Characterization of *Fusarium solani* Populations Associated with Spanish Strawberry Crops

M. Villarino,¹ E. De la Lastra,² M. J. Basallote-Ureba,² N. Capote,² I. Larena,¹ P. Melgarejo,¹ and A. De Cal^{1,†}

¹ Department of Plant Protection, INIA, Carretera de La Coruña Km 7,5. 28040 Madrid, Spain

² IFAPA Centro Las Torres, Apdo. Oficial, 41200 Alcalá del Río, Sevilla, Spain

Abstract

Fusarium solani is an emerging pathogen reported on Spanish strawberry crops both in nurseries and in fruit production fields, causing wilt and root rot. Pathogenicity, morphocultural characteristics, and sensitivity to biocides of 103 *F. solani* isolates recovered from symptomatic strawberry plants and soils from both Spanish strawberry areas were determined. The differences of isolates within and between nurseries and field crops in relation to these parameters were analyzed. Considerable variability in morphological and pathogenic characteristics was observed among the isolates in both areas. The majority of isolates were not pathogenic (62%), and only 38 *F. solani* isolates (37.62%) caused disease on strawberry plants under controlled conditions; 52.63% of pathogenic isolates induced low severity symptoms. Almost

70% of pathogenic isolates caused stunting on plants. The morphological characters that best explain the *F. solani* variability (86.85%) were colony color and the presence of macroconidia on culture medium. The sensitivity to the fumigants tested was similar between the isolates from nurseries and fruit production fields, showing greater sensitivity to the field doses of dazomet and chloropicrin. However, the isolates were less sensitive to metam sodium and poorly sensitive to 1,3-dichloropropene. This work can contribute to the advancement of sustainable production of strawberry.

Keywords: pathogenesis, color colony, macroconidia, secondary pathogen, emergence disease

Both fruit and runner plants of strawberry (*Fragaria × ananassa* Duchesne) are important crops in Spain. In 2016, there were 6,867 ha of fruit production, yielding 377,596 metric tons (t) of strawberry fruit. Huelva (southwestern Spain) is the main producer under plastic macro-tunnels. In 2016, this province contributed 97% of the production, of which more than 80% was exported with a wholesale price of 1,037 euros/t from December to May–June. Overall, the 2016 strawberry fruit production was valued at 392 million euros in Spain (Anuario Estadística Agraria 2017). Each year, strawberry runners (transplants) are produced throughout October in high-elevation nurseries in Castilla-León (north central Spain) under continental climatic conditions, to be subsequently transplanted in fruit production areas in Spain and other countries, mainly belonging to the European Union (EU). In 2015, more than 650 million strawberry runners were produced on an area of approximately 1,500 ha, which is one of the main runner production areas in the EU (Anuario Estadística Agraria 2016). Preplant soil fumigation is a critical point in the production process of the Spanish strawberry crops to control soil-borne plant pathogens (De Cal et al. 2004).

Diseases caused by soil-borne plant pathogens such as *Phytophthora cactorum*, *Verticillium dahliae*, *Rhizoctonia* spp., *Pythium* spp., and *Fusarium* spp. are the most important diseases in Spanish strawberry nurseries without soil disinfestation, particularly in fields where there were previously horticultural crops (De Cal et al. 2004). *P. cactorum* (de los Santos et al. 2002), *Colletotrichum acutatum* (Garrido et al. 2008), *Macrophomina phaseolina* (Avilés et al.

2008), *Pestalopsis clavispora* (Chamorro et al. 2016), *Fusarium oxysporum* (Arroyo et al. 2009), and *Fusarium solani* (Pastrana et al. 2014) are the most important pathogens on strawberry fruit crops in the south of Spain. *F. solani* was associated recently with symptomatic strawberry plants both in fruit production fields (Pastrana et al. 2014) and in nurseries (Redondo et al. 2012). *F. solani* has also been described as a strawberry pathogen in Italy and Mexico along with other pathogens (Ceja-Torres et al. 2008; Manici et al. 2005). However, the recent emergence of *F. solani* in both Spanish strawberry production areas has unknown origin.

The *F. solani* species complex (FSSC) is an important pathogenic ascomycetes group (Leslie and Summerell 2006) from soil and rhizosphere that causes vascular wilt or root rot in more than 100 plant species (Michielse and Rep 2009). The main symptoms of this disease on strawberry are plant-stunting, wilting and chlorosis of leaves, and root rot. The pathogenic *F. solani* isolates are morphologically indistinguishable from nonpathogenic strains (Lievens et al. 2008). Some molecular markers have been developed to identify *F. solani* (Chehri 2016; Costa et al. 2017; Laurence et al. 2016; Mehl and Epstein 2007; O'Donnell et al. 1998). Among them, EF1- α (translation elongation factor 1-alpha), RPB2 (RNA polymerase II second largest subunit), and the ITS rDNA region + the nuclear large-subunit (LSU) rDNA gene sequences, have demonstrated to be a potent phylogenetic marker within *F. solani* (Ke et al. 2016; O'Donnell et al. 2012). Real-time PCR has also been described as a significant detection and identification tool of different *F. solani* (De la Lastra et al. 2018; Gao et al. 2004; Li et al. 2014; Muraosa et al. 2014).

Higher genetic diversity was identified within members of *F. solani* based on EF1- α sequence analysis from Spanish strawberry fruit production fields than strawberry nurseries (E. De la Lastra, *personal communication*). However, there are no reports concerning the morphological and pathogenic variability present in *F. solani* populations in both Spanish strawberry nurseries and fruit production fields. Consequently, further work is needed to distinguish the phenotype of isolates in these Spanish areas in association with strawberry to develop effective control strategies. Here, we characterized *F. solani* isolates recovered from strawberry plants and soil in both Spanish areas (high-elevation nurseries and fruit production fields) based on morphological and pathogenic characteristics and sensitivity to soil fumigants, with

[†]Corresponding author: A. De Cal; cal@inia.es

Funding: This study was financed by Plan Nacional de I + D, Ministry of Economy and Competitiveness, Spain, project RTA2013-00062-C05-E, with co-financing from the 2014–2020 ERDF support: “Smart Growth” Operational Programme.

The author(s) declare no conflict of interest.

Accepted for publication 12 March 2019.

the aim of knowing the origin and structure of the populations from both Spanish strawberry production areas.

Materials and Methods

Fungal isolates. A total of 103 isolates of *F. solani* were selected for the present study. Strains were obtained from soil (57 isolates) and symptomatic strawberry plants (46 isolates) along prospections made in 2010/11, 2011/12, 2012/13, 2014/15, 2015/16, and 2016/17 strawberry seasons. The isolates were conserved as single spores at the INIA (Madrid, Spain) and IFAPA Las Torres (Sevilla, Spain) collections (Table 1).

Five nurseries were sampled in 2011, 2015, and 2016 located in Navalmanzano and Arévalo localities, in Segovia and Ávila provinces, respectively. Twelve fruit production fields were sampled in Huelva and one more in Cádiz during 2010 to 2016 (Table 1).

