SYMPTOMLESS RESERVOIRS OF *FUSARIUM OXYSPORUM* f. sp. *FRAGARIAE* AND ALTERNATIVE HOSTS OF *FUSARIUM SOLANI* PATHOGENIC TO STRAWBERRY

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SUMMARY

Fusarium oxysporum f. sp. *fragariae* and *F. solani* are soilborne fungal pathogens in strawberry crops in southwestern Spain. The pathogenicity of isolates recovered from strawberry plants was determined in three important regional crops, garlic, asparagus and tomato. In addition, *F. oxysporum* f. sp. *cepae*, *F. oxysporum* f. sp. *asparagi,* and *F. oxysporum* f. sp. *radicis-lycopersici* were assessed for pathogenicity in strawberry plants. Garlic, asparagus, and tomato were shown to be symptomless reservoirs of *Fusarium oxysporum* f. sp. *fragariae* although mild symptoms were shown in plants inoculated in test tubes. So, these species could serve as carriers and source of inoculum of this pathogen. Similarly, strawberry was shown to be a symptomless reservoir of *F. oxysporum* pathogenic to garlic, asparagus, and tomato, also causing mild symptoms in plantlets inoculated in test tubes. However, *F. solani* pathogenic for strawberry infected and caused disease on garlic, asparagus, and tomato, demonstrating that the host range of *F. solani* is not limited to one crop. Agronomic implications such as avoiding rotations involving these horticultural species are discussed.

Keywords: *Fragaria×ananassa*, *Allium sativum* L., *Asparagus officinalis* L., *Solanum lycopersicum* L.

INTRODUCTION

Fusarium oxysporum f. sp. *fragariae* Winks & Y.N. Williams (*Fof*), the causal agent of Fusarium wilt of strawberry (*Fragaria×ananassa*), is a devastating pathogen that causes substantial economic losses in strawberry production areas. It has been reported in Australia (Winks and Williams, 1965), Japan (Okamoto *et al*., 1970), Argentina (Mena *et al*., 1975), Korea (Kim *et al*., 1982), Chile (González *et al*., 2005), China (Huang *et al*., 2005), USA (Koike *et al*., 2009; Williamson *et al*., 2012), and Serbia (Stankovic *et al*., 2014). In addition, it was first detected in Spain in 2007 in fruit production fields causing wilt and death of strawberry plants (Arroyo *et al*., 2009). *Fof* infects strawberry plants through the roots, proliferates inside the plant and affects crowns and petioles, resulting in wilting, drying and stunting of plants, and reduced fruit production. Also, plants can collapse and die (Koike *et al*., 2009; Fang *et al*., 2012).

Fof is associated with the *F. oxysporum* species complex (FOSC), which includes a large number of host-specific forms, called *formae speciales* (Armstrong and Armstrong, 1981). Isolates of FOSC are distributed in soils worldwide and infect a broad range of agronomically important crops (Baayen *et al*., 2000; Lievens *et al*., 2008). In addition, FOSC includes non-pathogenic strains (Gordon and Martyn, 1997) that often cannot be distinguished from pathogenic ones by morphological analysis or vegetative incompatibility studies. In practice, isolates are usually assigned to a *forma specialis* after pathogenicity tests on a given crop. However, the pathogenicity of these isolates to other crops is rarely tested. Therefore, the *forma specialis* designation can be misleading (Summerell *et al*., 2001). To our knowledge there is no information concerning pathogenicity of *Fof* isolates from strawberry on other important horticultural crops in Southwestern Spain, such as garlic, asparagus, and tomato. Likewise, there are no reports on potential pathogenicity to strawberry of *F. oxysporum* isolates that cause disease on garlic (*F. oxysporum* f. sp. *cepae*), asparagus (*F. oxysporum* f. sp. *asparagi*), and tomato (*F. oxysporum* f. sp. *radicis-lycopersici*).

F. oxysporum f. sp. *cepae* (*Foc*) is pathogenic on species of *Allium* (garlic, onion, and leek) as well as asparagus (*Asparagus officinalis* L.) (Leoni *et al*., 2013). Symptoms in plants infected with *Foc* are a progressive curvature, chlorosis and/or necrosis of leaves from tips to the base, and brown-red discoloration of the basal plate, storage tissues and roots, which become necrotic as the disease progresses. When the fungus infects the plant at early developmental stages, reduced growth can be observed (Havey, 1995). *Fusarium oxysporum* f. sp. *asparagi* (*Foa*) was described as specific for asparagus based on pathogenicity tests. However, studies have shown that *Foa* also causes disease in

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garlic, celery, onion, gladiolus, and lupine (Summerell *et al*., 2001; Molinero-Ruiz *et al*., 2011). Symptoms caused by *Foa* in asparagus begin with progressive chlorosis, from the apex to the base, and appearance of necrotic lesions at the stems base and on storage and secondary roots. This necrosis becomes more extensive causing rot of roots and crowns until the plant dies (Corpas-Hervias *et al*., 2006). *F. oxysporum* f. sp. *radicis-lycopersici* (*Forl*) infects tomato (*Solanum lycopersicum* L.), but can also cause root and stem rot in numerous plant host families (Menzies *et al*., 1990). *Forl* penetrates tomato plants through root lesions or insertion branch points of secondary roots in the main root. Very often, the fungus is isolated near the lesions and does not spread systemically. Infected plants may wilt and die or remain in a state of weakness, with the consequent yield loss.

