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Short communication. Telluric pathogens isolated from bean plants with collar and root rots in northwest Spain

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Abstract

Pathogens belonging to the disease complex responsible for bean collar and root rots in northwest Spain were identified and their pathogenic behaviour studied over a two year period (2004-2005). The potential fungal and oomycete pathogens *Fusarium solani* f. sp. *phaseoli* (Burkh.) W.C. Snyder & Hansen, *Rhizoctonia solani* Kühn, *Pythium ultimum* Trow, *Pythium* Group G, *Fusarium avenaceum* (Fr.) Sacc., *F. culmorum* W. G. Sm. (Sacc.), *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary and *Sclerotium rolfsii* Sacc. were isolated from 419 bean (*Phaseolus vulgaris* L.) plants affected by collar or root rot. *Fusarium solani* f. sp. *phaseoli* was the most frequently isolated (found on 63.7% of the farms surveyed and isolated from 19.3% of the diseased plants), followed by *R. solani* and the *Pythium* species. Inoculating *Phaseolus vulgaris* cv. Musica and Zondra with 14 isolates of these fungi and oomycetes showed *F. solani* f. sp. *phaseoli* to be the most aggressive pathogen of the complex; *F. avenaceum* and *F. culmorum* were found not to be pathogenic for either cultivar. The results confirm that *F. solani* and *R. solani* are the main pathogens of the bean collar/root rot disease complex in northwest Spain, affecting crops in their early growth stages. The complex also includes *P. ultimum* and *Pythium* Group G.

Additional key words: *Fusarium solani* f. sp. *phaseoli, Rhizoctonia solani, Pythium, Phaseolus vulgaris.*

Resumen

Nota corta. Patógenos telúricos aislados de planta de judía (*Phaseolus vulgaris* L.) con síntomas de mal de pie en Galicia

Durante los años 2004 y 2005 se llevó a cabo en Galicia la identificación de los patógenos que formaban parte del complejo parasitario responsable del mal de pie en el cultivo de la judía, así como la evaluación de su poder patógeno. Los hongos y oomicetos patógenos potenciales aislados sobre un total de 419 plantas de judía (*Phaseolus vulgaris* L.) con síntomas de mal de pie, muestreados durante dicho período en Galicia, fueron los siguientes: *Fusarium solani* f. sp. *phaseoli* (Burkh.) W.C. Snyder & Hansen, *Rhizoctonia solani* Kühn, *Pythium ultimum* Trow, *Pythium* Grupo G, *Fusarium avenaceum* (Fr.) Sacc., *F. culmorum* W. G. Sm. (Sacc.), *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary y *Sclerotium rolfsii* Sacc. El patógeno más frecuentemente aislado fue *F. solani* f. sp. *phaseoli,* detectado en el 63,7% de las explotaciones muestreadas, así como en el 19,3% de las plantas analizadas, seguido de *R. solani* y de *Pythium* spp. Las pruebas de inoculación de 14 aislamientos de estos hongos y oomicetos sobre las variedades de judía Musica y Zondra indicaron que *F. solani* f. sp *phaseoli* posee el mayor poder patógeno, mientras que *F. avenaceum* y *F. culmorum* no resultaron ser patogénicos sobre ninguna de las dos variedades inoculadas. Los resultados de este trabajo confirman que *F. solani* y *R. solani* son los principales patógenos responsables del mal de pie de la judía en Galicia, complejo parasitario que incluye también a *P. ultimum* y *P.* Grupo *G* en los primeros estadíos del cultivo.

Palabras clave adicionales: *Fusarium solani* f. sp. *phaseoli, Rhizoctonia solani, Pythium, Phaseolus vulgaris.*

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Collar and root rots affect bean (*Phaseolus vulgaris* L.) in the USA (Keenan *et al.,* 1974; Hall, 1991; Estevez de Jensen *et al.,* 1998), Egypt (Yousseff *et al.,* 1975), Japan (Furuya, 1982), Argentina (Frezzi, 1950), France (Messiaen *et al.,* 1995) and Australia (Allen *et al.,* 1987). They are also a serious problem in the southern (Cuadrado and Gómez, 1983; Tello *et al.,* 1985), central (Sinobas *et al.,* 1994) and Basque regions (Berra and Arteaga, 1989) of Spain. No published information exists, however, on the incidence of this problem in Galicia (northwest Spain).