F. solani isolates were also obtained from soil before fumigation and symptomatic strawberry plants along seasons in nurseries and fruit production fields. The number of prospected plants was about 800 plants per nursery and about 2,150 plants per fruit production field in each season. Symptomatic plants were incubated for 2–5 days at 22–25°C before *Fusarium* isolation.

Twenty soil samples (0 to 20 cm in depth) in each of the four cardinal directions per nursery and year were randomly taken and pooled in a composite sample for further analysis. Soil samples in fruit production fields consisted of soil immediately below the collected plants (0 to 20 cm depth). Aliquots (10 g) of each soil mixture were suspended in 90 ml of sterile distilled water in 250-ml flasks and shaken for 30 min at 150 rpm; 10- to 100-fold dilutions were made, and 100- μ l aliquots from undiluted and diluted suspensions were spread onto petri dishes containing potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI, U.S.A.) amended with streptomycin at 0.5 g liter⁻¹ or *Fusarium* selective medium (Nash and Snyder 1962). Three replicate dishes were used for each soil sample, dilution, and media. Petri dishes were maintained at 20–25°C for 5–7 days and then *Fusarium* fungal colonies were isolated.

Fifty isolates were from high-elevation nurseries in north central Spain (Segovia and Ávila provinces), and 53 isolates were from southwestern Spain (Huelva and Cádiz provinces).

The isolates were grown on Czapek Agar (CA, Difco) in the dark at 22–25°C for mycelium and spore production for 7 days (De Cal et al. 1995), and each isolate was stored in sterile sand at 4°C (Delcán et al. 2002) until required. For production of microconidia to be used in pathogenicity tests, isolates were grown on Czapek Broth (CB, Difco) with agitation (150 rpm) for 5 days.

DNA isolation of each *F. solani* isolate was performed using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), in accordance with the manufacturer's instructions. The isolates were identified as *F. solani* by a specific PCR-based assay (Arif et al. 2012) and confirmed by sequencing of a portion of the translation elongation factor-1 alpha (EF-1 α) gene using primers EF1 and EF2 (Table 1) (O'Donnell et al. 1998).

Pathogenicity test. One hundred and one *F. solani* isolates (Table 1) were tested for pathogenicity on strawberry plants under growth chamber conditions. Pathogenic characterization of *F. solani* isolates was carried out by comparing their ability to cause disease on strawberry plants ('Fortuna' and 'Camarosa', both of them extensively grown in Spain) that were artificially inoculated by immersing the roots and crowns for 30 min in a suspension of microconidia (10⁶ conidia ml⁻¹) of each isolate (groups of 10 plants per isolate were inoculated). The controls consisted of the same number of plants with their roots and crowns immersed in sterile distilled water (SDW). After inoculation, the plants were transplanted into pots with sterilized mixture of peat:perlite (9:1, vol/vol). Plants were incubated in a controlled environment chamber adjusted to 25/18°C (day/night), 70% RH, and a photoperiod of 16 h for 6–8 weeks. At the end of the experiment, pathogenic characterization was recorded by fresh plant weight reduction and disease severity rate. Disease severity was rated as follows: no symptoms (0%), plant with yellow lower leaves (25%), plant with necrotic lower leaves and yellow top leaves (50%), wilted plant with lower dead leaves and top wilted leaves (75%), and dead

plant (100%). The crowns of plants were cut longitudinally and placed in humidity chambers. After 7 days of incubation at 22 $\pm$ 3°C, the presence or absence of *Fusarium* was confirmed by observing *Fusarium* structures from diseased crown segments under a light microscope (Zeiss Axioplan; Carl Zeiss). Pathogenicity test was conducted twice.

Cultural and morphological characterization. Monospore cultures of 103 isolates were morphologically characterized on PDA. Plugs (~5 mm) of 7-day-old actively growing mycelia from each isolate were placed in the center of a sterile PDA petri dish (~9 cm) and incubated at 22 $\pm$ 2°C in the dark for 7 days. The colony diameter of the isolates (cm) was measured from two perpendicular sides of the colony growth at 3, 4, 5, 6, and 7 days after inoculation in each plate. The growth rate (cm day⁻¹) was calculated from daily measurements of the colony's diameter using regression analysis. The presence or absence of microconidia, macroconidia, and chlamydospores of each isolate on PDA and agar-KCl (0.6%) was observed under the optical microscope (Leslie and Summerell 2006). Colony color from top view and reverse on PDA were recorded as HTML colors (<https://html-color-codes.info>). Five replicates per isolate were used and the complete assay was repeated twice.

Fumigant sensitivity tests. The sensitivity of each *F. solani* isolate (Table 1) to four fumigants was tested as described in Raposo et al. (1995). The automated quantitative method consists of the growth of each isolate in microplate wells (Nunc-Immuno plate, Thermo Scientific), which is spectrophotometrically monitored in a range of 0.60–1.98. Four active substances usually applied to soil in nurseries and fruit production fields were tested: Dazomet (tetrahydro-3, 5-dimethyl-2H-1, 3, 5-thiadiazine-2-thione, Sigma-Aldrich), metam sodium (sodium N-methyldithiocarbamate, Supleco), chloropicrin (trichloronitromethane, Symta), and 1,3-dichloropropene (Symta). Each fumigant was evaluated at nine different doses according to literature recommendation (0.25, 0.5, 2.5, 5.0, 50, 75, 100, 125, and 150 mg a.i. liter⁻¹). One hundred μ l of each fumigant solution was mixed with 100 μ l of spore suspension (10⁴ microconidia ml⁻¹) in potato dextrose broth (PDB; Difco Laboratories) in each of the microwells (100). Three replicates per isolate, fumigant, and dose were made. Absorbance was measured at 492 nm with a microplate reader (Multiskan Plus PV. 2.01). The first measurement was made just after filling the plate (A₀), before incubation in the dark at 22°C for 46 h, with one daily monitoring of absorbance as described Guijarro et al. (2017). The concentration of fumigant at which 50% of the colony growth was inhibited was determined at least twice.

Data analysis. All data were analyzed by an analysis of variance (ANOVA) (Snedecor and Cochran 1980) using STATGRAPHICS program (XVII Centurion. Ver. 17.2.00). When the *F* test was significant at *P* = 0.05, means from each isolate were compared by Student-Newman-Keuls multiple range test (Snedecor and Cochran 1980).

Significant means for each pathogenic, morphological, and fumigant sensitivity characteristic were used to design range values for each one. These significant ranges were: confirmed *Fusarium* disease severity (%) (0%; 0 to 15%; 15 to 20%; 20 to 25%; 25 to 35%; 35 to 50%; >50%); presence (1) or absence (0) of stunting (plant weight [g]); colony growth rate (cm day⁻¹) on PDA (<0.65; 0.7–0.75; 0.75–0.85; 0.85–0.95; 0.95–1; 1–1.09; >1.09); presence (1) or absence (0) of macroconidia, microconidia, and chlamydospores; colony color from top view on PDA [white (#FFFFFF), cream (#FFFFF0), pink (#FFE4E1), and yellow (#FFFACD)], reverse colony color on PDA [white (#FFFFFF), cream (#FFFFF0), pink (#FFE4E1), and yellow (#FFFACD)], orange (#FE9A2E), and dark orange (#DF7401)]; concentration of the fumigant at which 50% of the growth is inhibited (mg liter⁻¹) (0–0.25; 0.25–0.5; 0.5–2.5; 2.5–5; 5–50; 50–75; 75–100; 100–125; 125–150; >150).