Fusarium solani (Mart.) Sacc. (*Fs*) is a widely distributed soilborne fungus. It has been described as pathogenic of more than 111 plant species (Bogale *et al*., 2009; Aoki *et al*., 2014). In strawberry, it was first detected causing crown rot in nursery plants in Spain (Redondo *et al*., 2012) and then in fruit production fields causing stunting, wilting and/or death (Pastrana *et al*., 2014). *F. solani* is also a complex (*Fusarium solani* species complex, FSSC) that comprises at least 60 phylogenetically distinct species (Nalim *et al*., 2011; O'Donnell *et al*., 2008) including both pathogenic and non-pathogenic strains that can be morphologically indistinguishable (O'Donnell *et al*., 2008). Currently, 12 *formae speciales* are described for *F. solani* (Aoki *et al*., 2014). *Fusarium solani* can cause disease in one or more plant hosts from different botanical families (Chung *et al*., 2011; Romberg and Davis, 2007). In addition to strawberry (Pastrana *et al*., 2014), it has been described in Spain as pathogenic to asparagus (Corpas-Hervias *et al*., 2006), garlic (Basallote-Ureba *et al*., 2011) and tomato (Tello and Lacasa, 1988), and also to other crops: bean (Tello *et al*., 1985), pea (Tello *et al*., 1988), squash (García-Jiménez *et al*., 1997), watermelon (Armengol *et al*., 2000), melon (Gómez *et al*., 2014), and zucchini squash (Gómez *et al*., 2008). As for *Fof,* it is unknown whether *F. solani* pathogenic to strawberry can cause disease in other crops or is specific for strawberry.

Isolates belonging to both FOSC and FSSC are abundant in soil and commonly associated with roots and other lower parts of plants. They are important root and crown rot pathogens and readily form chlamydospores, and hence are well adapted to long-term survival in soil (Schippers and van Eck, 1981). *Fusarium* species can also persist as hyphae colonizing organic residues (Booth, 1971). These features make organic waste and substrates reservoirs of inoculum for subsequent crops (Abawi and Lorbeer, 1972). Eradication of both *Fusarium* species is rendered more difficult by their ability to colonize asymptomatic species (Molinero-Ruiz *et al*., 2011).

The objectives of this work were: i) to determine whether *F. oxysporum* f. sp. *fragariae* and *F. solani* isolates from strawberry are able to infect and cause disease in garlic, asparagus, and tomato, three economically important crops in Southern Spain, and ii) to examine whether *F. oxysporum* isolates pathogenic to garlic (*F. oxysporum* f. sp. *cepae*), asparagus (*F. oxysporum* f. sp. *asparagi*) or tomato (*F. oxysporum* f. sp. *radicis-lycopersici*) can also infect and cause disease in strawberry plants. This will offer information for future crop rotations in strawberry fields and will open the possibility of extending strawberry growing areas to fields previously cropped with these vegetables.

MATERIALS AND METHODS

Fungal isolates. The fungal isolates used were: *F. oxysporum* f. sp. *fragariae,* MAFF 744009 (NIAS Genebank) recovered from a symptomatic strawberry plant (Suga *et al*., 2013); *F. solani,* TOR-11 isolate (IFAPA Collection, Spain) recovered from a nursery strawberry plant (Pastrana *et al*., 2014); *F. oxysporum* f. sp. *cepae, Foc* Bal isolate, pathogen of garlic (Basallote-Ureba and Melero-Vara, 2012), *F. oxysporum* f. sp. *asparagi, Foa* 4 isolate, pathogen of asparagus (Corpas-Hervias *et al*., 2006) and *F. oxysporum* f. sp. *radicis-lycopersici, Forl* 411 isolate, pathogen of tomato and provided by Dr. Gómez-Vázquez.

Inoculum production. Conidial suspensions for *in vitro* tests and strawberry pot experiments were prepared by transferring five 8 mm-Potato Dextrose Agar (PDA) discs of actively growing mycelium to flasks containing 100 ml Potato Dextrose Broth (PDB) and incubated at 150 rpm at 25°C for 10 d in a 12 h photoperiod. The spore suspension concentration, after filtering through four layers of sterile cheesecloth, was determined using a haemocytometer, and the suspensions were diluted with sterile water to obtain a final concentration of 1×10^6 conidia/ml.

