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Short communication. Telluric pathogens isolated from blighted pepper (*Capsicum annuum* L.) plants in northwestern Spain

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Abstract

A survey of blighted pepper (*Capsicum annuum* L.) plants covering 120 farms in northwestern Spain was performed between 2001 and 2004 with the aim of identifying the main telluric pathogens associated with the disease in this part of the country. The following potential telluric pathogens were isolated from the 755 diseased plants inspected: *Phytophthora capsici* Leonian, *P. nicotianae* Breda de Haan, *Verticillium dahliae* Kleb., *Rhizoctonia solani* Kühn, *Sclerotium rolfsii* Sacc., *Fusarium solani* Mart. (Sacc.) and *Botrytis cinerea* Pers. *Rhizoctonia solani* was the most commonly isolated pathogen; this was detected on 38% of the farms and isolated from 16% of the plants analysed. Inoculation tests were performed with isolates of *P. capsici, P. nicotianae* and *F. solani* on *C. annuum* cv. Yolo Wonder. These confirmed *P. nicotianae* as a pepper pathogen, but with weaker pathogenic behaviour than *P. capsici. Fusarium solani* was confirmed as a secondary pathogen.

Key words: collar rot, *Fusarium solani,* phytopathogenic fungi, *Phytophthora nicotianae,* wilts.

Resumen

Nota corta. Patógenos telúricos aislados de plantas de pimiento (*Capsicum annuum* L.) con síntomas de marchitamiento en el noroeste español

Entre los años 2001 y 2004 se llevó a cabo una prospección de plantas de pimiento (*Capsicum annuum* L.) con síntomas de marchitamientos y amarilleos en 120 explotaciones de Galicia, con la finalidad de identificar los patógenos telúricos asociados a estos síntomas. Se analizaron 755 plantas de las que se aislaron los siguientes hongos telúricos potencialmente patógenos: *Phytophthora capsici* Leonian, *P. nicotianae* Breda de Haan, *Verticillium dahliae* Kleb., *Rhizoctonia solani* Kühn, *Sclerotium rolfsii* Sacc., *Fusarium solani* Mart. (Sacc.) y *Botrytis cinerea* Pers. *R. solani* resultó ser el hongo más frecuente, ya que se detectó en el 38% de las explotaciones muestradas y en el 16% del total de plantas analizadas. Las pruebas de inoculación realizadas con cepas de *P. capsici, P. nicotianae* y *F. solani* sobre la variedad Yolo Wonder confirmaron que *P. nicotianae* es un patógeno de pimiento, si bien resulta menos virulento que *P. capsici,* y que *F. solani* se comporta como un patógeno secundario.

Palabras clave: *Fusarium solani,* hongos fitopatógenos, marchiteces, *Phytophthora nicotianae,* podredumbres de cuello.

Pepper blight is one of the most serious worldwide threats to *Capsicum* production (Tian and Babadoost, 2004). Though the disease has long been known in Spain, the first published reference dates only from 1964 (Davila, 1964). It was not until 1970 when the disease was considered an epidemic in this country (Bartual *et al.,* 1991). The aetiology of the disease has been a focus of discussion since *Phytophthora capsici* Leonian (Alfaro and Vegh, 1971; Palazón *et al.,* 1978; Palazón and Palazón, 1989; Nuez *et al.,* 1996), *Verticillium dahliae* Kleb. (Palazón *et al.,* 1978; Palazón and Palazón, 1989; Nuez *et al.,* 1996) and *Fusarium* sp. (Palazón *et al.,* 1978) have all been referenced as responsible for blight symptoms in this crop. *Phytophthora nicotianae* Breda de Haan has been confirmed responsible for this disease in Tunisia (Allagui *et al.,*

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1995) and northwestern Spain (Andrés *et al.,* 2003). In addition, *Rhizoctonia solani* Kühn is a well known pathogen that causes damping-off in pepper seedlings as well as blight in adult plants (Muhy and Bosland, 1987; Nuez *et al.,* 1996). *Sclerotium rolfsii* Sacc. is responsible for blight and collar rot in adult pepper plants (CAB, 1974; Nuez *et al.,* 1996). Other fungal pathogens isolated from diseased plants are *P. cryptogea* Pethybr. and Laff. (Larregla *et al.,* 1996) and *Botrytis cinerea* Pers. (Tello, 1984; Pomar *et al.,* 2001).

The aim of the present work was to identify the fungal pathogens associated with blighted pepper plants in Galicia (northwestern Spain) and to assess their pathogenic behaviour.

A total of 755 adult pepper plants with symptoms of blight (brown-black discoloured collar and root rots causing permanent wilting and plant death; some of these symptoms may be associated with vascular browning) were sampled from 120 farms in 41 survey sessions between 2001 and 2004 (Table 1). These farms were located in the most important pepper producing regions of the provinces of A Coruña, Pontevedra and Ourense. Fragments of the collar of affected plants were disinfected with 0.6% sodium hypochlorite for 4 min and then plated on PDA (potato dextrose agar) (Rapilly, 1968) at 22-24°C for fungal isolation. Microscopic observations were made every 24 la for one week. *Fusaria* and *Phytophthora* isolates were classified according to Nelson *et al.* (1983) and Stamps *et al.* (1990) respectively.

