 m (Fig. 2).

Pathogenicity test. Barley was the most susceptible host; 61.0% of the isolates caused preemergence damping off on the seedlings. Melon followed in susceptibility, with 40.8% of the isolates caused preemergence damping off on the seedlings. On kohlrabi, 39.4% of the tested isolates were pathogenic. Tomato was least susceptible, with 36.6% of the tested isolates causing preemergence damping off. Post-emergence damping off was caused by 73.2% of the isolates inoculated on melon, 41.5% on barley, and 14.6 and 19.5% on kohlrabi and tomato, respectively.

F. acuminatum caused seed coat decay and root rot on barley and kohlrabi seedlings but did not cause symptoms on seedlings once they emerged (Table 4). In the case of melon and tomato, only one isolate of *F. acuminatum* produced a reaction on the emergence of pregerminated seed but both isolates caused significant death of seedlings once they emerged.

None of the three isolates of *F. chlamydosporum* were pathogenic on barley. On kohlrabi, only one isolate caused significant decrease in seedling emergence. On melon, one isolate of *F. chlamydosporum* caused severe post-emergence damping off.

Most of the isolates of *F. culmorum* (eight of nine) caused pronounced damping off on barley post emergence. Conversely, kohlrabi seedlings were affected

by *F. culmorum* causing damping off prior to emergence. Four isolates of *F. culmorum* were pathogenic on melon. On tomato, only one isolate was pathogenic after emergence.

Barley and kohlrabi seedlings were affected by *F. equiseti* causing damping off during preemergence but it did not cause symptoms once seedlings emerged. Only one isolate of *F. equiseti* caused preemergence damping off on melon but four isolates were pathogenic after emergence. On tomato, three isolates caused damping off in preemergence.

Only one *F. verticillioides* isolate produced significant decrease in seedling emergence on barley. No pathogenicity was detected on kohlrabi either pre- or post emergence. On melon, one isolate reduced emergence significantly and two of three isolates caused damping off after emergence. On tomato, one isolate of *F. verticillioides* significantly reduced emergence but all three isolates caused significant damping off post emergence.

None of the two isolates of *F. oxysporum* were pathogenic on barley and

kohlrabi. On melon, *F. oxysporum* isolates were pathogenic after emergence. On tomato, one of the isolates reduced emergence significantly. Seed and root rot were observed in not-emerged seedlings.

Four isolates of *F. proliferatum* produced an extensive preemergence damping off on barley but none of the isolates produced symptoms after emergence. Three isolates produced a significant decline in emergence on kohlrabi but did not cause symptoms once seedlings emerged. On melon, two isolates of *F. proliferatum* reduced emergence but they were slightly pathogenic after emergence. Those isolates that drastically reduced emergence in barley also did so on tomato, and two more strains of *F. proliferatum* slightly reduced emergence on tomato seedlings. Results showed no pathogenicity on tomato post emergence.

No isolates of *F. solani* were pathogenic on barley. Only one *F. solani* isolate produced significant decrease in seedling emergence on kohlrabi. All the isolates of *F. solani* caused damping off on melon seedlings during preemergence but did not

Table 2. Origin of the isolates of species of *Fusarium* used in greenhouse seedling inoculation studies

Sample code	Code of isolate	Sample and origin of the isolate	Species of <i>Fusarium</i>
Riv5	Fac1–Fac2	Andarax River water	<i>F. acuminatum</i>
Riv5	Fch11–Fch13	Andarax River water	<i>F. chlamydosporum</i>
Riv5	Fcu1–Fcu9	Andarax River water	<i>F. culmorum</i>
Sea3	Feq1–Feq2	Sea water mouth of the Andarax River	<i>F. equiseti</i>
Riv15	Feq3–Feq7	Andarax River water	<i>F. equiseti</i>
Sea25	Fox1–Fox2	Sea water. Mouth of the Albuñol River	<i>F. oxysporum</i>
Sea24	Fver1	Sea water. Mouth of the Albuñol River	<i>F. verticillioides</i>
Sea3	Fver2–Fver3	Sea water. Mouth of the Andarax River	<i>F. verticillioides</i>
Sea24	Fpro1–Fpro2	Sea water. Mouth of the Albuñol River	<i>F. proliferatum</i>
Riv5	Fpro3	Andarax River water	<i>F. proliferatum</i>
Sea24	Fpro4–Fpro5	Sea water. Mouth of the Albuñol River	<i>F. proliferatum</i>
Riv13	Fcu10–Fcu11	Andarax River water	<i>F. sambucinum</i>
Sea3	Fso1–Fso8	Sea water. Mouth of the Andarax River	<i>F. solani</i>