For pot experiments with garlic, asparagus, and tomato, 16 PDA disks with actively growing mycelium were added to flasks containing a cornmeal:sand:water (CMSW) mixture (9 : 1 : 2, v : v : v) previously sterilized for 70 min at 121°C in two consecutive days. Flasks were incubated at 25°C in darkness for 15 d and they were shaken every 2 d to facilitate substrate aeration and colonization by the fungus. Infested CMSW was mixed with sterile peat (1 : 4, v : v). Final concentration in the substrate was 1×10^5 CFU g^{-1} soil. Substrate mixture for control pots was prepared in the same way with non-inoculated PDA disks.

***In vitro* tests.** Strawberry, garlic, asparagus, and tomato plants were aseptically grown in test tubes containing Hoagland-Knop agar medium (Tuite, 1969).

Strawberry plants consisted on one-month-old plants cv. Camarosa micro-propagated under sterile conditions in individual test tubes (15×2.2 cm) containing 20 ml of Hoagland-Knop agar medium. Plantlets were inoculated by adding to the rooting medium 2 ml conidial suspension

at 10^6 conidia/ml of *Fof*, *Fs*, *Foc*, *Foa*, and *Forl* isolates, and incubated at 25°C and a 16 h photoperiod for three weeks.

Garlic cloves cv. Morado de Castilla-La Mancha were cleaned under tap water, surface disinfested in 1% sodium hypochlorite for 5 min and dried on sterile filter paper in a laminar flow hood. Cloves were aseptically introduced into 100 ml-glass flasks containing 40 ml of Hoagland medium. Glass flasks were capped and incubated for 7-10 d at 18°C with a 12 h photoperiod until inoculation. Garlic gloves were inoculated by adding 2 ml conidial suspension at 10^6 conidia/ml of *Fof*, *Fs*, and *Foc* isolates and maintained at 18°C with a 12 h photoperiod for three weeks.

Asparagus seeds cv. Ciprés were surface disinfested in 1% sodium hypochlorite for 2 min, rinsed twice with sterile distilled water (SDW) for 2 min, and a third time for 17 h. After drying on sterile filter paper, seeds were transferred to 0.6% water agar (WA) (6-7 seeds/ plate) and kept at 28°C in the dark for 7-10 d until emerged roots reached 1-1.5 cm length. Germinated seeds were individually transferred under sterile conditions to test tubes (15×2.2 cm) containing 20 ml of Hoagland medium. Tubes were capped and incubated in a growth chamber at 25°C under 12 h photoperiod for two weeks. Seedling were inoculated by adding 1 ml conidial suspension at 10^6 conidia/ml of *Fof*, *Fs*, and *Foa* isolates and maintained at 25°C with a 12 h photoperiod for three weeks.

Tomato seeds cv. Marmande Cuarenteno were surface disinfested and directly germinated in test tubes containing Hoagland medium, and incubated and inoculated as described for asparagus with 1×10^6 conidia/ml of *Fof*, *Fs*, and *Forl* isolates. Inoculated seedlings were kept at 25°C with a 12 h photoperiod for three weeks.

One or 2 ml of sterile water were added to control plants of each crop. Eight replicated plants were inoculated with each *Fusarium* isolate and arranged in a completely randomized design. Experiments were performed twice.

Pot experiments. Fresh strawberry runner plants cv. Camarosa from nursery were inoculated by dipping roots and crowns in a suspension adjusted to 1×10^6 conidia/ml of *Fof*, *Foc*, *Foa*, *Forl* or *Fs* isolates for 30 min and then transplanting to 1.3 l plastic pots containing sterile peat. The pathogenicity test was established over three months in a growth chamber adjusted to 25/18°C, 60/40% relative humidity (day/night) and 16 h photoperiod.

Garlic cloves were sown in 1.3 l plastic pots (two gloves per pot) containing CMSW:sterile peat (1 : 4, v : v) substrate mixture infested with *Fof*, *Foc* or *Fs* isolates, and incubated in the same conditions than strawberry plants.

Germinated asparagus seeds were transferred into trays containing sterile vermiculite, incubated for 21 d at 25/18°C and 60/40% relative humidity (day/night) and then, individually transplanted to 1.3 l pots containing CMSW:sterile peat (1 : 4, v : v) substrate mixture infested with *Fof*, *Foa* or *Fs* isolates, and incubated in the same conditions than strawberry plants.