Pathogenic, morphological, and fumigant sensitivity ranges were also analyzed using a numerical taxonomy and multivariate analysis system NTSYS-pc version 2.10b (Exeter Software, Setauket, New York, U.S.A.) (Rohlf 2002) such as is described in Villarino et al. (2016). Only morphological ranges selected by principal component analysis (PCA) using STATGRAPHICS program (Snedecor and

Table 1. Characterization of *Fusarium solani* isolates: isolation source, year and geographical origin of isolation, fumigant concentration range (mg liter⁻¹) to 50% growth inhibition on culture media, and sequence of a portion of the translation elongation factor-1 alpha (EF-1α) gene

Isolate	Strawberry isolation source	Isolation year	Geographical origin	Fumigant concentration range (mg liter ⁻¹) to 50% growth inhibition				EF-1α GenBank accession no.
				Dazomet	Metam sodium	Chloropicrin	1,3-Dichloropropene	
MA	Nursery plant	2015	Navalmanzano, Segovia, Spain	0-0.25	0-0.25	2.5-5	>150	MH300515
MAA	Nursery plant	2015	Navalmanzano, Segovia, Spain	0-0.25	125-150	5-50	>150	MH300518
MAB	Nursery plant	2015	Navalmanzano, Segovia, Spain	0-0.25	125-150	5-50	>150	MH300526
MAC	Nursery plant	2015	Navalmanzano, Segovia, Spain	5-50	0.25-0.5	0.25-0.5	125-150	MH300522
MAD	Nursery plant	2015	Navalmanzano, Segovia, Spain	5-50	0-0.25	5-50	>150	MH300511
MAE	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	50-75	5-50	>150	MH300534
MAF	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	0-0.25	50-75	>150	MH300535
MAG	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	100-125	5-50	>150	MH300540
MAH	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	125-150	5-50	>150	MH300536
MAI	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	50-75	5-50	>150	MH300544
MAJ	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	50-75	50-75	>150	MH300537
MAK	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	50-75	50-75	>150	MH300541
MAL	Nursery plant	2016	Navalmanzano, Segovia, Spain	5-50	50-75	5-50	>150	MH300504
MAM	Nursery plant	2016	Navalmanzano, Segovia, Spain	5-50	75-100	5-50	-	MH300506
MAN	Nursery plant	2016	Navalmanzano, Segovia, Spain	5-50	50-75	5-50	>150	MH300507
MAO	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	50-75	50-75	>150	MH300532
MAP	Nursery soil	2016	Navalmanzano, Segovia, Spain	0-0.25	50-75	5-50	>150	MH300538
MAQ	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	75-100	50-75	>150	MH300531
MAR	Nursery soil	2016	Navalmanzano, Segovia, Spain	50-75	50-75	5-50	>150	MH300530
MAS	Nursery soil	2016	Navalmanzano, Segovia, Spain	50-75	50-75	5-50	>150	MH300545
MAT	Nursery soil	2016	Navalmanzano, Segovia, Spain	50-75	0-0.25	0-0.25	>150	MH300543
MAV	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	0.25-0.5	5-50	>150	MH300503
MAW	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	50-75	5-50	>150	MH300542
MAX	Nursery soil	2016	Navalmanzano, Segovia, Spain	50-75	125-150	75-100	>150	MH300529
MAY	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	75-100	5-50	>150	MH300528
MAZ	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	75-100	50-75	>150	MH300539
MB	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	125-150	-	-	MH300501
MBA	Nursery soil	2016	Navalmanzano, Segovia, Spain	5-50	50-75	5-50	>150	MH300527
MBB	Nursery soil	2016	Navalmanzano, Segovia, Spain	-	>150	0.25-0.5	>150	MH300505
MBC	Nursery soil	2016	Navalmanzano, Segovia, Spain	50-75	50-75	50-75	>150	
MF	Nursery plant	2015	Navalmanzano, Segovia, Spain	5-50	50-75	0.25-0.5	>150	MH300510
MG	Nursery plant	2015	Navalmanzano, Segovia, Spain	5-50	0.25-0.5	0.25-0.5	>150	MH300514
MGN	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	125-150	0.25-0.5	>150	MH300502
MH	Nursery plant	2015	Navalmanzano, Segovia, Spain	5-50	5-50	0.25-0.5	>150	MH300521

(Continued on next page)

Table 1. (Continued from previous page)

