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Influence of temperature and bark injuries on the development of *Phytophthora cactorum* and *P. Citrophthora* on peach trees

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Abstract

The effect of temperature and bark injuries on the occurrence of crown rot of peach trees caused by *P. cactorum* and *P. citrophthora* were examined in field and laboratory. Lesions developed at 35 °C (the complete range of temperatures tested) but maximum development occurred at 20–25 °C. Greatest growth of these fungi on cornmeal agar (CMA) also occurred between 15 and 30 °C. Both pathogens could infect injured trees up to 20 days after wounding, but could not infect uninjured plants or plants wounded 40 and 30 days before inoculation, respectively. This study showed that temperature is a critical factor for the development of *Phytophthora* crown rot of peach trees. In addition, crown rot developed from recent wounds inoculated with agar plugs of *Phytophthora*.

Keywords: Bark injury; *Phytophthora*; Crown rot; Peach trees; Temperatures

1. Introduction

One of the most serious diseases of peach tree is crown rot caused by *Phytophthora* spp. In Greece, this disease is caused by *Phytophthora cactorum*, *P. citrophthora*, *P. megasperma*, and *P. syringae* (Chitzanidis and Stylianides, 1987; Kouyeas, 1971, 1977; Sarejanni, 1935; Stylianides et al., 1985). Other species, such as *P. innamomi*, *P. cryptogea*, *P. cambivora* (Flores and Hindal, 1983; Kim et al., 1985; Wilcox and Ellis, 1989), have also been found to be associated with crown rot in other parts of the world. In Greece, two types of *Phytophthora* crown rot can be distinguished on stone fruit trees. The first occurs during the hot summer period and is usually caused by *P. cactorum* and also *P. citrophthora*

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(Kouyeas, 1971) leading more serious damage in Greece. Its symptoms are sudden death of the trees sometimes but not always, preceded by mild leaf chlorosis (Kouyeas, 1971, 1977). The second type of *Phytophthora* crown rot occurs in late winter or early spring with more gradual wilting because water stress on the trees is less in this period. *P. syringae* has been isolated from this form of the disease (Kouyeas, 1977).

Temperature is one of the most important factors influence growth and sporulation of *Phytophthora* (Duncan, 1985; Grove et al., 1985; Jacobi et al., 1983; Kenerley and Bruck, 1983; MacDonald, 1991; Matheron and Mateika, 1992; Phillips and Weste, 1985; Wong et al., 1986). Soil temperatures at certain times of the year in orchards can be inhibitory to sporulation and colonization of citrus roots by *P. citrophthora* and *P. parasitica* (Matheron and Porchas, 1996). Extreme temperature may be useful indicators of periods of pathogen inactivity or arrested disease development and thus periods when fungicides are not essential for disease control. According to Tooley and Grau (1982), the expression of soybean resistance to *P. glycinea* is affected by temperature.

Most fungi can enter plants through various types of wounds. Wounds in the bark, if not fatal, are repaired with fresh bark tissue, which may provide a repaired barrier to infection by the pathogens. Development of *Phytophthora* was also associated with bark injuries created by mechanical implements or other causes and wounded trees are more susceptible to crown rot caused by *Phytophthora* spp. (Erwin and Ribeiro, 1996).

In this study, the influence of temperature and the importance of wounds as entry sites for natural infection on the pathogenicity of *P. cactorum* and *P. citrophthora* on peach trees were investigated.

2. Material and methods

2.1. Inoculum

One isolate of *P. cactorum* and one of *P. citrophthora* obtained from Benaki Phytopathological Collection were used. The isolate of *P. cactorum* was recovered from an almond tree showing the typical crown rot symptom and the *P. citrophthora* isolate from a citrus trees. In previous studies, both were highly pathogenic on peach trees (Thomidis, 2000). Both isolates were maintained on cornmeal agar (CMA) under paraffin oil at 21 °C. Fresh inoculum was prepared by placing an agar disk with mycelium of fungus on CMA.

2.2. Rootstocks

GF677 is a rootstock that was produced by Bernhard in France at the Grand Ferrad Research Station. It is an interspecific hybrid between beach and almond and is important to the peach industry in the Mediterranean basin. GF677 is clonally propagated and is particularly useful to control replant diseases. It is well suited to the cultural practices used in peach orchards in Greece and therefore, has become the primary rootstock used for most peach cultivars.