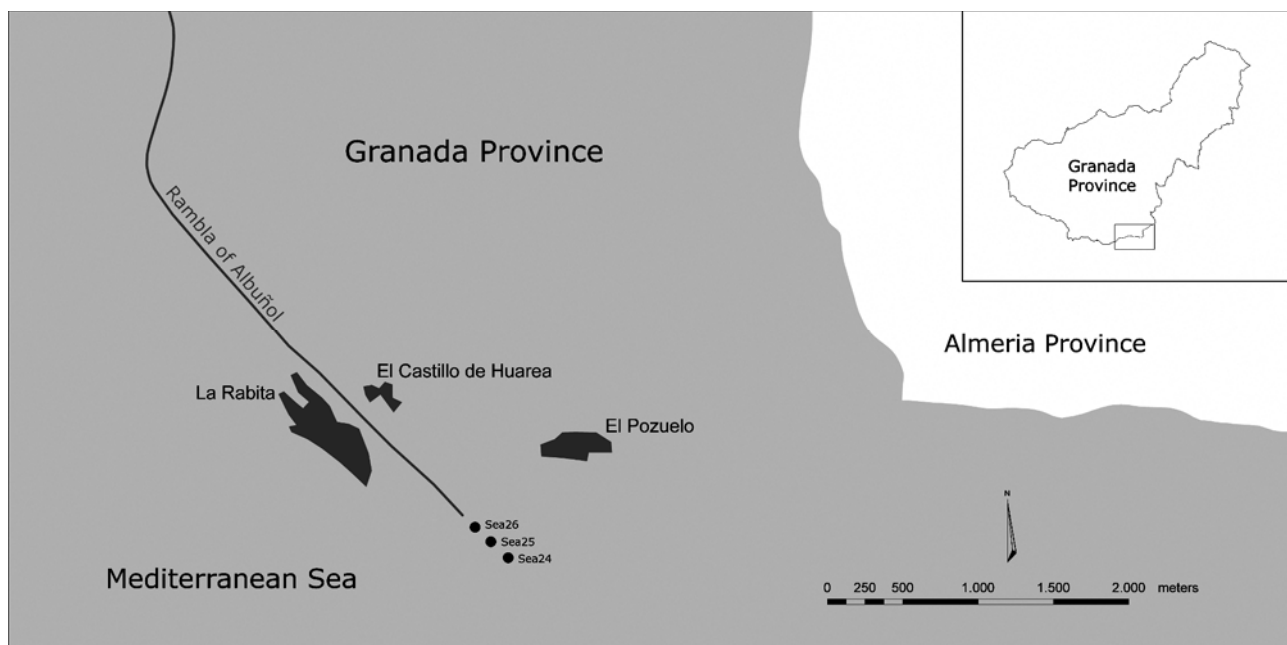


Fig. 3. Location of marine water samples collected from the coast of Granada in southeastern Spain.

cause symptoms once seedlings emerged. On tomato, two isolates showed significant effects in emergence and four isolates caused significant damping off post emergence.

Two isolates of *F. sambucinum* caused pronounced damping off on barley post emergence. Neither of the two isolates of *F. sambucinum* were pathogenic on kohlrabi, tomato, and melon.

Sterile agar controls developed no symptoms on seedlings. The respective *Fusarium* isolates were recovered and identified from all infected, inoculated plants.