Isolate	Strawberry isolation source	Isolation year	Geographical origin	Fumigant concentration range (mg liter ⁻¹) to 50% growth inhibition				EF-1 α GenBank accession no.
				Dazomet	Metam sodium	Chloropicrin	1,3-Dichloropropene	
MI	Nursery soil	2015	Navalmanzano, Segovia, Spain	5-50	50-75	0.25-0.5	>150	MH300513
MJ	Nursery plant	2011	Arévalo, Ávila, Spain	5-50	50-75	0.25-0.5	>150	MH300509
MK	Nursery plant	2015	Navalmanzano, Segovia, Spain	5-50	75-100	0.25-0.5	>150	MH300519
ML	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	50-75	0-0.25	-	MH300508
MM	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	5-50	5-50	>150	MH300533
MN	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	75-100	0-0.25	-	MH300499
MO	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	50-75	0.25-0.5	>150	MH300500
MP	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	-	0.25-0.5	>150	MH300497
MQ	Nursery soil	2016	Arévalo, Ávila, Spain	5-50	125-150	0.25-0.5	125-150	MH300498
MR	Nursery plant	2015	Navalmanzano, Segovia, Spain	0-0.25	50-75	0.25-0.5	>150	MH300524
MS	Nursery plant	2015	Navalmanzano, Segovia, Spain	5-50	0-0.25	0.5-2.5	>150	MH300523
MT	Nursery plant	2015	Navalmanzano, Segovia, Spain	0-0.25	0-0.25	-	>150	MH300525
MU	Nursery plant	2011	Arévalo, Ávila, Spain	5-50	125-150	5-50	>150	MH300516
MV	Nursery plant	2011	Arévalo, Ávila, Spain	50-75	75-100	5-50	>150	MH300520
MY	Nursery plant	2016	Arévalo, Ávila, Spain	0-0.25	0-0.25	5-50	125-150	MH300517
MZ	Nursery plant	2016	Arévalo, Ávila, Spain	100-125	-	5-50	>150	MH300512
TOR-1	Fruit production soil	2010	Moguer, Huelva, Spain	5-50	50-75	0-0.25	125-150	KF275034
TOR-7	Fruit production soil	2010	Moguer, Huelva, Spain	5-50	50-75	0-0.25	0-0.25	KY486705
TOR-397	Fruit production soil	2012	Moguer, Huelva, Spain	5-50	5-50	5-50	>150	KX215049
TOR-725	Fruit production soil	2012	Moguer, Huelva, Spain	50-75	125-150	0.5-2.5	125-150	KX215050
AB-G1	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	50-75	50-75	>150	KY486691
AB-D4	Fruit production soil	2014	Moguer, Huelva, Spain	50-75	50-75	5-50	>150	KY486690
AB-C2	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	5-50	>150	KY486688
AB-D2	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	5-50	125-150	KY486689
DB-A1	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	50-75	>150	KY486692
DB-C2	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	-	>150	KY486693
DB-F3	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	0-0.25	0-0.25	KY486694
DB-G1	Fruit production soil	2014	Moguer, Huelva, Spain	50-75	50-75	5-50	>150	KY486695
OCCI-C1	Fruit production soil	2014	Moguer, Huelva, Spain	-	-	-	-	KY486696
Fs-1S	Fruit production soil	2014	Almonte, Huelva, Spain	50-75	125-150	2.5-5	>150	KY486652
Fs-2S	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	0-0.25	>150	KY486653
Fs-4S	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	50-75	5-50	125-150	KY486654
Fs-10S	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	0.5-2.5	-	KY486655
Fs-19S	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	50-75	>150	KY486657
Fs-22S	Fruit production soil	2014	Lucena del Puerto, Huelva, Spain	50-75	50-75	5-50	>150	KY486658
Fs-23S	Fruit production soil	2014	Moguer, Huelva, Spain	0-0.25	5-50	2.5-5	-	KY486659
Fs-32S	Fruit production soil	2014	Bonares, Huelva, Spain	50-75	125-150	5-50	>150	KY486660
Fs-35S	Fruit production soil	2014	Bonares, Huelva, Spain	5-50	5-50	5-50	>150	KY486661
Fs-40S	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	5-50	0.5-2.5	0-0.25	KY486663
Fs-52S	Fruit production soil	2014	Moguer, Huelva, Spain	50-75	50-75	5-50	125-150	KY486668
Fs-54S	Fruit production soil	2014	Moguer, Huelva, Spain	5-50	75-100	5-50	-	KY486670
Fs-57S	Fruit production soil	2014	Moguer, Huelva, Spain	0-0.25	-	-	>150	KY486673
Fs-70S	Fruit production soil	2015	Moguer, Huelva, Spain	2.5-5	50-75	5-50	-	KY486674
TOR-11	Fruit production plant	2010	Moguer, Huelva, Spain	5-50	5-50	5-50	125-150	KF275033
TOR-393	Fruit production plant	2012	Moguer, Huelva, Spain	5-50	0.5-2.5	5-50	>150	KX215048
FPOST-164	Fruit production plant	2013	Cartaya, Huelva, Spain	50-75	125-150	5-50	125-150	KX215052
FPOST-169	Fruit production plant	2013	Cartaya, Huelva, Spain	50-75	125-150	5-50	>150	KX215053
FPOST-174	Fruit production plant	2013	Palos de la Frontera, Huelva, Spain	0-0.25	-	5-50	>150	KX215054
FPOST-176	Fruit production plant	2013	Cartaya, Huelva, Spain	5-50	50-75	5-50	>150	KX215055
FPOST-81	Fruit production plant	2012	Cartaya, Huelva, Spain	-	50-75	0-0.25	>150	KF275032
E-III	Fruit production plant	2014	Cartaya, Huelva, Spain	-	-	-	-	KX215056
Fs-1P	Fruit production plant	2015	Almonte, Huelva, Spain	5-50	50-75	5-50	-	KY486675
Fs-67P	Fruit production plant	2015	Moguer, Huelva, Spain	5-50	5-50	5-50	-	KY486676
Fs-71P	Fruit production plant	2015	Moguer, Huelva, Spain	50-75	50-75	-	>150	KY486678
Fs-76P	Fruit production plant	2015	Moguer, Huelva, Spain	5-50	50-75	0.5-2.5	0-0.25	KY486679
Fs-145P	Fruit production plant	2015	Moguer, Huelva, Spain	50-75	50-75	5-50	>150	KY486682
Fs-147P	Fruit production plant	2015	Cartaya, Huelva, Spain	50-75	50-75	5-50	>150	KY486684

(Continued on next page)

Cochran 1980) were used in numerical taxonomy and multivariate analysis.

Results

Pathogenicity test. Some *F. solani* isolates caused visible symptoms such as yellowing of older leaves and wilting and death on strawberry plants. Stunting was also evident on infected plants as a decrease in plant weight. No symptoms were observed on all uninoculated plants.

Significant differences in disease severity were observed among the 101 *F. solani* isolates. Based on presence or absence of stunting, the tested isolates grouped into two clusters (Fig. 1, $r = 0.91$). The results showed the existence of 63 nonpathogenic isolates from both Spanish strawberry areas (NP, 62.37%). Most *F. solani* were non-pathogenic isolates recovered from the soils of the two Spanish strawberry areas (66%).

Only 38 *F. solani* isolates (37.62%) caused disease on strawberry plants under controlled conditions (Fig. 1), showing variability of *Fusarium* symptoms (Fig. 2; $r = 0.95$). Of these 38 pathogenic strains, 18 isolates were from nurseries and 20 isolates from fruit production fields (Fig. 2). According to the isolation origin, 23 and 15 pathogenic isolates from plant and soil were obtained, respectively. Almost 70% of these pathogenic strains gave rise to stunted inoculated plants and 34.21% showed disease symptoms (Fig. 2). There were 13 *F. solani* isolates that caused stunting but not disease severity, and all of them were recovered from both Spanish strawberry areas diseased plants except isolate MAR_S from a strawberry nursery soil (Fig. 2). No relationship was found between pathogenic ranges and isolation origin (soil and plant).

Most of the pathogenic isolates (50%) showed slight severity (SP < 25%), mainly from nursery soils. Isolates clustered as high severity (HP > 50%) (one isolate) and moderate (MP = 25 to 50%) (four isolates) only came from fruit production field soils and plants (13.15%) (Fig. 2).

Cultural and morphological characterization. Cultural and morphological characterizations were carried out with 103 *F. solani* isolates from both Spanish strawberry areas.

Colony diameter showed variation between *F. solani* isolates. The minimum growth at 7 days of incubation was between 4.5 and 5 cm from nursery isolates, and between 5 and 5.5 cm from fruit production fields, where both ranges were significant. On the contrary, isolates reached a maximum growth of 7.71 to 8.12 cm from fruit production fields and nurseries, respectively. No significant differences were observed on these maximum growths. All isolates showed a growth rate ranging from 0.7 to 1 cm day⁻¹ on PDA at 25°C (data not shown).

Macroconidia were present on culture media with most isolates with some exceptions. Out of 50 isolates analyzed from nurseries, only five with white color on front of the colony did not present macroconidia (ML_S, MAL_P, MAM_P, MAN_P, and MAV_S).

However, only three isolates with cream color on front of the colony from fruit production fields did not present macroconidia (EC-1P_P, Fs-23S_S, and Fs-40S_S). On the other hand, microconidia existed in all *F. solani* isolates. Chlamydospores were present in all isolates from both areas, except in five from nurseries (MA_P, MAC_P, MP_S, MR_P, and MV_P) and eight from fruit production fields (FPOST-174_P, Fs-23S_S, Fs-32S_S, Fs-35S_S, Fs-40S_S, Fs-52S_S, Fs-57S_S, and TOR-397_S).

