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RESEARCH PAPERS

Characterization and pathogenicity of *Plectosphaerella* spp. collected from basil and parsley in Italy

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Summary. From 2012–2016 plants of basil (*Ocimum basilicum* L.) and parsley (*Petroselinum sativum* Hoffm.) showing decline symptoms were collected from local markets in Foggia Province (southern Italy) and assessed to determine their main fungal pathogens. These plants showed symptoms including leaf yellowing, necrotic lesions on stems, collar and roots, and in some cases, stunting of entire plants. Mycological analyses revealed fungal isolates mainly belonging to the *Plectosphaerella* genus. Molecular and morphological studies identified four species of *Plectosphaerella*: *Plectosphaerella cucumerina*, *P. pauciseptata*, *P. plurivora*, and *P. ramiseptata*. To understand the pathogenic roles of these fungi, and five other reference *Plectosphaerella* spp., pathogenicity tests were performed *in vitro* and *in-vivo* using, respectively, detached leaves and 30-d-old plants of basil (cv. Napoletano) and parsley (cv. Gigante di Napoli). All the fungal species isolated produced host symptoms, including necrotic spots, parenchymatic patches, hydropic areas, collar and root discolouration on leaves and young plants, with varying severity. The most aggressive species on both plants were *P. pauciseptata* and *P. ramiseptata*, while *P. alismatis*, *P. citrulli*, *P. cucumerina*, *P. delsorboi*, *P. melonis*, and *P. plurivora* gave less disease severity on both plants. This is the first report worldwide of *P. cucumerina, P. pauciseptata*, and *P. ramiseptata* as pathogens of parsley, and *P. pauciseptata*, *P. plurivora*, and *P. ramiseptata* as pathogens of basil.

Key words: *Ocimum basilicum*, *Petroselinum sativum*, endophytes, hemibiotrophs, necrotrophs.

Introduction

Foggia Province of southern Italy is a major area for horticultural production in Italy, although basil (*Ocimum basilicum* L.) and parsley (*Petroselinum sativum* Hoffm.) are considered minor crops (Istat, 2011). For this reason, little attention has been paid to diseases that affect quality and quantity of their production. The most important fungal pathogens for basil and parsley are *Alternaria* spp., *Fusarium* spp., *Pythium* and *Phytophthora* spp., *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, (Garibaldi *et al*., 1997; Minchinton *et al*., 2006).

Carlucci *et al*. (2012) described *Plectosphaerella* spp. as a new group of soilborne pathogens of several horticultural crops in Italy, including the four new species *P. citrulli*, *P. pauciseptata*, *P. plurivora*, and *P. ramiseptata*, along with four that were renamed as *P. alismatis*, *P. delsorboi*, *P. oratosquillae*, and *P. melonis*. As the main focus of their study was a taxonomic reassessment of the *Plectosphaerella* genus, no investigation was carried out on the disease symptoms incited by these fungi. *Plectosphaerella oligotrophica* from soil, *P. populi* from poplar, *P. sinensis* from cucurbits and *P. niemeijerarum* from soil have also been recently described (Liu *et al*., 2013; Crous *et al*., 2015; Crous *et al*., 2017; Su *et al*., 2017). Based on previous reports, with the inclusion of *P. cucumerina* (syn. *Plectosporium tabacinum*), the *Plectosphaerella* genus currently includes 13 species.

Over the last 30 years, *Plectosphaerella* isolates have been obtained from different plant hosts from many countries, with the most common species being *P. cucumerina*. For instance, *P. cucumerina* has been reported

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as a root and collar pathogen of economically important plants, including tomato, sunflower, soybean, melon, pumpkin and other cucurbits, white lupin, peanut, ranunculus, *Hydrilla verticillata*, *Campanula isophylla*, and *Aconitum*. *Plectosphaerella cucumerina* was also reported as a pathogen of basil (Mersha *et al*., 2012), and *P. pauciseptata* as a pathogen of coriander (*Coriandrum sativum* L.) (Usami *et al*., 2012). However, infections by *Plectosphaerella* spp. are not well defined, and they have also been reported as causal agents of wilt (Xu *et al*., 2014) and root rot diseases (Carrieri *et al*., 2014).

In the present study, the main aim was to identify and characterize a collection of fungal isolates from basil and parsley plants collected in local markets in the Foggia Province (Apulia, southern Italy). A detailed study was carried out using morphological and molecular tools to identify *Plectosphaerella* species. Pathogenicity tests were also carried out to determine if *Plectosphaerella* spp. caused disease symptoms on basil and/or parsley.

