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Short communication

Pathogenicity of 11 *Phytophthora* species on CAB-6P cherry rootstock

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Abstract The pathogenicity and virulence of 11 *Phytophthora* spp. isolated from various hosts were evaluated on CAB-6P cherry rootstock. Isolates of *P. cactorum*, *P. cryptogea*, *P. citrophthora*, *P. citricola*, and isolate 1143 of *P. nicotianae* were pathogenic by all three test methods. An *in vivo* test using bark strips did not distinguish between the isolates in relative virulence and an excised twig test showed *P. cactorum* and *P. cryptogea* to be more virulent than the other species. Trunk inoculation ranked the pathogenic species in the order given above. *P. capsici*, *P. cambivora*, *P. boehmeriae*, *P. drechsleri*, *P. palmivora*, *P. erythroseptica*, and isolate 1258 of *P. nicotianae* were not pathogenic on CAB-6P cherry rootstock. The identity and variability of *Phytophthora* spp. is important when considering strategies for applying an integrated program to control crown rot of cherry trees.

Keywords crown rot; host specificity; isolates; *Prunus cerasus*

INTRODUCTION

Crown rot, caused by *Phytophthora* spp., is a serious disease of stone fruit trees including peach, plum, and cherry (Erwin & Ribeiro 1996). In Greece, *P. citrophthora*, *P. cactorum*, and *P. syringae* were reported as causal agents of crown rot diseases on GF677, KID I, PR 204, and GF305 stone fruit rootstocks (Kouyeas 1971, 1977; Chitzanidis & Stylianides 1987; Elena & Tsipouridis 2000; Thomidis 2000a,b). Host specificity among isolates of some *Phytophthora* species has been reported (Hamm & Hansen 1981; Oyarzun et al. 1998). Variation in virulence among isolates of *P. infestans*, *P. clandestina*, *P. cinnamomi*, and *P. sojoe* has also been reported (Yang et al. 1996; Purwantara et al. 1998; Robin & Desprez-Loustau 1998; Peters et al. 1999).

Clone CAB-6P is possibly the best of those selected from wild *Prunus cerasus* material collected in the Emilia Romana region in Italy. This clone may be propagated by softwood cuttings or micro-propagated. It is reported to give 20–30% reduction in scion vigour compared with Mazzard rootstocks. CAB-6P is widely used in cherry orchards in Greece. A number of *Phytophthora* species have been associated with the symptoms of crown rot of cherry trees in different parts of the world (Mircetich & Matheron 1976; Wilcox & Mircetich 1985). Recently, *P. cactorum* and *P. syringae* isolates from almond trees and *P. citrophthora* isolate from citrus have been found to cause crown rot on different peach, plum, and cherry rootstocks after artificial inoculations (Thomidis 2001). However, *Phytophthora* spp. have not been isolated from naturally infected cherry trees. Therefore, studies of the role of *Phytophthora* spp. in the development of crown rot diseases on cherry trees are required.

The purpose of this study was to evaluate the pathogenicity and host specificity of 11 *Phytophthora* species on CAB-6P cherry rootstock.

MATERIALS AND METHODS

Isolates

Eleven Greek *Phytophthora* species, previously isolated from different hosts, were used in this study (no *Phytophthora* isolate originating from a cherry tree was available in the Greek collections) (Table 1). The *P. cactorum* isolate 1168 and the

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P. citrophthora isolate 1133 were pathogenic on Gisela 5 cherry rootstock in previous work (Thomidis 2001). These *Phytophthora* species are most commonly isolated from Greek fields.

Pathogens were isolated from infected plant material on cornmeal agar (CMA) amended with antibiotics (100 mg mycostatin, 50 mg polymyxin, and 20 mg penicillin per litre of CMA) from 1998 to 2000. Isolates were maintained on CMA at 22°C in the culture collection of the Benaki Phytopathological Institute, Athens, Greece. Fresh cultures were prepared by transferring an agar disc bearing actively growing mycelium of *Phytophthora* to plates containing fresh CMA.

Excised twig assay

The excised twig assay, developed by Jeffers et al. (1981), was used in these experiments. CMA amended with antibiotics (10 mg pimaricin; 250 mg ampicillin; 10 mg rifampicin) was dispensed into jars (9 cm diam. and 12 cm height) to a depth of c. 10 mm. Jars were seeded with an agar plug containing mycelium of a test *Phytophthora* isolate and sealed with parafilm to maintain a moist atmosphere. Two jars for each isolate were used to inoculate excised twigs of CAB-6P cherry rootstock. Two jars without inoculum were used as controls.