All isolates showed a wide variation with respect to the colony color, and pigmentation of the top view of the colony varied from white to yellow, while the colony reverse side showed colors from white to dark orange. Most isolates from fruit production fields showed cream color on the front (94.3%) and on the back of the colony (47.1%). However, most isolates from nurseries showed white color on the front (52%) and cream (36%) or pink (32%) color on the back of the colony. Yellow color on the front of colonies or cream color on the back of colonies was only observed in *F. solani* isolates from strawberry nurseries.

The results of the PCA revealed that the mycelial color and the presence or absence of macroconidia were the most important morphological variables that distinguished Spanish *F. solani* populations with 86.85% weight in variability (Fig. 3). Components 1, 2, and 3 explained 36.24, 28.45, and 21.50% of the variance, respectively. Based on these characteristics, both Spanish strawberry populations were separated according to the presence or absence of macroconidia and colony color as shown in Figure 4 ($r = 0.87$).

F. solani pathogenic populations (38 isolates) from both Spanish strawberry production areas also showed clear differences between them according to the colony's front color and the presence of macroconidia, with the exception of two isolates from nurseries (MAC_P and MS_P) and two isolates from fruit production fields (Fs-4S_S and EC-1P_P) (Fig. 5; $r = 0.94$). Almost all pathogenic isolates presented macroconidia, except four isolates from nurseries (MAL_P, MAM_P, MAN_P, and MAV_S) and one from fruit production fields (EC-1P_P), or chlamydospores, except three isolates from nurseries (MS_P, MAC_P, MAP_S) and two from fruit production fields (FPOST-174P_P and TOR-397_S) (Fig. 5). All pathogenic isolates from fruit fields were cream colored on the front of the colony except Fs-4S_S (pink), while all different colors were present on pathogenic isolates from nurseries. All colors were presented on the back of colonies from both Spanish areas, except white color did not appear on the back colony of these pathogenic isolates.

Fumigant sensitivity tests. Dazomet concentration range of 5–50 ppm inhibited 50% growth of most *F. solani* isolates from strawberry nurseries, while a higher concentration of this product was needed to achieve 50% inhibition of growth on *F. solani* isolates from the fruit production fields, with the exception of isolates that inhibited their growth at concentrations lower than 0.25 ppm in both areas (Table 1).

The doses of metam sodium that inhibited 50% of the growth ranged between 50 and 100 ppm for isolates from nurseries and

Table 1. (Continued from previous page)

Isolate	Strawberry isolation source	Isolation year	Geographical origin	Fumigant concentration range (mg liter ⁻¹) to 50% growth inhibition				EF-1α GenBank accession no.
				Dazomet	Metam sodium	Chloropicrin	1,3-Dichloropropene	
CH-1P	Fruit production plant	2016	Chipiona, Cádiz, Spain	-	50-75	-	0-0.25	KY486697
CH-2P	Fruit production plant	2016	Chipiona, Cádiz, Spain	5-50	50-75	50-75	>150	KY486698
CH-3P	Fruit production plant	2016	Chipiona, Cádiz, Spain	0-0.25	5-50	5-50	-	KY486699
CH-4P	Fruit production plant	2016	Chipiona, Cádiz, Spain	5-50	50-75	5-50	125-150	KY486700
CH-5P	Fruit production plant	2016	Chipiona, Cádiz, Spain	50-75	50-75	-	0-0.25	KY486701
CH-6P	Fruit production plant	2016	Chipiona, Cádiz, Spain	5-50	75-100	-	0-0.25	KY486702
EC-1P	Fruit production plant	2016	Moguer, Huelva, Spain	75-100	50-75	0-0.25	-	KY486703
EC-2P	Fruit production plant	2016	Moguer, Huelva, Spain	5-50	5-50	-	>150	KY486704
Fs-143P	Fruit production plant	2015	Cartaya, Huelva, Spain	-	50-75	-	0-0.25	KY486680
Fs-144P	Fruit production plant	2015	Cartaya, Huelva, Spain	50-75	50-75	5-50	-	KY486681
Fs-150P	Fruit production plant	2015	Chipiona, Cádiz, Spain	-	-	-	-	KY486687
Fs-152P	Fruit production plant	2016	Chipiona, Cádiz, Spain	-	-	-	-	

between 5 and 75 ppm for isolates from fruit production fields. There were 20 isolates from nurseries that inhibited their growth at lower (<0.25 ppm) and higher concentrations (>75 ppm) (Table 1).

However, most of the *F. solani* isolates growth was inhibited by low chloropicrin concentrations. No *F. solani* isolate grew at concentration greater than 75 ppm. Particularly, the concentration of 0.25–0.5 ppm of this fumigant inhibited the growth of 13 isolates from nurseries (Table 1).

On the other hand, *F. solani* isolate growth was only inhibited at 1,3-dichloropropene concentrations up to 125 ppm. There were only eight *F. solani* isolates from fruit production fields that showed growth inhibition at concentrations below 0.25 ppm.

Discussion

Pathogenic and morphology variability and sensitivity to fumigants of *F. solani* isolates from soils and strawberry plants from both

Spanish strawberry populations have been studied. The presence of *F. solani* was recently detected for the first time in the area of Huelva, as a pathogen causing stunting, wilting, necrosis, and death in strawberry plants (Pastrana et al. 2014). *F. solani* was also detected in 2012 in strawberry nursery plants with symptoms of crown rot (Redondo et al. 2012). *F. solani* also has been described as soil-borne fungal pathogen to several crops in Spain (Pastrana et al. 2017). *F. solani* caused different disease incidence on a great host range with diversity of colony morphology (Brasileiro et al. 2004).

The majority of *F. solani* isolates studied in both Spanish strawberry areas was not pathogenic (62%). Only 38 *F. solani* isolates (37.62%) caused Fusarium wilt on strawberry plants under controlled conditions. *F. solani* usually persists and survives in soil for a long time, and some strains may become pathogenic or nonpathogenic (Bahar and Shahab 2012). *F. solani* and other *Fusarium* spp. include different biological groups such as endophytes (Leslie et al. 1990),

Fig. 1. UPGMA (unweighted pair-group method with arithmetic average) dendrogram generated from the severity disease and plant weight on plants infected with each of the 101 isolates of *Fusarium solani*. The UPGMA tree was generated using the SAHN (sequential, agglomerative, hierarchical, and nested) clustering program (NTSYS-pc version 2.10b) after calculating the simple matching (SM) coefficient. The cophenetic coefficient, $r = 0.91$. Isolation source: soil (_S); diseased plant (_P).

Fig. 2. UPGMA (unweighted pair-group method with arithmetic average) dendrogram generated from combined severity disease and plant weight data. Plants were infected with each of the 38 pathogenic isolates of *Fusarium solani*. The UPGMA tree was inferred using the SAHN (sequential, agglomerative, hierarchical, and nested) clustering program (NTSYS-pc version 2.10b) after calculating the simple matching (SM) coefficient. The cophenetic coefficient, $r = 0.95$. Pathogenicity categories of isolates were: HP: highly pathogenic, MP: moderately pathogenic, and SP: slightly pathogenic. Isolation source: soil (_S); diseased plant (_P).

saprophytes (Bushelman et al. 1995; Fracchia et al. 2000), opportunists (Alves-Santos et al. 2002), and plant pathogens (Chandra et al. 2011), with a high level of polymorphism inside them (Alves-Santos et al. 1999). Pathogenic strains of *Fusarium* spp. can come from nonpathogenic strains, and vice versa, due to mutations in a few genes (Baayen et al. 2000).