DISCUSSION

In total 1,114 isolates and 10 different species of *Fusarium* obtained from 51 samples of river and marine water collected in southeastern Spain were identi-

fied. Of these, 95.06% originated from river water, and 77.8% of the river water samples yielded at least one species of *Fusarium*, with *F. oxysporum* comprising 72.2% of the total isolates. No isolates of *Fusarium* were obtained at the headwaters of the river basin. Most of the isolates were obtained from samples at Gador during two different freshets occurring in March and June 2004 comprising 12.7 and 0.3 mm of total precipitation, respectively, and also from the mouth of the river in October 2004 with 1.5 mm (precipitation data provided by meteorological station no. 84870-LEAN); however, no seasonal or geographic pattern of *Fusarium* distribution was discernable.

The presence of *Fusarium* spp. 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 crops were being grown.

Samples Riv17 and Riv18 were collected in areas very close to the line separating the sea from the river channel. These samples possibly represented water carried by either the river or the sea. Most remarkable was the absence of any species of *Fusarium* in riverbed soil at the point where river water enters the sea. This finding supports the hypothesis that these species which are carried by river water do not originate from dry riverbeds but, rather, from the run-off of cultivated fields adjacent to the riverbanks.

During this study, 4.9% of isolates were collected from sea water. In the case of marine water, 45.5% of the samples yielded at least one species of *Fusarium*, with *F. solani* comprising 36.3% of the

Table 3. Presence of species of *Fusarium* in river water and dry riverbed samples obtained from the Andarax River and in marine water samples obtained at the mouth of the Andarax and Albuñol Rivers in southeastern Spain

Origin ^b	<i>Fusarium</i> spp. recovered ^a									
	<i>F. oxy</i>	<i>F. sol</i>	<i>F. vert</i>	<i>F. pro</i>	<i>F. anth</i>	<i>F. cul</i>	<i>F. samb</i>	<i>F. chlmy</i>	<i>F. acum</i>	<i>F. eq</i>
River										
Riv1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Riv2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Riv3	0 ± 0	0 ± 0	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Riv4	14 ± 8	2 ± 1	1 ± 1	1 ± 1	0 ± 0	6 ± 2	0 ± 0	0 ± 0	2 ± 1	8 ± 3
Riv5	64 ± 8	3 ± 1	5 ± 4	2 ± 2	1 ± 1	11 ± 3	2 ± 1	6 ± 3	3 ± 2	44 ± 7
Riv6	66 ± 23	6 ± 6	5 ± 2	0 ± 0	0 ± 0	9 ± 3	1 ± 1	2 ± 1	0 ± 0	5 ± 2
Riv7	42 ± 18	1 ± 2	1 ± 1	0 ± 0	0 ± 0	9 ± 4	0 ± 0	1 ± 1	0 ± 0	3 ± 1
Riv8	37 ± 8	2 ± 2	1 ± 1	1 ± 1	0 ± 0	6 ± 2	0 ± 0	2 ± 1	2 ± 1	3 ± 2
Riv9	2 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1	1 ± 1
Riv10	0 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Riv11	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 1
Riv12	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 1
Riv13	8 ± 4	2 ± 1	1 ± 1	1 ± 1	0 ± 0	21 ± 5	2 ± 2	12 ± 3	3 ± 2	30 ± 6
Riv14	48 ± 12	81 ± 16	0 ± 0	0 ± 0	0 ± 0	12 ± 4	3 ± 2	9 ± 3	5 ± 3	36 ± 7
Riv15	37 ± 16	105 ± 22	0 ± 0	1 ± 2	0 ± 0	0 ± 0	0 ± 0	13 ± 3	0 ± 0	53 ± 11
Riv16	35 ± 15	91 ± 26	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	7 ± 2	0 ± 0	47 ± 8
Riv17	1 ± 1	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 1
Riv18	1 ± 1	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 1
Dry1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dry2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dry3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dry4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dry5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sea										
Sea1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sea2	0 ± 0	1 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sea3	2 ± 3	15 ± 4	1 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 2
Sea4	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Sea8	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sea9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Sea13	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Sea14	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 2
Sea15	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Sea18	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sea19	0 ± 0	1 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sea20	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 2
Sea21	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 1
Sea22	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Sea23	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 2
Sea24	1 ± 3	1 ± 3	5 ± 4	4 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sea25	3 ± 3	3 ± 6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 3
Sea26	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