Materials and methods

Plant sampling and fungal isolations

From 2012 to 2016, 75 basil and 86 parsley plants that showed various degrees of decline were collected from local markets in the Foggia region of the Apulia Province in southern Italy. The symptoms consisted of leaf yellowing, necrotic lesions on collars and roots, and in some cases, stunting of plants. Each plant sample was washed and the surface sterilized (Fisher *et al*., 1992), and then subjected to mycological analysis to ascertain the main fungi present. Five tissue portions (3 × 3 mm) were cut from each sample of plant roots, collars, stems or leaves, were plated onto potato dextrose agar (PDA; Oxoid Ltd) supplemented with 400 ppm streptomycin sulphate (Sigma-Aldrich), and were then incubated in the dark at 23 ± 2°C. Conidium suspensions from any fungal colony were spread onto agar plates, and after 24 to 36 h of incubation, single germinated conidia were transferred to fresh PDA plates and incubated from 10 to 21 d, at 23 ± 2°C, in the dark. Initially, the morphological and culture features (i.e., type and colour of colonies, presence, shape and size of conidia, ascomata or conidiomata formations) were used to distinguish the fungal genera and species isolated from symptomatic basil and parsley plants. A total of 136 isolates resembling *Plectosphaerella* genus were identified using morphological and molecular methods, according to Carlucci *et al.* (2012) and Raimondo and Carlucci (2018). Reference isolates are maintained in the culture collection of the Department of Sciences of Agriculture, Food and the Environment, of the University of Foggia, Italy. The isolation frequencies (IFs) relative to basil or parsley plants were calculated, as the number of tissue portions that were infected by a given fungus divided by the total number of tissue segments that were incubated, with the data expressed as percentages.

Molecular and morphological analyses

Genomic DNA of all *Plectosphaerella* isolates was extracted from 10-d-old colonies grown on PDA plates in the dark at 23 ± 2°C, using the methods of Carlucci *et al.* (2013). All isolates were used for amplifying the ITS1 and ITS2 regions flanking the 5.8S ribosomal DNA, using the ITS1 and ITS4 primers (White *et al*., 1990). PCR amplifications and purification of amplicons were performed according to Raimondo and Carlucci (2018). Both strands of the PCR products were sequenced by Eurofins Genomics Service. Representative isolates were used to perform phylogenetic analysis according to Raimondo and Carlucci (2018). To confirm the molecular identifications, the isolates were subjected to microscopic and morphological analyses, based on methods described by Carlucci *et al.* (2012).

Pathogenicity tests

In vitro *assays*

Five fungal isolates for each *Plectosphaerella* species were used for the pathogenicity tests, including one reference strain and two isolates from basil and two from parsley plants. Five fungal isolates of *P. melonis* and *P. citrulli*, and reference strains of *P. alismatis*, *P. delsorboi*, and *P. oratosquillae* were also included in the pathogenicity tests.

To assess if the *Plectosphaerella* isolates were hemibiotrophic or necrotrophic pathogens, detached leaves from 30-d-old plants of basil (cv. Napoletano) and parsley (cv. Gigante di Napoli), grown from seed in a greenhouse, were used for artificial inoculations, using methods of Raimondo and Carlucci (2018). Conidium suspensions were obtained from 10-d-old colonies of the following fungi (and isolates): *P. alismatis*

(CBS113362), *P. citrulli* (CBS131741, Plect 517, Plect 189, Plect 649, Plect 806), *P. cucumerina* (CBS131739, Plect 662, Plect 711, Plect 736, Plect 802), *P. delsorboi* (CBS116708), *P. melonis* (CBS131859, CBS131858, Plect 60, Plect 148, Plect 212), *P. oratosquillae* (NJM0662), *P. pauciseptata* (CBS131745, Plect 683, Plect 685, Plect 696, Plect 690), *P. plurivora* (CBS131742, Plect 698, Plect 722, Plect 936, Plect 1023), and *P. ramiseptata* (CBS131861, Plect 660, Plect 652, Plect 655, Plect 671). Control (nil fungus) inoculations were performed with sterile distilled water.

The experimental design included 'two independent batches', with each host × isolate combination replicated five times. The same experiment was repeated after 1 month, and the means from each host × isolate combination were used in the statistical analyses of data obtained. Individual disease severities were determined after 15 d at 23 ± 2°C, using a scale of 0 to 5, where 0 = no symptoms observed; 1 = 1 to 20%; 2 = 21 to 40%; 3 = 41 to 60%; 4 = 61 to 80%; and 5 = 81 to 100%, of leaf surface area showing necrotic symptoms. The overall disease severities (*DS*) were calculated according to Raimondo and Carlucci (2018).

In vivo *assays*

A mixture of soil and peat (3:1) was sterilized twice at 121°C for 30 min, and then kept for 20 d in a greenhouse at 25±3°C, 70% relative humidity, under natural light. Young (35-d-old) plants of basil cv. Napoletano and parsley cv. Gigante di Napoli were transplanted into pots (180 mL capacity) containing the soil/peat mixture. Seven days after transplanting of the seedlings, the soil in each pot was inoculated with fungus isolates, using the methods of Raimondo and Carlucci (2018). Treatments for the control pots were with sterile distilled water instead of inoculum. The experimental design and the replication were performed as described for the *in vitro* assays. The same experiment was repeated after 1 month, and the means from each host × isolate combination were used in the statistical analyses of the data obtained. The pots were then kept in a greenhouse at 25 ± 3°C, 70% relative humidity, and under natural light, for up to 35 d. Subsequently, each seedling was removed from its pot, and the roots and collar were carefully washed. The presence of root and collar browning symptoms was then evaluated and defined using the following 0 to 5 severity scale: 0 = no symptoms observed; 1 = 1 to 20%; 2 = 21 to 40%; 3 = 41 to 60%; 4 = 61 to 80%; and 5 =, 81% to 100% of tissue affected, and the disease severities on the roots and collars were determined. All fungi were isolated from root and collar tissues of inoculated plants to fulfil Koch's postulates.