Most *F. solani* pathogenic isolates from Spanish strawberry areas showed low severity (52.63%) on strawberry plants under controlled conditions, mainly those from nursery soils. The most pathogenic isolates came from diseased plants from fruit production fields. However, in both Spanish strawberry areas, *F. solani* seemed a weak pathogen, which caused mostly low wilting and stunting. Pathogenicity of *F. solani* isolates obtained from plants was more variable than that of those isolated from soils: more than 50% of pathogenic isolates came from plants, especially those causing only stunting. Almost 70% of pathogenic isolates caused stunting in strawberry plants in

the tests carried out under controlled conditions. Stunting usually was the most pronounced symptom in in vitro tests against *F. solani*, reducing fresh plant weight (Pastrana et al. 2017).

On the other hand, both Spanish *F. solani* populations were morphologically different. The morphological characteristics that best explain the *F. solani* variability (86.85% weight in the variability) were the colony color and the presence of macroconidia on culture medium which divided both Spanish strawberry areas into two different clusters. *F. solani* isolates from fruit production fields presented mostly macroconidia on culture and showed cream color on the front of the colonies, while the *F. solani* population from plant nurseries presented some isolates without macroconidia (16%) and their colonies showed variable colors (white, yellow, pink). *F. solani* pathogenic isolates (38 isolates) from both Spanish strawberry production areas also showed clear differences between them according to the colony's front color and the presence of macroconidia. These characteristics have already been used for the differentiation of *Fusarium* spp. (Albores et al. 2014). No relationship among presence of macroconidia or chlamydospores on culture media and pathogenesis of each Spanish strawberry isolates was recorded. In the same way, Berrueto et al. 2018 found notable variability in morphological characteristics and virulence in *F. solani* isolates from tobacco plants being part of the nonpathogenic strains.

The exchange of plant material from nurseries to fruit production areas does not appear to be the main source of inoculum. Currently, they are two unequal *F. solani* populations depending on Spanish strawberry production area whose origin could be their own soil or other hosts. These results confirmed those obtained by Trabelsi et al. (2017) where a source of transmission of *F. solani* isolates to several horticultural crops may be olive groves, increasing the soil-borne pathogen population (Bejarano-Alcázar et al. 1996). Therefore, Fusarium wilt control of strawberry mainly depends on soil-free pathogen in this crop and other infected hosts, since the strawberry *F. solani* isolates do not present host specificity

Fig. 3. Principal component analysis (PCA) of the morphological data from the 103 *F. solani* isolates growing on potato dextrose agar (PDA). PCA showed the main factors that differentiated both Spanish *Fusarium solani* populations. Components 1, 2, and 3 accounted for 36.24, 28.45, and 21.50% of the variance, respectively.

Fig. 4. UPGMA (unweighted pair-group method with arithmetic average) dendrogram inferred from the color of the front and back of the colony and the presence of macroconidia in colonies of the 103 isolates of *Fusarium solani* when they were grown on potato dextrose agar (PDA). The UPGMA tree was generated by the SAHN (sequential, agglomerative, hierarchical, and nested) clustering program (NTSYS-pc version 2.10b) after calculating the simple matching (SM) coefficient. The cophenetic coefficient, $r = 0.87$. Isolation source: soil (S); diseased plant (P).

Fig. 5. UPGMA (unweighted pair-group method with arithmetic average) dendrogram inferred from the color of the front of the colony and the presence of macroconidia in colonies of the 38 pathogenic isolates of *Fusarium solani* when they were grown on potato dextrose agar (PDA). The UPGMA tree was generated using the sequential, agglomerative, hierarchical, and nested (SAHN) clustering program (NTSYS-pc version 2.10b) after calculating the simple matching (SM) coefficient. The cophenetic coefficient, $r = 0.94$. Isolation source: soil (_S); diseased plant (_P).

and are able to infect other crops (Pastrana et al. 2017). Therefore, more knowledge of the susceptibility of rotational crops in strawberry production areas to *F. solani* would be necessary.

The strategies used against *F. solani* in other crops are usually aimed at decreasing inoculum density in the soil by fumigants, surface removal by disinfection of plant material, the use of moderately tolerant varieties (Ahmad et al. 2012; Burton et al. 2012), crop rotation (Oddino et al. 2008; Rupe et al. 1997), and biological control (Rojo et al. 2007). *F. solani* sensitivity to the fumigants tested was similar in the isolates from both Spanish areas, showing greater sensitivity to the field doses of dazomet and chloropicrin. However, the isolates were less sensitive to metam sodium and poorly sensitive to 1,3-dichloropropene. The main chemical alternatives to methyl bromide (MB), 1,3-dichloropropene, and chloropicrin, have been banned (in the EU) since 2010 and 2013, respectively (Domínguez et al. 2014). Chloropicrin might not have been used correctly against the fungal soil pathogen since the commercial field dose was higher than necessary to control the population of *F. solani*. The same would happen with 1,3-dichloropropene, a product whose field doses were 114–146 ppm, which would be ineffective for Spanish *F. solani* isolates that showed a high resistance (>150 ppm) within both strawberry crop areas. Comparing the results used in the dazomet test with the doses used in the commercial fields for this biocide (137.2–196 ppm), this fumigant has proved to be an effective control strategy against *F. solani*. However, metam sodium doses used in commercial fields (43.42–91.42 ppm) were too low to control the disease, so a resistant population of *F. solani* could be developed in the soil. Although dazomet and metam sodium are soil fumigants that generate methyl-isothiocyanate (MITC) in soil, we obtained different results after its application, as in De Cal et al. 2004. Therefore, likely the ineffectiveness of metam sodium in the last years may be due to a poor distribution of the product (De Cal et al. 2004) added to an inadequate dose used in commercial fields.

The emergence and re-emergence of soil-borne pathogens in strawberry cultivation is mainly caused by the phaseout of MB and its alternative fumigants. *F. solani* as an opportunistic strawberry pathogen could have emerged in both Spanish strawberry crop areas due to the phasing out of MB and the use of reduced doses of alternative fumigants. The evolution of the populations will be subject to soil fumigants that are being used in commercial fields. The results obtained to date suggest that only dazomet at the applied doses can be effective against *F. solani*, especially taking into account that chloropicrin has been removed from the field. *F. solani* populations may evolve depending on the sensitivity of the products applied in

each area. Biosolarization, an alternative technology to soil fumigation, combining biofumigation amended, e.g., chicken manure and soil solarization, could be also used to control *Fusarium* wilt of strawberry (Domínguez et al. 2014, 2016; Teruhiko et al. 2001).

Acknowledgments

M. Villarino held postdoctoral contracts that were associated to project RTA2013-00062-C05-01-E. The authors are grateful to R. Castillo, Y. Herranz, B. Larena, and M. C. Sánchez for the technical support.

