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RESEARCH ARTICLE

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Pathogenic and biological characterization of *Phytophthora capsici* isolates from zucchini and pepper in Southeast Spain

Miguel de-Cara-García, Marta Fernández-Plaza and Julio Gómez-Vázquez
IFAPA-La Mojonera. Camino San Nicolás 1, 04745 La Mojonera, Almería, Spain.

Abstract

In this work, isolates from pepper and zucchini were tested for pathogenicity on crops commonly cultivated in Mediterranean greenhouses. Also, morphological and growing features and mating types have been studied to find any differences linked with the plant origin and pathogenicity of each isolate. Three isolates were highly pathogenic on all cucurbitaceous and solanaceous hosts tested and caused root rot and wilting. Eggplant and pepper were moderately susceptible, while zucchini, tomato and cucumber were highly susceptible. No root rot symptoms occurred on the fabaceous plants inoculated with *Phytophthora capsici*, including bean and pea. Moreover, the pathogen was never reisolated from the roots of fabaceous plants. Other isolate of *P. capsici* was less pathogenic on cucurbitaceous and solanaceous crops, causing only slight root damages. None of the isolates tested produced chlamydospores, and all belonged to A1 mating type. These findings suggest that beans or peas may be a feasible alternative crop for those Mediterranean greenhouses with a history of root rot due to *P. capsici*.

Additional keywords: fabaceae; rotation; bean; pea; root rot.

Abbreviations used: AUDPS (area under disease progress stairs); dpi (days post inoculation); DSI (disease severity index); ITS (internal transcribed spacer); PCR (polymerase chain reaction).

Authors' contributions: MdeC and JG participated in the conception and design of the research. The three co-authors participated in the other stages of the work, including the performing of experiments, data analyzing, revision of the intellectual content and the drafting of the paper.

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Correspondence should be addressed to Miguel de Cara: franciscom.cara@juntadeandalucia.es

Introduction

Mediterranean countries offer favorable conditions for growing plants in a protected environment, by means of structures covered with plastic film and without fixed installations for winter heating. These structures are called "Mediterranean greenhouses" and are different from those in Central and Northern Europe (Marucci *et al.*, 2012). Mediterranean greenhouse crops are influenced by the subtropical temperatures in the winter, which often allow two crops per year (autumn to mid-winter and late winter to spring cycles); either with two different species or repeating with one (Castilla, 2002). Southeastern Spain, has the highest concentration of Mediterranean greenhouses. Here, the growing intensity ratio (crop growing area related to greenhouse area) is around 1.45, and thus crops cover more than 50,000 ha/year (Marucci *et al.*,, 2012; CAPDER, 2016). These crops include mainly Solanaceae and Cucurbitaceae, but also Fabaceae (Valera *et al.*, 2014; CAPDER, 2016; García-García *et al.*, 2016; Valera *et al.*, 2017). Such crop diversity favors the presence of diverse plant pathogens in the same area, such as soil-borne fungi and oomycetes, including *Phytophthora* species. *Phytophthora nicotianae* (*sensu* Waterhouse, 1963) and *P. capsici* Leonian are considered to be the main *Phytophthora* species associated to horticultural crops in this area (Herrero *et al.*, 2002; De Cara *et al.*, 2011b; Gómez *et al.*, 2013). Both species have a wide host range and are found worldwide (Erwin & Ribeiro, 1996). The pathogenicity of *P. nicotianae* isolates from southeastern Spain has been studied and isolates recovered from tomato plants with root and crown rot showed high host specificity (De Cara *et al.*,

2011a; Boix-Ruiz *et al.*, 2016). This species has not been associated with crops other than tomato in eastern Andalusian greenhouses, while the pathogen has been linked to pepper in the nearby region (of Murcia) (Blaya *et al.*, 2014).

Recent surveys in eastern Andalusian greenhouses consistently recovered *P. capsici* from diseased sweet pepper and zucchini (Gómez *et al.*, 2013; De Cara-García *et al.*, 2017). *P. capsici* is a devastating crown and root rot pathogen which has become a serious threat to Solanaceae and Cucurbitaceae cultivation worldwide, including the greenhouse crops (Hwang & Kim, 1995; Erwin & Ribeiro, 1996; Tian & Babadoost, 2004; Meitz *et al.*, 2010). Isolates recovered from pumpkin caused disease on a wide range of hosts (Tian & Babadoost, 2004), including the most common eight Mediterranean greenhouse crops. While isolates from pumpkin and pepper were pathogenic to pumpkin cultivars, pumpkin isolates were more aggressive than those recovered from pepper (Lee *et al.*, 2001). The snap (green) bean, family Fabaceae, also has been reported as a host of *P. capsici* (Gevens & Hausbeck, 2004; Gevens *et al.*, 2008) and symptoms associated with infection include damping off (Tian & Babadoost, 2004). Therefore, most Mediterranean greenhouse crops are at risk for infection by *P. capsici*.

Field studies have demonstrated the long-term persistence of *P. capsici* in soils of fields where susceptible hosts (cucumber, tomato) were grown in rotation (Lamour & Hausbeck, 2003). Crop rotation is commonly used in the Mediterranean greenhouse system, and the sequence of crops may be key to avoiding losses due to continual presence of this oomycete in soils.