Statistical analyses

Normal distributions and homogeneity of variance of data were verified, respectively, following Shapiro-Wilk tests (W tests) and Levene tests, using the Statistica software (version 6, StatSoft). Factorial analysis of variance (ANOVA) was carried out for the factors on both datasets (i.e., leaves and seedlings) considering each fungus species and the two plant hosts (basil and parsley). This allowed determination of the significance of the isolates considering the same fungal species of the plant hosts inoculated, and detection of interactions between these factors (i.e., plant host × fungus species), when possible. One-way ANOVA was performed for determination of statistically significant differences in disease severities caused by each inoculated fungus species, and any differences due to plant species (i.e., basil or parsley). Fischer's tests defined the comparisons of the treatment means, with statistical significance accepted at $P < 0.01$.

Results

Fungus isolation and isolates

Table 1 contains information of the number of basil and parsley samples which were infected by *Plectosphaerella* isolates, and the main host plant parts involved. The isolation analysis revealed that 44 basil and 48 parsley plants were infected by *Plectosphaerella* species. Different organs of individual plants were often infected, so more *Plectosphaerella* isolates resulted than numbers of infected plants. Seventy-nine *Plectosphaerella* isolates were obtained from basil and 57 were obtained from parsley samples. From 15 basil and 11 parsley plants, *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., or bacteria were rarely isolated (Table 1). The other 16 basil and 27 parsley plants were not affected by any microorganisms.

Table 2 outlines the numbers and IFs of microorganisms isolated from root, collar, stem or leaf tissues of basil and parsley plants. Fungi belonging to the *Plectosphaerella* genus were isolated with IFs ≥75% from basil and parsley plants. IFs from 16 to 25% were obtained for other fungal genera, including *Aspergillus, Fusarium* and *Penicillium* spp., and bacteria. *Plec-*

Table 1. Infections occurring on collected basil and parsley plants.

Fungal species on	Number of infected plants	Infected organs and tissues				Total number of *Plectosphaerella* isolates*
		Root	Collar	Stem	Leaf	
Basil						
Plectosphaerella spp.	7	+	-	-	-	7
	15	-	+	-	-	15
	2	-	+	+	-	4
	2	-	-	+	+	4
	5	+	+	+	+	20
	10	+	+	-	-	20
	3	+	+	+	-	9
Sub-total	44	-	-	-	-	79
Aspergillus spp.	2	+	+	-	-	2
	1	+	-	-	-	1
Penicillium spp.	1	+	+	-	-	1
	3	+	-	-	-	3
Fusarium spp.	1	+	-	-	-	1
Bacteria	4	+	-	-	-	4
	3	-	+	-	-	3
No fungal and bacterial growth	16	-	-	-	-	-
Sub-total	31	-	-	-	-	15
Total plants collected	75					
Parsley						
Plectosphaerella spp.	30	+	-	-	-	30
	6	-	+	-	-	6
	2	-	+	+	-	4
	2	-	+	+	+	6
	5	-	+	-	-	5
	3	+	+	-	-	6
Sub-total	48	-	-	-	-	57
Aspergillus spp.	2	+	+	-	-	4
	1	+	-	-	-	1
Penicillium spp.	1	+	+	+	-	3
	1	+	+	-	-	2
	2	+	-	-	-	2
Fusarium spp.	1	+	-	-	-	1
Bacteria	3	+	+	-	-	6
No fungal and bacterial growth	27	-	-	-	-	-
Sub-total	38	-	-	-	-	19
Total plants collected	86					

\+ *Plectosphaerella* infection.
\- No *Plectosphaerella* infection.
* sum of *Plectosphaerella* isolates from organs infected.

Table 2. Isolation Frequencies of *Plectosphaerella* and other microbial species from basil and parsley plants sampled in this study.