Literature Cited

- Ahmad, S., Zaman, N., and Khan, S. N. 2012. Response of groundnut genotypes against root rot disease in district mianwali. Pak. J. Bot. 44:383-386.
- Albores, L., Baños, S., Herrera, J., Necha, L., López, M., and Hernández, A. 2014. Morphological and molecular characterization of pathogenic isolates of *Fusarium* spp. obtained from gladiolus corms and their sensitivity to *Jatropha curcas* L. oil. Afr. J. Microbiol. Res. 8:724-733.
- Alves-Santos, F. M., Benito, E. P., Eslava, A. P., and Diaz-Minguez, J. M. 1999. Genetic diversity of *Fusarium oxysporum* strains from common bean fields in Spain. Appl. Environ. Microbiol. 65:3335-3340.
- Alves-Santos, F. M., Cordeiro-Rodrigues, L., Sayagués, J. M., Martín-Domínguez, R., García-Benavides, P., Crespo, M. C., Díaz-Minguez, J. M., and Eslava, A. P. 2002. Pathogenicity and race characterization of *Fusarium oxysporum* f. sp. *phaseoli* isolates from Spain and Greece. Plant Pathol. 51:605-611.
- Anuario Estadística Agraria. 2016. Ministerio de Agricultura, Pesca y Alimentación. <https://www.mapama.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica/>
- Anuario Estadística Agraria. 2017. Ministerio de Agricultura, Pesca y Alimentación. <https://www.mapama.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica/>
- Arif, M., Chawla, S., Zaidi, N. W., Rayar, J. K., Variar, M., and Singh, U. S. 2012. Development of specific primers for genus *Fusarium* and *F. solani* using rDNA sub-unit and transcription elongation factor (TEF-1 α) gene. Afr. J. Biotechnol. 11:444-447.
- Arroyo, F. T., Llergo, Y., Aguado, A., and Romero, F. 2009. First report of *Fusarium* wilt caused by *Fusarium oxysporum* on strawberry in Spain. Plant Dis. 93:323.
- Avilés, M., Castillo, S., Bascón, J., Zea-Bonilla, T., Martín-Sánchez, P. M., and Pérez-Jiménez, R. M. 2008. First report of *Macrophomina phaseolina* causing crown and root rot of strawberry in Spain. Plant Pathol. 57:382.
- Baayen, R. P., O'Donnell, K., Bonants, P. J. M., Cigelnik, E., Kroon, L. P. N. M., Roebroeck, E. J. A., and Waalwijk, C. 2000. Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. Phytopathology 90:891-900.
- Bahar, M., and Shahab, H. 2012. Analysis of Iranian isolates of *Fusarium solani* using morphological, pathogenicity and microsatellite DNA marker characterization. Afr. J. Biotechnol. 11:474-482.

