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# Short communication. *Phytophthora nicotianae,* the causal agent of root and crown rot (*Tristeza* disease) of red pepper in La Vera region (Cáceres, Spain)

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## Abstract

Root and crown rot (*Tristeza* disease) is an increasing problem for red pepper crop in La Vera region (Cáceres, western Spain). Field surveys were carried on in 2006 and 2007 to identify the causal agents of this disease. A *Phytophthora* species was isolated from diseased plants in most of the surveyed fields (27 of 36 in 2006 and 15 of 16 in 2007), while *Verticillium* spp. were not detected. Fifteen *Phytophthora* isolates were examined and identified as *P. nicotianae*, all of them were heterothalic isolates of mating type A2. Pathogenicity tests conducted on 'Jaranda' red pepper plants developed symptoms of wilt and root and crown rot, although disease severity differed significantly ($P < 0.001$) among isolates. Results indicate that *P. nicotianae* is the principal causal agent of the *Tristeza* disease of red pepper plants in La Vera region and this has several implications for the development of future disease management strategies. The host range of isolates from red pepper plants should be studied in order to establish suitable crop rotation in this region.

**Additional key words**: 'Jaranda' cultivar, paprika, *Phytophthora parasitica.*

## Resumen

**Comunicación corta. *Phytophthora nicotianae,* agente causal de la *Tristeza* (podredumbre de cuello y raíz) del pimiento en la comarca de La Vera (Cáceres, España)**

La enfermedad de la *Tristeza* del pimiento, que causa podredumbre radicular y de cuello, es un problema creciente en los cultivos de pimiento para pimentón de la región de La Vera (Cáceres, oeste de España). En 2006 y 2007 se realizaron una serie de prospecciones en campo para identificar los agentes causales de esta enfermedad. En la mayoría de las parcelas prospectadas (27 de 36 en 2006 y 15 de 16 en 2007) se aisló una única especie de *Phytophthora* de las plantas enfermas, mientras que en ningún caso se detectó *Verticillium* spp. Se examinaron 15 aislados de *Phytophthora*, que se identificaron como *P. nicotianae*. Todos ellos eran heterotálicos del tipo de compatibilidad A2. Se realizaron pruebas de patogenicidad en plantas de pimiento 'Jaranda' y aunque todos los aislados causaron síntomas de marchitamiento y podredumbre en raíces y cuello, la severidad de la enfermedad difirió significativamente ($P < 0,001$) entre aislados. Los resultados indican que *P. nicotianae* es el principal agente causal de la enfermedad de la *Tristeza* del pimiento para pimentón en la región de La Vera, aspecto importante para el desarrollo de estrategias de control de la enfermedad. Es necesario estudiar el rango de hospedadores de los aislados de plantas de pimiento para el establecimiento de rotaciones de cultivo adecuadas en esta región.

**Palabras clave adicionales**: cultivar 'Jaranda', *Phytophthora parasitica,* pimentón.

Red pepper (*Capsicum annuum* L.) is one of the main horticultural crops in La Vera region (Cáceres, western Spain), with a total cultivated area of about 1,000 ha. Red peppers are dried according to a traditional system that uses holm oak firewood, and the Protected Designation of Origin «Pimentón de La Vera»

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Abbreviations used: PARP (pimaricin-ampicillin-rifampicin-PCNB), PDA (potato-dextrose-agar), V8A (V8 juice-agar).

is given to the smoked paprika that is elaborated with these red peppers. Root and crown rot (*Tristeza* disease) is an increasing problem for red pepper production in this region, as recent surveys show. This is the main disease of pepper crops in the Mediterranean area (Palazón and Palazón, 1989), and an important disease worldwide (Erwin and Ribeiro, 1996). *Phytophthora capsici* Leonian has been reported as the causal agent of root and crown rot of *C. annuum* in many countries (Erwin and Ribeiro, 1996), but other *Phytophthora* species and even other genera have been also associated with this disease. Thus *P. nicotianae* Breda de Haan (= *P. parasitica* Dastur) has been reported as a pathogen to pepper plants in United States, Puerto Rico, Japan, Italy, Mauritius and India (Erwin and Ribeiro, 1996 and references therein) and this is the species that causes the disease in Tunisia (Allagui *et al*., 1995). In Spain *P. nicotianae* causes root and crown rot of peppers in Ciudad Real and Toledo (Castilla-La Mancha) (Bartual *et al*., 1991) and has been reported in association with *P. capsici* in Andalucía (Larregla, 2003) and Galicia (Pomar *et al*., 2001; Andrés-Ares *et al*., 2003). Moreover, *Verticillium dahliae* Kleb. is the main causal agent of the disease in the Ebro Valley (Palazón, 1988; Gil and Gutiérrez, 2001; Gil *et al*., 2001) and it is also found infecting plants in Galicia (Bernal *et al.*, 2000) and País Vasco (Larregla, 2003).

