Fungal microbiota from rain water and pathogenicity of *Fusarium* species isolated from atmospheric dust and rainfall dust

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Abstract In order to determine the presence of *Fusarium* spp. in atmospheric dust and rainfall dust, samples were collected during September 2007, and July, August, and October 2008. The results reveal the prevalence of airborne *Fusarium* species coming from the atmosphere of the South East coast of Spain. Five different *Fusarium* species were isolated from the settling dust: *Fusarium oxysporum*, *F. solani*, *F. equiseti*, *F. dimerum*, and *F. proliferatum*. Moreover, rainwater samples were obtained during significant rainfall events in January and February 2009. Using the dilution-plate method, 12 fungal genera were identified from these rainwater samples. Specific analyses of the rainwater revealed the presence of three species of *Fusarium*: *F. oxysporum*, *F. proliferatum* and *F. equiseti*. A total of 57 isolates of *Fusarium* spp. obtained from both rainwater and atmospheric rainfall dust sampling were inoculated onto melon (*Cucumis melo* L.) cv. Piñonet and tomato (*Lycopersicon esculentum* Mill.) cv. San Pedro. These species were chosen because they are the main herbaceous crops in Almeria province. The results presented in this work indicate strongly that spores or propagules of *Fusarium* are able to cross the continental barrier carried by winds from the Sahara (Africa) to crop or coastal lands in Europe. Results show differences in the pathogenicity of the isolates tested. Both hosts showed root rot when inoculated with different species of *Fusarium*, although fresh weight measurements did not bring any information about the pathogenicity. The findings presented above are strong indications that long-distance transmission of *Fusarium* propagules may occur. Diseases caused by species of *Fusarium* are common in these areas. They were in the past, and are still today, a problem for greenhouses crops in Almería, and many species have been listed as pathogens on agricultural crops in this region. Saharan air masses dominate the Mediterranean regions. The evidence of long distance dispersal of *Fusarium* spp. by atmospheric dust and rainwater together with their proved pathogenicity must be taken into account in epidemiological studies.

Keywords Airborne fungi · Aeromycobiota · *Fusarium*

Introduction

Most studies conducted on atmospheric dust have considered it to originate mainly in the Sahara and Sahel regions of Africa [11, 12]. Some authors consider the Sahara as the main source of sediments transported by dust to far away areas such as Caribbean Islands [25, 26], Russia, Germany or Great Britain [6]. The importance of these deposits (2 million metric tons of dust each year) can even influence the geological evolution of many soils [12]. But there are many aspects that need further investigation. One of these concerns the microbiological aspects of such large movements of dust and the role of rainfall in its deposition. Particulate microorganisms in air have been broadly studied [4, 12]. A statistical correlation between the presence of

desert dust and observed increases in the number of colony forming units (cfu) has also been reported [10, 12, 13]. Infectivity and respiratory tract penetration in humans has also been studied for pathogenic organisms [9]. The genus *Fusarium* has regularly been isolated in these works but no specific studies have been performed and frequently only the genus is indicated without species assignation [9, 15, 32]. *Fusarium* spp. propagules have been recovered from grain dust [8]. Evidence of long-distance transport in the atmosphere has been reported for *Gibberella zeae* (teleomorph of *Fusarium graminearum* and causal agent of *Fusarium* head blight of wheat and barley and *Giberrella* ear rot on maize). Atmospheric populations have been proposed as the origin of the inoculum causing epidemics of these diseases [8, 27]. Abdel-Hafez et al. [1] recovered 24 genera and 57 species of fungi from air dust samples in Egypt; 1.26% of the total were *Fusarium* spp. isolates. Some *F. oxysporum* and *F. solani* isolates were toxic to brine shrimp and some of them proved to be zearalenone producers. A total of 44 fungal genera and 102 species were recovered from airborne mycobiota from the Egyptian desert [16], of which only two species within the *Fusarium* genus were isolated (*F. dimerum* and *F. oxysporum*). *Fusarium* genus was recovered as only 0.3% of the overall sample.