- Bejarano-Alcázar, J., Blanco-López, M. A., Melero-Vara, J. M., and Jiménez-Díaz, R. M. 1996. Etiology, importance, and distribution of Verticillium wilt of cotton in southern Spain. *Plant Dis.* 80:1233-1238.
- Berrueto, L. A., Cárdenas, G. E., Harries, E. M., Stenglein, S. A., Curti, R. N., Rodríguez, M. S., and Galván, M. Z. 2018. Characterization of *Fusarium* species associated with tobacco diseases in Northwestern Argentina. *Eur. J. Plant Pathol.* 151:1065-1079.
- Brasileiro, T. R. V. B., Coimbra, M. R. M., Antonio de Moraes, M., Jr., and Tinti de Oliveria, N. 2004. Genetic variability within *Fusarium solani* species as revealed by PCR-fingerprinting based on PCR markers. *Braz. J. Microbiol.* 35:205-210.
- Burton, J. W., Miranda, L. M., Carter, T. E., Jr., and Bowman, D. T. 2012. Registration of 'NC-Miller' Soybean with High Yield and High Seed-Oil Content. *J. Plant Regist.* 6:294-297.
- Bushelman, S. J., Callen, J. P., Roth, D. N., and Cohen, L. M. 1995. Disseminated *Fusarium solani* infection. *J. Am. Acad. Dermatol.* 32:346-351.
- Ceja-Torres, L. F., Mora-Aguilera, G., Teliz, D., Mora-Aguilera, A., Sánchez-García, P., Muñoz-Ruiz, C., Tlapal-Bolaños, B., and De La Torre-Almaraz, R. 2008. Fungi prevalence and etiology of strawberry dry wilt under different crop management systems. *Agrociencia* 42:451-461.
- Chamorro, M., Aguado, A., and De los Santos, B. 2016. First report of root and crown rot caused by *Pestalotiopsis clavispora* (*Neopestalotiopsis clavispora*) on strawberry in Spain. *Plant Dis.* 100:1495.
- Chandra, N. S., Wulff, E. G., Udayashankar, A. C., Nandini, B. P., Niranjana, S. R., Mortensen, C. N., and Prakash, H. S. 2011. Prospects of molecular markers in *Fusarium* species diversity. *Appl. Microbiol. Biotechnol.* 90: 1625-1639.
- Chehri, K. 2016. Molecular identification of pathogenic *Fusarium* species, the causal agents of tomato wilt in western Iran. *J. Plant Prot. Res.* 56:143-148.
- Costa, S. S., Moreira, G. M., and Pfenning, L. H. 2017. Development of a PCR protocol for the identification and detection of *Fusarium solani* f. sp. *piperis* from soil and roots of black pepper (*Piper nigrum*). *Trop. Plant Pathol.* 42: 55-59.
- De Cal, A., Martínez-Trecheño, A., López-Aranda, J. M., and Melgarejo, P. 2004. Chemical alternatives to methyl bromide in Spanish strawberry nurseries. *Plant Dis.* 88:210-214.
- De Cal, A., Pascual, S., Larena, I., and Melgarejo, P. 1995. Biological control of *Fusarium oxysporum* f. sp. *lycopersici*. *Plant Pathol.* 44:909-917.
- De la Lastra, E., Basallote-Ureba, M. J., De los Santos, B., Miranda, L., Vela-Delgado, M. D., and Capote, N. 2018. A TaqMan real-time polymerase chain reaction assay for accurate detection and quantification of *Fusarium solani* in strawberry plants and soil. *Sci. Hortic. (Amsterdam)* 237:128-134.
- de los Santos, B., Porras, M., Blanco, C., Barrau, C., and Romero, F. 2002. First report of *Phytophthora cactorum* on strawberry plants in Spain. *Plant Dis.* 86:1051.
- Delcán, J., Moyano, C., Raposo, R., and Melgarejo, P. 2002. Storage of *Botrytis cinerea* using different methods. *J. Plant Pathol.* 84:3-9.
- Domínguez, P., Miranda, L., Medina, J. J., de los Santos, B., Talavera, M., Daugovish, O., Soria, C., Chamorro, M., and López-Aranda, J. M. 2016. Evaluation of non-fumigant alternative soil treatments for strawberry production in Huelva (Spain). *Int. J. Fruit Sci.* 16 (supl):28-36.
- Domínguez, P., Miranda, L., Soria, C., de los Santos, B., Chamorro, M., Romero, F., Daugovish, O., López-Aranda, J. M., and Medina, J. J. 2014. Soil biosolarization for sustainable strawberry production. *Agron. Sustain. Dev.* 34:821-829.
- Fracchia, S., García-Romera, I., Godeas, A., and Ocampo, J. A. 2000. Effect of the saprophytic fungus *Fusarium oxysporum* on arbuscular mycorrhizal colonization and growth of plants in greenhouse and field trials. *Plant Soil* 223:177-186.
- Gao, X., Jackson, T. A., Lambert, K. N., Li, S., Hartman, G. L., and Niblack, T. L. 2004. Detection and quantification of *Fusarium solani* f. sp. *glycines* in soybean roots with real-time quantitative polymerase chain reaction. *Plant Dis.* 88: 1372-1380.
- Garrido, C., Carbu, M., Fernández-Acero, F. J., Budge, G., Vallejo, I., Colyer, A., and Cantoral, J. M. 2008. Isolation and pathogenicity of *Colletotrichum* spp. causing anthracnose of strawberry in south west Spain. *Eur. J. Plant Pathol.* 120:409-415.
- Guijarro, B., Larena, I., Melgarejo, P., and De Cal, A. 2017. Adaptive conditions and safety of the application of *Penicillium frequentans* as a biocontrol agent on stone fruit. *Int. J. Food Microbiol.* 254:25-35.
- Ke, X., Lu, M., and Wang, J. 2016. Identification of *Fusarium solani* species complex from infected zebrafish (*Danio rerio*). *J. Vet. Diagn. Invest.* 28: 688-692.
- Laurence, M. H., Howard, C., Summerell, B. A., and Liew, E. C. Y. 2016. Identification of *Fusarium solani* f.sp. *phalaenopsis* in Australia. *Australas. Plant Dis. Notes* 11:15.
- Leslie, J. F., Pearson, C. A. S., Nelson, P. E., and Toussoun, T. A. 1990. *Fusarium* species from corn, sorghum, and soybean fields in the central and eastern United States. *Phytopathology* 80:343-350.
- Leslie, J. F., and Summerell, B. A., eds. 2006. *The Fusarium Laboratory Manual*, 1st ed. Backwell Publishing, Ames, IA.
- Li, Y., Mao, L., Yan, D., Ma, T., Shen, J., Guo, M., Wang, Q., Ouyang, C., and Cao, A. 2014. Quantification of *Fusarium oxysporum* in fumigated soils by a newly developed real-time PCR assay to assess the efficacy of fumigants for *Fusarium* wilt disease in strawberry plants. *Pest Manag. Sci.* 70:1669-1675.
- Lievens, B., Rep, M., and Thomma, B. P. 2008. Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. *Pest Manag. Sci.* 64:781-788.
- Manici, L. M., Caputo, F., and Baruzzi, G. 2005. Additional experiences to elucidate the microbial component of soil suppressiveness towards strawberry black root rot complex. *Ann. Appl. Biol.* 146:421-431.
- Mehl, H. L., and Epstein, L. 2007. Identification of *Fusarium solani* f. sp. *cucurbitae* race 1 and race 2 with PCR and production of disease free pumpkin seeds. *Plant Dis.* 91:1288-1292.
- Michielse, C., and Rep, M. 2009. Pathogen profile update: *Fusarium oxysporum*. *Mol. Plant Pathol.* 10:311-324.
- Muraosa, Y., Schreiber, A. Z., Trabasso, P., Matsuzawa, T., Taguchi, H., Moretti, M. L., Mikamia, Y., and Kameia, K. 2014. Development of cycling probe-based real-time PCR system to detect *Fusarium* species and *Fusarium solani* species complex (FSSC). *Int. J. Med. Microbiol.* 304:505-511.
- Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology* 52: 567-572.
- O'Donnell, K., Humber, R. A., Geiser, D. M., Kang, S., Park, B., Robert, V. A. R. G., Johnston, P. R., Aoki, T., Rooney, A. P., and Rehner, S. A. 2012. Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium* MLST. *Mycologia* 104:427-445.
- O'Donnell, K., Kistler, H. C., Cigelnik, E., and Plötz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. Natl. Acad. Sci.* 95:2044-2049.
- Oddino, C., Marinelli, A., Zuza, M., and March, G. J. 2008. Influence of crop rotation and tillage on incidence of brown root rot of peanut caused by *Fusarium solani* in Argentina. *Can. J. Plant Pathol.* 30:575-580.
- Pastrana, A. M., Basallote-Ureba, M. J., and Capote, N. 2017. Symptomless reservoirs of *Fusarium oxysporum* f. sp. *fragariae* and alternative hosts of *Fusarium solani* pathogenic to strawberry. *J. Plant Pathol.* 99:141-148.
- Pastrana, A. M., Capote, N., De los Santos, B., Romero, F., and Basallote-Ureba, M. J. 2014. First report of *Fusarium solani* causing crown and root rot on strawberry crops in Southwestern Spain. *Plant Dis.* 98:161.
- Raposo, R., Colgan, R., Delcán, J., and Melgarejo, P. 1995. Application of an automated quantitative method to determine fungicide resistance in *Botrytis cinerea*. *Plant Dis.* 79:294-296.
- Redondo, C., Villarino, M., Larenas, J., Melgarejo, P., and De Cal, A. 2012. Enfermedades emergentes en los viveros de altura de fresa tras la retirada total del bromuro de metilo en 2009. [Emerging diseases in high-altitude strawberry nurseries after the total removal of methyl bromide in 2009]. Poster session presented at: XVI Congreso Nacional de la Sociedad Española de Fitopatología, September 2012, Málaga, Spain.
- Rohlf, E. J. 2002. NTSYS-pc: Numerical taxonomy and multivariate analysis system. New York: Applied Biostatistics Inc. Exeter Software Setauket. Ref Type: Computer Program.
- Rojo, F. G., Reynoso, M. M., Ferez, M., Chulze, S. N., and Torres, A. M. 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. *Crop Prot.* 26:549-555.
- Rupe, J. C., Robbins, R. T., and Gbur, E. E., Jr. 1997. Effect of crop rotation on soil population densities of *Fusarium solani* and *Heterodera glycines* and on the development of sudden death syndrome of soybean. *Crop Prot.* 16: 575-580.
- Snedecor, G. W., and Cochran, W. G. 1980. *Statistical Methods*, 7th ed. Iowa State University, Ames, IA.
- Teruhiko, S., Masahiro, N., and Keiichi, H. 2001. Control of *Fusarium* wilt of strawberry by soil solarization using mulching and tunnel-covering combining with irrigation of solar-heated water. *Bull. Nara Prefectural Agric. Exp. Stn.* 32:1-7.
- Trabelsi, R., Sellami, H., Yáakoub, G., Krid, S., Cheffi, M., Kammoun, S., Dammak, M., Mseddi, A., Gdoura, R., and Triki, M. A. 2017. Morphological and molecular characterization of *Fusarium* spp. associated with olive trees dieback in Tunisia. *3 Biotech.* 7:28.
- Villarino, M., Melgarejo, P., and De Cal, A. 2016. Growth and aggressiveness factors affecting *Monilinia* spp. survival peaches. *Int. J. Food Microbiol.* 224: 22-27.