The objective of this study was to identify the causal agents of the *Tristeza* disease of red pepper in La Vera region (Cáceres).

During the growing season of 2006 a total of 36 fields growing red pepper were surveyed in the agricultural area (200-600 m above the sea level and with average annual precipitation between 600 and 800 mm) of La Vera region, located at 40° 16'-39° 56'N, 5° 59'-5° 20' W. In 2007, three of the fields visited in 2006 were surveyed again, together with 12 new sites. Two plants showing *Tristeza* symptoms (general wilt without yellowing of leaves) and soil samples from their rizospheres were collected from each of the fields. Vegetal material and soil were processed in laboratory.

Roots and crowns of plants were carefully washed with tap water and root fragments 8 mm in length were plated on potato-dextrose-agar (PDA) and on pimaricin-ampicillin-rifampicin-PCNB (PARP) medium, that is selective for *Phytophthora* and *Pythium* species (Jeffers and Martin, 1986). Soil from rizosphere was analysed following the baiting technique described by Ponchet *et al.* (1972) using immature carnation petals floated on soil suspension. After incubation for 2-4 days at 24°C petals were transferred to PARP medium to isolate *Phytophthora* spp.

Stems were surface-sterilised by dipping into 96% ethanol and flaming, and fragments were plated on PDA to detect *Verticillium* spp.

Fifteen *Phytophthora* isolates from diseased red pepper plants (10 of them isolated in 2006 and 5 isolated in 2007) were used for taxonomic identification following the characters indicated below.

Colony morphology was examined on two different media, PDA and V8 juice-agar (V8A) (Erwin and Ribeiro, 1996). Isolates were grown at 25°C for 7 days in the dark and colony morphology was described. Hyphal swellings and chlamydospore production were observed on V8 juice broth medium incubated at 25°C in the dark.

To study the morphological characteristics of sporangia the isolates were grown on V8A and 1 $cm^2$ sections were cut from the edge of the colony and floated on distilled water together with immature carnation petals in Petri dishes (90 mm diameter). Plates were incubated for 3-4 days at room temperature and under continuous fluorescent light to induce sporangium formation. Edges of petals were examined under the light microscope (100x) for the presence of sporangia. Portions of the petals with sporangia were removed, mounted in lactophenol-cotton blue stain and observed under the light microscope (200x and 400x), recording the shape of sporangia and the presence/absence of papillae. For each isolate the lenghts and widths of 50 mature sporangia were measured and the average lenght:width ratio of sporangia was calculated.

Growth rate at 36 and 37°C was studied placing 7 mm diameter inoculum plugs in the centre of 9 cm Petri dishes of V8A. Four replicates of each isolate were prepared. Plates were incubated in the dark and colony diameters were measured after 3, 4 and 5 days (two perpendicular diameters per Petri dish). When no growth occurred after 5 days plates were incubated at 25°C during 5 additional days to determine if temperature was lethal.

Isolates on V8A were incubated for three months at 25°C in the dark and examined for the presence of sexual structures, indicating homothallic properties. Besides each isolate was paired with reference isolates of *P. nicotianae* of mating type A1 (isolate CBS 535.92) and type A2 (isolate CBS 534.92) to determine its mating type. When sexual structures were formed the morphology of oogonia and antheridia was recorded,

indicating the position of the antheridia in relation to the oogonia (amphigynous or paragynous).

