Plant Pathology (2008) **57**, 84–91 Doi: 10.1111/j.1365-3059.2007.01702.x

Branch cankers on citrus trees in Spain caused by *Phytophthora citrophthora*

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Considerable losses of citrus trees have been observed in the major citrus-growing areas of Spain. Samples were collected from 132 orchards, and isolations and pathogencity tests were conducted to determine the aetiology of a serious canker disease. Affected trees showed cankers on the scion that frequently began on the branches. Three *Phytophthora* species were identified based on their morphological, cultural, physiological and molecular profiles. *Phytophthora citrophthora* was the main species associated with this new syndrome in 114 orchards. *Phytophthora nicotianae* (syn. *P. parasitica*) was isolated from nine orchards as the sole *Phytophthora* species and in coinfection with *P. citrophthora* from another nine orchards. *Phytophthora citricola* was isolated only from one orchard. In stem-inoculation studies conducted under greenhouse conditions, clementine mandarin cv. Hernandina and sweet orange cv. Navel Late were more susceptible to *P. citrophthora* than sour orange and Carrizo citrange rootstocks. Clementine cv. Hernandina was also highly susceptible in field inoculation experiments. In agreement with field surveys, clementine mandarin cultivars were the most affected, their rootstocks remaining healthy. *Phytophthora citrophthora* was found to be the predominant species in orchard soils; however, *P. nicotianae* was also isolated. This information changes the scenario of diseases caused by *Phytophthora* spp. in Spain and consequently, the present knowledge of epidemiology and the effectiveness of the current control measures should be reassessed.

Keywords: aetiology, clementine mandarin, *Phytophthora parasitica*, rootstock, scion, soil

Introduction

Spain produces more than 5·7 million tonnes of citrus fruits, cultivated over 300 000 ha, and is Europe's leading producer and the world's foremost exporter of fresh citrus (FAO, 2005). Spanish citrus-growing areas are mainly located along the eastern and southern coasts of the country. Since 2002, an unusual cause of death of citrus trees has been detected in these areas. The disease is characterized by the presence of cankers and gum on trunks and main branches of several citrus cultivars. These cankers girdle affected branches and the trunk, frequently leading to the death of the tree. In some cases, cankers are very noticeable, while in others, very limited external symptoms are observed and it is only after removal of the outer bark that extensive necrotic areas are revealed. Because of the extensive losses, this new disease has caused considerable concern among growers.

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Accepted 7 May 2007

Several abiotic and biotic factors are considered responsible for rots and gumming on the trunk and main branches in citrus. Frost damage, sunscald or water injury can induce wood rot caused by several ascomycetes and basidiomycetes (Fawcett, 1936; Oren *et al*., 2001). However, wood rot affects mainly the xylem and it is not often associated with gum formation. In contrast, Rio Grande gummosis affects the bark and wood of large limbs and trunks in several American citrus-growing areas (Powell *et al*., 1998). Affected trees usually show cracks in the bark that release a pale yellow gum. Although a complex of fungi have been associated with Rio Grande gummosis, its aetiology is uncertain (Sonoda, 2000). Additionally, some viral and viroid diseases, such as psorosis, exocortis and cachexia, are also characterized by the presence of bark scaling and gumming (Wallace, 1978).

Phytophthora spp. are the main pathogens associated with gum diseases of citrus trees. Ten species of *Phytophthora* have been reported as pathogenic to citrus, causing several diseases that can affect roots, trunk, branches, fruits and shoots (Erwin & Ribeiro, 1996). However, the most widespread and important are *P. citrophthora* and

P. nicotianae (syn. *P. parasitica*) (Erwin & Ribeiro, 1996). *Phytophthora citrophthora* causes gummosis and root rot in Mediterranean climates and is the most common cause of brown rot in these areas. *Phytophthora nicotianae* is more common in subtropical areas of the world and causes foot rot and root rot. Occasionally, *P. nicotianae* attacks aerial parts of the tree and causes a brown rot of fruit (Graham & Menge, 1999).