Fungus and other microbe species isolated	Number (and Isolation Frequencies) [n (%)][a]										Total isolates from basil and parsley
	Basil				Total	Parsley				Total	
	Root	Collar	Stem	Leaf		Root	Collar	Stem	Leaf		
Plectosphaerella cucumerina	11 (11.7)	15 (16.0)	7 (7.4)	4 (4.3)	37 (39.4)	16 (21.1)	12 (15.8)	1 (1.3)	0 (0.0)	29 (38.2)	66
P. pauciseptata	5 (5.3)	4 (4.3)	2 (2.1)	1 (1.1)	12 (12.8)	2 (2.6)	1 (1.3)	1 (1.3)	1 (1.3)	5 (6.6)	17
P. plurivora	6 (6.4)	13 (13.8)	1 (1.1)	1 (1.1)	21 (22.3)	13 (17.1)	3 (3.9)	1 (1.3)	0 (0.0)	17 (22.4)	38
P. ramiseptata	3 (3.2)	3 (3.2)	2 (2.1)	1 (1.1)	9 (9.6)	2 (2.6)	2 (2.6)	1 (1.3)	1 (1.3)	6 (7.9)	15
Total of *Plectosphaerella* species isolated	25 (26.6)	35 (37.2)	12 (12.8)	7 (7.4)	79 (84.0)	33 (43.4)	18 (23.7)	4 (5.3)	2 (2.6)	57 (75.0)	136
Aspergillus spp.	2 (2.1)	1 (1.1)	0 (0.0)	0 (0.0)	3 (3.2)	3 (3.9)	2 (2.6)	0 (0.0)	0 (0.0)	5 (6.6)	8
Penicillium spp.	3 (3.2)	1 (1.1)	0 (0.0)	0 (0.0)	4 (4.3)	4 (5.3)	2 (2.6)	1 (1.3)	0 (0.0)	7 (9.2)	11
Fusarium spp.	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)	2
Bacteria and others	4 (4.3)	3 (3.2)	0 (0.0)	0 (0.0)	7 (7.4)	3 (3.9)	3 (3.9)	0 (0.0)	0 (0.0)	6 (7.9)	13
Total of other fungi and bacteria isolated	10 (10.7)	5 (5.3)	0 (0.0)	0 (0.0)	15 (16.0)	11 (14.5)	7 (9.2)	1 (1.3)	0 (0.0)	19 (25.0)	34

[a] Isolation Frequency per host was calculated as the number of segments infected by a given fungus divided by the total number of segments incubated, expressed as a percentage.

tosphaerella cucumerina and *P. plurivora* were the most commonly isolated *Plectosphaerella* species from basil (IFs, respectively, 39.4 and 22.3%) and parsley (IFs, respectively, 38.2 and 22.4%). *Plectosphaerella ramiseptata* was the least frequently isolated species from basil (IF = 9.6%), while *P. pauciseptata* was the least isolated from parsley (IF = 6.6%). The greatest IF values for *Plectosphaerella* spp. recorded were those from root and collar tissues; from basil (root = 26.6%; collar = 37.2%) and from parsley (root = 43.4%; collar = 23.7%).

Morphological and molecular identifications

Based on the molecular, phylogenetic and morphological protocols of Carlucci *et al.* (2012) and Raimondo and Carlucci (2018), 136 isolates were attributed to four species in the Plectosphaerellaceae, as *P. cucumerina*, *P. pauciseptata*, *P. plurivora*, and *P. ramiseptata*. ITS sequences generated for 136 isolates of the *Plectosphaerella* spp. were compared with reference sequences retrieved from GenBank. Sixty-six isolates showed 99% of similarity with the reference strain of *P. cucumerina* (acc. no. HQ238980), 17 showed 100% similarity with *P. pauciseptata* (acc. no. HQ238971), 38 showed 100% similarity with *P. plurivora* (acc. no. HQ238975), and 15 showed 100% similarity with *P. ramiseptata* (acc. no. JQ246953). The phylogenetic analysis resulted in a tree with similar topology to that reported by Raimondo and Carlucci (2018) (no data or tree presented). The morphological identification of isolates confirmed the results obtained from molecular analysis.

Pathogenicity tests

In vitro *assays*

Shapiro-Wilk and Levene tests showed the data from the *in vitro* pathogenicity tests with basil and parsley leaves followed normal distributions, and the homogeneity of the variances was statistically significant. Factorial ANOVA demonstrated that, except for *P. delsorboi* and *P. oratosquillae*, which did not produce any symptoms on host leaves, there were significant differences between the two plant hosts inoculated with the different *Plectosphaerella* spp. (Table 2). However, no significant differences were detected among the isolates of each fungus species inoculated. The interactions between host and fungus species factors were not statistically significant (Table 3).

One-way analysis of variance carried out for data from basil and parsley leaves showed that the *Plectosphaerella* spp. used in this study differed in virulence (Table 4). Symptoms on basil leaves 15 d after inoculation with the nine *Plectosphaerella* spp. showed different mean disease severity scores, from 2.9 for *P. plurivora* to 5.0 for *P. citrulli* and *P. ramiseptata*. No symptoms were observed for the basil or parsley leaves inoculated with *P. delsorboi* or *P. oratosquillae*, while *P. melonis* showed very low virulence, giving a mean severity score of 0.5. All fungi that were pathogenic on basil leaves produced necrotic symptoms, as necrotic parenchyma patches or spots with light discoloured rings (i.e., *P. alismatis, P. citrulli*, *P. cucumerina, P. melonis*, *P. ramiseptata*), as well as vascular necrosis (i.e., *P. pauciseptata*, *P. plurivora*) (Figure 1).