^a *F. oxy*: *Fusarium oxysporum*; *F. sol*: *F. solani*; *F. vert*: *F. verticillioides*; *F. pro*: *F. proliferatum*; *F. anth*: *F. anthropilum*; *F. cul*: *F. culmorum*; *F. samb*: *F. sambucinum*; *F. chlmy*: *F. chlamydosporum*; *F. acum*: *F. acuminatum*; and *F. eq*: *F. equiseti*.

^b Origin and sampling code for river and sea samples. Presence of species of *Fusarium* expressed in number of colonies per milliliter (UFC/ml of water) for Riv and Sea samples and number of colonies per gram (UFC/g of dry soil) for Dry samples, followed by the average standard deviation.

total isolates. In sea water, *F. oxysporum*, *F. solani*, *F. equiseti*, *F. proliferatum*, and *F. verticillioides* were recovered at a depth of 4 m near the Andarax River delta 15 days after the last freshet. If we assume that river water was the only influx of *Fusarium* spp., this indicates that these species came from the river and were able to survive in saline water during that period.

In a larger sampling made 78 days after the last freshet from the river to the sea, *F. solani* was found in the surface sample. *F. equiseti* was present at depths of 2 and 4 m and *F. solani* and *F. equiseti* were present at 6 m. Of the species isolated from water gathered 15 days after the last freshet, *F. solani* and *F. equiseti* appeared again 78 days later. Such results suggest that these species might be able to remain alive in a saline aquatic medium for at least 2 months.

These results agree partly with those found by Tello et al. (58) regarding beach sands from the coast of Spain, with the difference that the former authors found *F. merismoides* and *F. reticulatum* var. *majus* in their samples, which were not identified in our study. The former study did not find *F. culmorum*, *F. sambucinum*, and *F. proliferatum*, which were obtained in our study. Our findings also agree with some of the species found by Núñez et al. (34) for coastal waters of the Mediterranean Sea at depths of 27.9 and 7.2 m: *F. oxysporum*, *F. equiseti*, and *F. acuminatum*.

In the samples collected at the mouth of the Albuñol River, *F. oxysporum* and *F. solani* were isolated from depths of 1.5 and 4.5 m, *F. equiseti* at 1.5 m, and *F. verticillioides* and *F. proliferatum* at 4.5 m. Both sampling areas are close to antropic areas with intense agricultural activity where marine winds are frequent and strong. These winds carry dust particles that can fall on the surface of the sea, which could also explain the presence of *Fusarium* spp. in the sea samples. Tello and Lacasa (54) showed that, on a 8.75-m² surface exposed to wind south of the Canary Islands, 12,598 UFC of *F. solani* and 8,712 UFC of *F. verticillioides* were recovered. In this sense, Burgess (5) wrote that, although the traditional ecological categories, soilborne

and airborne, can be applied to the majority of *Fusarium* spp., evidence indicates that some populations can colonize substrates outside their accepted habitat. Based on our results, it is likely that the aquatic media under study do consistently harbor various *Fusarium* spp.

Certainly the populations we report are small compared with what is common in agricultural soils in the area (*F. oxysporum* at 2,602 UFC/g, *F. solani* at 1,759 UFC/g, and *F. equiseti* at 221 UFC/g on carnation crops; or *F. oxysporum* at 53 UFC/g of dry soil and *F. equiseti* at 131 UFC/g of dry soil on tomato crops) (53,54), but they could be comparable with soils found in pine groves (54) and to beach sand (34,58). Supposing that these species came only from the water flowing from the river to the sea, the first sampling was collected 19 days and the second 78 days, respectively, after the last freshet. The fact that the river might be the source of species of *Fusarium* in sea water can be supported by the absence of *Fusarium* spp. in seven sea water samples collected at Cabo de Gata, located in the Cabo de Gata—Nijar Natural Park, where there are no channel mouths or agricultural activities. In these samples, no species of *Fusarium* were isolated.