The aim of this work is to evaluate the susceptibility to *P. capsici* on nine greenhouse crops that may be grown as an alternative crop to sweet pepper and zucchini, focusing on Fabaceae crops. Susceptibility was tested for two isolates recovered from symptomatic peppers and two isolates recovered from diseased zucchinis. All four isolates were biologically characterized and the differences reported.

Material and methods

Isolates selection and maintenance

Four representative *Phytophthora capsici* isolates were used in the work: two recovered from zucchini (*Cucurbita pepo* ssp. *pepo*) and two recovered from pepper (*Capsicum annuum*) cultivated in greenhouses in the province of Almería (southeastern Spain). Isolates 'Pcl-612' and 'Pcl-811' were obtained from the basal stems of zucchinis showing leaf wilting and crown- and root-rot disease. These isolates were purified, and Koch's postulates were demonstrated in a previous work (Gómez *et al.*, 2013). Isolates 'MI0211' and 'MI2311' were recovered from rotten epidermic basal stem tissue of sweet peppers showing blight, in a recent survey; Koch's postulates were demonstrated for sweet pepper elsewhere (De Cara-García *et al.*, 2017). Cultures were maintained on potato dextrose agar (PDA) (Biolife, Italy) at 25ºC in the dark and transferred every 6 months.

Colony growth patterns and cardinal temperatures of isolates

To monitor the colony growth pattern, three replicates per isolate were transferred onto V8-agar (Tello *et al.*, 1991) and PDA plates, and incubated at 25 ºC in the dark for two weeks. Growth ratios were determined after incubation (Mod. Hotcold B, P Selecta, Spain) at different temperatures (5, 10, 15, 20, 25, 30, 35, 38 and 40 ºC). One-week-old PDA colonies were used to obtain 6 mm-diameter plugs of young mycelia that were transferred to five Petri dishes containing 12 mL of PDA per isolate. Each plate was randomly located at different points inside the incubator. Temperature inside the incubator was recorded with a HOBO data logger (Onset Computer Co., Bourne, MA, USA). Colony growth was measured at four or seven days depending on the temperature tested. Four perpendicular radii were taken per dish and the average value was used to calculate linear growth per day.

Morphological characterization of isolates

All isolates were morphologically characterized according to the methods of Waterhouse (1963) and Erwin & Ribeiro (1996). Young mycelium plugs were transferred from PDA to Lima Bean Agar (LBA) plates (Gallegly & Hong, 2008) and incubated in the dark for 2-3 weeks at 25ºC until enough sporangia were visible. Then, sterile distilled water (SDW) was poured onto the colonies, and the mycelium was shaken until sporangia were detached. Fifty sporangia per isolate were mounted for microscopic examination. The presence and length of the pedicels were recorded as well as papilla presence and sporangia shape and size. As the resulting numerical data did not show normality or homoscedasticity, the comparison among isolates was subjected to Kruskal-Wallis one-way non-parametric analysis of variance ($p = 0.05$).

The presence of chlamydospores was checked on LBA and V8-agar media. Additionally, liquid V8-juice-submerged cultures were used to test chlamydospore

formation (Uchida & Aragaki, 1985). Twenty mycelial mats per *P. capsici* isolate were produced in clarified 10% V8 broth containing 3 g/L $CaCO_3$ for 5 days at 25ºC in a rotary shaker (125 rpm). Then the mats were rinsed with SDW and submerged in 150 mL SDW for 10 weeks at 16ºC in darkness. The mats were checked every 2 weeks for the presence of chlamydospores. An isolate of *P. palmivora*, known to produce abundant chlamydospores, was used as the control. This test was repeated.

Isolates were tested for mating type by pairing with known mating types of *P. capsici 59* and *P. capsici 50* (A1 and A2 types, respectively, from the INRA collection, Antibes, France) on LBA-agar plates. Fifty sexual structures per isolate were classified and sized after 45 days of incubation in the dark.

Molecular characterization of isolates

The four *P. capsici* isolates, as well as the *P. palmivora* isolate used for testing the formation of chlamydospores, were analyzed by polymerase chain reaction (PCR) sequencing of amplicons of the internal transcribed spacer region (ITS) rDNA using the primers ITS4 and ITS5 (White *et al.*, 1990) and subsequent database searches using BLASTN software. The PCR conditions were: five min at 94 ºC, 35 cycles of one min at 94 ºC, one min at 55 ºC, two min at 72 ºC, and a final elongation of seven min at 72 ºC. Purified PCR products were sequenced using Sanger sequencing method (Instituto de Biología Molecular y Celular de Plantas, Valencia, Spain) and edited by base calling on BioEdit program. The forward and reverse sequences of all the isolates were aligned to create a consensus sequence, which was deposited at the NCBI GenBank with accession number MG012233.

Host range test 1: Most common species of Mediterranean greenhouses

The eight most common greenhouse horticultural species were studied for their susceptibility to *P. capsici* (Table 1). All seeds were disinfected with Na hypochlorite (35 g/L active chloride) for 20 min and subsequently rinsed with tap water and incubated at 28 ºC in the dark. Only germinated seeds were potted. Previous tests were arranged to estimate the sowing date for each species in order to inoculate them simultaneously when all plants had two to four true leaves. The substrate used for the experiments was vermiculite at field capacity with a standard nutrient solution (1.5 dS/m). The experiment was performed in a growth chamber (14 h photoperiod, >12,000 lux, 23-33 ºC). Fertigation with the above-mentioned nutrient solution was applied until the end of the tests according to plant needs, trying to maintain the substrate close to saturation. The inoculum was

Table 1. Experimental design of host range tests.