The jars were placed in chambers in darkness at 25°C until colony growth nearly covered the agar surface. In November and again in December 2000, 1-year-old woody shoots were collected from 4-year-old CAB-6P cherry rootstock trees planted in the experimental field of the Pomology Institute, Naoussa, Greece. Segments (7 cm long and 1 cm in diam.) were cut from the central part of the shoots and were disinfected in a 10% solution of domestic bleach (4.89% sodium hypochlorite). Segments were then rinsed in sterile water and blotted dry. The bark from the basal end of each twig was removed on opposite sides to expose the cambium. Ten of these stripped twig segments were inserted vertically, distal end up, into the agar medium in each jar at the periphery of the fungal colony. The jars were resealed and incubated for 6 days in darkness at 25°C. After incubation, the twigs were removed and examined for cambium necrosis. By subtracting the depth of agar from the total length of necrosis, a value of necrosis length was obtained.

Bark strip assay

Two vertical strips of bark (10 cm long and 1.5 cm wide) were removed from the trunk of 4-year-old CAB-6P cherry rootstock trees in November and again in December 2000 and inoculated by placing

Table 1 Isolates of *Phytophthora* used to inoculate CAB-6P cherry rootstock.

Species*†	Isolates	Host		Disease
P. boehmeriae	1909*	Cotton	*Gossypium hirsutum*	Boll blight
	1923	Cotton	*Gossypium hirsutum*	Boll blight
P. cactorum	1128	Almond tree	*Prunus amygdalus*	Crown rot
	1168	Almond tree	*Prunus amygdalus*	Crown rot
P. cambivora	1172	Chestnut	*Castanea* spp.	Crown rot
P. capsici	1131	Green pepper	*Capsicum annuum* var.	Stem blight
	1134	Green pepper	*Capsicum annuum* var.	Stem blight
P. citricola	1177	Pistachio tree	*Pistacia vera*	Crown rot
	1178	Lemon	*Citrus limon*	Fruit rot
P. citrophthora	1133	Almond tree	*Prunus amygdalus*	Crown rot
	1183	Plum tree	*Prunus domestica*	Crown rot
P. cryptogea	1191	Carnation	*Dianthus caryophyllus*	Stem blight
	1195	Almond tree	*Prunus amygdalus*	Crown rot
P. drechsleri	1196	Almond tree	*Prunus amygdalus*	Crown rot
P. erythroseptica	1136	Potato	*Solanum tuberosum*	Pink tuber rot
	1198	Tulip	*Tulipa*	Stem blight
P. palmivora	1140	Coconut	*Cocus nucifera*	Bud rot
P. nicotianae	1143	Pistachio tree	*Pistacia vera*	Crown rot
	1258	Pistachio tree	*Pistacia vera*	Crown rot

*Serial number of isolate in Benaki Phytopathological Institute Collection, Greece.
†No *Phytophthora* isolate originating from a cherry tree was available in the Greek collections.

a 4-mm-diam. disk of CMA containing mycelium of a *Phytophthora* isolate into the centre of the bark strip on the cambium side. Inoculated areas were covered with wet cotton and wrapped with adhesive tape to avoid desiccation. Inoculated bark strips were incubated for 4 days in darkness at 25°C in moist chambers after which the vertical length of necrosis was measured. There were 20 strips (replicates) for each isolate. Strips treated with an agar plug without mycelium served as controls.