Phytophthora spp. are commonly present in citrus soils, causing the decay of fibrous roots in susceptible rootstocks; lesions on structural roots or crown rot may also occur (Fawcett, 1936; Sandler *et al*., 1989; Graham & Menge, 1999). Foot rot and gummosis occur when *Phytophthora* propagules are splashed onto the trunk near ground level, infect wounds or growth cracks and produce lesions which extend down to the bud union (Graham & Menge, 1999). Affected trees show reduced growth flushes, defoliation and twig dieback. If the disease is severe, trees may eventually die. Young trees are more often affected than mature trees.

Phytophthora gummosis and foot-rot infections are typically initiated at the base of the trunk and there are few reports of *Phytophthora* spp. producing branch cankers in citrus trees. Fawcett (1936) described damage caused by *Phytophthora* spp. affecting all parts of the tree from the crown roots to the topmost branches on grapefruit in the Cape Province in South Africa. A similar case was observed in Egypt, where the lesions on affected trees started at the crown roots and progressed up into the branches (Fawcett, 1936). In Belize, under certain conditions, *Phytophthora* spp. may cause rot and gummosis on the main branches (Gutiérrez, 2003). This was observed on trees totally covered by water during floods. Graham & Menge (1999), in reference to canopy blight of citrus, indicated that in rainy areas, cankers may be formed on large branches on susceptible species such as lemons.

A *Phytophthora* sp. was isolated from cankers on the trunks and branches of citrus trees in a preliminary survey of this new syndrome conducted in some Spanish citrus-growing areas (Vicent *et al*., 2003, 2004). Further to this, a large-scale study was conducted in the main citrus-growing areas in Spain in order to better understand the cause of this new epidemic. The objectives of this work were: (i) to characterize the symptomatology of the disease with the purpose of determining the infection sites on the tree; (ii) to determine the range of cultivars affected by this disease; and (iii) to study the aetiology of the disease and its relationship with *Phytophthora* spp. present in the soil.

Materials and methods

Disease surveys

From March 2003 to December 2004 trees in 132 citrus orchards (Table 1) located in the main Spanish citrus-growing areas were visited when the syndrome was observed by growers, farm advisors or plant health authorities. All citrus orchards visited were severely affected by the disease, with 5–75% of trees having symptoms. For each affected orchard the province, citrus cultivar, rootstock and age of the trees were recorded. At least five trees with symptoms were examined carefully in each orchard. A tree was defined as cankered when it had any of the following symptoms: discoloration of the bark surface, discoloration of the underlying tissues and exudation of gum from infected tissues. Cankers were categorized either as present on the rootstock or on the scion, or on both. Cankers from the scion were categorized according to their apparent origin on the tree: (i) infections initiated on the trunk; (ii) infections initiated on the branches; and (iii) undetermined infections, when the lesion could not be assigned to any of the previous categories because of their advanced state of development. Only when root lesions were observed, were root samples taken and later processed.

Table 1 Numbers of Spanish citrus orchards sampled according to geographic location and location of *Phytophthora*-cankers on the tree

	Number of orchards			
		Scion		
Province	Rootstock	Trunk	Branches	Undetermined[a]
Alicante	1	2	3	–
Castellón	1	5	10	–
Valencia	4	16	29	3
Huelva	3	11	32	2
Murcia	–	3	4	–
Tarragona	1	2	–	–
Total	10	39	78	5
Percentage[b]	7·6	29·5	59·1	3·8

[a]Undetermined infection site – the lesion could not be assigned to any category because of its advanced state of development.
[b]Of the 132 surveyed orchards.

