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# Pathogenicity and relative virulence of 11 Greek *Phytophthora* species on apple and pear rootstocks

T. THOMIDIS
C. TSIPOURIDIS
J. CULLUM

National Agricultural Research Foundation
Pomology Institute
Naoussa, P.C. 59200, Greece
email: tomgr@otenet.gr

**Abstract** The pathogenicity and virulence of 11 *Phytophthora* spp. isolated from various host plants were examined on an apple (*Malus domestica* Borkh.) (MM106) and a pear (*Pyrus communis* L.) (O.H.F. 333) rootstock. Only *P. cactorum* and *P. citricola* isolates were pathogenic to these rootstocks. The isolates of *P. cactorum* were the most aggressive. The severe crown rot caused by isolates of *P. cactorum* and *P. citricola* suggests that these pathogens pose a potential threat to apple and pear orchards. *P. citrophthora*, *P. boehmeriae*, *P. erythroseptica*, *P. capsici*, *P. cryptogea*, *P. drechsleri*, *P. cambivora*, *P. palmivora*, and *P. parasitica*, isolated from various hosts, were not pathogenic to MM106 and O.H.F. rootstocks, suggesting that these pathogens may not be a serious threat to apple and pear trees grafted on these rootstocks.

**Keywords** crown rot; pathogenicity; *Phytophthora*; virulence

## INTRODUCTION

Crown rot is an important soil-borne disease of apple (*Malus domestica* Borkh.) and pear trees (*Pyrus communis* L.) in Macedonia, Greece. This disease is caused primarily by *Phytophthora cactorum* around the world (Harris 1990). Other species of *Phytophthora* have been associated with crown rot of apple or pears trees such as *P. cambivora*, *P. cryptogea*, *P. citricola*, *P. syringae*, *P. megasperma*, *P. drechsleri*, *P. cinnamomi*, *P. parasitica*, and *P. citrophthora* (Jeffers et al. 1981; Jeffers & Aldwinckle 1986; Jeffers & Aldwinckle 1988; Browne et al. 1995). Host specificity and variation in pathogenicity among isolates of *Phytophthora* have also been reported (Hamm & Hansen 1981; Wilcox & Mircetich 1987; Lebreton & Andrivon 1998; Robin & Desprez-Loustau 1998).

In Greece, few studies have been conducted on the virulence of different *Phytophthora* species on apple and pear trees. The purpose of this study was to evaluate the pathogenicity and virulence of *P. cactorum*, *P. cambivora*, *P. cryptogea*, *P. citricola*, *P. erythroseptica*, *P. capsici, P. drechsleri*, *P. palmivora*, *P. boehmeriae*, *P. parasitica*, and *P. citrophthora* on an apple and pear rootstocks.

## MATERIALS AND METHODS

Eleven *Phytophthora* species, originating from various hosts, were used in this study (Table 1). Pathogens were isolated on cornmeal agar (CMA) amended with antibiotics (mycostatin 100 mg, polymyxin 50 mg, and penicillin 20 mg litre$^{-1}$ CMA). Isolates were maintained on CMA at 22°C in the culture collection of the Benaki Phytopathological Institute. Fresh cultures were prepared by transferring an agar disk bearing actively-growing mycelium of *Phytophthora* to plates containing fresh CMA.

### Excised stem inoculation: Method 1

These experiments were based on methods described by Jeffers et al. (1981). CMA amended with antibiotics (pimaricin 10 mg, ampicillin 250 mg, rifampicin 10 mg) was dispensed to c. 10 mm depth in sterilised pyrex jars (9 cm diam. and 12 cm high). Two agar plugs bearing mycelium of a tested isolate were added to each jar. Jars were sealed with lids and placed in incubators at 25°C in the dark. One-year-old twigs, c. 1 cm in diameter and 7 cm long, were

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collected from 5-year-old apple (MM106) and pear (O.H.F. 333) rootstocks. Using a flamed sharp knife, segments were trimmed to a slant at the base and inserted upright in the periphery of the growing culture. Two jars for each isolate were used to inoculate 20 segments of each rootstock. Additionally, two jars containing CMA without mycelium were used as controls for each rootstock. Then, jars were resealed and returned to incubators (25ºC) for 4 days. By subtracting the depth of agar from the total length of necrosis that developed, a value for necrosis was obtained.

### Excised stem inoculation: Method 2

A second laboratory experiment was conducted using an excised shoot assay described by Matheron & Matejka (1985). Segments of woody shoots of each rootstock, 6 cm in length and 1.5–2 cm in diameter, were collected. Twenty segments (10 from each of the two tested rootstocks) were inoculated with each isolate tested. In addition, 10 segments were inoculated with CMA without mycelium and served as controls. The inoculum, which consisted of a 6-mm-diameter agar plug bearing mycelium from a 5-day-old culture of *Phytophthora*, was inserted in the middle of excised shoot pieces under the bark. The wound was covered with petroleum jelly and wrapped with adhesive tape to prevent desiccation. Inoculated shoot segments were incubated for 4 days at 25°C in moist chambers, after which the length of resulting necrosis was recorded.

### Tree trunk inoculation

A field experiment was conducted using a rootstock assay (Thomidis 2001). Two-year-old apple (MM106) and pear (O.H.F. 333) tree rootstocks were used in these experiments. Plants were wounded using a flamed knife c. 10 cm above the soil surface by removing a 6 mm length of bark periderm to expose the cambium. Inoculation was performed by placing an agar plug containing mycelium of the pathogen onto the wound. The mean temperatures fluctuated between 24 and 28ºC in the day and between 16 and 18ºC at night. The wound was then covered with petroleum jelly and wrapped with adhesive tape to prevent desiccation. Fifteen days later, the length of resulting necrosis was measured. Twenty plants (10 of each rootstock) were inoculated with each isolate. Also, 10 plants for each rootstock were inoculated with CMA agar without mycelium to serve as controls. $P_5$ARP selective medium was used (Jeffers & Martin 1986) for re-isolation of *Phytophthora*.