Factor 1			Factor 2			
Level	**Isolate code**	**Original host**	**Level**	**Species common name**	**Scientific name**	**Cultivar**
				Test 1		
1	MI0211	Sweet pepper	1	Zucchini	*Cucurbita pepo* spp. *pepo*	Milenio F1
2	MI2311	Sweet pepper	2	Sweet pepper	*Capsicum annuum*	Melchor F1
3	Pcl-612	Zucchini	3	Cucumber	*Cucumis sativus*	Marumba F1
4	Pcl-811	Zucchini	4	Melon	*Cucumis melo*	Piel de Sapo
5	Non-inoculated		5	Watermelon	*Citrullus lanatus*	Mara F1
			6	Tomato	*Solanum lycopersicum*	RAF
			7	Eggplant	*Solanum melongena*	Alegría F1
			8	Green bean (round)	*Phaseolus vulgaris*	Emilia
				Test 2		
1	MI0211	Sweet pepper	1	Zucchini	*Cucurbita pepo*	Milenio F1
2	Pcl-612	Zucchini	2	Green bean (round)	*Phaseolus vulgaris*	Emilia
3	Non-inoculated		3	Green bean (flat)	*Phaseolus vulgaris*	Donna
			4	Pea (round)	*Pisum sativus*	Altesse
			5	Pea (flat)	*Pisum sativus*	Tirabeque

prepared by grinding several colonies fully covering the dish surface of the isolate previously grown in PDA in sterile water. The rate was one plate per 400 mL of final suspension. Inoculum density was ca. 2×10^4 CFU per pot. The inoculum consisted mainly of mycelia, and rates were calculated *a posteriori* by means of a dilution-plate technique (Tello *et al.*, 1991) on selective medium P_5ARP (Jeffers & Martin, 1986). Sets of three plants at the growth stage above mentioned per 1-L pot with vermiculite were inoculated adding 50 mL of inoculum suspension to each pot. The control tests were plants not inoculated but watered with an aqueous homogenize of non-colonized PDA. For each isolate and plant species, one pot with three plants was the elementary replication. Four replications were distributed in a factorial design (factor 1: isolate (5 levels); factor 2: plant species, (8 levels) (Table 1). The trials lasted 37 days post-inoculation (dpi). The temperature and relative humidity were measured using a HOBO data logger.

Disease incidence was determined as the percentage of symptomatic plants (showing wilting, chlorosis, crown rot, and/or death was recorded every three to four days until 37 dpi). This was used to calculate the area under the disease progress stairs (AUDPS) (Simko & Piepho, 2012). At the end of the experiments, plants were removed from the pots and the roots examined for symptoms. Then, a disease severity index (DSI), from "0" to "4", according to the volume of roots showing necrosis (0 = 0-5%; 1 = 6-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%), was used. Also, the root samples from two randomly assigned pots per treatment were analyzed for *P. capsici* by means of the carnation-petals baiting technique (De Cara *et al.*, 2011b) in order to re-isolate the strains. Negative re-isolation samples were repeated using the two remaining pots.

The entire experiment was repeated, and data from both experiments were pooled and analyzed using the statistical software program Statistix (Version 9; Analytical Software, Tallahassee, FL, USA). AUDPS was submitted to the analysis of variance (ANOVA) using the factorial procedure. Interactions were sliced when found to be significant at p=0.05 in an ANOVA to analyze simple main effects, and LSD all-pairwise comparison test. AUDPS data were normally distributed and presented homoscedasticity, as checked by Bartlett's test. DSI data were subjected to two-way non-parametric analysis of variance via the Friedman method (p=0.05) for each level of factor 1 and were subjected to Kruskal-Wallis one-way non-parametric analysis of variance (p=0.05) for each level of factor 2.

Data from non-inoculated control tests, as well as bean-level for factor 2, were not included in the analyses because no disease symptoms were detected and root isolations did not yield *P. capsici*. Isolate 811-level for factor 1 was not included in the analyses for AUDPS, but it was for DSI, as root damage was found and consistently re-isolated from solanaceous and cucurbitaceous plants.

Host range test 2: Fabaceae greenhouse species

As green bean did not show any symptoms in the previous tests, a specific test was applied for the two legume species cultivated in southeastern Spain greenhouses, *i.e.* beans and peas. The procedure and conditions of the trial were the same as above mentioned, but the number of levels changed for factor 1: isolate, (3 levels); factor 2: plant species, (5 levels) (Table 1). Round and flat bean and pea were tested to increase the probability of finding non-susceptible hosts. Zucchini was included in the test as a reference control. The most pathogenic isolates from sweet pepper and zucchini were used separately as the inoculum (Table 1). This test lasted 30 days after inoculation. The reisolation tests were made as explained for the previous host range test. The entire experiment was repeated.

Results

Colony growth patterns and cardinal temperatures of isolates

All isolates exhibited slight radial growth pattern on PDA and uniform moderately fluffy pattern on V8. All of them grew well at 35 ºC but were unable to grow at 10 or 40 ºC. At optimal temperatures, the isolates showed moderately slow growth, ranging from 3.8 to 5.2 mm/d (Fig. 1). All the isolates grew well between 23 and 33 ºC, the temperature range of the pathogenicity tests.

