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Susceptibility of Thirty Cherry Genotypes on *Phytophthora cactorum*, *P. citrophthora*, *P. citricola* and *P. parasitica*

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Abstract

The diseases *Phytophthor*a crown and root rot consist of the most important problems in cherry cultivation. In this study, the susceptibility of 30 cherry genotypes to *Phytophthora cactorum*, *P. citrophthora, P. parasitica* and *P. citricola* was evaluated by using excised twig assay, excised shoot method and stem inoculation method. The results showed that all cherry genotypes tested were susceptible to all *Phytophthora* isolates used. *Phytophthora parasitica* and *P. citrophthora* were the most aggressive species. Host specificity of the *Phytophthora* isolates used in this study was not found although these isolates were from different plant species. In conclusion, because none of the cherry genotype showed a level of resistance to these pathogens, caution should be taken when these genotypes are used in locations, where these diseases are endemic.

Introduction

Cherry cultivation is one of the most popular of tree crops in Greece (Koukourojiannis, 1996). The highest quantity of cherries is produced in northern Greece, where climatic conditions are excellent for the cultivation of cherry trees. Unfortunately these climatic conditions encourage the development of *Phytophthora* crown and root rot resulting in destruction of many cherry trees by these pathogens every year. In Greece, *P. cactorum*, *P. cryptogea*, *P. citrophthora*, *P. citricola*, *P. nicotianae* and *P. syringae* were reported as causal agents of crown rot diseases on cherry trees (Thomidis, 2001; Thomidis and Sotiropoulos, 2003; Exadaktylou and Thomidis, 2005), while a number of *Phytophthora* species (*P. citricola*, *P. nicotianae, P. cryptogea, P. citrophthora*, *P. alni*, *P. megasperma* and *P. cinnamomi*) have also been associated with the symptoms of crown rot of cherry trees in different parts of the world (Wilcox and Mircetich, 1985; Wicks, 1989; Barzanti et al., 2004; Santini et al., 2006).

So far, control of *Phytophthora* diseases has been mainly based on the use of chemicals (Thomidis and Elena, 2001; Thomidis et al., 2002). Using of resistant trees is the most important available method of controlling because the growers need not expend funds for extraneous control measures such as fungicides. Even where fungicides must be used, resistance is a useful complementary control measure.

At the Pomology Institute Naoussa, a research programme was carried out on the selection of cherry genotypes appropriate for the Greek conditions. Thirty of these were recognized as promised genotypes based on their agronomic characteristics (productivity, fruit quality, etc.). However, there were no data on the susceptibility of these genotypes to Phytopththora crown rot. The purpose of this study was to evaluate the susceptibility of these genotypes to *P. cactorum, P. citrophthora, P. citricola* and *P. parasitica*.

Materials and Methods

Isolates

Isolates of *P. cactorum* (two isolates originated from the trunk of infected almond trees) *P. citrophthora* (one isolate originated from the trunk of citrus and one from the trunk of pear tree), *P. parasitica* (one isolate originated from the trunk of citrus) and *P. citricola* (one isolate originated from lemon fruit) were used in this study. All isolates were cultured onto cornmeal agar (CMA). Unfortunately, no *Phytophthora* isolates originated from cherry orchard was available in any Greek collection. The above isolates had been pathogenic in cherry trees in previous works (Exadaktylou and Thomidis, 2005).