Dispersal in aquatic systems has also been previously proved for *Fusarium culmorum* [29], and spread of *Fusarium* spp. via irrigation water has been described for some species causing plant diseases in greenhouses [7]. Wind and rain dispersal of *Fusarium moniliforme* have also been studied in corn fields [22]. But research focussing on the occurrence of this genus in aquatic habitats is not common, perhaps because *Fusarium* spp. are generally considered to be soil-borne fungi. Tello and Lacasa [30] and Palmero et al. [23] studied the presence of species of *Fusarium* in uncultivated land, finding a high proportion of *F. solani* and *F. oxysporum*; however, the authors questioned the ability of the isolates (especially *F. oxysporum*) to cause diseases in plants cultivated close by.

The transport of atmospheric dust is a phenomenon with global character. From the microbiological point of view, its effects have been studied for certain genera, including several foliar pathogens such as rusts, powdery mildew, etc., but rarely for soil-borne pathogens [5]. This study attempts (1) to determine possible new sources of inoculum of *Fusarium* species by studying both rainwater and atmospheric settling dust collected in Almeria, Spain, and (2) to determine their pathogenicity by studying the effect of the recovered *Fusarium* strains on damping off in seedlings of the two main species cultivated in the area: tomato and melon.

Materials and methods

Almeria province is located on the Spanish Mediterranean coast at a mid-altitude position that is influenced by high-latitude and subtropical wind systems. It is an arid region characterised by intensive agriculture in plastic greenhouses.

Dust sampling

In order to determine whether *Fusarium* spp. are present in atmospheric dust and rainfall dust (downfall dust), samples were collected during September 2007, and July, August, and October 2008 in disinfected trays (0.192 and 0.322 m^2) (Table 1). Analysis of dust samples consisted of adding 0.02 g atmospheric dust to a *Fusarium* selective medium as described by Komada [17] and modified by Tello et al. [31]. Twenty Petri dishes per sample were used, and divided into four blocks of five 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. Downfall dust samples collected during rainfall events were analysed following the same methodology as indicated for atmospheric dust but samples were previously dried under aseptic conditions at room temperature (20–25°C).

Rain water sampling

Water samples were obtained during significant rainfall events in January and February 2009 (Table 1). All water samples were collected in plastic trays (0.322 m^2) and rain water transferred to a 200 ml sterile plastic jars (Eurotubo Deltalab: Plaza de la Verneda, 1, 08191 Rubí, Barcelona). Samples were analysed within 24 to 48 h after collecting them. Sampling dates are listed in Table 1.

Analysis of the fungal microbiota in water samples involved gently shaking each jar, transferring 1 ml of the rain water sample to a 9 cm Petri dish containing 10 ml non-selective medium (Malta agar), cooled to 35°C, then gently agitating the mixture to assure fusion of the sample water and agar. A total of 20 dishes per sample were set up in this way, and divided randomly into four blocks of 5 dishes. Plates were incubated for 10 days at laboratory temperature (25°C) under continuous fluorescent light. The mean cfu per Petri dish and the standard deviation of the mean were calculated for all colonies, and used as the basis for comparisons. Using this same methodology, specific analysis with a selective medium for *Fusarium* genus were conducted with the rainwater samples [17].

Table 1 Characteristics of rainfall dust and rain water samples collected in Almería (Spain) from 2007 to 2009

Sample code	Sampling date	Sample type	Exposed area	Rainfall (date and quantity)
Lluv1	11 September 2007	Rainfall dust	0.1924 m^2	10 September 2007 → 0.51 ml
				11 September 2007 → 1.02 ml
Ven 1	17 July 2008	Atmospheric dust	0.1924 m^2	No rain was recorded
	30 August 2008			
Ven 2	1 October 2008	Rainfall dust	0.322 m^2	9 October 2008 → 3.3 mm
AG.Fu	30 October 2008	Rainfall dust	0.322 m^2	10 October 2008 → 12.7 mm
				11 October 2008 → 0.25 mm
Lluv 2	5 January 2009	Rain water	0.322 m^2	5 January 2009 → 8.89 mm
	9 January 2009			7 January 2009→ 0.25 mm
				8 January 2009 → 1.27 mm
				9 January 2009 → 6.10 mm
Lluv 3	6 February 2009	Rain water	0.322 m^2	1 February 2009 → 0 mm
				2 February 2009 → 7.87 mm
				3 February 2009 → 2.29 mm
				4 February 2009 → 1.27 mm
				5 February 2009 → 2.54 mm
				6 February 2009 → 4.06 mm