Morphological characterization of isolates

All the isolates produced abundant sporangia, many displaying umbellate sporangiophores. Sporangia varied in shape, but no differences between isolates were noticed (Table 2). All sporangia were papillated and caducous. Sporangia of zucchini-recovered isolates (Pcl-612, Pcl-811) were larger than those isolates recovered from pepper (MI0211, MI2311). Also, stalks of zucchini-recovered isolates were longer (more than double) than pepper-recovered ones. None of the isolates produced chlamydospores on V8-agar or LBA after 2 months incubation. After 10 weeks incubation in the liquid-medium test, the formation of chlamydospores was not detected. *P.*

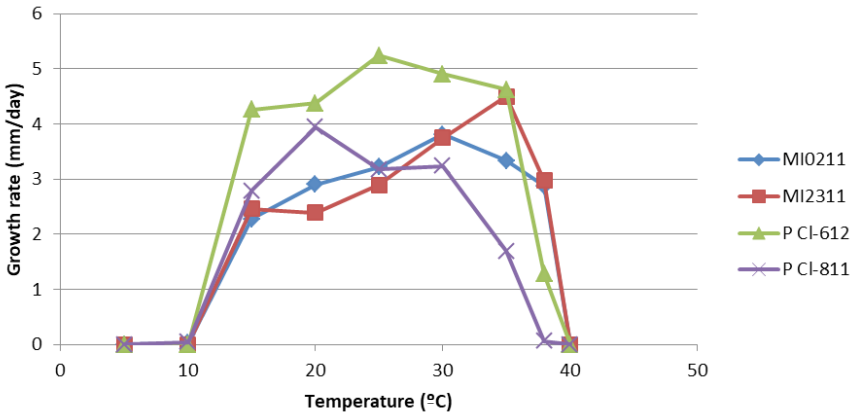

Figure 1. Temperature growth curves on PDA for *P. capsici* isolates obtained from pepper (MI0211, MI2311) and from zucchini (Pcl612, Pcl811).

Table 2. Morphological characteristics of *P. capsici* isolates from pepper and zucchini.

	Isolate				*p*-value (Chi approximation)	Reference[a]
	MI0211 (pepper)	**MI2311 (pepper)**	**Pcl-612 (zucchini)**	**Pcl-811 (zucchini)**		
SPORANGIA (%)						
Shapes Ovoid	51.0	35.1	51.1	46.3		
Limoniform	35.3	61.4	26.7	43.3		
Distorted	11.8	1.8	20.0	9.0		
Sphaerical	1.8	1.8	2.2	1.5		
Pedicel length (µm)	4.4-64.9 (25.0) b	8.4-42.1 (24.8) b	4.8-130.3 (50.3) a	15.4-178.5 (81.7) a	0.00	10-200
Length (µm)	17.2-32.6 (27.0) bc	12.7-31.9 (20.9) c	27.3-64.8 (39.6) a	20.2-40.2 (31.2) ab	0.00	30-100
Breadth (µm)	13.2-22.1 (18.9) b	8.9-17.7 (13.2) c	18.2-34.2 (26.6) a	15.0-27.4 (21.4) ab	0.00	25-90
L/B ratios	1.2-1.7 (1.4)	1.1-2.1 (1.6)	1.2-2.2 (1.5)	1.2-1.7 (1.5)	0.1227	1.3-2.1
SEX ORGANS						
Oogonium diameter (µm)	25-32.6 (29.2) b	21.3-32.1 (28.4) b	25.6-39.1 (33.3) a	21.5-40.3 (28.4) b	0.00	18-50
Oospore diameter (µm)	20.6-29 (24.1) b	20.1-30.4 (25.6) ab	21.3-33.6 (27.9) a	18.6-36.6 (25.2) b	0.00001	15-40 (26)
Length (L) antheridium (µm)	10.4-16.4 (13.3)	8.9-14.4 (12.9)	10.0-16.5 (13.4)	8.3-16.1 (13.1)	0.6768	14-19
Breadth (B) antheridium (µm)	10.8-16.2 (13.2)	10.7-14.5 (12.7)	6.3-14.7 (12.1)	7.2-16.9 (11.8)	0.085	13-15
L/B ratio antheridium	0.7-1.3 (1.0)	0.8-1.4 (1.0)	0.8-1.8 (1.1)	0.7-1.5 (1.1)	0.4986	

[a] Erwin & Ribeiro (1996). Numbers in brackets indicate average value. Different letters in the same row means significant differences (Kruskal Wallis-All pairwise comparison test (α=0.05)). Absence of letters indicates no differences among isolates.

palmivora control isolate produced chlamydospores from the first week of incubation at 16ºC.

The four isolates tested were heterothallic, belonging to group A1. All antheridia were amphyginous and oospores plerotic. Antheridia of these isolates were similar in size. Pcl-612 produced the largest oogonia and oospores, but no significant differences appeared when isolates were compared. All organs measurements ranged as expected for *P. capsici*.

Molecular characterization of isolates

The PCR product of the ITS-rDNA region for all the isolates yielded the consensus 759-bp sequence (Fig. S1 [suppl]), which showed 99% identity with the sequence of two *P. capsici* representative isolates from Database of the World Phytophthora Collection, PD_00009 and PD_00091, with accession numbers GU259440 and GU259417, respectively (www.phytophthoradb.org).