The study of the pathogenicity of 41 isolates from nine species of *Fusarium* showed that most of the isolates caused preemergence damping off on the seedlings.

The least pathogenic species of *Fusarium* were *F. chlamydosporum* and *F. sambucinum*, both prior to and after emergence. All the isolates within the two species used in the inoculation test were obtained from river water.

F. chlamydosporum could be considered a saprophyte, although Elmer (13) isolated it in association with asparagus root and crown disease and moderate pathogenicity has been reported in pea seedlings (18). *F. sambucinum* has been described as a cause of root rot and storage rot. It has also been reported attacking cereal seedlings (barley, maize, and oat), forest trees (3), potato (25), lupin, tomato, and strawberry (4).

The most pathogenic species on barley were *F. culmorum* and *F. equiseti*, both

pre- and post emergence. All the *F. culmorum* isolates were obtained from river water whereas most of the inoculated isolates of *F. equiseti* (five of seven) were obtained from sea water. Booth (4) observed that *F. culmorum* can cause serious damage to cereals, including wheat, rye, barley, oat, and maize. *F. culmorum* has been associated with head blight of wheat and barley (12,16,46) and it is considered to be the cause of Fusarium foot rot disease in cereals (6). Analytical results are consistent with these; isolates of *F. culmorum* from river water caused pronounced damping off on barley. In addition, *F. culmorum* has been mentioned as the source of diseases in other crops (3,13,31).

F. equiseti can be found worldwide (28,32). Messiaen and Casini (29) considered it to be typically soilborne. *F. equiseti* has been isolated from 17 plant species. It causes stem rot in maize and root rot in winter wheat (4). Isolates of *F. equiseti* were pathogenic on melon and tomato; these results agree with Joffe and Palti (24), who reported that *F. equiseti* is pathogenic on cucurbits. Conversely, Gerlach and Nirenberg (18) believe *F. equiseti* to be only mildly parasitic and of minor economic importance. *F. equiseti* pathogenicity did not seem to be related to the origin of the isolates (sea water or fresh water).

F. proliferatum showed pathogenicity on all four plant species prior to emergence. According to Gerlach and Nirenberg (18), *F. proliferatum* var. *proliferatum* occurs in rice, fig, and orchid and it is responsible for foot rots, fruit rots, and leaf spot. It is a major pathogen in Fusarium crown and root rot diseases in asparagus plants (11,13). As in the case of *F. equiseti*, pathogenicity did not seem to be related to the origin of the isolates.

F. solani showed pathogenicity on tree plant species prior to emergence. *F. solani* was not pathogenic on barley but it caused significant decrease in seedling emergence on melon. *F. solani* has been described as a pathogen of a large number of plant species, including trees (33), legumes (20), vegetables (42), potato (45), and other tropical plants (37).

Table 4. Incidence of damping off on locally grown plant species inoculated with isolates of *Fusarium* recovered from river and sea water of south-eastern Spain^a