Table 2 *Fusarium* species isolated from settling atmospheric dust and rainwater samples

Sample code	*Fusarium* species recovered[c]					
	F.oxy	*F.sol*	*F.eq*	*F. pro*	*F. dim*	*Acre*
Lluv[a]	41.31 ± 33.59	86.38 ± 43.23	2,054.46 ± 616.66	15.02 ± 24.48	0	0
Ven 1[a]	32.49 ± 27.38	57.71 ± 24.91	181.05 ± 132.71	4.64 ± 11.37	27.85 ± 35.23	0
Ven 2[a]	45.59 ± 60.02	55.72 ± 44.74	303.95 ± 54.37	10.13 ± 15.69	15.19 ± 25.43	0
AG.Fu[a]	24.87 ± 49.75	1,305.97 ± 522.38	1,990.04 ± 495.85	2,599.50 ± 907.76	87.06 ± 47.63	746.26 ± 211.07
Lluv 2[b]	0.15 ± 0.36	0	2.55 ± 2.45	0	0	2.35 ± 2.75
Lluv 3[b]	0.13 ± 0.34	0	0.23 ± 0.50	0.93 ± 1.17	0	0

[a] Presence of species of *Fusarium* expressed in number of colonies per gram (cfu g^{-1} dry dust) followed by the average standard deviation

[b] Presence of species of *Fusarium* expressed in number of colonies per millilitre (cfu ml^{-1} water) followed by the average standard deviation

[c] *F. oxy Fusarium oxysporum*, *F.sol F. solani*, *F.pro F. proliferatum*, *F.dim F. dimerum*, *F.eq F. equiseti*, *Acre Acremonium*

Maintenance and identification of fungal colonies

The entire collection of *Fusarium* isolates 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 Technical University of Madrid. The identification procedures and the taxonomic criteria of Barnet and Hunter [2] within the division of Deuteromycetes, and those of Nelson et al. [21] and Leslie and Summerell [18] within the *Fusarium* genus were followed to assign isolates to species level.

Pathogenicity tests

A total of 57 isolates of *Fusarium* obtained during both rainwater and atmospheric rainfall dust sampling were inoculated onto melon (*Cucumis melo* L.) cv. Piñonet and tomato (*Lycopersicon esculentum* Mill.) cv. San Pedro.

Inoculation tests were conducted following a modification of the technique proposed by Messiaen et al. [19] used previously for pathogenicity testing of *Fusarium* isolates from fluvial water [24]. 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, Moncada, 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. Seeds were first disinfected with sodium hypochlorite (active chlorine 4–5%) for 15 min, then washed with sterile water. Thereafter, ten germinating seeds (with a root length of 1–2 cm at the time

Table 3 Fungal aeromicrobiota isolated from rainwater in Almería (Spain) expressed in colony forming units per millilitre (cfu ml^{-1}) water followed by the average standard deviation

Morphological ID for Fungi	January 2009 cfu ml^{-1}	February 2009 cfu ml^{-1}
Acremonium sp.	6.9 ± 7.56	0.06 ± 0.36
Aspergillius spp.	0.1 ± 0.30	0.03 ± 0.18
Alternaria sp.	1.8 ± 1.64	0.03 ± 0.18
Aureobasidium sp.	0.15 ± 0.36	8.5 ± 8.45
Beauveria bassiana	0.9 ± 1.68	0 ± 0.00
Botrytis cinerea	0.4 ± 0.59	0.06 ± 0.25
Cladosporium sp.	11.45 ± 3.77	0.76 ± 1.04
Epicoccum sp.	0.1 ± 0.30	0 ± 0.00
Fusarium sp.	0.4 ± 0.75	0 ± 0.00
F. oxysporum	0.15 ± 0.67	0.16 ± 0.37
F. proliferatum	0.9 ± 1.61	0.96 ± 1.54
F. equiseti	0.5 ± 0.68	0 ± 0.00
Penicillium spp.	0.7 ± 0.97	0.13 ± 0.34
Phoma sp.	0.3 ± 0.47	0.1 ± 0.40
Rhizopus sp.	0.15 ± 0.36	0 ± 0.00
Sthemphyllium sp.	0 ± 0.00	0.03 ± 0.18
Unidentified	0.15 ± 0.67	0.4 ± 0.56