Also, our consensus sequence showed 99% identity with each of the 5 sequences (FJ944088, FJ944089, FJ944091, FJ944092 and GU109334) belonging to the 5 genotypes of *P. capsici* based on ITS PCR-RFLP patterns described by Meitz *et al.* (2010).

Host range test 1: Most common species in Mediterranean greenhouses

All species tested but bean proved susceptible to all *P. capsici* isolates. Isolates recovered from pepper were pathogenic to solanaceous and cucurbitaceous hosts, as it was isolate Pcl-612 (original from zucchini). The three isolates induced the same symptoms. Symptomatic plants showed chlorosis and brown discoloration of basal stem, followed by wilting and plant dead (Fig. 2). Pathogenicity of isolate Pcl-811 was weak: slight wilting was recorded only in the first of the experiments and only for the cucurbitaceous species tested: zucchini, cucumber, melon, and watermelon. However, none of the plants tested in the second experiment exhibited wilt. Only moderate root rot was observed when isolate Pcl-811 was inoculated in both experiments, and DSI was consistently higher for the other three isolates (Table 3). Bean roots were not damaged in any case, and eggplant showed lower root damage in comparison with other plant species depending on the isolate tested (Table 3).

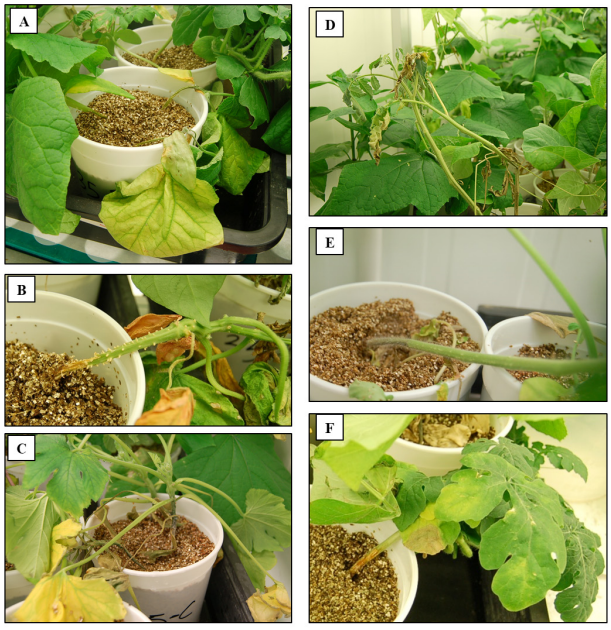

Figure 2. Symptoms induced by *P. capsici* on artificially inoculated horticultural species at 14 days post inoculation. **A:** Cucumber (chlorosis, crown rot); **B:** Melon (crown discoloration, adventitious roots); **C:** Zucchini (chlorosis, crown rot, wilting); **D:** Tomato (wilting); **E:** Tomato (crown rot); **F:** Eggplant (crown rot).

When AUDPS was calculated and analyzed, differences among host response and isolate pathogenicity, as well as interactions between host and isolate (factors 1 and 2), were revealed (Table 4). Zucchini, cucumber, and tomato were the most susceptible hosts, followed by melon and watermelon, which displayed intermediate susceptibility. Pepper and eggplant showed a late susceptible response in comparison with the other species, but symptoms expressed unequivocally and at least 40% of plants died following inoculation with some of the isolates, before the end of the trials (Fig. 3). At least one plant of each of these solanaceous and cucurbitaceous crops was dead before 18 dpi.

Strains MI0211, MI2311, and Pcl-612 were reisolated from all the samples of symptomatic roots, while it was not possible to reisolate them from bean root samples. Isolate Pcl-811 was reisolated only from plants with damaged roots.

Host range test 2: Fabaceae greenhouse species

None of the inoculated fabaceous plants expressed any symptoms. However, all zucchini controls wilted, and an average of 83% and 100% zucchini plants died after being inoculated with isolates MI0211 and Pcl-612, respectively (Fig. 4). Both strains were reisolated only from zucchini samples.

Discussion

Continuous cropping of the same plant host is often considered the bane of sustainable crop production due to increased outbreaks of plant diseases resulting from augmented pathogen populations (Chellemi *et al.*, 2016). Mediterranean protected farms, normally host two crops per year (Castilla, 2002). There, typical rotations alternate between solanaceous and cucurbitaceous, or even between species of the same family. However, pepper and zucchini are very infrequent rotation crops (García-García *et al.*, 2016). *Phytophthora capsici* was isolated from zucchini and pepper greenhouse crops in Almeria (Spain), and pathogenicity to both hosts was demonstrated (Gómez *et al.*, 2013; De Cara-García *et al.*, 2017). In this work, isolates recovered from symptomatic pepper and zucchini plants were tested for their pathogenicity on the other plant species commonly cultivated in Mediterranean greenhouses. Also, biological features were studied in order to find any differences linked with the plant origin and pathogenicity of each isolate.

According to the results, the four isolates included in this work clearly belong to the species *P. capsici*. ITS

Table 3. Pathogenicity of each *P. capsici* isolate to different Mediterranean greenhouse crops measured by root damage.