Trunk inoculation

Inoculations were made on the trunk of 4-year-old CAB-6P trees, 10 cm above the soil surface, in May and again in September 2001 when temperatures (c. 21–28°C) favoured disease development (Erwin & Ribeiro 1996). There were 10 trees for each isolate tested. Trunks of tree were wounded by removing a 6 mm disc of bark (using a cork borer) to expose the cambium. The inocula, consisting of 6-mm-diam. plugs from 5-day-old cultures on CMA, were inserted directly on the cambium of the trees. The wounds were covered with petroleum jelly and wrapped with adhesive tape to prevent desiccation. Ten additional trees served as controls and were treated with a sterile plug of CMA. Fifteen days after inoculation, the adhesive tape was removed from wounds and the trunk bark was scraped with a knife blade to reveal margins between healthy (white to yellow) and necrotic (brown) tissues in the underlying periderm and secondary phloem. The vertical distance of necrosis (both up and down) development was measured. For recovery of *Phytophthora*, Jeffers & Martin's (1986) selective medium (P_5ARP) was used. Autoclaved CMA, after cooling to 45°C, was amended with 5 mg pimaricin, 250 mg ampicillin, and 10 mg rifampicin. After lesions were measured, sections from the margin of each lesion were placed in a 10% solution of domestic bleach (4.89%) for 1–3 min then washed 3 times with sterile distilled water. Tissue sections were blotted with a sterile paper towel and placed on selective medium in Petri dishes, which then were sealed with parafilm and incubated at the appropriate temperature (c. 23–26°C) for each pathogen.

Statistical analysis

A completely randomised experimental design was used throughout the laboratory and field experiments. All experiments were conducted twice. Data were analysed by one-way analyses of variance. Before combining data for both runs of an experiment, Bartlett's test was used to confirm homogeneity of variances. Treatment means were separated by least significant diference ($P = 0.05$).

RESULTS

Excised twig assay

Isolates of *P. cactorum*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, and isolate 1143 of *P. nicotianae* caused necrosis on CAB-6P cherry rootstock (Table 2). *P. cactorum* and *P. cryptogea* isolates caused the longest lesions. No significant difference was observed in the length of necrosis among isolates of *P. cactorum* or *P. cryptogea*. The virulence of *P. citrophthora* isolates did not differ significantly from those of *P. citricola* or isolate 1143 of *P. nicotianae* (Table 2). Isolates of *P. cambivora*, *P. erythroseptica*, *P. capsici*, *P. drechsleri*, *P. palmivora*, *P. boehmeriae*, and isolate 1258 of *P. nicotianea* did not produce necrosis on excised twigs of CAB-6P cherry rootstock. No necrosis was evident on control twigs.

Bark strip assay

Isolates of *P. cactorum*, *P. cryptogea*, *P. citricola*, *P. citrophthora*, and isolate 1143 of *P. nicotianae* caused necrosis on CAB-6P cherry rootstock bark tissue. No significant difference in necrosis length was observed among those isolates with this assay. Isolates of *P. cambivora*, *P. erythroseptica*, *P. capsici*, *P. drechsleri*, *P. palmivora*, *P. boehmeriae*, and isolate 1258 of *P. nicotianea* did not cause necrosis on CAB-6P bark tissue. No necrosis developed on control bark strips.

Trunk inoculation

Tested isolates of *P. cryptogea*, *P. cactorum*, *P. citrophthora*, *P. citricola*, and isolate 1143 of *P. nicotianae* caused necrosis on the trunk of tested cherry rootstock trees. Both isolates of *P. cactorum* and isolate 1191 of *P. cryptogea* caused the longest necrosis of all isolates tested. The length of lesions caused by *P. citrophthora* isolates were longer than those caused by *P. citricola* which in turn was longer than that caused by isolate 1143 of *P. nicotianae*. Significant differences among isolates were observed in the length of lesions caused by *P. cryptogea* and *P. citricola*. Isolate 1191 of *P. cryptogea* caused significantly longer necrosis than isolate 1195. Similarly, the length of necrosis caused by isolate 1177 of *P. citricola* was longer than that

Table 2 Use of the excised twig, bark strip, and trunk inoculation methods to test the pathogenicity of 11 *Phytophthora* species isolated from various hosts on CAB-6P cherry rootstock.

Isolates		Lesion length (cm)		
		Excised twigs	Bark strips	Trunk inoculation
P. cactorum	1128[*]	3.4[†] a[‡]	2.2 a	17.1 a
	1168	3.3 a	2.3 a	17.2 a
P. cryptogea	1191	3.2 a	2.4 a	17.0 a
	1195	3.1 a	2.4 a	14.2 b
P. citrophthora	1133	2.3 b	2.2 a	12.0 c
	1183	2.2 b	2.2 a	12.3 c
P. citricola	1177	2.2 b	2.1 a	10.4 d
	1178	2.2 b	2.1 a	8.3 e
P. nicotianae	1143	2.1 b	2.3 a	6.5 f
	1158	0 c	0 b	0 g
P. capsici	1134	0 c	0 b	0 g
	1131	0 c	0 b	0 g
P. boehmeriae	1909	0 c	0 b	0 g
	1923	0 c	0 b	0 g
P. cambivora	1172	0 c	0 b	0 g
P. drechsleri	1196	0 c	0 b	0 g
P. erythroseptica	1136	0 c	0 b	0 g
	1198	0 c	0 b	0 g
P. palmivora	1140	0 c	0 b	0 g
Control	–	0 c	0 b	0 g
$LSD_{0.95}$		0.365	0.396	0.641