Tomato seeds were sown in plastic trays with sterile vermiculite, maintained for 15 d at 25/18°C and 60/40% relative humidity (day/night) and 16 h photoperiod and then transplanted (two plants per pot) to 1.3 l plastic pots containing CMSW:sterile peat (1:4, v:v) substrate mixture infested with *Fof*, *Forl* or *Fs* isolates, and incubated in the same conditions than strawberry plants.

For each combination crop species/*Fusarium* isolate or non-inoculated control, eight replications (pots) were arranged in a complete randomized-block design. Plants were watered as needed, and fertilized with 0.3 g Osmocote® (garlic, strawberry, and tomato) or with 0.2 g Floranid® Permanent (asparagus) 15 d after transplanting.

Disease symptoms assessment. Plants were rated for disease incidence over three weeks (for *in vitro* assays) or three months (for experiments in pots). At the end of each experiment, plants were removed from the substrate, rinsed in tap water, and symptom severity assessed based on a numeric scale that varied depending on the crop. In addition, the percentage of new roots and crown necrosis, the number of fruits produced, and plant fresh weight were determined for strawberry plants in pot experiments. The length of garlic plants and the length of stems and roots for asparagus and tomato plants were measured. Mean ± standard error was based on 16 (*in vitro*) or 8 (in pots) inoculated plants with each isolate. In pots with two plants (asparagus and tomato), the average data of both were used.

Strawberry disease severity was assessed on a 1-to-5 scale, where 1 = no symptoms, 2 = apical necrosis on roots, 3 = <50% root necrosis and noticeable wilt symptoms, 4 = >50% root necrosis and petiole necrosis and/or majority of leaves wilted/dead, stunted plant, 5 = dead plant.

Garlic plants were rated separately for lesions on a 1-to-5 scale where 1 = no symptoms, 2 = apical necrosis on roots, 3 = apical necrosis on roots, necrosis on basal plate and <50% necrosis on aerial system, 4 = extensive necrosis on roots and >50% necrosis on aerial system, 5 = all the previous symptoms plus lesions on storage tissue, or dead plants.

Assessment of asparagus plant symptoms was according to a 1-to-5 scale where 1 = no symptoms, 2 = apical necrosis on roots, 3 = extensive necrosis on roots, 4 = extensive necrosis on roots, necrosis on crown and chlorosis on the aerial system, 5 = necrosis on underground and aerial systems or dead plant.

Tomato plants were rated on a 1-to-5 scale where 1 = no symptoms, 2 = apical necrosis on roots, 3 = necrosis affecting <50% roots and chlorosis and/or defoliation, 4 = necrosis affecting >50% roots, necrotic flecks on stem base, and 5 = dead plant.

Re-isolation of the pathogens on PDA plates was performed from different tissues: roots, crowns, and petioles from strawberry; roots, basal plates, and storage tissues from garlic; storage and secondary roots and stems base

Table 1. Pathogenicity of four *formae speciales* of *F. oxysporum* and *Fusarium solani* on strawberry 'Camarosa'.

Fusarium spp.[c]	*In vitro*[a]	In pot[b]				
	Severity[d] ($P=0.0001$)	Severity[d] ($P=0.0001$)	% New roots[e] (ns)	% Crown rot[e] ($P=0.0090$)	No. fruits/plant[e] ($P=0.0001$)	Plant fresh weight (g)[e] ($P=0.0001$)
Control	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	50.0 ± 9.8	10.0 ± 3.4^{bc}	7.5 ± 1.2^{a}	32.4 ± 3.7^{c}
Fof	4.9 ± 0.1^{c}	4.6 ± 0.2^{b}	38.1 ± 3.8	26.3 ± 11.1^{ab}	1.9 ± 1.4^{b}	10.8 ± 3.5^{d}
Foc	3.1 ± 0.2^{abc}	1.0 ± 0.0^{a}	54.4 ± 5.9	8.8 ± 2.1^{bc}	9.3 ± 2.0^{a}	44.8 ± 4.9^{b}
Foa	3.0 ± 0.3^{ab}	1.0 ± 0.0^{a}	60.6 ± 9.5	8.2 ± 4.1^{c}	9.8 ± 2.1^{a}	63.0 ± 5.4^{a}
Forl	3.3 ± 0.3^{bc}	1.0 ± 0.0^{a}	61.3 ± 3.0	8.2 ± 3.6^{c}	2.8 ± 1.0^{b}	57.5 ± 3.1^{a}
Fs	3.2 ± 0.4^{bc}	3.6 ± 0.4^{b}	36.3 ± 16.4	38.1 ± 11.8^{a}	1.4 ± 0.5^{b}	8.1 ± 1.8^{d}