Of the nine *Plectosphaerella* species inoculated on parsley leaves, *P. alismatis*, *P. citrulli*, *P. cucumerina*, *P. pauciseptata*, *P. plurivora*, and *P. ramiseptata* produced symptoms 15 d after inoculation, with variable mean severity scores, from 0.9 (*P. cucumerina*) to 3.5 (*P. pauciseptata*, *P. ramiseptata*). *Plectosphaerella delsorboi*, *P. melonis* and *P. oratosquillae* did not produce any symptoms on parsley leaves. All of the fungi that were pathogenic on parsley leaves produced necrotic symptoms, as necrotic parenchyma patches with hydropic rings (Figure 2).

In vivo *assays*

Shapiro-Wilk and Levene tests showed that the data from the *in vivo* pathogenicity tests with basil and parsley seedlings followed normal distributions, and the homogeneity of the variances was statistically significant.

Factorial ANOVA demonstrated that except for *P. oratosquillae*, which did not produce symptoms on inoculated seedlings, and *P. citrulli* and *P. cucumerina*, there were statistically significant differences between the plant hosts inoculated with the different *Plectosphaerella* spp. Conversely, there were neither significant differences among the isolates of each inoculated fungus species, nor between host and isolates of the same fungus species (Table 3).

One-way analysis of variance results for the basil and parsley seedlings are outlined in Table 3. The symptoms observed on basil seedlings after inoculation with the nine *Plectosphaerella* spp. isolates were determined, with mean disease severity scores from 1.6 (*P. alismatis*) to 5.0 (*P. ramiseptata*). Among the fungi inoculated, only *P. oratosquillae* did not produce symptoms. All of the fungi that were pathogenic produced collar and root browning on host plants, although with different disease severities (Figure 1).

The symptoms observed on parsley seedlings after inoculation with the nine *Plectosphaerella* spp. isolates also varied, with mean severity scores from 1.6 (*P. delsorboi*) to 4.5 (*P. ramiseptata*). Among the fungi inoculated, only *P. oratosquillae* did not produce symptoms. All of the fungi that were pathogenic produced collar and root browning on parsley plants. The isolates of *P. alismatis*, *P. citrulli*, *P. cucumerina*, *P. pauciseptata*, and *P. ramiseptata* also caused consistent disease on parsley roots (Figure 2).

All of the *Plectosphaerella* spp. were re-isolated from respective inoculated basil and parsley seedlings at high frequencies, from 92 to 100% (Table 4).

Discussion

This study highlights that minor cultivated crops such as basil and parsley can often be affected by *Plectosphaerella* spp. as soilborne pathogens (Garibaldi *et al.*, 1997; Egel *et al.*, 2010; Carlucci *et al.*, 2012; Mersha *et al.*, 2012). *Plectosphaerella cucumerina* (= *Plectosporium tabacinum*) is the most well-known of these species, and this fungus was first reported on basil by Garibaldi *et al.* (1997), as *Microdochium tabacinum*, who considered this fungus as a saprophyte that could cause black leg symptoms. Egel *et al.* (2010) and Mersha *et al.* (2012) also implicated *P. cucumerina* as causal agent of black leg. Carlucci *et al.* (2012) first reported *P. plurivora* from parsley plants, although no information about its pathogenic role was described. The isolations carried out in the present study demonstrated that different *Plectosphaerella* spp. have been isolated from symptomatic basil and parsley plants.

Table 3. Factorial analysis of variance (ANOVA) between the main factors (hosts, isolates) and their interactions in the pathogenicity tests with different *Plectosphaerella* spp. carried out *in vitro* (leaves) and *in vivo* (seedlings) of basil and parsley.