'Jaranda' red pepper plants were inoculated with the 15 *Phytophthora* isolates previously identified to evaluate their pathogenicity. Plants were grown in a sterilized (autoclaved for 1 h at 120°C) vermiculite: peat (1:1 by volume) mixture and inoculated at the two- to four-true-leaf stage. To produce inoculum, the isolates were grown in 90 mm diameter Petri dishes containing 18 mL V8A until the micelium enterely colonized each plate. A suspension of propagules was prepared by blending in 100 mL distilled water the isolate grown on one Petri dish. The resultant suspension was the inoculum unit (Tello *et al*., 1991) used to inoculate 10 pepper plants by pouring 10 mL of the suspension into the substrate besides each plant. Ten control plants were treated with 10 mL of a suspension of 18 mL of V8A blended in 100 mL distilled water. Plants were grown in a growth chamber with a 16 h light at 28°C/8 h dark at 24ºC cycle. Disease severity was recorded every week during 28 days after inoculation using the following scale: 0 = no symptoms; 1 = light wilting and/or light damage on the base of stem; 2 = severe wilting and/or severe damage on the base of the stem; 3 = dead plant. Fragments of roots and crown were analysed on PDA and also on PARP to re-isolate the pathogen.

Data of sporangia dimensions and disease severity were analysed by the analysis of variance (ANOVA) procedure by using the software package SYSTAT version 10.0 (SYSTAT, 2000).

During the field surveys carried on in 2006 and 2007 the symptoms observed on the diseased pepper plants were general wilt without yellowing of leaves, root and crown rot. V*erticillium* spp. were not isolated from any sample from the surveys done in 2006, while *Phytophthora* isolates were obtained from roots and rhizosphere soil in most of the surveyed fields (27 of 36), sometimes associated with *Rhizoctonia solani* or *Sclerotium rolfsii*. Analysis of samples of surveys in 2007 corroborated the former observations and *Phytophthora* isolates were obtained from roots and rhizosphere soil from 15 of 16 fields.

Fifteen *Phytophthora* isolates from diseased plants were examined for taxonomic identification and pathogenicity. The colonies of the fifteen examined isolates showed a distinctive arachnoid (spider web) growth on PDA. However, colonies were fluffy without any clear pattern when grown on V8A. Hyphal swellings and abundant globose chlamydospores, both terminal and intercallary, were formed.

All fifteen isolates grew at 36°C with a radial growth rate on V8A of 0.3-9.3 mm day$^{-1}$. However no isolate grew at 37°C, although this temperature was not lethal since all isolates did grow when re-incubated at 25°C.

All the isolates formed terminal, spherical to ovoid, non caducous sporangia with prominent papilla (occasionally with two papillae).

Data of sporangium dimensions (length, width and the length:width ratio) are presented in Table 1. Isolates were significantly different in the length ($F = 16.87$; $P < 0.001$) and width ($F = 15.76$; $P < 0.001$) of the sporangia, as well as in the length:width ratio ($F = 8.86$; $P < 0.001$), although this ratio was always < 1.4.

None of the isolates formed sexual structures in single culture after incubation for three months at 25°C in dark. However, all of them formed sexual structures when paired with the reference isolate of mating type A1, and were treated as heterothalic isolates of mating type A2. Antheridia were amphigynous and ornamented oogonia were not observed.

Species identification was based on the Stamps *et al*. (1990) key, the latest revision of Watherhouse (1963) taxonomic key. Redescription of *P. nicotianae* by Hall (1993) was also considered. The presence of papillated sporangia delimits identification of isolates to Group I or II, and the amphigynous position of antheridia to Group II. The production of non caducous sporangia, the shape and dimensions of sporangia and the length: width ratio (lower than 1.4 in all isolates) and the distinctive arachnoid morphology of colonies are clear morphological characteristics of *P. nicotianae* that distinguish it from *P. capsici*. The characteristics indicated above and the presence of chlamydospores and hyphal swellings of the mycelia, the growth at 36°C and the heterothalism are characters that distinguish *P. nicotianae* from the other species included in Group II. Therefore, the fifteen isolates examined were identified as *P. nicotianae*.

All fifteen inoculated *P. nicotianae* isolates were pathogenic on 'Jaranda' red pepper plants, although the disease severity varied significantly ($F = 12.98$; $P < 0.001$) between isolates 28 days after inoculation (Table 1). Plants showed wilt and root and crown rot and *P. nicotianae* was re-isolated from roots and crowns, thus fulfilling Koch´s postulates.