The aetiology of this disease has long been a matter of discussion. Several pathogens have been reported involved in this disease complex in different parts of the world. The most commonly cited are *Fusarium solani* f. sp. *phaseoli* (Burkh.) W.C. Snyder & Hansen (Maloy, 1959; Gupta and Saharan, 1973; Hoch *et al.,* 1975; Steadman *et al.,* 1975; Davet *et al.,* 1980), *Rhizoctonia solani* Kühn (Luttrell and Garren, 1952; Hoch *et al.,* 1975; Steadman *et al.,* 1975), *Thielaviopsis basicola* (Berk. and Broom) Ferraris (L'Échappe *et al.,* 1988), and a number of species belonging to the genus *Pythium* (Hoch *et al.,* 1975; Davet *et al.,* 1980; Rusuku *et al.,* 1997). In Spain, *F. solani* (Tello *et al.,* 1985; Berra and Arteaga, 1989; Sinobas *et al.,* 1994), *R. solani* (Tello *et al.,* 1985; Berra and Arteaga, 1989; Sinobas *et al.,* 1994) and *T. basicola* (Tello *et al.,* 1985; Berra and Arteaga, 1989) have been associated with the complex. *Pythium* has been reported responsible for the disease only in the Basque region (Berra and Arteaga, 1989).

The aim of the present work was to identify the pathogens associated with the disease complex responsible for bean collar and root rots in northwest Spain, and to evaluate their pathogenic behaviour.

During 2004 and 2005, 419 bean plants with collar or root rot symptoms were sampled from 58 farms in seven regions belonging to the provinces of A Coruña, Lugo, Ourense and Pontevedra (Table 1). The sampled plants had characteristic red streaks, dry brownish or soft humid rots along the base of the hypocotyls and collars, and showed discoloration and deterioration of the main taproot and lateral roots. Severely diseased roots were associated with visible symptoms on the aerial part of the plants, such as chlorosis, defoliation and stunting. Fragments of the collar and roots of affected plants were disinfected with 0.6% sodium hypochlorite for 4 min, washed with sterile distilled water for 1 min and then plated on potato dextrose agar (PDA) (Rapilly, 1968) at 22-24°C for fungal and oomycete isolation. Microscopic observations were made every 24 h over the period of one week. *Fusarium, Pythium* and *Rhizoctonia* isolates were classified according to Nelson *et al.* (1983), Van der Plaats-Niterink (1981) and Sneh *et al.* (1994) respectively.

Fusarium solani and *R. solani* were the most commonly isolated potential pathogens (Table 2). *Fusarium solani* f. sp. *phaseoli* has been reported by

Table 1. Farms surveyed and plants inspected in northwest Spain

Province/year	Region	Nr. sessions	Nr. farms	Nr. plants	Type of bean
A Coruña	Ferrol	2	3	19	fresh
	Vedra	3	12	90	fresh
Pontevedra	Umia	4	10	66	fresh
	Salnés	2	5	23	fresh
	Baixo Miño	6	13	108	fresh
Ourense	Ourense	2	7	75	fresh
Lugo	Lourenzá	2	8	38	dry
Total 2004		12	30	219	
Total 2005		9	28	200	
Total (2004-05)		21	58	419	

Table 2. Potential telluric pathogens isolated from bean plants *(Phaseolus vulgaris* L.) with collar and/or root rots in northwest Spain

Potential pathogens	2004		2005		Total (2004-2005)			
	A[1]	B[2]	A	B	A	B	C[3]	D[4]
Botrytis cinerea	1.4	3.3	0.0	0.0	0.7	1.7	1	0.0
Fusarium solani	18.7	67.0	20.0	60.7	19.3	63.7	37	17.2
Fusarium spp.[5]	1.8	13.3	4.5	32.1	3.1	22.4	13	0.0
Pythium spp.[6]	10.5	40.0	11.0	42.8	13.1	41.4	24	8.6
Rhizoctonia solani	18.7	47.0	17.5	57.1	18.1	51.7	30	6.8
Sclerotinia sclerotiorum	0.9	3.3	0.5	3.6	0.7	3.4	2	0.0
Sclerotium rolfsii	0.9	3.3	0.0	0.0	0.5	1.7	1	0.0
Nr. of analysed plants	219		200		419			
Nr. of surveyed farms		30		28		58		