[*]Serial number of isolate in Benaki Phytopathological Institute Collection, Greece.
[†]Values are the means of two experiments; results were similar according to the Bartletts's test of homogeneity of variance, so data were combined.
[‡]Values followed by the same letters are not significantly different according to least significant difference (LSD) ($P < 0.05$).

caused by isolate 1178. *P. capsici*, *P. cambivora*, *P. boehmeriae*, *P. drechsleri*, *P. palmivora*, *P. erythroseptica*, and isolate 1258 of *P. nicotianae* did not cause necrosis on cherry rootstock trunks. *P. cryptogea*, *P. cactorum*, *P. citrophthora*, *P. citricola*, and isolate 1143 of *P. nicotianea* were recovered from, at least, one inoculated tree. No necrosis was observed on control trees.

DISCUSSION

The pathogenicity of the most common *Phytophthora* species isolated in Greek fields on the CAB-6P cherry rootstock was examined. In Greece, this is the first report of *P. cryptogea*, *P. citricola*, and *P. nicotianae* as pathogens of cherry trees. Thomidis (2001) reported *P. cactorum* and *P. citrophthora* originating from almond and citrus trees, respectively, as pathogens of cherry trees in Greece. *P. cryptogea* and *P. citricola* also caused serious damage in commercial cherry orchards in the United States (Mircetich & Matheron 1976; Wilcox & Mircetich 1985). Although *Phytophthora* spp. have not been isolated from naturally infected cherry trees in Greece, this study demonstrated that *P. cactorum*, *P. citrophthora*, *P. cryptogea*, *P. citricola*, and *P. nicotianae* could be a threat to cherry trees. The ability of *Phytophthora* spp. to infect cherry trees grown in infested soil should be investigated in the future.

Isolates of *P. cactorum* and *P. cryptogea* caused the longest lesions, suggesting that these isolates pose a serious threat to cherry orchards in Greece (Table 2). Furthermore, the high virulence on cherry of *Phytophthora* isolates originating from different plant species, suggests that these isolates may not be host specific. This lack of host specificity should be considered in decisions involving the use of recycled irrigation water, selection, and preparation of new orchard planting sites, choice of tree species to be planted, and the movement of equipment between fields and orchards suspected of having a *Phytophthora* disease problem.

This is the first time that host specificity may have been detected among two isolates of *P. nicotianae* from Greece. Isolate 1143 caused necrosis on cherry rootstock CAB-6P whereas isolate 1258 of the pathogen was not pathogenic (Table 2). Tested isolates of *P. boehmeriae*, *P. cambivora*, *P. capsici*, *P. drechsleri*, *P. erythroseptica*, and *P. palmivora* did not infect CAB-6P rootstock. In contrast, *P. cambivora* and *P. drechsleri* were reported as pathogens of cherry trees in the United States (Wilcox & Mircetich 1985). Apparently, *P. cambivora* and *P. drechsleri* isolates can have various host ranges. Host specificity among isolates of *P. infestans* and *P. megasperma* has been found (Hamm & Hansen 1981; Oyrzun et al. 1998). Host specificity has major consequences for disease management. For example, common strategies designed to control disease through the reduction or exclusion of inoculum (e.g., sanitation or crop rotation) is fundamentally dependent on knowledge of what potentially constitutes inoculum.

In field experiments, variability in virulence on cherry trees was noted among isolates of *P. cryptogea* and *P. citricola* isolated from different hosts. These results should also be considered when applying control strategies against *Phytophthora* diseases. Variation in virulence among isolates of *P. cactorum* and *P. parasitica* has been reported (Hantula et al. 1997; Lebreton & Adrivon 1998; Matheron & Matejka 1990). Some differences were observed between laboratory and field experiments. Differences among laboratory and field experiments exist, and the reasons for these differences are unknown. Variability among fungal isolates should also be considered when evaluating rootstocks for resistance to certain *Phytophthora* spp. This research demonstrates importance of the correct identification of *Phytophthora* spp. when considering strategies for applying an integrated program to control *Phytophthora* of cherry trees.