of sowing, which takes 6 days for melon and 5 days for tomato) were placed in each pot, and covered with 1 cm layer of disinfected vermiculite. Once the pot was full, it was watered to saturation, and then with 250 ml water every 2 days. Care was taken to prevent 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 24–27°C under a photoperiod of 16 h at 12,000 lx.

Plants in plots were rated every 5 days for percent emergence. After 20 days, plants were evaluated for the percentage of damping off [28] and non-emerged germinating seeds were uncovering and symptoms observed. Re-isolation and identification of *Fusarium* species was done on PDA medium for all plants and isolates. The experiment was repeated.

Results and discussion

The results show the prevalence of airborne *Fusarium* species coming from the atmosphere in the South East coast of Spain (Table 2). Five different *Fusarium* species were isolated from settling dust: *Fusarium oxysporum*, *F. solani*, *F. equiseti*, *F. dimerum*, and *F. proliferatum*. Previous studies have shown that Basidiomycetes and Ascomycetes can be transported passively by wind, and that the ambient concentration and number of spores of

Table 4 Incidence of damping off on locally grown plant species inoculated with isolates of *Fusarium* recovered from atmospheric dust of Almería (south-eastern Spain)

Code of isolate	*Fusarium* species	Tomato[f]	Melon[f]
FV1	*F. solani*	53.33[g] ± 23.15 bcd	98.33 ± 22.72 de
FV2	*F. solani*	36.67 ± 23.09 abcd	100 ± 23.12 e
FV3	*F. solani*	46.67 ± 23.4 bcd	88.33 ± 23.5 bcde
FV4	*F. solani*	56.67 ± 23.86 bcd	91.67 ± 23.58 bcde
FV13	*F. proliferatum*	65 ± 23.63 d	96.67 ± 24.08 de
FV14	*F. dimerum*	53.33 ± 23.53 bcd	100 ± 24.62 e
FV15	*F. dimerum*	46.67 ± 23.76 bcd	95 ± 25.09 cde
FV20	*F. oxysporum*	36.67 ± 23.95 abcd	91.67 ± 25.7 bcde
FV21	*F. oxysporum*	38.33 ± 24.08 abcd	88.33 ± 26.38 bcde
FV22	*F. oxysporum*	40 ± 24.38 abcd	98.33 ± 27.17 de
FV23	*F. oxysporum*	33.33 ± 24.96 ab	90 ± 27.85 bcde
FV27	*F. equiseti*	41.67 ± 24.59 ab	65 ± 28.68 b
FV28	*F. equiseti*	40 ± 22.42 abcd	83.33 ± 27.85 bcde
FV29	*F. equiseti*	35 ± 22.44 abc	90 ± 29.05 bcde
FV30	*F. equiseti*	61.67 ± 23.02 bcd	78.33 ± 30.33 bcd
FV31	*F. equiseti*	48.33 ± 23.03 bcd	93.33 ± 29.32 bcde
FV32	*F. equiseti*	53.33 ± 22.74 bcd	93.33 ± 31.16 bcde
FV33	*F. equiseti*	51.67 ± 22.11 bcd	100 ± 33.37 e
FV38	*F. proliferatum*	33.33 ± 23.15 ab	76.67 ± 33.59 bc
FV39	*F. proliferatum*	18.33 ± 26.91 a	35 ± 37.53 a
Control[h]		63.33 ± 17.95 cd	75 ± 25 bcd

Values with the same lower case letter did not differ significantly

[f] Inoculation studies were repeated

[g] Percent germination (value ± SD)

[h] Non inoculated

fungi such as *Penicillium*, *Aspergillus* and *Nigrospora* increase significantly during sandstorm episodes. This is a possible explanation for the observed increase in *Fusarium* colonies in sample "AG.Fu", when a sandstorm episode took place. However, *Fusarium* species appeared regularly in analysed samples of settling dust so this cannot be the only explanation. These results are in agreement with those reported by Ismail et al. [16] for airborne mycobiota in the desert of Egypt for *F. dimerum* and *F. oxysporum*, and with the observations of Abdel-Hafez et al. [1], who recovered *F. oxysporum* and *F. solani* from Upper Egypt.