Species	Factor	ANOVA[a]					
		Leaves			Seedlings		
		DF	F	P	DF	F	P
P. alismatis	Host[b]	1	55.7	0.001	1	33.33	0.001
	CBS133362[c]	–	–	–	–	–	–
	Host × CBS133362[d]	–	–	–	–	–	–
P. citrulli	Host	1	113.50	0.001	1	1.29	0.27
	CBS131741/ Plect 189[e]/ Plect 517/ Plect 649/ Plect 806	4	0.04	0.96	4	0.43	0.66
	Host × CBS131741/ Plect 189/ Plect 517/ Plect 649/ Plect 806	1	0.05	0.95	1	1.29	0.30
P. cucumerina	Host	1	460.93	0.001	1	0.16	0.70
	CBS131859/ Plect 662/ Plect 711/ Plect 736/ Plect 802	4	0.34	0.72	4	0.35	0.70
	Host × CBS131859/ Plect 662/ Plect 711/ Plect 736/ Plect 802	1	0.11	0.89	1	1.22	0.31
P. delsorboi	Host	1	0.00	1.00	1	7.54	0.03
	CBS116708	–	–	–	–	–	–
	Host × CBS116708	–	–	–	–	–	–
P. melonis	Host	1	6.69	0.01	1	6.67	0.01
	CBS131858/ CBS131859/ Plect60/ Plect 148/ Plect 212	4	1.99	0.16	4	0.27	0.77
	Host × CBS131858/ CBS131859/ Plect 60/ Plect 148/ Plect 212	1	1.84	0.18	1	0.26	0.77
P. oratosquillae	Host	1	0.20	0.67	1	1.00	0.35
	NJM0662	–	–	–	–	–	–
	Host × NJM0662	–	–	–	–	–	–
P. pauciseptata	Host	1	11.07	0.003	1	32.67	0.001
	CBS131745/ Plect 683/ Plect 685/ Plect 690/ Plect 696	4	0.07	0.93	4	0.56	0.97
	Host × CBS131745/ Plect 683/ Plect 685/ Plect 690/ Plect 696	1	0.23	0.80	1	1.27	0.36
P. plurivora	Host	1	56.31	0.001	1	145.80	0.001
	CBS131742/ Plect 698/ Plect 722/ Plect 936/ Plect 1023	4	0.5	0.62	4	0.60	0.56
	Host × CBS131742/ Plect 698/ Plect 722/ Plect 936/ Plect 1023	1	2.63	0.92	1	0.62	0.55
P. ramiseptata	Host	1	143.50	0.001	1	5.44	0.03
	CBS131861/ Plect 660/ Plect 652/ Plect 655/ Plect 671	4	1.82	0.18	4	0.44	0.65
	Host × CBS131861/ Plect 660/ Plect 652/ Plect 655/ Plect 671	1	2.11	0.14	1	0.44	0.65

[a] DF = degrees of freedom; F = F value; *P* = P value ($P < 0.01$).
[b] Hosts, basil × parsley.
[c] Isolate used in pathogenicity tests.
[d] Hosts × isolate.
[e] Code of Culture Collection at Dept. Sciences of Agriculture, Food and Environment, University of Foggia.

Table 4. Mean disease severity scores from pathogenicity assays carried out with different species of *Plectosphaerella* inoculated onto basil and parsley leaves or seedlings.

Fungal species	Leaves				Seedlings				Re-isolation (%)
	Disease severity score (0–5 scale)			Symptoms description	Disease severity score (0–5 scale)			Symptoms description	
	Mean	SD	Min–Max[a]		Mean	SD	Min–Max		
Basil									
Control	0.0 A	–	–	No symptoms	0.0 A	–	–	No symptoms	0
P. alismatis	3.4 B	1.1	2.0–5.0	Necrotic patches (PPB[b])	1.6 B	0.5	1.0–2.0	Reduced collar and browning, light root browning	92
P. citrulli	5.0 D	0.0	5.0–5.0	Necrotic patches (PPB)	2.8 BC	0.6	2.0–4.0	Blackish necrosis of collar, root browning, reduced root growth	100
P. cucumerina	4.9 CD	0.3	4.0–5.0	Necrotic patches (PPB)	2.5 BC	1.1	1.0–4.0	Collar browning, light root browning	96
P. delsorboi	0.0 A	–	–	No symptoms	3.0 BC	1.0	2.0–4.0	Blackish necrosis of collar, light root browning	100
P. melonis	0.5 A	0.8	0.0–2.0	Necrotic spots (PPB)	3.40C	0.5	3.0–4.0	Blackish necrosis of collar, light root browning	100
P. oratosquillae	0.0 A	–	–	No symptoms	0.0 A	–	–	No symptoms	0
P. pauciseptata	4.3 C	0.7	3.0–5.0	Necrosis (PTB[c])	2.6 BC	1.2	1.0–4.0	Blackish necrosis of collar, reduced root growth	96
P. plurivora	2.9 B	0.4	2.0–3.0	Necrosis (PTB)	4.8 D	0.4	4.0–5.0	Blackish necrosis of collar, root browning	100
P. ramiseptata	5.0 D	0.0	5.0–5.0	Necrotic patches (PPB)	5.0 D	0.0	5.0–5.0	Blackish necrosis of collar, root browning	100
Parsley									
Control	0.0 A	–	–	No symptoms	0.0 A	–	–	No symptoms	0
P. alismatis	2.6 C	0.5	2.0–3.0	Necrotic patches (PPB)	3.6 D	0.5	3.0–4.0	Collar browning, destruction of root	100
P. citrulli	1.7 BC	1.1	0.0–3.0	Necrotic patches (PPB)	3.0 CD	0.4	2.0–4.0	Light collar browning, browning, reduction and destruction of root	96
P. cucumerina	0.9 A	0.6	0.0–2.0	Necrotic spots (PPB)	2.3 BC	0.7	1.0–3.0	Blackish necrosis of collar, and destruction of root	96
P. delsorboi	0.0 A	–	–	No symptoms	1.6 B	0.5	1.0–2.0	Reduced root growth	92
P. melonis	0.0 A	–	–	No symptoms	2.7 C	0.8	2.0–4.0	Light collar browning, root browning	96
P. oratosquillae	0.0 A	–	–	No symptoms	0.0 A	–	–	No symptoms	0

(Continued)

Table 4. (Continued).