Phytophthora-isolation from infected trees and from soil

A total of 299 samples, obtained from the 132 surveyed orchards, were processed. Tissues selected at the margins of canker lesions were removed. Samples were washed under running tap water, surface-sterilized by a 5- to 10-s immersion in 70% ethanol and dried on filter paper. Affected tissues from the edge of the lesions were selected by cutting 2- to 4-mm-wide pieces which were placed on modified PARBPH selective agar containing cornmeal agar (CMA) amended with 10 μg pimaricin, 200 μg ampicillin, 10 μg rifampicin, 25 μg pentachloronitrobenzene (PCNB), 10 μg benomyl and 50 μg hymexazol mL^{-1} (Jeffers & Martin, 1986). Plates were incubated at 24°C in the dark and examined within 2–3 days. Pure cultures of *Phytophthora* were obtained by transferring hyphal tips onto potato dextrose agar (PDA). This culture medium was also used for colony pattern description, while V8 juice agar (2 g $CaCO_3$, 200 mL V8 juice and 15 g agar in 800 mL distilled water) was used for morphological description.

A total of 67 soil samples were obtained from the orchards with the highest incidence of the disease. These samples were taken at different times during 2003 and 2004. The method of Hendrix & Campbell (1970) was used to isolate *Phytophthora* species from soil. Soil samples (about 500 cm^3) were taken from four quadrants under the dripline of the affected trees from a depth of 10–20 cm. In each orchard, the four quadrant samples were pooled and mixed and a small portion placed into two 10-mm-diameter × 15-mm-deep wells cut into an apple. Two apples per sample were prepared following this method. Infected fruit tissue at the margin of the necrosis was removed aseptically and placed on PARBPH medium. The resulting fungal growth was transferred to PDA and V8 juice agar for identification.

Identification of *Phytophthora* isolates

Isolates of *Phytophthora* were identified on the basis of colony morphology, mycelial characteristics, cardinal growth temperatures, and production, morphology and dimensions of sporangia, oogonia and antheridia (Erwin & Ribeiro, 1996). For colony morphology and growth temperature studies, a 5-mm-diameter mycelial plug of each isolate was transferred to PDA and incubated at 5, 24 or 35°C for 7 days in the dark. Sporangia were produced by cutting 5-mm-diameter disks from the advancing margin of a colony grown on V8 and floating these disks on 10 mL of 1·5% sterile soil extract for 4–5 days at 24°C under fluorescent light. Isolates of heterothallic species were paired on V8 juice agar with A1 and A2 tester strains of *P. citrophthora* and *P. nicotianae*. Reference testers were kindly provided by F. Panabières (INRA, France) and A. Ippolito (University of Bari, Italy). Production of sex organs was studied after 2–6 weeks of incubation at 24°C in the dark.

Molecular identification was performed by sequencing the internal transcribed spacer (ITS) region and the 5·8S rRNA gene, using the conserved primers ITS5 and ITS4 (White *et al*., 1990). PCR products were purified with the High Pure PCR Product Purification Kit (Roche Diagnostics GmbH) and directly sequenced using the Tag DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems), in an Applied Biosystems automatic DNA sequencer (model 373A). The primers ITS5 and ITS4 were used to obtain the sequence of both strands. Sequences were aligned with the program CLUSTAL × and compared with *Phytophthora* sequences available in the EMBL/GenBank database.

Pathogenicity tests

Greenhouse pathogenicity tests were carried out on 3-year-old citrus trees grown in plastic pots (20 cm diameter × 25 cm deep) containing potting mix (75% peat, 25% sand, v/v). The rootstocks: Carrizo citrange (*Poncirus trifoliata* × *C. sinensis*) and sour orange (*C. aurantium*); and the scions: clementine mandarin cv. Hernandina (*C. clementina*) and sweet orange cv. Navel Late (*C. sinensis*), both grafted on Carrizo citrange; were used as host material.

Four isolates of *P. citrophthora* Phy 028, Phy 033 (Huelva province) and Phy 051, Phy 075 (Valencia province) were used for inoculation. All isolates were obtained from branch cankers on clementine mandarin cv. Clemenules.

Plants were stem-inoculated by removing a 5-mm-diameter disc from the bark with a cork borer to expose the cambium, and placing a PDA agar plug of similar size containing mycelium of *P. citrophthora* on the exposed cambium. After inoculation, the wound was moistened with a drop of sterile water, sealed with a strip of Parafilm® and wrapped with foil paper to prevent drying.