[1] A: Percentage of plants positive for the potential pathogen. [2] B: Percentage of farms affected by the potential pathogen. [3] C: N.º of positive samples of the potential pathogen. [4] D: Percentage of positive samples with a single potential pathogen. [5] *Fusarium culmorum* and *F. avenaceum.* [6] *Pythium ultimum* and *P.* Group G.

some authors as the most important pathogen of the disease complex in the USA (Maloy, 1959; Steadman *et al.,* 1975), Lebanon (Davet *et al.,* 1980), Brittany (France) (L'Échappe *et al.,* 1988) and Spain (Berra and Arteaga, 1989; Sinobas *et al.,* 1994). However, others did not consider this pathogen as the main pathogenic agent of the complex (Tello *et al.,* 1985), and some have even failed to isolate this fungus from diseased plants (Luttrel and Garren, 1952; Rusuku *et al.,* 1997). Similarly, *R. solani* has been reported both as a strong, primary pathogen (Luttrell and Garren, 1952; Hoch *et al.,* 1975; Davet *et al.,* 1980; Tello and Lacasa, 1985; Berra and Arteaga, 1989) and as a secondary pathogen of the complex (Steadman *et al.,* 1975; L'Échappe *et al.,* 1988; Rusuku *et al.,* 1997).

In the present work, the incidence of *Pythium* was high (Table 2). This agrees with surveys performed in the USA (Hoch *et al.,* 1975), Lebanon (Davet *et al.,* 1980), France (L'Échappe *et al.,* 1988) and Rwanda (Rusuku *et al.,* 1997). However, only in the last of these countries is the *Pythium* group considered to be the main pathogenic group of the bean collar/root rot disease complex. Only two groups of *Pythium* species were found to be part of the complex in the present work: *P. ultimum* —reported as belonging to the complex in the USA (Drechsler, 1952; Hoch *et al.,* 1975; Lumsden *et al.,* 1976)— and *Pythium* Group G. This is the first report of *Pythium* Group G as a pathogen of bean in Spain.

Thielaviopsis basicola, which has been reported as a bean pathogen in the Basque region (Berra and Arteaga, 1989) and southern Spain (Tello *et al.,* 1985), was not isolated in the present work.

It is important to note that several pathogens were frequently isolated simultaneously from the same sample (Table 2). This reinforces the hypothesis of the existence of a bean disease complex formed by several pathogens that can infect plants at the same time, which has been reported in other regions (Davet *et al.,* 1980, L'Échappe *et al.,* 1988). Further studies are required to determine which pathogens have synergic activity.

For pathogenicity studies, the bean cultivars Musica and Zondra were inoculated with the following 14 isolates: *R. solani* (five different isolates), *F. solani* (four isolates), *F. culmorum* (W. G. Sm.) Sacc. (two isolates), *F. avenaceum* (Fr.) Sacc. (one isolate), *Pythium ultimum* Trow (one isolate) and *Pythium* Group G (one isolate).

Rhizoctonia solani and *Fusarium* isolates were grown on PDA at 22-24°C for 7 days. Inocula were prepared by blending each isolate (from four Petri dishes) with 400 ml of sterile distilled water at low speed for 1 min. The suspensions were then adjusted to 10^5 propagules or macroconidia per ml using a Burker-Turk chamber. Each bean plant was inoculated at the two-leaf stage by dropping 10 ml of inoculum onto the collar using a sterile micropipette (Jones and Belmar, 1989; Schneider and Kelly, 2000).

Pythium isolates were inoculated at pre or post-emergence. The post-emergence inocula were prepared after growing each isolate on V8 juice agar (Erwin and Ribeiro, 1996) at 22-24°C for four days.

Plants at the two-leaf stage were inoculated by 'seeding' pieces (10 mm diameter) of the isolate onto the collar (Moorman and Kim, 2004). Pre-emergence inoculation was performed according to Ricci *et al.* (1976). This consisted of placing four bean seeds on the surface of the oomycete culture and then plating both seeds and fungal culture onto a sterilized substrate in a plastic tray. Two cultures of the same strain and eight seeds of the same cultivar were included in each plastic tray and covered with sterile substrate.