Isolate	Host	DSI[a]	Homogeneous groups[b]
MI0211	Zucchini	3.67	
	Cucumber	4	
	Tomato	3.75	
	Watermelon	2.75	
	Melon	2.92	
	Pepper	4	
	Eggplant	2.88	
MI2311	Zucchini	3.21	ab
	Cucumber	3.71	ab
	Tomato	4	a
	Watermelon	2.71	ab
	Melon	3	ab
	Pepper	1.71	ab
	Eggplant	1.17	b
Pcl-612	Zucchini	3.71	a
	Cucumber	3.96	a
	Tomato	3.75	a
	Watermelon	2.92	ab
	Melon	2.96	ab
	Pepper	2.92	ab
	Eggplant	0.63	b
Pcl-811	Zucchini	1.29	
	Cucumber	1	
	Tomato	0.42	
	Watermelon	1.46	
	Melon	1.42	
	Pepper	0.58	
	Eggplant	0.08	

[a]Pathogenicity is expressed by a disease severity index (DSI) ranging from 0 to 4 (0 = 0-5% roots necrotic; 1 = 6-24% roots necrotic; 2 = 26-50% roots necrotic; 3 = 51-75% roots necrotic; 4 = 76-100% roots necrotic). DSI data are mean ranks calculated through Kruskal-Wallis nonparametric AOV. [b]Homogeneus groups by host generated when differences were found by Kruskal-Wallis. Kruskal-Wallis all-pairwise comparison test used.

sequence study strongly supports this finding. However, colony growth and sporangia length of isolate Pcl-811 differed from the other *P. capsici* isolates. Pcl-811 grew very slow at 35 ºC (1.7 mm/day), in contrast to all 47 and 57 isolates from pepper and pumpkin studied by Bartual *et al.* (1991) and Islam *et al.* (2005), respectively (over 4.0 mm/day at 35 ºC). Ristaino (1990) proposed sporangium length as character suitable to be used for separating cucurbit-recovered from pepper-recovered isolates, as he found mean lengths of sporangia among cucurbit-recovered isolates were greater than 55 µm, whereas 10 out of 17 pepper-recovered isolates studied were significantly shorter. In our case, sporangium length did not significantly differentiate the zucchini-recovered isolate Pcl-811 from pepper-recovered isolates, all sporangia measuring less than 55 µm long. In addition, isolate Pcl-811 showed the lowest pathogenicity to any of the hosts in the current study, which could be linked to these differential features. Non-pathogenic or low aggressive isolates of *P. capsici* have been reported by other researchers (Polach & Webster, 1972; Islam *et al.*, 2005; Foster & Hausbeck, 2010; Wang *et al.*, 2013). The role of these isolates in the agrosystem is unknown, but it should be the object of future studies.

According to Erwin & Ribeiro (1996), the formation of chlamydospores of *P. capsici* depends on the cultural methods and the host origin of each isolate. The lack of chlamydospores formation is usual in *Capsicum* isolates (Uchida & Aragaki, 1985; Bartual *et al.*, 1991; Andrés *et al.*, 2003). However pumpkin isolates were easily able to form chlamydospores (Islam *et al.*, 2005). In the present work, we tested chlamydospore production on agar and liquid media, as recommended by Uchida & Aragaki (1985), but isolates did not form chlamydospores in any case. This result agrees with those of Ristaino (1990), and can determine lack of survival of the isolates in soils or plant debris.

The production of oospores also can improve the survival of this oomycete, but requires the coexistence of the A1 and A2 mating types. In contrast to other areas where both mating types of *P. capsici* are present (Lamour & Hausbeck, 2003; Islam *et al.*, 2005; Gevens *et al.*, 2007; Meitz *et al.*, 2010), all our isolates belong to the A1 type. This finding is complemented with 57 additional isolates belonging to the A1 type in a recent study in the area (De Cara-García *et al.*, 2017), and is consistent with the results of other works in Spain, where only the A1-type isolates of *P. capsici* has been reported (Tello, 1984; Bartual *et al.*, 1991; Andrés *et al.*, 2003; Larregla del Palacio, 2003; Silvar *et al.*, 2006; Guerrero, 2012).

The absence of chlamydospores as well as oospores reduces the persistence of the pathogen in soil, so that crop rotation could be a feasible option to manage the disease in the area, using non-susceptible plants. Among the eight most common species cultivated in Mediterranean greenhouses, only green bean (round and flat types) proved non-susceptible in our assays for all the isolates. Additionally, other minor fabaceous Mediterranean greenhouse crop such as green pea (round and flat types) showed full incompatibility with *P. capsici.* This could suggest that fabaceous

Table 4. Pathogenicity of different *P. capsici* isolates to different Mediterranean greenhouse crops measured by area under disease progress stairs (AUDPS).

Host	Isolate	AUDPS[a]	Group[b]
Zucchini	MI0211	26.24 a	Group 1 (a,b)
Cucumber	MI2311	24.52 ab	
Tomato	MI0211	23.49 ab	
Cucumber	MI0211	22.51 ab	
Zucchini	Pcl-612	22.20 ab	
Tomato	MI2311	22.13 ab	
Zucchini	MI2311	21.92 ab	
Tomato	Pcl-612	21.74 ab	
Cucumber	Pcl-612	21.04 b	
Watermelon	Pcl-612	15.99 c	Group 2 (c,d)
Melon	MI2311	15.16 cd	
Melon	Pcl-612	14.84 cd	
Pepper	MI0211	14.06 cd	
Watermelon	MI0211	13.45 cd	
Melon	MI0211	12.98 cde	
Watermelon	MI2311	10.45 def	
Eggplant	MI0211	8.37 ef	Group 3 (e-h)
Pepper	Pcl-612	6.37 fg	
Eggplant	MI2311	2.80 gh	
Eggplant	Pcl-612	2.17 gh	
Pepper	MI2311	1.26 h	