Each isolate was inoculated individually onto five plants of each host following a complete factorial design. Five additional plants of each host were inoculated with sterile PDA discs to serve as controls. Inoculated plants were placed in random order on benches in a greenhouse and fertilized and watered as needed. Two experiments were conducted in spring of 2005.

Three weeks after inoculation, the bark was removed both above and below the wound of each plant. Canker area was traced on a transparent plastic sheet and quantified by means of the software ASSESS (American Phytopathological Society). The size of the inoculation wound was subtracted from the total area to give the real size of the canker. Isolations onto PARBPH were made in order to confirm that cankers were caused by *P. citrophthora*. Analysis of variance (ANOVA) was used to study the effects of cultivar, isolate and their interaction on canker area. Means were separated according to Fisher's least significant difference test ($P < 0{\cdot}05$) using the software STATGRAPHICS PLUS 5·1 (Manugistics Inc.).

Field inoculations were conducted in two commercial clementine mandarin cv. Hernandina orchards on Carrizo citrange rootstock at El Puig (Valencia province) and Castellón (Castellón province) in spring of 2004. The trees were 7 years old in El Puig and 17 years old in Castellón. *Phytophthora citrophthora* isolates Phy 033 and Phy 051 were used in this study. Four branches per tree (~25 mm diameter) were inoculated following the procedure described above. Each isolate was inoculated onto four trees arranged in a completely randomized design. Four additional trees were inoculated with sterile PDA discs to serve as controls. Average canker area for each tree was evaluated 4 weeks after inoculation as previously described. Isolations were made onto PARBPH in order to confirm the presence of *P. citrophthora*. ANOVA was used to study the effect of isolate on canker area in each orchard using STATGRAPHICS PLUS 5·1.

Results

Disease symptoms and affected cultivars

Affected citrus trees in the field displayed narrow cracks and cankers in the bark, which produced a pale yellow water-soluble gum. Frequently, these cankers were found on the main branches (Fig. 1), from which the lesion expanded to affect the main scaffold branches, secondary branches and the trunk in the lower parts of the tree. The

Figure 1 Canker affecting the main branches of a clementine mandarin cv. Hernandina tree.

rot progressed to a point of totally girdling the branch or the tree, causing a sudden wilt of trees that still retained their fruits and leaves. Advanced symptoms showed a line of demarcation between healthy rootstock tissues and the discoloured tissue of the affected scion.

The disease affected mainly mature citrus trees. Cankers were more frequent on scions than rootstocks (92·4 and 7·6% of surveyed orchards, respectively) (Table 1). Scion cankers had their origin more often on branches than on the trunk (59·1 and 29·5% of surveyed orchards, respectively), whereas in 3·8% of surveyed orchards, the origin could not be determined. There was a broad range of citrus cultivars affected by the disease (Table 2). Clementine mandarins and their hybrids were the most affected, comprising 57·6 and 23·5% of surveyed orchards, respectively. The number of affected orchards was lower for sweet orange and other citrus species and cultivars.

Phytophthora-identification from infected trees and from soil

Phytophthora spp. were isolated from all 132 orchards surveyed. Three species: *P. citrophthora*, *P. nicotianae* and *P. citricola*, were identified on the basis of morphological, physiological and cultural characteristics. These results were in agreement with sequencing data of internal transcribed spacer regions (ITS5 and ITS4) of the ribosomal DNA. *Phytophthora citrophthora* isolates failed to produce oospores, even after prolonged incubation, and were therefore considered sterile. In contrast, 62% of *P. nicotianae* isolates were identified as mating type A1 and the other 38% as A2.

In 114 orchards (86·4%) across the six provinces studied, *P. citrophthora* was the only species recovered, with infected scions in 109 of these orchards and infected rootstocks in five. Scion cankers caused by this species had their origin on the branches in 59·1% of the surveyed orchards, on the trunk in 19·7% and could not be included in any of these categories in 3·8% (Table 3). There were nine orchards from which *P. nicotianae* was the only species recovered; seven of them with trunk cankers and two with rootstock cankers. Coinfections of *P. citrophthora* and *P. nicotianae* were observed in nine orchards; six with trunk cankers and three with rootstock cankers. *Phytophthora citricola* was isolated from affected roots in one orchard from Huelva province.