Excised twig assay

These experiments were based on method described by Jeffers et al. (1981). CMAamended with the antibiotics pimaricin 10 mg, ampicillin 250 mg, rifampicin 10 mg for *Phytophthora* were dispensed to ca. 10-mm depth in sterilized pyrex jars (9 cm diameter 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 for a week. One-year-old twigs, ca. 1-cm diameter and 7-cm long were collected from 6-year-old mother trees of cherry genotypes in the second half (15 days) of December. Twigs taken from the cherry rootstock Gisela 5 were used as susceptible control (Exadaktylou and Thomidis, 2005; Exadaktylou, 2007). Twigs were surface disinfected in domestic chlorine bleach 10% (4.8% Sodium hypochloride) for 10 min and then rinsed three times with sterile water. Using a flamed sharp knife, twigs were trimmed to a slant at the base and inserted upright into the growing culture. Two jars for each isolate were used to inoculate 20 twigs of each genotype tested. Additionally, two jars containing CMAwithout mycelium were used as control. Jars were resealed and returned to incubators (25°C) for 4 days. By subtracting the depth of agar from the total length of necrosis that developed (Fig. 1), a value for necrosis was obtained. This experiment was conducted twice.

Excised shoot method

A second laboratory experiment was conducted using an excised shoot method described by Matheron and Matejka (1988). Twigs of woody shoots of each genotype tested, 6 cm in length and 1.5–2 cm in diameter were collected. They were disinfected in domestic chloride bleach 10% (4.8% Sodium hypochloride) for 10 min and then rinsed three times with sterile water. Ten twigs from each genotype were inoculated with each isolate of *Phytophthora*. In addition, 10 twigs were inoculated with CMA without mycelium and served as controls. Twigs from the cherry rootstock Gisela 5 were used as susceptible control. 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 twigs were incubated for 4 days at 25°C in moist chambers, after which the length of resulting necrosis (Fig. 2) was recorded. This experiment was conducted twice.

Tree trunk inoculation

A glasshouse experiment was conducted using a trunk inoculation method (Thomidis, 2001). One-year-old tree of each genotype was used in these experiments. Trees were wounded using a flamed knife ca. 10 cm above the soil surface by removing a 6-mm length of periderm of bark to expose the cambium. Inoculation was performed by placing an agar plug containing mycelium of *Phytophthora* onto the wound. The wound was then covered with petroleum jelly and wrapped with adhesive tape to prevent desiccation. The mean temperatures fluctuated 24–28°C in day and 16–18°C at night, which are appropriate for developing of studied *Phytophthora* species. The length of resulting necrosis was measured 15 days later. Eight plants from each genotype were inoculated with each *Phytophthora* isolate. Plants inoculated with agar without mycelium and plants of the cherry rootstock Gisela 5 inoculated with each isolate served as controls. Pimaricin 5 mg, ampicillin 250 mg, rifampicin 10 mg, pentachloronitrobenzene (PCNB) 100 mg (P_5ARP) selective medium was used for re-isolation of *Phytophthora*. This experiment was conducted in May 2006 and repeated in September 2006 (by using different trees).

Statistical analysis

A completely randomized experimental design was used throughout the laboratory and field experiments. Correlation analysis was used to compare the different

Fig. 1 Necrosis on excised twigs of cherry genotypes after artificial inoculations

Fig. 2 Necrosis on excised shoots of cherry genotypes after artificial inoculations

inoculation methods. Data were analyzed by 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 Least Significant Difference ($P > 0.05$).

Results

Statistical analysis showed that there was correlation between laboratory methods used. Besides, correlation was also found between excised shoot method and stem inoculation method (Table 1). In contrast, statistical analysis showed no correlation between excised twig assay and stem inoculation method.

Table 1
Correlation ('*r*' value) between excised twig assay, excised shoot method and stem inoculation method

	Excised twig	Excised shoot	Stem inoculation
Excised twig	1	0.734 (*)	0.400 (*)
Excised shoot	–	1	0.482 (*)
Stem inoculation	–	–	1.000

*Correlation is significant at the 0.01 level (two-tailed).
Pearson Correlation significance (two-tailed).

In all experiments, the results showed no significant difference in length of necrosis caused by *Phytophthora,* between cherry genotypes and susceptible control (Tables 1–3). Among the *Phytophthora* species used in this study, the most aggressive were *P. parasitica* and *P. citrophthora*. These species developed significant longer necrosis than *P. cactorum* and *P. citricola*. No significant difference in aggressiveness was found between *P. cactorum* and *P. citricola*. Similarly, *P. parasitica* and *P. citrophthora* showed similar aggressiveness (Tables 2–4).