Yolo Wonder pepper plants were inoculated with 10 fungal isolates, including four of *P. capsici,* two of *P. nicotianae* and four of *F. solani,* to study fungal and oomycete pathogenicity.

Phytophthora inocula were prepared after growing each isolate on V8 juice agar (Erwin and Ribeiro, 1996) at 22-24°C for 7 days. Each inoculum was prepared by seeding pieces of the isolate in sterile 1% potassium nitrate solution distributed in several Petri dishes (20 ml per Petri dish). This culture was grown under ultraviolet light at 24°C for seven days to stimulate sporangium formation. When abundant sporangia were formed, the potassium nitrate solution was replaced by sterile distilled water and the Petri dishes maintained at 5°C for 30 min, and then at 24°C for 3 h, to stimulate zoospore discharge. The zoospore suspension was then filtered through Whatman paper, vibrated for 1 min and adjusted to 20,000 zoospores per ml using a Burker chamber (Bartual *et al.,* 1991). Each plant was inoculated at the 6-leaf stage by dropping 5

Table 1. Farms surveyed and plants inspected

Province	Locality	N.º survey sessions	N.º farms	N.º plants	TCS[1]
A Coruña	**Padrón**	3	8	42	
	Ferrol	4	10	91	
	Vedra	5	14	75	
	Betanzos	1	6	27	
	Total	**13**	**38**	**235**	**91.0**
Pontevedra	**Rosal**	8	41	224	
	Salnés	12	30	220	
	Total	**20**	**71**	**444**	**375.0**
Ourense	**Arnoia**	3	6	39	
	Ourense	5	5	37	
	Total	**8**	**11**	**76**	**235.0**
Galicia (NW-Spain)	**Total**	**41**	**120**	**755**	**744.0**
Years	**2001**	8	26	156	
	2002	10	39	173	
	2003	8	24	280	
	2004	15	31	146	

[1] TCS: Total cultivated area in ha (MAPA, 2002).

ml of the zoospore suspension onto the collar of each plant using a sterile micropipette (Gil Ortega *et al.,* 1995).

Fusaria isolates were grown on PDA (Rapilly, 1968) at 22-24°C for 7 days. Inocula were prepared by shaking 100 ml of sterile water per Petri dish with each isolate for 1 min and adjusting to 10^5 macroconidia per ml using a Burker chamber. Each plant was inoculated at the 6-leaf stage by dropping 10 ml of the macroconidia suspension onto the collar of the plants using a sterile micropipette.

Yolo Wonder pepper plants were grown on plastic trays in a glasshouse at 18 (night temperature) to 22°C. The rooting medium was a mixture of peat and sand (1:1, v v^{-1}) previously sterilised at 120°C for 45 min. The inoculation tests had a completely randomised design with 3 replicates per isolate and 10 plants per unit and replicate. Disease severity for *Phytophthora* and *F. solani* was determined 28 days after the inoculation according to Kim and Hwang (1992) and Schneider and Kelly (2000) respectively.

Mean comparisons were made using Duncan′s multiple range test after transforming the disease severity data as follows:

$$Y = \text{arc sin } \sqrt{X/100},$$

where X is the disease index of each plant expressed as a percentage. All calculations were performed using SAS software v. 8.2 (SAS, 1999).

Rhizoctonia solani and *F. solani* were the most commonly isolated potential pathogens (Table 2). *Rhizoctonia solani* has previously been isolated from blighted pepper plants in Mexico (González-Pérez *et al.,* 2004), Pakistan (Mushtaq and Hashmi, 1997), Australia (Stirling *et al.,* 2004) and Spain (Tello, 1984; Pomar *et al.,* 2001; Tello and Lacasa, 2004) but was not the predominant fungus in any of these surveys. Two *Phytophthora* species were also isolated, *P. capsici* and *P. nicotianae,* which confirms the results of previous studies performed in northwestern Spain (Andrés *et al.,* 2003). The incidence of both species was similar, both in terms of the percentage of blighted plants infected and the percentage of affected farms. *Verticillium dahliae* was detected on more farms than *P. capsici,* a result inconsistent with previous studies performed in this part of the country (Pomar *et al.,* 2001) and indeed in other parts of Spain (Tello, 1984) (Table 2).

It is important to note that several pathogens usually affected the same pepper plant simultaneously (Table 2). This was particularly true of *P. nicotianae* and *F. solani* (of all positive samples only 7% were infected by *P. nicotianae* alone, and only 13% were infected by *F. solani* alone) (Table 2).