[a]Pathogenicity is expressed by AUDPS. Combinations are ordered by decreasing AUDPS. Different letters following numbers indicate significance (LSD, p=0.05). [b]Results are grouped according to LSD test. Group 1: High susceptible combination; Group 2: Moderate susceptible combination; Group 3: Low susceptible combination.

crops are a suitable family of rotation crops to avoid *P. capsici* damage. In fact, green pea was cited as non-host of *P. capsici,* showing inhibitory effects on this pathogen (Hwang & Kim, 1995). Green bean was reported to be a susceptible crop in Michigan, but damage was not detected in the roots, but only in aerial tissues of the plants (Gevens & Hausbeck, 2004). Another work reproduced Koch's postulates for foliar and pod symptoms, but not for root rot (Gevens *et al.*, 2008). Under controlled inoculations, snow pea (*Pisum sativum*), lima bean (*Phaseolus lunatus*), and snap bean (*Ph. vulgaris*) expressed *P. capsici* root rot (Tian & Babadoost, 2004). Another fabaceous species (*Lathyrus latifolius*) was non-susceptible to *P. capsici* in controlled trials, while *Lupinus polyphyllus*, and *Glycine max* were susceptible (Tian & Babadoost, 2004; Enzenbacher *et al.*, 2015). It is worthy to mention that beans and peas are also non-hosts of the most recent devastating disease reported for zucchini in the Mediterranean area: ToLCNDV (Ruiz *et al.*, 2017).

Regarding the other crops assessed, isolates MI0211, MI2311 and Pcl-612 damaged more of the 50% of roots systems of all the cucurbitaceous and solanaceous crops tested. However, pepper and eggplant showed lower susceptibility to *P. capsici*, with low number of dead plants at 18 dpi (Table 4 & Fig. 3). Similar results were found in melon and watermelon, but the percentage of symptomatic plants was higher, and the disease was expressed more evenly. Zucchini, cucumber, and tomato expressed the highest susceptibility with an average over 50% of plants dead at 18 dpi, and more than 90% at 37 dpi.

Tompkins & Tucker (1941) studied the host range of *P. capsici* isolates recovered from pepper and zucchini. These authors found high susceptibility on pumpkin (including zucchini), squash, tomato, and pepper, but 3 of 5 eggplant varieties inoculated proved non-susceptible. Notably, the incubation period for inoculated pepper ranged from 5 to 49 days, in contrast to pumpkin (4 to 33 days). Using different inoculation

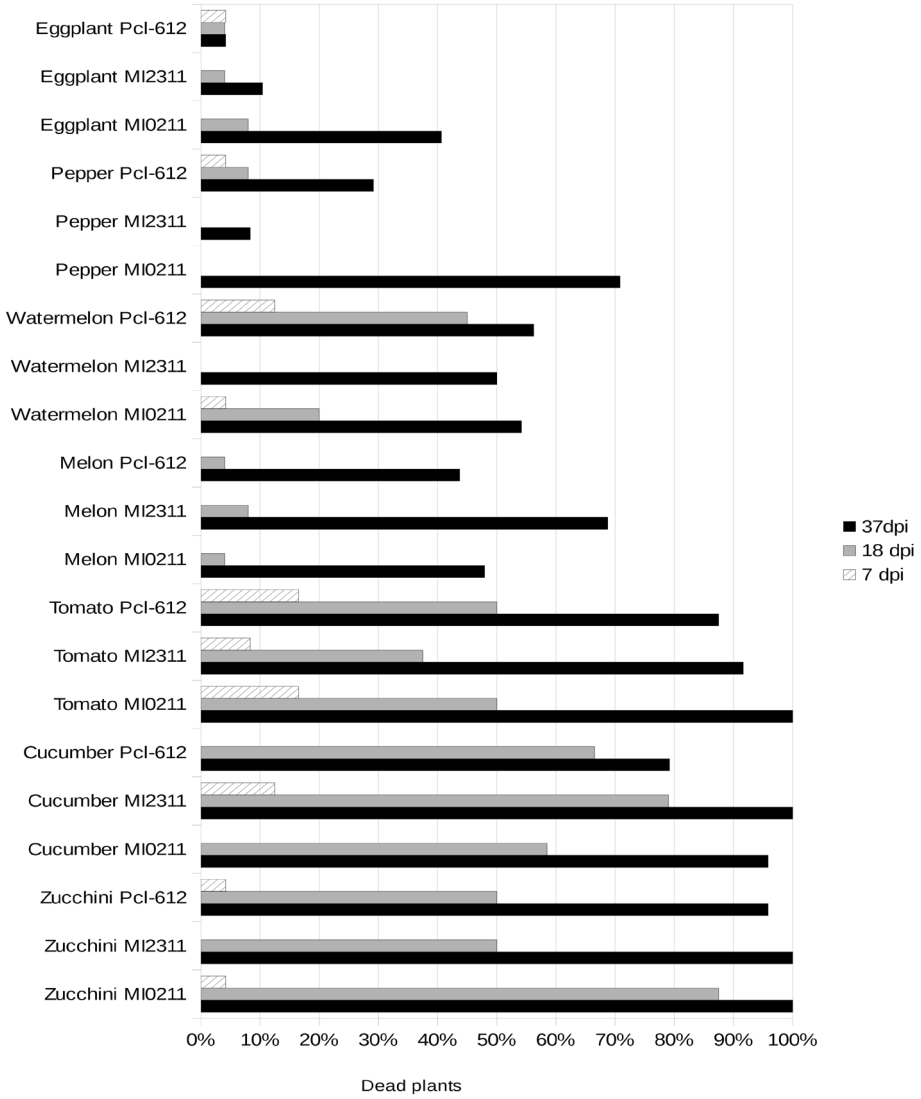

Figure 3. Pathogenicity of *P. capsici* isolates to Mediterranean greenhouse crops expressed by percentage of dead inoculated plants at different times after inoculation.