Phytophthora spp. were recovered from 82·1% of soil samples (Table 4). *Phytophthora citrophthora* and *P. nicotianae* were identified in 55·2 and 26·8% of sampled orchards respectively. *Phytophthora citrophthora* was isolated from soil in all the provinces studied. *Phytophthora nicotianae* was also widely isolated from soil, with the exception of samples from Murcia province.

Pathogenicity tests

Results of greenhouse pathogenicity tests are shown in Fig. 2. Data of two assays were combined because of the lack of significant difference between the two tests and its interaction with the studied factors ($P > 0{\cdot}05$). Cultivar effect was statistically significant in ANOVA analysis ($P < 0{\cdot}05$); however, isolate and its interaction with cultivar were not significant ($P > 0{\cdot}05$). Fisher's mean separation test showed a LSD of ±4·14. Average canker areas in the rootstocks ranged from 0·65 cm^2 in sour orange to

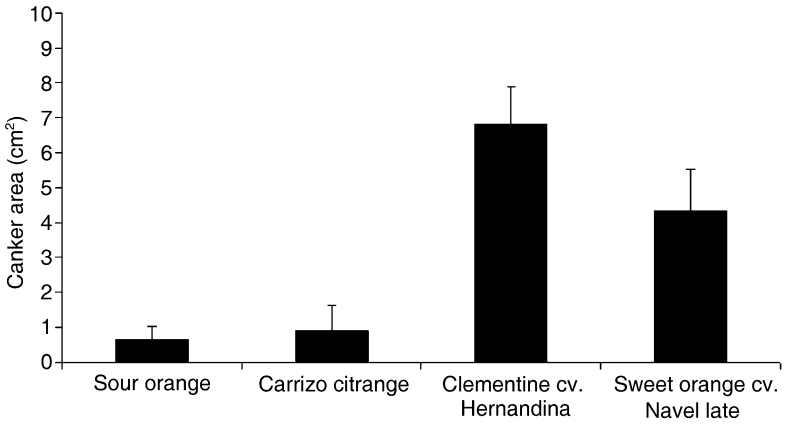

Figure 2 Canker area in 3-year old trees of several citrus genotypes 3 weeks after inoculation with *Phytophthora citrophthora* (average of 40 replicates; LSD = ±4·14).

Table 2 Numbers of Spanish citrus orchards from which *Phytophthora* spp. were isolated according to cultivar and geographic location

Affected citrus scions[a]		Province						
Scion	Rootstock	Alicante	Castellón	Valencia	Murcia	Huelva	Tarragona	Total (%)[b]
Clementine								76 (57·58)
Clemenules	Carrizo citrange	–	–	5	–	6	–	16
	Troyer citrange	–	–	–	–	1	–	
	Sour orange	–	–	–	–	4	–	
Hernandina	Carrizo citrange	1	4	20	–	4	–	38
	Swingle citrumelo	–	5	–	–	–	–	
	Cleopatra mandarin	–	4	–	–	–	–	
Arrufatina	Carrizo citrange	–	–	2	–	–	–	2
Marisol	Carrizo citrange	–	–	4	–	–	–	4
Loretina	Carrizo citrange	–	–	1	–	–	–	1
Orogrande	Carrizo citrange	–	–	–	–	1	–	1
Nour	Carrizo citrange	–	–	–	–	14	–	14
Satsuma								2 (1·51)
Okitsu	Troyer citrange	–	–	–	2	–	–	
Lemon								3 (2·27)
Verna	Sour orange	–	–	–	3	–	–	
Sweet orange								10 (7·58)
Navelina	Carrizo citrange	–	–	5	–	–	–	6
	Troyer citrange	–	–	1	–	–	–	
Navel-late	Carrizo citrange	–	–	–	–	1	–	2
	Volkamer lemon	–	–	–	–	1	–	
Newhall	Sour orange	–	–	–	2	–	–	2
Hybrids								31 (23·48)
Nova	Carrizo citrange	–	–	–	–	5	–	5
Fortune	Carrizo citrange	1	–	9	–	5	–	26
	Swingle Citrumelo	–	–	–	–	–	2	
	Cleopatra mandarin	3	2	1	–	–	–	
	Volkamer lemon	–	–	–	–	3	–	
Total percentage (scions)[c]		3·79	11·37	36·36	5·30	34·09	1·51	
Affected citrus rootstocks		Provinces						
Rootstock	Scion	Alicante	Castellón	Valencia	Murcia	Huelva	Tarragona	Total
Carrizo citrange	Hernandina	–	1	2	–	–	–	10 (7·58)
	Clemenules	–	–	–	–	2	–	
	Fortune	1	–	1	–	1	1	
	Navelina	–	–	1	–	–	–	
Total percentage (rootstocks)[c]		0·76	0·76	3·03	0	2·27	0·76	
Total orchards (scions + rootstocks)		6	16	52	7	48	3	132