<i>Fusarium</i> spp.	No. ^b	Barley		Kohlrabi		Melon		Tomato	
		Emerg. (%)	Survival (%)	Emerg. (%)	Survival (%)	Emerg. (%)	Survival (%)	Emerg. (%)	Survival (%)
<i>F. acuminatum</i>	2	45.2 ± 16.82	42.85 ± 13.50	28.85 ± 32.59	28.85 ± 32.59	90.0 ± 14.14	36.7 ± 0.0	65.3 ± 40.44	55.1 ± 34.64
<i>F. chlamydosporum</i>	3	77.76 ± 11.02	74.6 ± 5.54	94.9 ± 5.54	94.9 ± 5.54	84.46 ± 3.86	64.43 ± 9.64	110.2 ± 2.0	103.56 ± 8.02
<i>F. culmorum</i>	9	42.33 ± 20.94	14.27 ± 9.50	71.73 ± 18.79	72.01 ± 18.85	89.62 ± 9.04	72.23 ± 12.56	97.05 ± 9.63	94.11 ± 11.70
<i>F. equiseti</i>	7	34.01 ± 19.12	33.32 ± 19.04	45.71 ± 25.81	45.87 ± 25.92	89.04 ± 6.30	56.67 ± 14.79	76.98 ± 14.32	74.34 ± 12.91
<i>F. oxysporum</i>	2	54.75 ± 10.11	52.35 ± 6.71	70.85 ± 5.44	71.15 ± 5.44	93.35 ± 4.73	65.0 ± 2.40	78.55 ± 4.31	74.5 ± 4.38
<i>F. proliferatum</i>	5	33.32 ± 32.63	33.32 ± 32.63	63.08 ± 35.57	63.08 ± 35.57	88.0 ± 7.67	78.66 ± 9.02	39.74 ± 44.48	42.46 ± 45.99
<i>F. sambucinum</i>	2	57.15 ± 6.71	16.65 ± 10.11	97.7 ± 0.0	98.1 ± 0.0	88.35 ± 2.33	66.7 ± 14.14	100.0 ± 8.62	95.9 ± 14.42
<i>F. solani</i>	8	100 ± 0.0	100 ± 0.0	78.75 ± 20.66	75.63 ± 21.45	18.13 ± 9.58	16.89 ± 9.62	80.41 ± 27.45	70.0 ± 37.92
<i>F. verticillioides</i>	3	55.56 ± 10.96	55.56 ± 10.96	90.3 ± 16.80	90.4 ± 16.62	86.66 ± 13.35	66.66 ± 21.84	77.56 ± 18.73	49.66 ± 11.63

^a Greenhouse inoculation studies were repeated; Emerg. = emergence; value ± standard deviation.

^b Number of isolates tested.

Isolates of *F. oxysporum* were not pathogenic to barley or kohlrabi and only slightly pathogenic on melon and tomato. *F. oxysporum* is distributed worldwide and is extremely common in a wide range of soils (28,32). *F. oxysporum* is the causal agent of vascular wilts, damping off, and rots on hundreds of different host plants (18) but its pathogenic specialization can be very limited (i.e., formae speciales and races causing wilt diseases). Research has also highlighted specialized forms that induce foot and root rot disease in plants (23,61). Our results suggest that the acquired isolates were slightly pathogenic.

F. acuminatum affected barley and kohlrabi seedlings during emergence and melon and tomato once they emerged. *F. acuminatum* has been reported as causing stem rot in maize. It is also widespread as a foot and root rot of legumes and crown rot in lucerne (4). Elmer (13) described it as a saprophyte in asparagus and Sanders and Cole (43) associated it with *Fusarium* head blight of wheat and barley.

F. verticillioides isolates caused damping off on barley, melon, and tomato. *F. verticillioides* is a major parasite of several members of Gramineae, including rice, sugar cane, maize, and sorghum. Additionally, *F. verticillioides* may cause diseases such as seedling blights, scorch, foot rot, stunting, and hypertrophy in 31 plant families (4,18), including asparagus (11,13,51) and forest tree nurseries (3). As for other species, *F. verticillioides* pathogenicity did not seem to be related to the origin of the isolates (sea water or fresh water).

This work has revealed some epidemiological information about the genus *Fusarium* in natural environments. Ten species of *Fusarium* were isolated from river water. Some of the *Fusarium* spp. studied are potential mycotoxin producers which exhibit high solubility. The ecotoxicological effects of the presence of mycotoxins in surface waters remain to be elucidated.

Five species of *Fusarium* were isolated from sea water samples from the Mediterranean Sea 78 days after the last freshet from the river to the sea. There were no algae or vegetation in the sampled area. Our results suggest that the acquired isolates could probably survive in the aquatic habitats as saprophytes. This finding also supports previous hypothesis about the ability to grow in saline media as an adaptive advantage for *Fusarium* spp. in warm, saline soils (35,36). The increased use of saline water or water coming from desalination plants for crop irrigation in the studied areas underlines the importance of the research.

The results provide information on the biology and distribution of *Fusarium* spp. and could indicate that pathogenic isolates of *Fusarium* might be dispersed long distances in water.

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