The inoculated bean plants were grown on plastic trays in a glasshouse at 18-26°C during the months of July and August. The rooting substrate was a mixture of peat and sand (1:1, v:v) previously sterilised at 120°C for 45 min. The inoculation tests for *Pythium* spp., *R. solani, F. solani* and the other *Fusarium spp.* had a split plot design with randomised isolate subplots for the different cultivars. Three replications were undertaken per isolate-cultivar interaction. Each subplot included nine plants. In post-emergence inoculated plants, disease severity was determined according to Schneider and Kelly (2000) 30 days after inoculating the plants. The disease severity of plants inoculated with *Pythium* isolates at pre-emergence was determined using the method of Ricci *et al.* (1976) 15 days after the inoculation. Duncan's multiple range test was used to compare the means after transforming the disease severity values 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).

Table 3 shows that *Fusarium solani* f. sp. *phaseoli* was the most aggressive pathogen for both cultivars, followed by *R. solani, P. ultimum* and *Pythium* Group G. The *Fusarium* spp. isolates —*F. avenaceum* and *F. culmorum*— showed only very weak pathogenicity and

Table 3. Pathogenic behaviour of *Pythium* spp., *Rhizoctonia solani, Fusarium solani* and *Fusarium* spp. strains, isolated from bean plants (cv. Musica and Zondra) with collar and/or root rots

Species	Strain	Origin	Disease index[1]			Re-isolation of the pathogen	
			A[2]	B[3]	A		
			Musica		Zondra	Musica	Zondra
Pythium ultimum	Pyt4/04	Pontevedra	1.46 ab	3.91 a	2.18 a	+	+
Pythium Group G	Pyt1/04	Pontevedra	1.70 a	4.00 a	1.58 a	+	+
Control *Pythium*	Control		1.15 b	0.13 b	1.44 a	–	–
R. solani	Riz1/04	Pontevedra	1.27 bc		1.73 bc	+	+
R. solani	Riz2/04	Pontevedra	1.59 b		1.95 b	+	+
R. solani	Riz3/04	A Coruña	1.07 c		1.67 bc	–	+
R. solani	Riz4/04	A Coruña	2.25 a		2.70 a	–	+
R. solani	Riz5/04	A Coruña	1.41 bc		1.50 bc	+	+
Control *R. solani*	Control		1.07 c		1.25 c	–	–
F. solani	Fsol1/04	Pontevedra	2.88 ab		3.81 a	–	+
F. solani	Fsol3/04	Pontevedra	3.39 a		3.58 a	–	+
F. solani	Fsol4/04	A Coruña	2.11 c		2.75 a	+	+
F. solani	Fsol5/05	A Coruña	2.37 bc		2.89 a	+	+
Control *F. solani*	Control		1.04 d		1.16 b	–	–
F. avenaceum	Fus1/4	Pontevedra	1.41 a		1.82 a	+	+
F. culmorum	Fus2/4	Pontevedra	1.37 a		1.43 ab	+	+
F. culmorum	Fus4/4	A Coruña	1.26 a		2.00 a	+	+
Control *Fusarium* spp.	Control		1.20 a		1.22 b	–	–

[1] Within each column and between isolates of the same group (*Pythium* group - *Rhizoctonia solani* group - *Fusarium solani* group - *Fusarium* spp. group), different letters express statistical significance (Duncan's multiple range test) ($P<0.05$). [2] A: Disease index used for bean root rots obtained after post-emergence inoculation (Schneider and Kelly, 2000): values varied from 1 (asymptomatic plant) to 7 (dead plant). [3] B: Disease index used for bean *Pythium* rots obtained after pre-emergence inoculation (Ricci *et al.,* 1976): values varied from 0 (asymptomatic plant) to 4 (dead plant in pre-emergence).

were therefore concluded not to be primary pathogens in this disease complex (Table 3). This situation differs from that described by other authors working with *Fusarium* species of the roseum group *sensu* Messiaen and Cassini (1968). *Fusarium semitectum* Berk & Rav. has been reported as the cause of considerable losses to bean seed production in Brazil under prolonged humid conditions (Dhingra and Muchovej, 1979). The *Pythium* species were significantly more aggressive when inoculated at pre-emergence, probably due to the early receptivity of the crop to these pathogens (Hall, 1991).

The present results confirm that *F. solani* and *R. solani* are the main pathogens of the bean collar/root rot disease complex in northwest Spain, a complex that also includes *P. ultimum* and *Pythium* Group G, affecting bean crops at early growth stages.

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