[a] For *in vitro* experiments, one-month strawberry plants cv. Camarosa produced by meristem culture were used in individual test tubes containing Hoagland-Knop medium. Plantlets were inoculated by adding 2 ml of the appropriate *Fusarium* spp. conidial suspension to the rooting medium, tubes were capped and incubated at 25°C and 16 h photoperiod for three weeks. [b] For pot experiments, strawberry runner plants cv. Camarosa were used. Inoculations were performed by dipping roots and crowns into the appropriate *Fusarium* spp. conidial suspension for 30 min, transplanted to pots containing sterile peat, and maintained in a growth chamber at 25/18°C and 60/40% relative humidity (day/night) and 16 h photoperiod over three months. [c] Control: non-inoculated; *Fof* (*Fusarium oxysporum* f. sp. *fragariae*); *Foc* (*Fusarium oxysporum* f. sp. *cepae*); *Foa* (*Fusarium oxysporum* f. sp. *asparagi*); *Forl* (*Fusarium oxysporum* f. sp. *radicis-lycopersici*). [d] Disease severity is on a 1-5 scale, where 1 = no symptoms and 5 = dead plant. Data were analyzed using Kruskal-Wallis non-parametric test. Values followed by the same letters were not significantly different according to Dunn's multiple comparison test ($P<0.05$). [e] Values are means ± standard error of 16 (*in vitro*) or 8 (in pot) plants. Different letters indicate significant differences among treatments according to LSD test at significance levels indicated for each column. ns = No significant differences found.

from asparagus; and roots, cotyledon insertion areas and stems base from tomato plants. Plant tissues were surface sterilized in 1% sodium hypochlorite for 2 min, rinsed in SDW for 2 min and air dried in a laminar flow cabinet. Small disinfested pieces were transferred to Petri dishes containing PDA and incubated for seven days at 25°C in a 12 h photoperiod. The identity of re-isolated fungi was determined by observation of morphological structures under the microscope.

Statistical analysis. The disease severity rating values were analyzed using the non-parametric Kruskal-Wallis test. Dunn's multiple comparison test was used for comparisons of the means at $P=0.05$. Analysis of variance (ANOVA) was used to test for significant effects of the rest of independent variables, following a completely randomized design. Data from repeated experiments were previously subjected to an ANOVA and when no statistical differences were found, they were combined into a single set of data. Percentage data were transformed (arcsine $\sqrt{Y/100}$) prior to analysis. Where significant F values were obtained, means were compared using Fisher's LSD test at $P=0.05$. All statistical analyses were performed with Statistix 9.0 (Analytical Software, Tallahassee, FL, USA).

RESULTS

Symptom development.

Strawberry. All *Fusarium* isolates caused necrosis in roots, leaves and petioles of strawberry plants inoculated in the *in vitro* tests. The most severe symptoms were caused by *Fof* (Table 1), although plants inoculated with *Fs* and *Forl* isolates also showed symptoms that significantly differ from the control. All inoculated fungi were re-isolated from roots, crowns and petioles of strawberry plants growing *in vitro.* No fungi were isolated from control plants.

In pot experiments, *Fof* and *Fs* isolates caused mortality in 62% and 37.5% of inoculated plants, respectively, significantly differing from the control. Both fungi caused crown rot, wilting and stunting. In addition, the number of fruits produced per plant and plant fresh weight were both significantly reduced in plants inoculated with *Fof* and *Fs*. The emergence of new roots was similar to control plants or plants inoculated with other *formae speciales* of *F. oxysporum* (Table 1). *F. oxysporum* isolates pathogenic to garlic (*Foc*), asparagus (*Foa*) and tomato (*Forl*) did not cause symptoms in strawberry, but *Forl* caused a reduction in the number of fruits per plant (Table 1). *Fof* and *Fs* were re-isolated on PDA from roots, crowns, and petioles of all inoculated strawberry plants. *Foc*, *Foa*, and *Forl* isolates were recovered from roots but not from crowns and petioles.

Garlic. Symptoms on garlic seedlings inoculated *in vitro* with *Foc* and *Fs* were significantly more severe than in seedlings inoculated with *Fof*, which did not significantly differ from the control (Table 2). The first symptoms were observed one week after inoculation. Plants inoculated with *Foc* and *Fs* showed apical necrosis of roots and necrosis on basal plates. In addition, plants inoculated with *Foc* showed progressive necrosis in storage tissues by 10 d after inoculation. In control plants, small necrotic spots were also observed in roots and basal plates at the end of the experiment, but no pathogens were isolated from these lesions. *Foc*, *Fof*, and *Fs* isolates were recovered from lesions in the roots, basal plates and storage tissues of the respectively inoculated plants.

At the end of the pot experiment, all inoculated garlic plants had apical or complete necrosis in leaves, although *Foc* and *Fs* caused significantly more severe symptoms

Table 2. Pathogenicity of *Fusarium oxysporum* from garlic and *F. oxysporum* and *F. solani* from strawberry on garlic 'Morado de Castilla-La Mancha'.