In excised twig assay, the genotypes A14, D3/99, B1/98 and C6/99 showed the smallest length of necrosis, while the genotypes A2, A11, A° Hybrid, 9° Hybrid and D1/99 showed the greatest length. Interactions among treatments were also found. The genotypes A4/01, A11 inoculated with *P. parasitica* and A7/01, 9° Hybrid, D1/99 inoculated with *P. citrophthora* showed the greatest length of necrosis, while the genotypes A14, D3/99, C6/99, B1/98 inoculated with *P. parasitica*, B1/98, D3/99 inoculated with *P. citrophthora*, A14, D3/99, C6/99, A6/01, A3, B1/98 inoculated with *P. cactorum* and A4/01, A6/01, D4/99, D3/99, A13 inoculated with *P. citricola* showed the smallest length of necrosis (Table 2). In excised shoot

Table 2
Testing the susceptibility of 30 cherry genotypes on *Phytophthora cactorum*, *P. citricola*, *P. cactorum* and *P. citricola* by using the excised twig assay

	Length of necrosis (mm)				
Genotypes	*P. parasitica*	*P. citrophthora*	*P. cactorum*[c]	*P. citricola*	Mean
Gisela 5	16.01	16.39[ab]	14.22	13.53	15.04
A13	18.70	11.44	10.95	9.55	12.66
A12	19.80	12.04	10.11	10.45	13.10
D1/99	20.95	22.34	14.45	11.31	17.26
C4/99	16.91	14.54	13.21	10.05	13.68
9° Hybrid	20.93	22.9	18.72	10.55	18.28
A4/98	10.69	14.86	13.99	10.77	12.58
4° Hybrid	21.93	20.68	19.82	11.17	18.40
A14	8.61	10.79	8.11	10.38	9.47
A5	20.53	14.05	12.43	10.79	14.45
D3/99	9.43	9.63	8.27	9.45	9.20
D4/99	19.02	14.08	14.28	9.58	14.24
C2/99	15.35	14.46	14.01	10.91	13.68
C5/99	18.2	14.94	12.86	10.79	14.20
C7/99	14.54	13.67	12.19	13.58	13.50
A11	22.01	19.04	17.75	12.44	17.81
A9	19.64	16.45	13.79	11.62	15.38
A10	13.79	11.43	10.09	10.82	11.53
1° Hybrid	17.33	14.12	13.06	11.19	13.93
B1/98	8.50	8.53	8.22	10.63	8.97
C6/99	8.29	11.57	6.79	11.44	9.52
A2	20.61	21.64	15.55	12.29	19.27
A6/01	18.74	19.56	9.68	8.66	14.16
A4/01	23.25	20.54	10.05	9.79	15.91
A7/01	15.38	22.15	11.25	11.48	15.07
A3/01	18.26	21.09	14.82	12.74	16.73
A1/01	21.35	15.44	10.05	10.47	14.33
A1	20.38	21.06	13.04	10.09	16.14
A3	16.59	20.89	9.77	11.38	14.66
A2/01	17.22	21.88	10.15	12.22	15.37
Mean	17.10	16.41	12.39	10.96	

[a]Values are the means of two experiments; results were similar to Bartlett's test of homogeneity of variance and the data were therefore combined.
[b]Treatment means were separated by using Least significant difference ($P = 0.05$). $LSD_{0.5}$ for genotypes = 6.35; $LSD_{0.5}$ for *Phytophthora* species = 3.11; $LSD_{0.5}$ for interactions = 8.89.
[c]The isolates of the same species (BPIC-1168 and BPIC-1128 for *P. cactorum*, and BPIC-1262 and BPIC-1267 for *P. citrophthora*) showed statistically similar aggressiveness, so data were combined.