Fungal species	Leaves				Seedlings				Re-isolation (%)
	Disease severity score (0–5 scale)			Symptoms description	Disease severity score (0–5 scale)			Symptoms description	
	Mean	SD	Min–Max[a]		Mean	SD	Min–Max		
P. pauciseptata	3.5 C	0.5	3.0–4.0	Necrotic patches (PPB)	4.0 DE	0.5	3.0–5.0	Collar browning, browning and destruction of root	100
P. plurivora	1.2 B	0.9	0.0–3.0	Necrotic patches (PPB)	3.0 CD	0.4	2.0–4.0	Root browning	100
P. ramiseptata	3.5 C	0.5	3.0–4.0	Necrotic patches (PPB)	4.5 E	0.7	3.0–5.0	Collar browning, browning and destruction of root	100

[a] Maximum and minimum values detected on the basis of five assessments.
[b] Putative parenchymatous behaviour.
[c] Putative tracheomycotic behaviour.

The molecular tools and observations of fungal morphology allowed identification of four *Plectosphaerella* spp. (*P. cucumerina*, *P. pauciseptata*, *P. plurivora* and *P. ramiseptata*) from collar and root browning, with the greatest IFs. This is the first report worldwide of *P. pauciseptata*, *P. plurivora*, and *P. ramiseptata* from basil, and the first report of *P. cucumerina*, *P. pauciseptata*, and *P. ramiseptata* from parsley.

Through the use of isolates of nine species of *Plectosphaerella* in pathogenicity assays, it was possible to determine whether these can infect basil and parsley, and to describe symptoms they produced on these hosts. The pathogenicity trials showed, that except for *P. oratosquillae*, all of the other *Plectosphaerella* spp. were pathogenic to basil and parsley, although they caused different degrees of disease severity on roots and collars of these hosts. Results from *in vitro* inoculation of detached host leaves should be regarded as preliminary confirmation of fungus pathogenicity. However, on inoculation of young intact plants, the resulting symptoms supported the *in vitro* results.

The most aggressive species was *P. ramiseptata*, which the greatest disease severity scores on basil and parsley seedlings. The symptoms observed indicated that *P. ramiseptata* caused cell wall degradation through pectinolytic enzymes, inducing collar and root browning, with root destruction and then death of host plants.

Plectosphaerella pauciseptata and *P. plurivora* isolated from basil and parsley were pathogenic when artificially inoculated, with high disease severity scores. This confirmed the isolation data. Although *P. alismatis*, *P. citrulli*, *P. delsorboi*, and *P. melonis* were not isolated from originally assayed diseased basil or parsley plants, when used in the pathogenicity tests, isolates of these fungi were also pathogens on both of these hosts, with severe disease resulting. The symptoms observed were browning of the plant collars and roots at low disease severity scores, while necrosis, root reduction and/or destruction occurred at greater disease severities.

On the basis of the results obtained in the present study, *P. alismatis*, *P. cucumerina*, *P. delsorboi*, and *P. melonis* can show necrotrophic behaviours. Similar results were reported for root rot on tomato and pepper, according to Raimondo and Carlucci (2018), and on curcuma according to Antignani *et al.* (2008). *Plectosphaerella citrulli*, *P. pauciseptata*, *P. plurivora*, and *P. ramiseptata* affected host vascular tissues, as they produced extended necroses of leaf veins when they were inoculated on leaf surfaces. This symptom was confirmed when these fungi were inoculated onto plants in pots, causing collar and root browning on basil and parsley seedlings that evolved into wilt diseases. Similar symptoms were associated with *P. pauciseptata* on lettuce, coriander, and chervil by Usami *et al.* (2012), with *P. pauciseptata* and *P. plurivora* on tomato and

Figure 1. Pathogenicity assays carried out on basil plants under artificial conditions. Leaves and seedlings were inoculated with sterile distilled water (a), or with conidium suspensions of nine *Plectosphaerella* species, including *P. alismatis* (b), *P. citrulli* (c), *P. cucumerina* (d), *P. delsorboi* (e), *P. melonis* (f), *P. oratosquillae* (g), *P. pauciseptata* (h), *P. plurivora* (i), or *P. ramiseptata* (j).