The results also agree with those of Tello and Lacasa [30], who reported the capacity of winds to carry dust particles and disseminate *Fusarium* spp. propagules. On a 8.75 m^2 surface exposed to wind south of the Canary Islands, they recovered 12,598 cfu of *F. solani* and 8,712 cfu of *F. verticillioides*. *F. equiseti* was collected from dust particles from the Saharan winds on the European coast. These studies demonstrate the ability of *Fusarium* spp. to be spread by the wind. The results presented in this work strongly indicate that spores or propagules of *Fusarium* spp. are able to cross the continental barrier carried by winds from the Sahara (Africa) to crop and coastal lands in Europe.

We cannot specifically discuss rainfall dust in samples obtained in 2007 and 2008 because it took several weeks and it was not raining all the time so settling atmospheric dust was also collected. For this reason we directly analysed rain water collected in 2009.

Using the dilution-plate method, 12 fungal genera were identified from rainwater samples collected during January and February 2009 in Almería (Spain). From our results, *Cladosporium*, *Acremonium* and *Aureobasidium* are the most predominant fungal spores, comprising more than 73% of total fungal spores per millilitre (Table 3). *Cladosporium* is one of the most dominant fungi recovered in outdoor atmospheric environments [12]. The rainwater analysis revealed a large content of airborne fungal spores (9,084 cfu of *Botrytis cinerea* were recovered per square metre). Some of these fungi are considered plant pathogens, for example *Acremonium*, *B. cinerea* and some species of *Fusarium* and *Alternaria*. Others, such as *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium* and *Rhizopus*, have been cited as the cause of postharvest losses. Others still have been used as biological agents to control pests in greenhouses (*Beauveria bassiana*). The number of fungal propagules per millilitre was seemingly small (Table 3), but if we consider that the total amount of rainfall in the sampling date was 22.74 L m^{-2}, a simple multiplication shows us that 9,084 cfu of *Botrytis cinerea*—an important pathogen in the studied area for tomatoes, peppers, zucchini, or eggplants—settled in 1 m^2.

Table 5 Incidence of damping off on locally grown plant species inoculated with isolates of *Fusarium* recovered from atmospheric downfall dust of Almería (south-eastern Spain)

Code of isolate	*Fusarium* species	Tomato[d]	Melon[d]
F1	*F. solani*	51.67[e] ± 30.47 bc	98.33 ± 8.39 b
F2	*F. proliferatum*	41.67 ± 30.29 abc	93.33 ± 8.57 b
F12	*F. solani*	35 ± 30.28 abc	93.33 ± 8.6 b
F13	*F. solani*	50 ± 30.66 bc	100 ± 8.62 b
F14	*F. solani*	6.67 ± 30.68 a	100 ± 8.89 b
F18	*F. equiseti*	38.33 ± 30.36 abc	100 ± 9.17 b
F36	*F. equiseti*	38.33 ± 30.81 abc	100 ± 9.49 b
F43	*F. solani*	58.33 ± 30.55 c	93.33 ± 9.84 b
F44	*F. oxysporum*	45 ± 30.69 bc	98.33 ± 10.02 b
F45	*F. solani*	50 ± 31.56 c	100 ± 10.46 b
F46	*F. proliferatum*	11.67 ± 31.38 ab	75 ± 10.98 a
F55	*F. equiseti*	48.33 ± 31.51 bc	98.33 ± 4.32 b
F66	*F. oxysporum*	33.33 ± 32.56 abc	98.33 ± 4.41 b
F67	*F. oxysporum*	33.33 ± 32.35 abc	96.67 ± 4.53 b
F68	*F. oxysporum*	36.67 ± 33.54 abc	98.33 ± 3.31 b
F88	*F. solani*	45 ± 31.89 bc	96.67 ± 3.14 b
F89	*F. solani*	40 ± 31.74 abc	100 ± 0 b
Control[f]		58.33 ± 31.84 c	100 ± 0 b

Values with the same lower case letter did not differ significantly

[d] Inoculation studies were repeated

[e] Percentage germination (value ± SD)

[f] Non inoculated

Specific analyses of rainwater to detect the genus *Fusarium* revealed the presence of three species of *Fusarium* (Table 2): *F. oxysporum*, *F. proliferatum* and *F. equiseti*.