Table 3
Testing the susceptibility of 30 cherry genotypes on *Phytophthora cactorum*, *P. citricola*, *P. cactorum* and *P. citricola* by using the excised shoot method

Genotypes	Length of necrosis (mm)				Mean
	P. parasitica	*P. citrophthora*[c]	*P. cactorum*[c]	*P. citricola*	
Gisela 5	36.33	35.82[ab]	20.07	21.11	28.33
A13	35.70	32.24	16.88	24.12	27.24
A12	27.25	26.67	23.51	19.28	24.18
D1/99	35.36	39.86	17.95	21.03	28.55
C4/99	23.93	26.49	22.34	22.16	23.73
9^o Hybrid	45.71	39.99	27.95	18.67	33.08
A4/98	30.40	25.20	26.42	25.46	26.87
4^o Hybrid	39.32	38.86	25.84	22.36	31.60
A14	21.88	22.23	16.96	24.08	21.29
A5	22.35	29.46	20.67	22.71	23.80
D3/99	22.99	26.62	16.25	22.45	22.08
D4/99	28.87	33.64	15.58	18.77	24.22
C2/99	29.15	27.57	23.16	19.47	24.84
C5/99	29.69	27.86	14.93	18.42	22.73
C7/99	28.47	30.71	15.53	19.56	23.57
A11	26.59	27.35	18.98	23.31	24.06
A9	35.34	33.89	25.19	18.58	28.25
A10	30.77	28.01	23.89	20.63	25.83
1^o Hybrid	33.08	30.81	20.30	25.01	27.30
B1/98	22.49	24.16	16.13	22.16	21.24
C6/99	22.82	22.99	15.66	19.82	20.32
A2	35.47	36.86	17.50	18.99	27.21
A6/01	31.29	34.71	24.54	20.01	27.64
A4/01	30.51	33.02	19.28	23.45	26.57
A7/01	33.71	35.49	18.88	21.06	27.29
A3/01	29.88	30.41	22.45	22.48	26.31
A1/01	33.94	34.74	19.05	19.38	26.78
A1	34.01	35.05	19.78	21.84	27.67
A3	32.88	31.77	18.98	20.91	26.14
A2/01	33.01	30.88	20.14	20.01	26.01
Mean	30.77	31.11	20.16	21.24	

[a]Values are the means of two experiments; results were similar according to Bartlett's test of homogeneity of variance and the data were therefore combined.
[b]Treatment means were separated by using Least significant difference (P = 0.05). $LSD_{0.5}$ for genotypes = 8.64; $LSD_{0.5}$ for *Phytophthora* species = 6.01; $LSD_{0.5}$ for interactions = 9.31.
[c]The isolates of the same species (BPIC-1168 and BPIC-1128 for *P. cactorum*, and BPIC-1262 and BPIC-1267 for *P. citrophthora*) showed statistically similar aggressiveness, so data were combined.

method, the cherry genotypes B1/98, A14 and C6/99 had the smallest length of necrosis, while the genotypes 9^o Hybrid and 4^o Hybrid showed the greatest length. Interactions were also found (Table 3). The genotypes 9^o Hybrid and 4^o Hybrid inoculated with *P. parasitica* and *P. citrophthora* and the genotype D1/99 inoclated with *P. citrophthora* showed the greatest length of necrosis, while the genotypes D4/99, A14, A13, D3/99, B1/98, C5/99, C7/99 and C6/99 inoculated with *P. cactorum* showed the smallest length of necrosis. Finally, in the tree trunk inoculation experiments, only interactions were found (Table 4). The cherry genotypes A13, D1/99 A14 and C7/99 inoculated with *P. citrophthora* showed the greatest length of necrosis, while the genotype A14, A11 inoculated with *P. cactorum* and A14, D3/99, A9, A10, C5/99 inoculated with *P. citricola* showed the smallest length of necrosis (Table 4).