***Fusarium* spp.**[c]	***In vitro***[a]		**In pot**[b]	
	Severity[d] ($P=0.0001$)	**Severity**[d] ($P=0.0001$)	**Plant length (cm)**[e] (ns)	**Plant fresh weight (g)**[e] ($P=0.0150$)
Control	2.5±0.2[a]	2.2±0.3[a]	31.0±2.7	13.6±0.5[a]
Foc	4.6±0.2[b]	3.9±0.2[b]	32.2±2.7	8.9±1.4[c]
Fof	3.6±0.3[ab]	2.8±0.3[a]	36.1±2.4	12.4±1.0[ab]
Fs	4.1±0.1[b]	4.1±0.1[b]	27.3±1.8	10.0±1.1[bc]

[a] For *in vitro* experiments, cloves were aseptically grown in glass flasks containing Hoagland-Knop medium. After 7-10 days, seedlings were inoculated by adding 2 ml of the appropriate *Fusarium* spp. conidial suspension to the rooting medium and incubated at 18°C with a 12 h photoperiod for three weeks. [b] For pot experiments, garlic cloves were sown in 1.3 l plastic pots containing substrate previously infested with the appropriate *Fusarium* spp. isolate and maintained in a growth chamber at 25/18°C and 60/40% relative humidity (day/night) and 16 h photoperiod over three months. [c] Control: non-inoculated; *Foc* (*Fusarium oxysporum* f. sp. *cepae*); *Fof* (*Fusarium oxysporum* f. sp. *fragariae*) y *Fs* (*Fusarium solani*). [d] Disease severity is on a 1-5 scale, where 1 = no symptoms and 5 = extensive necrosis on roots, >50% necrosis on aerial system and lesions on storage tissue or dead plants. Data were analyzed using Kruskal-Wallis non-parametric test. Values followed by the same letters were not significantly different according to Dunn's multiple comparison test ($P<0.05$). [e] Values are means ± standard error of 16 (*in vitro*) or 8 (in pot) plants. Different letters indicate significant differences among treatments according to LSD test at significance levels indicated for each column. ns = No significant differences found.

Table 3. Pathogenicity of *Fusarium oxysporum* from asparagus and *F. oxysporum* and *F. solani* from strawberry on asparagus 'Cipres'.

***Fusarium* spp.**[c]	***In vitro***[a]			**In pot**[b]			
	Severity[b] (P=0.0001)	**Stem length (cm)**[e] (ns)	**Root length (cm)**[e] (ns)	**Severity**[b] (P=0.0001)	**Stem length (cm)**[e] (ns)	**Root length (cm)**[e] (ns)	**Plant fresh weight (g)**[e] (ns)
Control	1.4±0.1 [a]	13.3±0.9	5.2±0.6	1.2±0.1 [a]	54.4±4.8	26.6±2.4	10.4±1.6
Foa	3.9±0.2 [b]	12.0±0.5	4.1±0.6	4.6±0.3 [b]	58.1±4.5	32.3±2.3	11.9±1.5
Fof	3.4±0.3 [b]	13.1±0.4	5.0±0.7	2.5±0.3 [a]	63.4±4.5	29.0±2.3	10.9±1.5
Fs	3.9±0.1 [b]	13.1±0.5	6.2±0.2	4.5±0.2 [b]	49.9±4.5	30.9±2.3	9.1±1.5

[a] For *in vitro* experiments, germinated seeds were individually transferred under sterile conditions to test tubes containing Hoagland-Knop medium. Tubes were incubated for two weeks until inoculation. Seedlings were inoculated by adding 1 ml of the appropriate *Fusarium* spp. conidial suspension to the rooting medium, and incubated at 25°C with a 12 h photoperiod for three weeks. [b] For pot experiments, germinated seeds were sown in trays containing sterile vermiculite, incubated for 21 d, transplanted to pots with substrate previously infested with the appropriate *Fusarium* spp. isolate and then maintained in a growth chamber at 25/18°C and 60/40% relative humidity (day/night) and 16 h photoperiod over three months. [c] Control: non-inoculated; *Foa* (*Fusarium oxysporum* f. sp. *asparagi*); *Fof* (*Fusarium oxysporum* f. sp. *fragariae*) y *Fs* (*Fusarium solani*). [d] Disease severity is on a 1-5 scale, where 1 = no symptoms and 5 = extensive necrosis on the underground and aerial system or dead plant. Data were analyzed using Kruskal-Wallis non-parametric test. Values followed by the same letters were not significantly different according to Dunn's multiple comparison test ($P<0.05$). [e] Values are means ± standard error of 16 (*in vitro*) or 8 (in pot) replicates. Different letters indicate significant differences among treatments according to LSD test at significance levels indicated for each column. ns = No significant differences found.

than *Fof*, which did not significantly differ from the control (Table 2). Symptoms in the root system showed more pronounced differences among isolates. Plants inoculated with *Fof* had scarce or no necrosis, whereas plants inoculated with *Foc* and *Fs* showed extensive necrotic areas on roots and basal plates. In addition, these plants showed a poor root system and significantly lower weights but no differences were observed in plant length as compared with the control plants (Table 2). All isolates were recovered from lesions of roots, basal plates and storage tissues from plants inoculated with the corresponding *Fusarium* isolate.