[a]Source for citrus species and cultivars: Saunt (1992).
[b]Total for each scion, across rootstocks if more than one shown. Figure in brackets is this total as a percentage of the 132 surveyed orchards.
[c]Each value is the sum for the province, shown as a percentage of the 132 surveyed orchards.

0·88 cm^2 in Carrizo citrange. Scion cultivars showed higher average canker areas, ranging from 4·35 cm^2 in sweet orange cv. Navel Late to 6·82 cm^2 in clementine mandarin cv. Hernandina. No symptoms were observed in control plants.

In field inoculations of both orchards of clementine mandarin cv. Hernandina, the isolate effect was not statistically significant in either orchard ($P < 0·05$). Average canker areas 4 weeks after inoculation ranged from 17·2 cm^2 in the El Puig orchard to 23·1 cm^2 in the Castellón orchard. No symptoms were observed in control plants.

Discussion

Phytophthora diseases in citrus have been known in Spain for a long time. Although the causal agent was not identified, a severe epidemic of root and foot rot was reported at the end of the19th century in the Balearic Islands and Castellón province (eastern Spain) (Bou, 1879; Rullán, 1896). At that time, citrus trees were grafted on susceptible rootstocks such as sweet orange, lemon (*C. limon*) and citron (*C. medica*). This disease was described as starting on the roots and progressing to the trunk. Consequently,

Table 3 Numbers of Spanish citrus orchards sampled according to *Phytophthora* species isolated, location of cankers on the tree, and geographic location

	Number of orchards											
	P. citrophthora				*P. nicotianae*				*P. citrophthora* + *P. nicotianae*			
		Scion				Scion				Scion		
Province	Rootstock	Trunk	Branches	Undetermined[a]	Rootstock	Trunk	Branches	Undetermined	Rootstock	Trunk	Branches	Undetermined
Alicante	1	2	3	–	–	–	–	–	–	–	–	–
Castellón	–	3	10	–	–	1	–	–	1	1	–	–
Valencia	2	11	29	3	1	4	–	–	1	1	–	–
Huelva	2	7	32	2	–	1	–	–	1	3	–	–
Murcia	–	3	4	–	–	–	–	–	–	–	–	–
Tarragona	–	–	–	–	1	1	–	–	–	1	–	–
Total	5	26	78	5	2	7	–	–	3	6	–	–
Percentage[b]	3·8	19·7	59·1	3·8	1·5	5·3	–	–	2·3	4·5	–	–

[a]Undetermined infection site – the lesion could not be assigned to any category because of its advanced state of development.
[b]Of the 132 surveyed orchards.