*P. capsici* is the *Phytophthora* species that usually causes root and crown rot of *C. annuum*, although in some countries *P. nicotianae* has been also reported pathogenic to pepper plants (Erwin and Ribeiro, 1996). In Spain *P. nicotianae* has been reported in association

**Table 1.** Dimensions of sporangia (lenght, width and length/width ratio) and disease severity on 'Jaranda' red pepper plants of 15 isolates of *Phytophthora nicotianae*

| Isolate | Sporangia[a] | | | Disease severity[b] |
|---|---|---|---|---|
| | Length (µm) | Width (µm) | Lengh/width | |
| P-4 | 51 ± 7 | 40 ± 7 | 1.28 ± 0.12 | 2.8 ± 0.4 |
| P-5 | 47 ± 4 | 39 ± 4 | 1.21 ± 0.07 | 3.0 ± 0.0 |
| P-10 | 48 ± 8 | 37 ± 7 | 1.30 ± 0.09 | 2.8 ± 0.4 |
| P-11 | 52 ± 8 | 42 ± 6 | 1.24 ± 0.08 | 3.0 ± 0.0 |
| P-12 | 52 ± 9 | 43 ± 6 | 1.20 ± 0.10 | 2.4 ± 0.8 |
| P-13 | 56 ± 5 | 45 ± 5 | 1.26 ± 0.09 | 3.0 ± 0.0 |
| P-15 | 48 ± 5 | 38 ± 4 | 1.26 ± 0.08 | 0.2 ± 0.4 |
| P-18 | 42 ± 5 | 35 ± 4 | 1.19 ± 0.07 | 3.0 ± 0.0 |
| P-21 | 49 ± 5 | 40 ± 4 | 1.20 ± 0.08 | 1.9 ± 1.5 |
| P-23 | 57 ± 7 | 46 ± 7 | 1.25 ± 0.11 | 2.4 ± 1.3 |
| P-26 | 51 ± 7 | 41 ± 4 | 1.24 ± 0.13 | 2.7 ± 1.0 |
| P-28 | 53 ± 5 | 44 ± 5 | 1.21 ± 0.07 | 2.0 ± 1.0 |
| P-29 | 47 ± 5 | 37 ± 5 | 1.25 ± 0.07 | 3.0 ± 0.0 |
| P-32 | 50 ± 6 | 38 ± 5 | 1.28 ± 0.10 | 0.9 ± 1.5 |
| P-35 | 51 ± 6 | 38 ± 6 | 1.37 ± 0.14 | 2.9 ± 0.3 |

[a] Values are means of 50 measurements ± standard deviation. [b] Disease severity using a scale from 0 = no symptoms to 3 = dead plant. Values are means of ten replicates ± standard deviation.

with *P. capsici* in Andalucía (Larregla, 2003) and Galicia (Saavedra and Collar, 1991; Pomar *et al*., 2001; Andrés-Ares *et al*., 2003). The results of this study indicate that *P. nicotianae* is the principal causal agent of the *Tristeza* disease of red pepper plants in La Vera region since neither *P. capsici* nor *V. dahliae* has been isolated from diseased plants. This situation is similar to that described in Tunisia by Allagui *et al*. (1995) and in Ciudad Real and Toledo (Castilla-La Mancha, Spain) by Bartual *et al*. (1991), where *P. nicotianae* was also the only species associated with the disease. Symptoms observed in diseased plants from La Vera region were also like those described by Allagui *et al.* (1995) in Tunisia.

Identification of *P. nicotianae* as the principal causal agent of *Tristeza* of red pepper in La Vera has several implications for the development of disease management strategies. A great number of *C. annuum* cultivars with genetic resistance to *P. capsici* have been developed (Erwin and Ribeiro, 1996), but there is no cultivar with resistance to *P. nicotianae*. Specific genetic breeding programs would be necesary to obtain red pepper cultivars suitable for elaborating paprika and resistant to *P. nicotianae*. On the other hand, *P. nicotianae* persists in the soil as chlamydospores and oospores in the abscence of a susceptible host and it is pathogenic to a wide range of plant species (Erwin and Ribeiro, 1996). These factors are important when crop rotation strategies are considered. All the studied isolates from La Vera are heterothalic of mating type A2 and this suggests that the pathogen survives mainly as chlamydospores in this region. Red pepper crop rotates frequently with tobacco crops in La Vera, and *P. nicotianae* is the causal agent of the black shank disease of tobacco, that is a serius problem in practically all places that grow tobacco. Although *P. nicotianae* as a species has a wide host range, considerable evidence supports host preference by some isolates (Erwin and Ribeiro, 1996). The host range of isolates from red pepper from La Vera should be studied in order to establish rational crop rotation in this region.

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