Asparagus. Asparagus plants inoculated *in vitro* with *Foa*, *Fs,* and *Fof* showed apical necrosis and shortening of roots one week after inoculation. Two weeks later, all inoculated plants presented extensive necrotic lesions in roots and crowns, independently of the inoculated isolate. These plants also showed above-ground symptoms, consisting of necrosis of stems 2 to 3 weeks after inoculation. Control plants did not show any symptoms. No significant differences were observed in stem and root lengths among inoculated and control plants (Table 3).

At the end of the pot experiment, asparagus plants inoculated with *Foa* and *Fs* showed symptoms of chlorosis and necrosis of stems and cladodes. Plants inoculated with *Fof* only showed apical necrosis on cladodes. Necrotic symptoms of the root system were only observed in plants inoculated with *Foa* and *Fs*; the rest of the plants exhibited completely symptomless root systems. There were no significant differences in stem and root length, or weight of the inoculated plants as compared to the control, although plants inoculated with *Fs* remained smaller and with reduced weight (Table 3). The three fungal isolates were recovered from all inoculated plants. *Foa* was re-isolated from roots, crowns, and stems. However, *Fs* and *Fof* were only recovered from roots and crowns and not from stems.

Tomato. Plants inoculated with *Forl* showed significantly more extensive necrosis than the rest of the inoculated

Table 4. Pathogenicity of *Fusarium oxysporum* from tomato and *F. oxysporum* and *F. solani* from strawberry on tomato 'Marmande Cuarenteno'.

***Fusarium* spp.**[c]	***In vitro***[a]			**In pot**[b]			
	Severity[d] (P=0.0001)	**Stem length (cm)**[e] (ns)	**Root length (cm)**[e] (P=0.0227)	**Severity**[d] (P=0.0001)	**Stem length (cm)**[e] (P=0.0001)	**Root length (cm)**[e] (ns)	**Plant fresh weight (g)**[e] (P=0.0003)
Control	2.3±0.2 [a]	5.6±0.4	4.4±0.5 [a]	2.0±0.3 [a]	43.7±0.9 [a]	24.9±1.4	25.0±1.5 [a]
Forl	5.0±0.0 [b]	4.1±0.7	2.8±0.4 [b]	3.9±0.2 [b]	38.6±1.4 [b]	20.5±1.7	23.9±0.7 [a]
Fof	2.9±0.1 [a]	6.0±0.1	3.8±0.3 [ab]	1.4±0.2 [a]	47.8±1.6 [a]	23.6±2.0	24.4±1.3 [a]
Fs	3.0±0.1 [a]	5.5±0.3	3.8±0.3 [ab]	1.6±0.2 [a]	37.0±1.9 [b]	21.6±0.8	18.0±0.8 [b]

[a] For *in vitro* experiments, germinated seeds were individually transferred under sterile conditions to test tubes containing Hoagland-Knop medium. Tubes were incubated for two weeks until inoculation. Seedlings were inoculated by adding 1 ml of the appropriate *Fusarium* spp. conidial suspension to the rooting medium, and maintained at 25°C with a 12 h photoperiod for three weeks. [b] For pot experiments, seeds were sown in plastic trays with sterile vermiculite, incubated for 15 d, transplanted to pots with substrate previously infested with the appropriate *Fusarium* spp. isolate and then, maintained in a growth chamber at 25/18°C and 60/40% relative humidity (day/night) and 16 h photoperiod over three months. [c] Control: non-inoculated; *Forl* (*Fusarium oxysporum* f. sp. *radicis-lycopersici*); *Fof* (*Fusarium oxysporum* f. sp. *fragariae*) y *Fs* (*Fusarium solani*). [d] Disease severity is on a 1-5 scale, where 1 = no symptoms and 5 = dead plant. Data were analyzed using Kruskal-Wallis non-parametric test. Values followed by the same letters were not significantly different according to Dunn's multiple comparison test (P<0.05). [e] Values are means ± standard error of 16 (*in vitro*) or 8 (in pot) replicates. Different letters indicate significant differences among treatments according to LSD test at significance levels indicated for each column. ns = No significant differences found.

plants and died two weeks after inoculation (Table 4). Although there were no significant differences in stem length of the inoculated plants, as compared to the control, plants inoculated with *Forl* remained smaller. They also showed significant lower root lengths than the control and the rest of inoculated plants (Table 4). All isolates were recovered from the roots, stem base and stem of inoculated tomato plants, but not from any tissue of the control plants.