A number of differences were seen in the pathogenicity profiles of fungal species either not well known as pathogens of this crop or considered secondary pathogens. *Phytophthora nicotianae* caused typical collar rot symptoms similar to those observed in the field, plus very mild blight symptoms. Isolates of *Phy-*

Table 2. Potential telluric pathogens isolated from wilted pepper (*Capsicum annuum* L.) plants in northwestern Spain

Potential pathogens	2001		2002		2003		2004		Total (2001-2004)			
	A[1]	B[2]	A	B	A	B	A	B	A	B	C[3]	D[4] (%)
Phytophthora capsici	19.8	23.0	3.5	2.6	1.8	12.5	0.7	3.2	**5.7**	**9.1**	**11**	**18**
P. nicotianae	13.4	19.2	5.2	12.8	5.0	20.8	0.0	0.0	**5.7**	**12.5**	**15**	**7**
Verticillium dahliae	7.1	23.0	12.7	25.6	11.4	29.2	13.0	22.6	**8.5**	**25.0**	**33**	**33**
Rhizoctonia solana	9.6	19.2	30.6	51.2	10.4	29.2	18.5	41.9	**16.4**	**37.5**	**48**	**40**
Sclerotium rolfsii	8.3	15.4	10.4	15.4	6.8	8.3	1.4	6.4	**6.9**	**11.7**	**14**	**86**
Fusarium solani[5].	33.3	53.8	17.3	35.8	15.0	29.2	3.4	12.9	**17.0**	**32.5**	**39**	**13**
Botrytis cinerea	0.0	0.0	10.4	23.0	2.1	12.5	3.4	12.9	**3.8**	**13.3**	**16**	**50**
N.º of analysed plants	156		173		280		146		**755**			
N.º of surveyed farms		26		39		24		31		**120**		

[1] A: Percentage of plants positive for the potential pathogen.
[2] B:Percentage of farms with the potential pathogen.
[3] C: N.º of samples positive for the potential pathogen.
[4] D:Percentage of positive samples with a single potential pathogen.
[5] Considered by some authors as a secondary pathogen.

tophthora capsici (PA-1 and RO-4) produced intense blight symptoms causing plant death 28 days after inoculation. These results contrast with those previously described for *P. nicotianae* on a different pepper cultivar (cv. California). This may be due to the differences in virulence of this pathogen in *Capsicum* germplasm of different origin (Andrés *et al.,* unpublished data).

Only two of the four *F. solani* isolates tested showed slight pathogenic behaviour (Table 3). The affected plants showed only small areas of rot at the base of the collar and did not develop clear blight symptoms. Such weak responses have previously been reported for this species in Spain (Palazón *et al.,* 1978) and elsewhere (Messiaen *et al.,* 1995). These results suggest that *F. solani* is a secondary pathogen that usually infects pepper plants already affected by some other pathogen or which are suffering abiotic stress (Gerlach and Nirenberg, 1982; Tello, 1984; Nuez *et al.,* 1996).

The present results strongly suggest that *P. capsici, V. dahliae, P. nicotianae* and *R. solani* are involved in pepper blight in northwestern Spain. Whether *F. solani,* which was isolated from diseased pepper plants but found to have very weak pathogenic behaviour, can form part of a complex with other pathogens and thus increase the injuries produced, remains to be determined.

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Table 3. Pathogenic behaviour of strains of *Phytophthora capsici* Leonian*, P. nicotianae* Breda de Haan and *Fusarium solani* Mart. (Sacc.) isolated from wilted pepper plants (*Capsicum annuum*) after inoculation of cv. Yolo Wonder plants

Strain	Pathogen species	Origin	Disease index[1]	Disease index[2]	Re-isolation of the pathogen
PA-1	*P. capsici*	A Coruña	4.49 b[3]		+
PA-5	*P. capsici*	A Coruña	2.05 cd		+
RO-4	*P. capsici*	Pontevedra	5.00 a		+
BE-4	*P. capsici*	A Coruña	2.21 c		+
Png01	*P. nicotianae*	Pontevedra	1.05 e		+
Png04	*P. nicotianae*	Ourense	0.80 e		+
1st Control			0.00 f		–
Prm-4-04	*Fusarium solani*	Pontevedra		1.21 A[3]	+
Hor-1-04	*F. solani*	Pontevedra		1.00 B	+
Fsol-9-04	*F. solani*	Ourense		1.10 AB	+
Fsol-10-04	*F. solani*	Ourense		1.17 A	+
2nd Control				1.00 B	–

[1] Disease index used for *Phytophthora* rots: from 0 (asymptomatic plant) to 5 (dead plant) (Kim and Hwang, 1992).
[2] Disease index used for root rots caused by *F. solani*: from 1 (asymptomatic plant) to 7 (100% root rot) (Schneider and Kelly, 2000).
[3] Figures within columns followed by the same letter are not significantly different (Duncan′s multiple range test) ($P < 0.05$).

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