REFERENCES

Chitzanidis, A.; Stylianides, D. C. 1987: Seasonal fluctuation in extent of colonization of rootstock GF677 by three *Phytophthora* species. *Options Mediterraneennes, CIHEAM 87*: 87–90.

Elena, K.; Tsipouridis, K. 2000: Evaluation of resistance of stone fruit rootstocks to *Phytophthora* crown rot. *Journal of Phytopathology 6*: 365–371.

Erwin, D.; Ribeiro, O. 1996: *Phytophthora* diseases worldwide. St Paul, MN, United States, APS.

Hamm, P. B.; Hansen, E. M. 1981: Host specificity of *Phytophthora megasperma* from Douglas-fir, soybean and alfalfa. *Phytopathology 71*: 65–68.

Hantula, J.; Lilja, A.; Parikka, P. 1997: Genetic variation and host specificity of *Phytophthora cactorum* isolated in Europe. *Mycological Research 5*: 565–572.

Jeffers, S. N.; Martin, S. B. 1986: Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease 70*: 1038–1043.

Jeffers, S. N.; Aldwinckle, H. S.; Burr, T. J.; Arneson, P. A. 1981: Excised twig assay for the study of apple tree crown rot pathogens in vitro. *Plant Disease 65*: 823–825.

Kouyeas, H. 1971: On the apoplexy of fruit trees caused by *Phytophthora* spp. *Annals Institute Phytopathological Benaki N.S. 10*: 163–170.

Kouyeas, H. 1977: Stone fruit tree apoplexy caused by *Phytophthora* collar rot. *EPPO Bulletin 7*: 117–124.

Lebreton, D.; Andrivon, D. 1998: French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype. *European Journal of Plant Pathology 104*: 583–594.

Matheron, M. E.; Matejka, J. C. 1990: Differential virulence of *Phytophthora parasitica* recovered from citrus and other plants to rough lemon and tomato. *Plant Disease 74*: 138–140.

Mircetich, M. S.; Matheron M. E. 1976: *Phytophthora* root and crown rot of cherry trees. *Phytopathology 66*: 549–558.

Oyarzun, P. J.; Pozo, A.; Ordonez, M. E.; Doucett, K.; Forbes, G. A. 1998: Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. *Phytopathology 88*: 265–271.

Peters, R. D.; Platt, H. W.; Hall R.; Medina, M. 1999: Variation in aggressiveness of Canadian isolates of *Phytophthora infestans* as indicated by their relative abilities to cause potato tuber rot. *Plant Disease 83*: 652–661.

Purwantara, A.; Flett, S. P.; Kane, P. J. 1998: Variation in pathogenicity among isolates of *Phytophthora cladestina*. *Journal of Phytopathology 146*: 587–591.

Robin, C.; Desprez-Loustau, M. 1998: Testing variability in pathogenicity of *Phytophthora cinnamomi*. *European Journal of Plant Pathology 104*: 465–475.

Thomidis, T. 2000a: Field susceptibility of four peach rootstocks to *Phytophthora citrophthora* and *P. syringae*. *Phytopathologia Mediterranea 39*: 404–409.

Thomidis, T. 2000b: Seasonal variation in crown rot of GF677 and KID I peach rootstocks by *Phytophthora cactorum*, *P. citrophthora* and *P. syringae*. *Phytopathologia Mediterranea 39*: 396–403.

Thomidis, T. 2001: Testing variability in pathogenicity of *Phytophthora cactorum*, *P. citrophthora* and *P. syringae* to apple, pear, peach, cherry and plum rootstocks. *Phytoparasitica 29*: 47–49.

Wilcox, W. F.; Mircetich, S. M. 1985: Pathogenicity and relative virulence of seven *Phytophthora* spp. on Mahaleb and Mazzard cherry. *Phytopathology 75*: 221–226.

Yang, X. B.; Ruff, R. L.; Meng, X. Q.; Workneb, F. 1996: Races of *Phytophthora sojoe* in Iowa soybean fields. *Plant Disease 80*: 1418–1420.