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. R. 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. F. 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., 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 h 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

### Table 1. Farms surveyed and plants inspected

<table>
<thead>
<tr>
<th>Province</th>
<th>Locality</th>
<th>No. survey sessions</th>
<th>No. farms</th>
<th>No. plants</th>
<th>TCS¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Coruña</td>
<td>Padrón</td>
<td>3</td>
<td>8</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ferrol</td>
<td>4</td>
<td>10</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vedra</td>
<td>5</td>
<td>14</td>
<td>75</td>
<td></td>
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<tr>
<td></td>
<td>Betanzos</td>
<td>1</td>
<td>6</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>13</td>
<td>38</td>
<td>235</td>
<td>91.0</td>
</tr>
<tr>
<td>Pontevedra</td>
<td>Rosal</td>
<td>8</td>
<td>41</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salnés</td>
<td>12</td>
<td>30</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>20</td>
<td>71</td>
<td>444</td>
<td>375.0</td>
</tr>
<tr>
<td>Ourense</td>
<td>Arnoia</td>
<td>3</td>
<td>6</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ourense</td>
<td>5</td>
<td>5</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>8</td>
<td>11</td>
<td>76</td>
<td>235.0</td>
</tr>
<tr>
<td>Galicia (NW-Spain)</td>
<td>Total</td>
<td>41</td>
<td>120</td>
<td>755</td>
<td>744.0</td>
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<table>
<thead>
<tr>
<th>Years</th>
<th>No. survey sessions</th>
<th>No. farms</th>
<th>No. plants</th>
</tr>
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<tr>
<td>2001</td>
<td>8</td>
<td>26</td>
<td>156</td>
</tr>
<tr>
<td>2002</td>
<td>10</td>
<td>39</td>
<td>173</td>
</tr>
<tr>
<td>2003</td>
<td>8</td>
<td>24</td>
<td>280</td>
</tr>
<tr>
<td>2004</td>
<td>15</td>
<td>31</td>
<td>146</td>
</tr>
</tbody>
</table>

¹ TCS: Total cultivated area in ha (MAPA, 2002).
inoculated at the 6-leaf stage by dropping 5 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 10⁵ 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:

\[ Y = \arcsin \sqrt{X/100} \]

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). *R. 
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. *V. 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 
affect the same pepper plant simultaneously 
(Table 2). This was particularly true for *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. *P. nicotianae* caused typical 
collar rot symptoms similar to those observed in the 
field, plus very mild blight symptoms. Isolates of *P.*

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

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A¹</td>
<td>B²</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td><em>Phytophthora capsici</em></td>
<td>19.8</td>
<td>23.0</td>
<td>3.5</td>
<td>2.6</td>
<td>1.8</td>
</tr>
<tr>
<td><em>P. nicotianae</em></td>
<td>13.4</td>
<td>19.2</td>
<td>5.2</td>
<td>12.8</td>
<td>5.0</td>
</tr>
<tr>
<td><em>Verticillium dahliae</em></td>
<td>7.1</td>
<td>23.0</td>
<td>12.7</td>
<td>25.6</td>
<td>11.4</td>
</tr>
<tr>
<td><em>Rhizoctonia solana</em></td>
<td>9.6</td>
<td>19.2</td>
<td>30.6</td>
<td>51.2</td>
<td>11.4</td>
</tr>
<tr>
<td><em>Sclerotium rolfsii</em></td>
<td>8.3</td>
<td>15.4</td>
<td>10.4</td>
<td>15.4</td>
<td>6.8</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>33.3</td>
<td>53.8</td>
<td>17.3</td>
<td>35.8</td>
<td>15.0</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td>0.0</td>
<td>0.0</td>
<td>10.4</td>
<td>23.0</td>
<td>2.1</td>
</tr>
<tr>
<td>No. of analysed plants</td>
<td>156</td>
<td>173</td>
<td>280</td>
<td>575</td>
<td></td>
</tr>
<tr>
<td>No. of surveyed farms</td>
<td>26</td>
<td>39</td>
<td>24</td>
<td>31</td>
<td>120</td>
</tr>
</tbody>
</table>

¹ A: Percentage of plants positive for the potential pathogen.
² B: Percentage of farms with the potential pathogen.
³ C: No. of samples positive for the potential pathogen.
⁴ D: Percentage of positive samples with a single potential pathogen.

* Considered by some authors as a secondary pathogen.
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.

### Acknowledgements

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### References


DAVILA M., 1964. La enfermedad de la «tristeza del pimiento». Boletín Informativo de Plagas de Campo 18, 10-11.


### 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pathogen species</th>
<th>Origin</th>
<th>Disease index¹</th>
<th>Disease index²</th>
<th>Re-isolation of the pathogen</th>
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</thead>
<tbody>
<tr>
<td>PA-1</td>
<td><em>P. capsici</em></td>
<td>A Coruña</td>
<td>4.49 b³</td>
<td>+</td>
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<tr>
<td>PA-5</td>
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<td>2.05 cd</td>
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<tr>
<td>RO-4</td>
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<td>Pontevedra</td>
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<td>+</td>
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<tr>
<td>BE-4</td>
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<td>A Coruña</td>
<td>2.21 c</td>
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<tr>
<td>Png01</td>
<td><em>P. nicotianae</em></td>
<td>Pontevedra</td>
<td>1.05 e</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Png04</td>
<td><em>P. nicotianae</em></td>
<td>Ourense</td>
<td>0.80 e</td>
<td>+</td>
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</tr>
<tr>
<td>1st Control</td>
<td></td>
<td></td>
<td>0.00 f</td>
<td></td>
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</tr>
<tr>
<td>Prm-4-04</td>
<td><em>F. solani</em></td>
<td>Pontevedra</td>
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</tr>
<tr>
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<td>Pontevedra</td>
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<td>+</td>
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<tr>
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<td>Ourense</td>
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<tr>
<td>Fsol-10-04</td>
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<td></td>
<td>1.17 A</td>
<td>+</td>
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<tr>
<td>2nd Control</td>
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<td></td>
<td>1.00 B</td>
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</tbody>
</table>

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).