The presence of species of *Fusarium* in river water and the seabed of southeastern Spain was previously reported [23, 24], and fluvial sediment transport was suggested as the main route for the transportation of lithogenic and fungal particles to the Alboran Sea. The results presented here clearly suggest that those species could also be deposited in the sea by dust carried by wind or directly by rain.

Fusarium spp. are considered as important soil-borne plan pathogens that cause negative economic impacts on crop yield. Previous studies on aeromicrobiology did not address the issue of whether or not the *Fusarium* species isolated from this habitat were pathogenic to crops in the sampled areas. Therefore, it seemed necessary to evaluate the pathogenicity of the recovered isolates.

Pathogenicity assays were conducted with a total of 57 of the collected isolates of *Fusarium* species. A total of 20 isolates from the collected downfall dust (Table 4): 7 isolates of *F. equiseti*, 5 of *F. oxysporum*, 4 of *F. solani*, 2 of

Table 6 Incidence of damping off on locally grown plant species inoculated with isolates of *Fusarium* recovered from rainwater in Almería (south-eastern Spain)

Code of isolate	*Fusarium* species	Tomato[g]	Melon[g]
Fml1	*F. proliferatum*	73.33[h] ± 20.82 def	86.67 ± 19.80 cd
Fml2	*F. proliferatum*	36.67 ± 20.56 abc	93.33 ± 19.84 cd
Fml3	*F. proliferatum*	33.33 ± 20.26 ab	96.67 ± 20.21 cd
Fml4	*F. proliferatum*	53.33 ± 19.56 abcdef	86.67 ± 20.55 bc
Fml5	*F. proliferatum*	43.33 ± 19.64 abcd	83.33 ± 21.00 bc
Fml6	*F. proliferatum*	50.00 ± 19.78 abcdef	86.67 ± 21.40 bcd
Fml7	*F. proliferatum*	73.33 ± 19.82 cdef	93.33 ± 21.77 cd
Fml8	*F. proliferatum*	60.00 ± 19.99 abcdef	100.00 ± 22.29 d
Fml9	*F. proliferatum*	66.67 ± 20.47 bcdef	96.67 ± 22.72 cd
Fml10	*F. proliferatum*	60.00 ± 20.98 abcdef	83.33 ± 23.25 bc
Fml11	*F. proliferatum*	50.00 ± 20.93 abcdef	86.67 ± 23.88 bcd
Fel1	*F. equiseti*	43.33 ± 21.09 abcd	100.00 ± 24.59 d
Fel2	*F. equiseti*	53.33 ± 21.36 abcdef	100.00 ± 25.20 d
Fel3	*F. equiseti*	53.33 ± 21.83 abcdef	96.67 ± 25.85 cd
Fel4	*F. equiseti*	73.33 ± 21.24 cdef	100.00 ± 26.66 d
Fel5	*F. equiseti*	60.00 ± 21.59 abcdef	100.00 ± 27.37 d
Fel6	*F. equiseti*	76.67 ± 20.53 ef	100.00 ± 28.07 d
Fol1	*F. oxysporum*	50.00 ± 20.06 abcdef	100.00 ± 28.70 d
Fol2	*F. oxysporum*	46.67 ± 20.47 abcde	90.00 ± 29.14 cd
Fol3	*F. oxysporum*	76.67 ± 20.70 def	26.67 ± 30.00 a
Fol4	*F. oxysporum*	53.33 ± 20.81 abcdef	36.67 ± 24.46 a
Fol5	*F. oxysporum*	26.67 ± 22.90 a	70.00 ± 13.82 b
Control[c]		70.00 ± 8.16 bcdef	100.00 ± 0.00 d

Values with the same lower case letter did not differ significantly

[g] Inoculation studies were repeated

[h] Percentage germination (value ± SD)

[i] Non inoculated

F. proliferatum, and 2 isolates of *F. dimerum*. And 17 isolates from the dust carried with rain water (after evaporation) (Table 5): 8 isolates of *F. solani*, 4 of *F. oxysporum*, 3 of *F. equiseti*, and 2 isolates of *F. proliferatum* were tested on tomato (*Lycopersicum esculentum* Mill), and melon (*Cucumis melo* L.), to evaluate their pathogenicity.