Table 4 Numbers of Spanish citrus orchards sampled according to *Phytophthora* species isolated from the soil and geographic location

		Number of successful isolations from soil	
Province	Number of orchards	*P. citrophthora*	*P. nicotianae*
Alicante	6	4	2
Castellón	15	8	4
Valencia	26	16	5
Huelva	8	2	1
Murcia	7	4	–
Tarragona	5	3	6
Total	67	37	18
Percentage[a]		55·2	26·8

[a]Of the 67 surveyed orchards.

sour orange rootstock was introduced by citrus growers because of its high tolerance to the disease. The importance of sour orange rootstock increased progressively during the following decades and it was almost the only rootstock used in Spain until the outbreak of the *Citrus tristeza virus* (CTV) in 1957 (Cambra *et al*., 2000). During the following 40 years, a total of 40 million trees grafted on sour orange were replaced progressively by CTV-tolerant rootstocks. Carrizo citrange represents about 75% of the rootstocks currently used in Spain (Agustí, 2000). During the 1970s and 1980s, some cases of crown and foot rot associated mainly with *P. nicotianae* were recorded in poorly drained or improperly irrigated areas (Tuset, 1983). During recent decades, brown rot of citrus fruit has been the disease of greatest economical importance in Spain. The main species associated with brown rot was *P. citrophthora*; but other *Phytophthora* species such as *P. nicotianae* and *P. hibernalis* were also reported (Tuset, 1977; Tuset *et al*., 1990).

This work is the first large-scale and geographically wide study of *Phytophthora* diseases on citrus trees conducted in Spain. The results confirm that *P. citrophthora* is the causal agent of a new and extremely damaging *Phytophthora* disease in the main Spanish citrus-growing areas. In contrast to previous reports, the disease was characterized by the presence of cankers on the scions, often starting on the branches, whereas rootstocks generally remained healthy.

Soil surveys showed a prevalence of *P. citrophthora* in citrus orchards. This fact is in agreement with previous work on *Phytophthora* species in Mediterranean citrus soils (Ricci *et al*., 1990). *Phytophthora nicotianae* was isolated in 26·8% of the samples and was described previously in Spain associated with foot and crown rot (Tuset, 1977, 1983). However, according to the present data, this species seems to be of less importance.

The re-emergence of *Phytophthora* in Spanish citrus-growing areas could be related to the use of more susceptible cultivars or an increase in pathogenicity of the *P. citrophthora* population. Production of clementine mandarin has undergone an important increase in Spain during the

last 30 years, rising from 8000 ha in 1965 to more than 100 000 ha in 2003 (González-Sicilia, 1968; FAO, 2005). Clementine mandarin and its hybrids were the most-affected genotypes in the field surveys in this study, occurring in 81·06% of surveyed orchards. Moreover, clementine mandarin cv. Hernandina had the greatest average canker area in pathogenicity tests in the greenhouse. This cultivar was also highly susceptible to *P. citrophthora* in the pathogenicity tests in the field. The role of the shift from sour orange to Carrizo citrange rootstock seems to be of minor importance, because of the lack of symptoms on the rootstocks in field surveys and because both rootstocks were highly tolerant to bark infection in pathogenicity tests. Nevertheless, more work is needed to understand the influence of Carrizo citrange on scion susceptibility and its effect on the soilborne population of *P. citrophthora*. No difference in pathogenicity between isolates was observed in the greenhouse and field tests, but further research is also needed to characterize the *P. citrophthora* population in Spain. In Corsica, new populations belonging to mating type A2 of *P. citrophthora* were reported to be more aggressive to scion cultivars than the resident sterile population (Cohen *et al*., 2003; Vernière *et al*., 2004).

In many of the affected orchards, scion cankers were clearly initiated on the branches, the mechanisms for dispersal of *P. citrophthora* to citrus branches are unknown. In general, airborne-disseminated *Phytophthora* species have caducous sporangia, whereas *P. citrophthora* produces non-caducous sporangia (Erwin & Ribeiro, 1996). Consequently, rain-splash is regarded as the main pathway of dissemination toward aerial parts of the tree (Fawcett, 1936; Graham *et al*., 1998; Graham & Menge, 1999). In Florida, USA, only lower fruits in the canopy are affected by *P. nicotianae*; however, brown rot caused by *P. palmivora* was usually found high (> 1·0 m) on the tree (Graham *et al*., 1998). In contrast to *P. nicotianae*, *P. palmivora* is able to produce abundant sporangia on affected fruits and they are recognized as an important inoculum source in brown rot epidemics (Timmer *et al*., 2000). Brown rot in Spain is mainly associated with *P. citrophthora* and there are some reports of its sporulation on affected fruits on the tree (Tuset, 1977, 1983; Tuset *et al*., 1990), but more work is needed to establish a relationship between fruit sporulation and branch infections. Some *Phytophthora*-induced diseases are known to be spread by pests, ants, rodents and snails from soil to aerial host sites (Taylor & Griffin, 1981; El-Hamalawi & Menge, 1996; Konam & Guest, 2004). Such mechanisms should also be considered in future epidemiological studies.