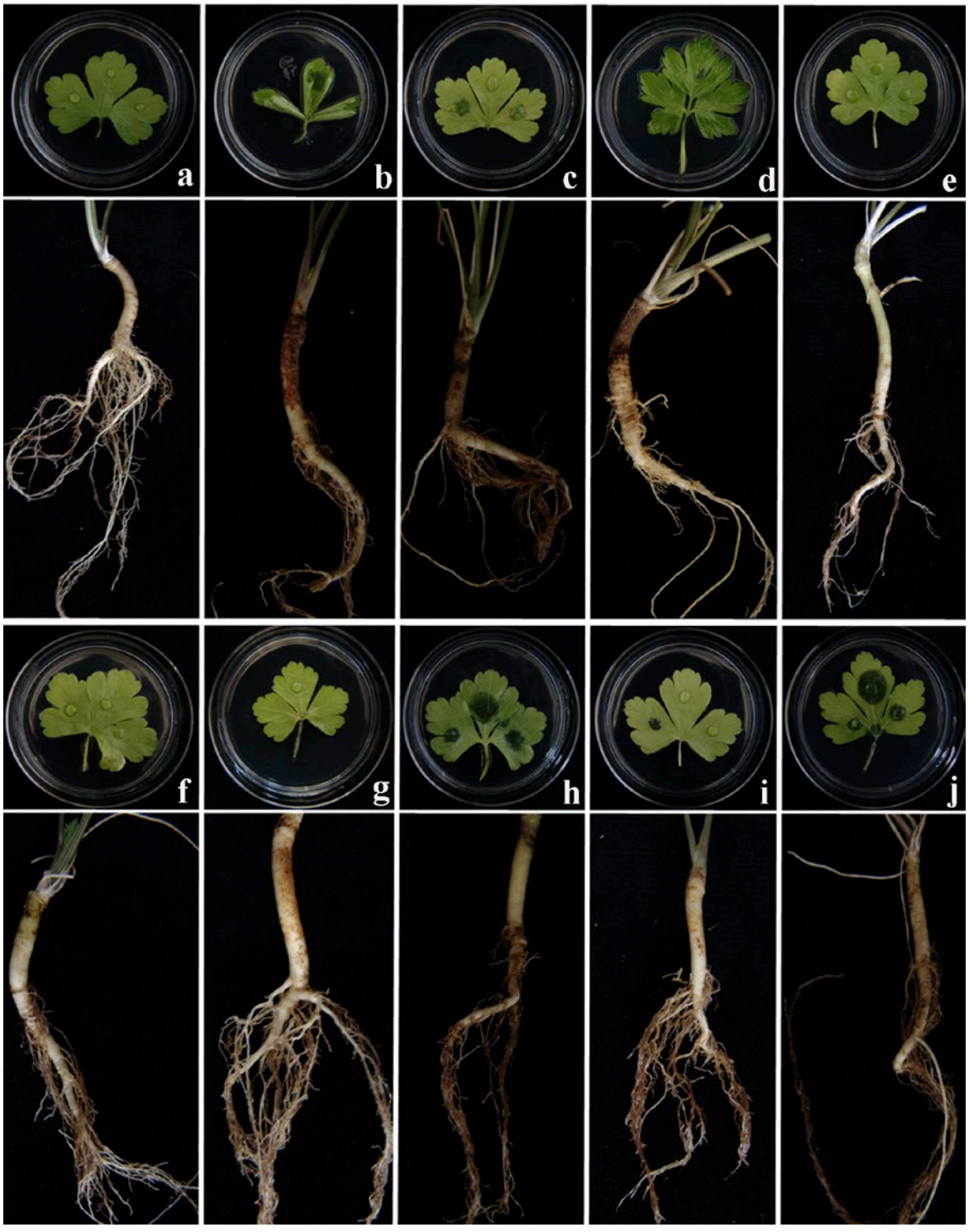

Figure 2. Pathogenicity assays carried out on parsley plants under artificial conditions. Leaves and seedlings were inoculated with sterile distilled water (a), or with conidium suspensions of nine *Plectosphaerella* species, including *P. alismatis* (b), *P. citrulli* (c), *P. cucumerina* (d), *P. delsorboi* (e), *P. melonis* (f), *P. oratosquillae* (g), *P. pauciseptata* (h), *P. plurivora* (i), or *P. ramiseptata* (j).

pepper by Raimondo and Carlucci (2018), and on lettuce by Usami and Katagiri (2017). Satou *et al.* (2010) associated black discolouration and decay of chrysanthemum cuttings with *P. tabacinum* (syn. *P. cucumerina*), although on the basis of the recent taxonomic revision (Carlucci *et al*., 2012), their strain MAFF 712335 (acc. no. AB537556) was *P. plurivora*. Therefore, the results described here are similar to those obtained by Raimondo and Carlucci (2018). These authors did not consider *Plectosphaerella* spp. as endophytic fungi, but as hemibiotrophs, able to become pathogens that are mainly necrotrophic, although these fungi produced different disease severities.

The data from the present study show that almost all *Plectosphaerella* species used in pathogenicity tests can cause severe disease symptoms on basil and parsley. These symptoms included discolouration, necrosis, reduction or destruction of collar and roots of young basil and parsley seedlings, root and collar rot, and vascular and leaf symptoms. Therefore, we consider that these symptoms can be summarized as 'stunting disease' of basil and parsley, as defined by Raimondo and Carlucci (2018).

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