**Table 1** Isolates of *Phytophthora* used in this study. (All isolates were Greek and kept in Benaki Phytopathological collection.)

| Isolates | | Host | Latin name | Disease |
|---|---|---|---|---|
| *P. boehmeriae* | 1909 | Cotton | *Gossypium* spp. | Boll blight |
| | 1923 | Cotton | *Gossypium* spp. | Boll blight |
| *P. cactorum* | 2000 | Apple tree | *Malus domestica* | Crown rot |
| | 2001 | Apple tree | *Malus domestica* | 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 tuberasum* | Pink tuber rot |
| | 1198 | Tulip | *Tulipa* spp. | Stem blight |
| *P. palmivora* | 1140 | Coconut | *Cocus nucifera* | Bud rot |
| *P. parasitica* | 1143 | Pistachio tree | *Pistacia vera* | Crown rot |
| | 1258 | Pistachio tree | *Pistacia vera* | Crown rot |

### Statistical analysis

A completely randomised experimental design was used in all laboratory and field experiments. All experiments were conducted twice. Data were analysed by one-way analyses of variance. For analysis of data from combined experiments, Bartlett's test was used to demonstrate homogeneity of variances. Treatment means were separated by Duncan's Multiple Range Test ($P > 0.05$).

## RESULTS AND DISCUSSION

Direct inoculation onto stems or beneath the bark only allows assessment of the ability of isolates to develop lesions once they are inside the host tissue. Stems instead of roots were inoculated with the different *Phytophthora* species because stem inoculation enhances differences in susceptibility among hosts (Tippet et al. 1985). Furthermore, inoculations of stems overcomes the problems of the large variation in root size and the large amount of labour needed to excavate enough roots for adequate replication.

Generally, results from laboratory and glasshouse experiments were in good agreement on tested apple (MM106) and pear (O.H.F. 333) rootstocks. Only *P. cactorum* and *P. citricola* isolates were pathogenic on apple and pear rootstocks. The isolates of *P. cactorum* were more aggressive than *P. citricola* (Table 2). Only *P. cactorum* and *P. citricola* isolates were recovered from inoculated trees. This study confirms earlier reports that *P. cactorum* and *P. citricola* are serious pathogens of apple trees around the world (Jeffers & Aldwinckle 1988; Utkhede & Smith 1994; Browne & Mircetich 1996). Futhermore, the aggressiveness on apple and pear of *P. citricola* isolates originating from different plant species indicates that these isolates are not host specific. *P. citricola* isolates used in this study were also pathogenic on cherry trees (Thomidis unpubl. data). Such non-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.

*Phytophthora citrophthora*, *P. boehmeriae*, *P. erythroseptica*, *P. capsici*, *P. cryptogea*, *P. drechsleri*, *P. cambivora*, *P. palmivora*, and *P. parasitica*, isolated from various hosts, were not pathogenic to the apple and the pear rootstocks tested in this study. In contrast, *P. citrophthora*, *P.*

**Table 2** Testing of the pathogenicity of 11 *Phytophthora* species isolated from various hosts on apple (MM106) and pear (O.H.F. 333) rootstocks in the laboratory and field. (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 Duncan's Multiple range Test ($P > 0.05$). Segments inoculated with cornmeal agar without mycelium did not develop any necrosis.)

| Isolates* | | Necrosis length (cm) | | |
|---|---|---|---|---|
| | | Excised stem inoculation | | Tree trunk inoculation |
| | | Method 1 | Method 2 | |
| **MM106 rootstock** | | | | |
| *P. cactorum* | 2000 | 2.82 a | 3.27 a | 12.85 a |
| | 2001 | 2.63 a | 3.38 a | 13.20 a |
| *P. citricola* | 1177 | 1.67 b | 2.18 b | 8.45 b |
| | 1178 | 1.89 b | 2.02 b | 8.70 b |
| **O.H.F. 333 rootstock** | | | | |
| *P. cactorum* | 2000 | 3.18 a | 4.46 a | 12.70 a |
| | 2001 | 2.93 a | 4.58 a | 12.55 a |
| *P. citricola* | 1177 | 1.87 b | 3.52 b | 9.65 b |
| | 1178 | 1.84 b | 3.24 b | 9.80 b |

*Remainder of the isolates listed in Table 1 were not pathogenic.

*cryptogea*, *P. drechsleri*, *P. cambivora*, and *P. parasitica* have been reported to cause crown rot on apple trees in other studies (Julis et al. 1979; Jeffers & Aldwinckle 1986; Matheron & Matejka 1988; Browne et al. 1995). One possible explanation for the differing findings is that isolates of these *Phytophthora* spp. may differ in host range. Host specificity among isolates of *Phytophthora* has been reported (Hamm & Hansen 1981; Oyarzum et al. 1998). Another possible reason could be that the apple rootstock used in this study (MM106) may be resistant to these pathogens, whereas plant material used in other referenced work may have been susceptible to one or more of the pathogens.

Host specificity has important implications for disease management. For example, the rootstocks evaluated in this study could be planted in fields where tested non-pathogenic *Phytophthora* isolates were established. In other words, the avirulence of *Phytophthora* isolates (only those tested in these experiments) suggests that those isolates are not a serious threat to apple and pear trees grafted on MM106 and O.H.F. 333 rootstocks. However, the possibility that these *Phytophthora* isolates may infect feeder roots should be investigated. Besides, other (virulent) populations of these species of *Phytophthora* may currently exist or be imported into Greece, which could develop into a serious problem in apple and pear orchards.

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