At the end of the pot experiment, aerial parts of all plants showed some chlorotic and/or necrotic leaves, although plants inoculated with *Forl* exhibited significantly more severe above-ground symptoms, extensive necrosis in the roots, and death of plants. Furthermore, *Forl* and *Fs* caused a significant reduction of the stem but not root length. In addition, *Fs* caused a decrease in plant weight as compared with control plants (Table 4). All isolates were recovered from the root, cotyledon insertion area and stem base of inoculated tomato plants.

DISCUSSION

The results obtained in potted plants experiments in this study indicated that *Fusarium oxysporum* f. sp. *fragariae* (*Fof*) could colonize garlic, asparagus, and tomato plants without causing disease symptoms. Similarly, *F. oxysporum* pathogenic to garlic, asparagus, and tomato (*Foc*, *Foa* and *Forl*) could colonize strawberry plants but were not able of causing disease. Results are consistent with previous studies indicating that strawberry was the only known host to *Fof* (Kodama, 1974). More recent studies demonstrated that some *formae speciales* of *F. oxysporum*, including those that infect garlic, asparagus, and tomato, have a wider host range (Menzies *et al*., 1990; Summerell *et al*., 2001; Molinero-Ruiz *et al*., 2011; Leoni *et al*., 2013). Our results indicated that the *formae speciales* of *F. oxysporum* pathogenic for garlic, asparagus, and tomato seem not to be a threat to strawberry plants. However, these three crops could act as symptomless reservoirs of *F. oxysporum* f. sp. *fragariae*. Therefore, different tissues of colonized garlic, asparagus, and tomato plants could serve as inoculum source of this pathogen. Epidemiological implications of these results allow us to conclude that it would not be advisable to use farms that have been previously cropped to garlic, asparagus, and tomato for strawberry cultivation and vice versa.

Conversely, we have demonstrated that *F. solani* pathogenic to strawberry is able to infect and cause disease on garlic, asparagus, and tomato. Stunting was the most pronounced symptom in tested crops, reducing plant height and fresh weight. In addition, *F. solani* isolated from strawberry was able to cause similar symptoms in garlic and asparagus plants to those described in strawberry (Pastrana *et al*., 2014), consisting of general wilt, necrosis on aerial system, roots and crowns in asparagus plants, as well as necrosis on roots, basal plate and storage tissue in infected garlic plants. These results are in agreement with previous studies that demonstrated a wider host range of *F. solani* pathogen for garlic and asparagus (Basallote-Ureba *et al*., 2011; Molinero-Ruiz *et al*., 2011; Basallote-Ureba and Melero-Vara, 2012). We can conclude that *F. solani* pathogenic to strawberry is not specific to this crop because it can infect and cause disease in other horticultural crops, reducing plant vigor and thereby affecting production. It would, therefore, be important to implement control strategies that prevent the increase of this emerging pathogen in soils where strawberries are produced. At the same time, growers should be advised not to use crops such as garlic, asparagus, and tomato as new or rotational crops in strawberry production fields and nurseries.

In vitro experiments have been accepted to evaluate Fusarium wilt of asparagus (Stephens and Elmer, 1988; Schreuder *et al*., 1995; Elena and Kranias, 1996; Corpas-Hervias *et al*., 2006). In our study, disease severity values obtained in the *in vitro* tests were usually higher that in pot experiments, according with the results obtained by

Corpas-Hervias *et al.* (2006) in asparagus plants inoculated with *Fusarium* spp. Variation in plant susceptibility to *Fusarium* between *in vitro* and in pot experiments can be attributed to experimental differences. *In vitro* plants were younger, smaller and with soft roots than plants grown in substrate, which may render them more susceptible to infection (Cumagun *et al.*, 2010). Besides, different type and density of inoculum and different methods of inoculation were used in the *in vitro* and pot experiments. *In vitro* garlic and tomato roots could be sensitive to added water since we can observe rot symptoms in control plants inoculated with sterile water. For these reasons, the confirmation of pathogenicity should be performed on mature plants.

Overall, our results showed garlic, asparagus and tomato plants as symptomless reservoirs of *F. oxysporum* f. sp. *fragariae* and confirm the ability of *F. solani* recovered from strawberry to cause disease in a wider range of plant species. In addition, symptomless plants which can be colonized by *F. oxysporum* could be potential reservoirs of these pathogens and hence, would constitute an inoculum source if they are used as new or rotational crops. Future studies should assess the ability of *Fof* and *Fs* to cause disease in raspberry and blueberry, two emerging crops in southern Spain that are being grown in what were previously strawberry production fields.

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