The results show differences in the pathogenicity of the isolates tested. Little pathogenicity was observed on tomato caused by *F. oxysporum*, *F. proliferatum* and *F. equiseti*, but none of the isolates of *F. solani* and *F. dimerum* were pathogenic on tomato (Table 4). On *Cucumis melo* L., two isolates of *F. proliferatum* caused significant decrease in seedling emergence. Regarding isolates from rainwater (Table 6), pathogenicity assays were conducted with 22 of the collected isolates: 11 isolates of *F. proliferatum*, 6 isolates of *F. equiseti* and 5 isolates of *F. oxysporum*. Pre and post-emergence pathogenicity was evaluated. This study of pathogenicity showed that some of these isolates caused pre-emergence damping-off on tomato seedlings. In the case of melon, most isolates of *F. oxysporum* (three out of five) caused important damping-off in seedlings. However, *F. proliferatum* and *F. equiseti* did not show pathogenicity on melon.

It could be concluded that a low number of recovered isolates show pathogenicity, but we must bear in mind that, in many cases, the introduction of new fungal pathogens has been evidenced to be airborne rather than carried by people or plants or plant products [5]. Most of these involved rust (with strong spores against environmental damage). The results presented here indicate that the same airborne dissemination pattern may occur for soil-borne fungi that have a markedly host specificity. This means that a single spore of a new race of a *formae specialis* of *F. oxysporum* can be transported over very long distances, and determine the original cause of a single-step pathogen invasion to new production areas far away from the pathogen origin.

Conclusions

The findings presented above are strong indicators that long-distance transmission of *Fusarium* can occur. Our results are coincident with those of Schmale et al. [27], who stated that inoculum can originate from multiple locations and, in practice, the existence of long-distance transport suggests that management of inoculum sources on a local scale will not be completely effective in managing fungal diseases.

The Mediterranean Sea acts as a reservoir in which evaporation exceeds freshwater input through precipitation and runoff [3]. Previous studies revealed the presence of five different *Fusarium* species in the river and sea water of

southeastern Spain [23]. Fluvial sediments transport was proposed as the primary route for the transportation of lithogenic and fungal particulates to the Alboran Sea [20, 24]. The results presented here allow us to speculate on a new source of inoculum for *Fusarium* genus: atmospheric dust, which can fall down directly or together with rain.

On the other hand, diseases produced by species of *Fusarium* are common in these areas. They were in the past, and are still today, a problem for greenhouses crops in Almería, and many species have been listed as pathogens on agricultural crops in this region. Saharan air masses dominate the Mediterranean regions. Dust deposition over the western Mediterranean has been estimated at 9–25 tons km^{-2} $year^{-1}$ [14]. The evidence for long-distance dispersal of *Fusarium* spp. by atmospheric dust and rainwater, together with their proved pathogenicity, must be taken into account in epidemiological studies. Rainwater not only spreads the inocula of this soil-borne pathogen to new areas on a scale of hundreds of kilometres but also provides the necessary humidity for germination of the fungal spores.

This work reveals some epidemiological aspects of plant pathogenic fungi in natural environments. The presence of pathogenic species within the *Fusarium* genus in rainwater could indicate long distance dispersal in natural environments.

The evidence of long distance aerial dispersal of pathogenic strains of *Fusarium* species also has to be taken into account as a survival strategy within the population dynamics of plant pathogens as well as for plant protection strategies. *Fusarium* spores dispersed by wind from infected crops to new cultivated areas may overcome effective resistance. On the other hand, some of the isolated *Fusarium* spp. are potential mycotoxin producers. The ecotoxicological significance of their presence within rainwater remains to be elucidated.

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