Discussion

Considering the importance of the diseases *Phytophthora* crown rot (D'Ascenzo et al. 2005; Santini et al., 2006; Thomidis, 2001; Thomidis and Sotiropoulos, 2003) for the cultivation of cherry trees, it is essential for growers to know the level of susceptibility of cultivars to these diseases. Resistance is defined as the ability of the host to hinder development of the pathogen. Host resistance is probably the most valuable control measure in agriculture and it is most successful if sanitation measures are also practiced. Resistance is effective during the colonization stage in the resistant cultivar (Purwantara et al., 1998; Widmer et al.,1998).

The main aims of this study were to investigate the susceptibility of 30 cherry genotypes on *P. parasitica, P. citrophthora, P. cactorum* and *P. citricola* by conducting experiments in laboratory (excised twig assay, excised shoot method) and glasshouse (stem inoculation method). These methods can be useful techniques to assess the susceptibility to *Phytophthora* (Thomidis and Sotiropoulos, 2003; Exadaktylou and Thomidis, 2005); they are reliable and quick, allow ample replication, and can be adapted to accommodate almost any type of woody plant host. However, inoculation of stems and excised twigs and shoots can be useful techniques for determining plant susceptibility to wood pathogens if relative and not absolute levels of disease are evaluated. The reason is that resistance of excised phloem tissue to colonization by wood pathogens may be altered by changes in the physiology of the tissue brought about by physical detachment from the grow-

Table 4
Testing the susceptibility of 30 cherry genotypes on *Phytophthora cactorum*, *P. citricola*, *P. cactorum* and *P. citricola* by using the stem inoculation method

Genotypes	Length of necrosis (mm)				Mean
	P. parasitica	*P. citrophthora*[c]	*P. cactorum*[c]	*P. citricola*	
Gisela 5	41.22[a,b]	55.05	24.27	26.50	36.76
A13	32.04	84.61	20.02	24.39	40.26
A12	34.33	43.99	22.96	19.96	30.31
D1/99	40.26	73.35	20.96	22.49	39.26
C4/99	44.83	33.51	24.07	27.40	32.45
9^{o} hybrid	55.00	24.96	22.67	18.48	30.28
A4/98	34.60	62.51	20.65	30.40	37.04
4^{o} hybrid	68.09	18.17	17.34	19.31	30.73
A14	61.75	77.56	15.28	16.18	42.69
A5	52.44	69.27	17.77	18.11	39.40
D3/99	46.01	61.52	18.62	15.06	35.30
D4/99	26.67	43.57	37.13	29.62	34.25
C2/99	26.71	32.48	21.69	26.95	26.96
C5/99	41.49	37.93	18.57	12.11	27.53
C7/99	26.73	79.30	17.60	17.85	35.37
A11	69.40	20.45	15.35	39.65	36.21
A9	65.13	51.77	17.05	15.33	37.32
A10	69.01	37.56	19.05	15.60	35.30
1^{o} hybrid	50.62	48.61	19.29	31.40	37.48
B1/98	35.68	34.21	20.24	24.22	28.59
C6/99	36.52	41.28	19.58	19.62	29.25
A2	49.12	30.61	18.55	24.87	30.79
A6/01	64.22	48.33	27.36	24.03	40.98
A4/01	46.15	61.15	20.73	22.67	37.67
A7/01	57.88	21.79	28.03	25.02	33.18
A3/01	73.58	56.25	24.42	22.11	44.09
A1/01	42.55	38.72	22.11	28.44	32.96
A1	56.34	44.39	25.52	17.13	35.85
A3	42.90	42.05	18.88	25.56	32.35
A2/01	43.31	41.16	19.41	24.38	32.07
Mean	47.82	47.20	21.17	22.83	

[a]Values are the means of two experiments; results were similar according to Bartlett's test of homogeneity of variance and the data were therefore combined.
[b]Treatment means were separated by using Least significant difference (P = 0.05). $LSD_{0.5}$ for genotypes = 21.57; $LSD_{0.5}$ for *Phytophthora* species = 13.89; $LSD_{0.5}$ for interactions = 29.01.
[c]The isolates of the same species (BPIC-1168 and BPIC-1128 for *P. cactorum*, and BPIC-1262 and BPIC-1267 for *P. citrophthora*) showed statistically similar aggressiveness, so data were combined.

ing plant. In addition, direct inoculation of the cambium and inner phloem tissues only evaluates resistance mechanisms operative once the pathogen has entered host tissue, thus bypassing defence mechanisms present in the outer phloem tissue (Agrios, 1988).