Other aspects to consider in the outbreak of this disease could be the variation in environmental factors and changes in citrus cultural practices. Spanish citrus areas are characterized by typical Mediterranean climate, with warm dry summers and cold wet winters. The dominant status of *P. citrophthora* compared to other *Phytophthora* species on citrus in the region is favoured by these environmental conditions (Tuset, 1977; Ricci *et al*., 1990; Erwin & Ribeiro, 1996). *Phytophthora citrophthora* is active at moderate temperatures (< 30°C) during the autumn, winter and spring, but not during the summer. Conversely, *P. nicotianae* is most active during the summer at high temperatures (> 30°C) and is the predominant species in tropical citrus areas (Ricci *et al*., 1990; Erwin & Ribeiro, 1996). However, recent studies indicate an increase in summer precipitation in the coastal plains (Millán *et al*., 2005) that are the main citrus-growing areas in Spain. The unusual rains during the dry season may be promoting favourable conditions for dispersal and development of *P. citrophthora*, but more work is needed to confirm this hypothesis.

Recently in Spain, there has been an important shift from furrow- to drip-irrigation systems. In fact, about 95% of the sampled orchards were drip-irrigated. Increased and continuous soil moisture during the dry period from July to September would enhance survival and dispersal of the pathogen. This was observed previously for other *Phytophthora* diseases (Brasier *et al*., 1993; Reilly *et al*., 1998). In addition, as a consequence of the introduction of clementine mandarin and its hybrids in Spain, ringing of branches is extensively used to improve fruit set (Agustí, 2000). Wounds produced in this way could facilitate *P. citrophthora* infection on branches as described for other *Phytophthora* diseases (Zaiger & Zentmeyer, 1965; Doster & Bostock, 1988).

Because of the increasing interest in clementine mandarins in the global market, more than 40% of Spanish citrus fruit exports are based on these cultivars (FAO, 2005). Since clementine mandarin and its hybrids were the genotypes most affected by *P. citrophthora* in this study, the Spanish citrus industry has to consider this new outbreak as a significant threat. Currently in Spain, control strategies have been designed only for foot rot and brown rot. *Phytophthora* tolerance is only considered in rootstock breeding programmes and, since the disease is now affecting the scions, more work is needed to evaluate scion susceptibility. Systemic fungicides are actually applied as soil drenches or foliar sprays and it is necessary to test the effectiveness of other application techniques. In addition, more epidemiological studies are needed to better understand the biology of *P. citrophthora*, its means of dissemination and the relationship with environmental factors.

Acknowledgements

This research was partially funded by Conselleria de Agricultura, Pesca y Alimentación de la Generalitat Valenciana, Asociacion de Citricultores de la Provincia de Huelva, and Bayer CropScience S.L. We thank F. Alfaro (Servicio de Sanidad Vegetal, Conselleria de Agricultura, Pesca y Alimentación de la Generalitat Valenciana), J. M. Soler (Bayer CropScience S.L.), S. Orta and J. Castro (Laboratorio de Sanidad Vegetal de Huelva) for assistance in the field surveys; and D. García-Rellán and S. P. Sanz for assistance in pathogenicity tests. We acknowledge L. W. Timmer (Citrus Research and Education Center. IFAS, University of Florida) and M. Agustí (Departamento de Producción Vegetal, Universidad Politécnica de Valencia) for reviewing the manuscript.

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