and irrigation techniques for *P. capsici* isolates from pumpkin, Tian & Babadoost (2004) also found a shorter incubation period for cucurbitaceous species (more than 75% of diseased plants per species at 3 dpi) than for solanaceous (ranged between 35.6% of diseased eggplants to 51.4% of diseased peppers, at 3dpi). These findings agree with our results, which show that pepper and eggplant seem to require a longer incubation period than the other species. However, this delay cannot be associated with the family level, as the other solanaceous (tomato) showed a short incubation period.

With regard to the isolate host origin and its host-pathogen response, previous studies have included inoculations with isolates of *P. capsici* from solanaceous and cucurbits. Ristaino (1990) inoculated pepper with isolates recovered from pepper and pumpkin, and concluded that all the isolates were pathogenic. Ezenbacher & Hausbeck (2012) found that *P. capsici* isolates recovered from solanaceous (eggplant and pepper) were more aggressive than those recovered from pickling cucumber, when inoculated in squash, pumpkin or zucchini. On the contrary, Lee *et al.* (2001) found more aggressiveness in pumpkin than pepper-recovered isolates, when inoculated in Korean pumpkin cultivars. In our work, significant differences among isolates were identified only for pepper host. Isolate MI0211 (pepper origin) was more aggressive on pepper than MI2311 and Pcl-612 (Tables 3 and 4). Nevertheless, the last two isolates were pathogenic to pepper, so that in terms of virulence (*sensu* VanderPlank, 1963) pepper could not be characterized as non-susceptible to MI2311 or Pcl-612. Based on our results and the literature, host susceptibility cannot be linked to the host origin of *P. capsici*. In our study aggressiveness of the isolates was also independent of the host origin of the isolate. Nonetheless, hosts were grouped by their

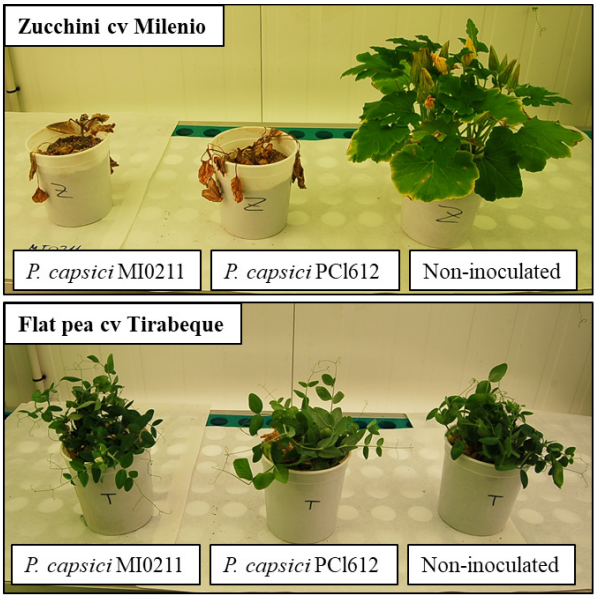

Figure 4. Comparisons of symptoms induced by *P. capsici* between Zucchini and Flat pea.

AUDPS, showing a quantitative response in terms of delay in the expression of the disease and number of affected plants.

In the area where the isolates were collected, *P. capsici* damage is more frequent on pepper than in other hosts (De Cara-García *et al.*, 2017), contrary to our results for the inoculations. This result may be a consequence of the cropping cycles for the different crops. Pepper crop is planted in June-August in the area, while cucumber, zucchini, eggplant, and tomato are planted later. Melon and watermelon are spring crops. The higher temperatures in soils and water from June to August can be decisive in the expression of the disease caused by *P. capsici*. On the other hand, soil solarization (including chemical or not) is widespread in Mediterranean greenhouses, but efficacy of solarization requires high environmental temperatures that are achieved only from mid-June to mid-September in southern Spain (Gómez & Pérez, 2017). If pepper is planted in June-July, solarization cannot be effectively accomplished, and *P. capsici* structures can survive in soils. It bears noting that we have not found the A2 mating type of *P. capsici* in the area, and our isolates did not produce any chlamydospores, and therefore *P. capsici* is not in the best situation to survive in these soils. Thus, crop rotation with non-hosts is a good option in order to reduce the level of inoculum in infested soils. Our findings indicate the role of bean and pea as non-susceptible alternatives to solanaceous and cucurbitaceous crops of the Mediterranean greenhouses. This finding is supported by previous surveys in the area, focused on root rot diseased beans, which never allowed the detection of *P. capsici*, while other soil-borne fungi were associated to the mentioned symptoms (Tello *et al.*, 1985; Serrano *et al.*, 2004).

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