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. Similarly, Thomidis and Sotiropoulos (2003) found no host specificity for *P. cactorum*, *P. citrophthora* and *P. citricola*. However, they found that although isolate of *P. nicotianea* originated from pistachio tree was pathogenic to the cherry rootstock CAB-6P, another isolate that originated from the same host was not pathogenic.

Differences among inoculation methods could due to different plant tissues used in each inoculation method and the incubation of inoculated tissues under different environmental conditions. The results that all *Phytophthora* species (*P. cactorum, P. citrophthora, P. citricola, P. parasitica*) used in this study were pathogenic to cherry genotypes tested suggesting that these isolates pose a serious threat to cherry orchards in Greece. These results agree with those reported in previous work. Thomidis (2001) found that *P. citrophthora* and *P. cactorum* were pathogenic on cherry trees. Thomidis and Sotiropoulos (2003) also reported that *P. nicotianea* and *P. citricola* can infect the cherry rootstock CAB-6P. It was also found that the level of susceptibility varied according to *Phytophthora* species. *Phytophthora parasitica* and *P. citrophthora* were the most aggressive. Similarly, Santini et al. (2006) found that *P. citrophthora* was highly aggressive to wild cherry. In contrast, Thomidis and Sotiropoulos (2003) found that *P. cactorum* was more aggressive than *P. citrophthora* and *P. nicotianea* on cherry rootstock CAB-6P. They also found that *P. citrophthora* was more aggressive than *P. nicotianea* and *P. citricola* on CAB-6P. The results from the pathogenicity tests used in this study were not in good agreement for the susceptibility of cherry genotypes to different *Phytophthora* species. Therefore, they will now have to be confirmed by using a more natural inoculation method.

The mechanisms of resistance of different tissues towards *Phytophthora* are poorly understood. Because

of the great differences in the type of tissues affected, it is probable that more than one resistance and/or tolerance mechanism is involved. Little is known about the mechanisms that control the pathogenicity and virulence of different *Phytophthora* isolates on a specific plant. Therefore, caution is needed when a plant or rootstock is characterized as resistant or susceptible because its behaviour may differ from species to species and even from isolate to isolate. The lack of host specificity should be considered in decisions involving the choice of tree species to be planted in fields and orchards suspected of having a *Phytophthora* disease problem.

The results of this study have important implications for the proper management of *Phytophthora* diseases under orchard conditions. Overall, all genotypes were susceptible to *Phytophthora* species tested. So far, no cherry rootstocks/cultivars have been found to show a level of resistance in Greek *Phytophthora* isolates (Thomidis, 2001; Thomidis and Sotiropoulos, 2003; Exadaktylou and Thomidis, 2005). Therefore, all these rootstocks/cultivars are unsuitable for orchards planted in fields where conditions are favourable for infection by *Phytophthora*. Chemical and cultural control measures must be integrated into an overall strategy for managing *Phytophthora* in cherry orchards in Greece. In contrast, Jacobs and Johnson (1996) evaluated the susceptibility of eight *Prunus* taxa on *P. cryptogea* and found that *P. takesimensis* had the highest survival rate of 100% and, along with *P. mahaleb* and *P. yedoensis*, it showed some tolerance to the pathogen, while *P. sargentii* had the lowest survival rate of 81% and appeared to be least tolerant to the pathogen. These results consist of a good encouragement to continue evaluation programmes to identify cherry genotypes with improved resistance to *Phytophthora* which could be included in integrated programme for the management of these diseases.

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