KID I is a rootstock that was developed in 1986 at the Pomology Institute in Naoussa, Greece, as an interspecific hybrid between peach and almond cultivars, Rutgers Red Lead

and Marcona, respectively. Because KID I is a relatively new peach rootstock, not much is known about its cultural characteristics.

2.3. Experiment 1

A 6 mm agar plug with mycelium of *P. cactorum* or *P. citrophthora* isolate was placed in the center of six replicate petri plates containing CMA. Mycelial growth (diameter) was measured after 7 days incubation period at 0, 5, 10, 15, 20, 25, 30, 35 °C.

2.4. Experiment 2

This method was based on that described by Jeffers et al. (1981). For preparing inoculum, CMA amended with antibiotics (pimaricin 10 mg/l, ampicillin 250 mg/l, rifampicin 10 mg/l) was added to sterilized Pyrex jars, about 12 cm in height and 9 cm in diameter, to give an agar depth about 10 mm. Agar plugs with mycelium were used to inoculate the agar surface. Jars were sealed with parafilm and placed in incubators at 23 °C for each *Phytophthora* species until mycelium growth covered the agar surface. There were four jars for each temperature, two for each *Phytophthora* species.

Dormant shoots were collected from 4-year-old GF677 mother trees in November and again in December 1999. Segments, about 7 cm in length and 10 mm in diameter were cut from the center of the shoots. Segments were then disinfested in 10% sodium hypochlorite (4.8%) solution for 3 min, thoroughly rinsed in distilled water and blotted dry. Using a sharp flamed knife, segments were trimmed to a slant at the base. Ten segments were inserted into CMA + A at the colony periphery. The lids of storage jars were re-sealed with parafilm and jars were incubated for 6 days at 0, 5, 10, 15, 20, 25, 30, 35 °C in incubators in the dark after which the lengths of resulting lesions were recorded.

2.5. Experiment 3

GF677 plants were bought from a tissue culture station (Bitro Hellas) and transplanted in methyl bromide fumigated potting soil. Plants were grown using standard cultural practices for 12 months prior to inoculation and were approximately 50 cm in height at the time of inoculation. They did not show any symptoms of disease or signs of injury at this time. Inoculations were made in May and again in September 1999 using different plants on each date. Plants were inoculated with *P. cactorum* and *P. citrophthora* as follows: using a flamed knife, bark and phloem tissues from the trunk were removed completely to expose cambium, leaving a wound about 6 mm in diameter and 5 cm above the soil surface. Agar plugs (4 mm diameter) from the edge of actively growing CMA cultures were placed directly onto the wounds, a wet cotton wool pad was placed on top and wrapped with adhesive tape to avoid desiccation. After inoculation, trees were maintained for 21 days at 0, 5, 10, 15, 20, 25, 30, 35 °C in illuminated growth chamber with a 12 h photoperiod. Disease severity was determined by measuring the length of necrosis. There were 30 plants for each temperature, 15 for each *Phytophthora* species. Pathogens were re-isolated onto the selective medium developed by Jeffers and Martin (1986).

2.6. Experiment 4

This experiment used GF677 and KID I rootstocks planted in an experimental field of the Pomology Institute, Naoussa. Forty, 30, 20, 10 and 1 day before inoculation, 2-year-old dormant shoots (about 60 cm in length and 2 cm in diameter) were wounded in the center by removing a 6 mm strip of periderm of bark to expose the cambium, using a flamed knife. Segments, 10 cm in length, were cut from the central part of each shoot. Inoculation was as described above. This experiment was conducted in December 1998 and again in February 1999.

Inoculated segments were placed in an incubator at 23 °C for 5 days, after which the remaining periderm was removed and the length of the lesion from the part of inoculation was recorded. There were 200 segments for each rootstock. One hundred of them were used for each *Phytophthora* isolate, 20 for each wounding date. Twenty non-wounded segments for each *Phytophthora* isolate were used as control.

2.7. Experiment 5

This experiment was conducted in the experimental field of the Pomology Institute, Naoussa. GF677 and KID I plants were bought from a commercial tissue culture station (Bitro Hellas) and were planted in a high density plantation (1 m × 1 m).

Forty, 30, 20, 10 and 1 day before inoculation, 20 plants were wounded about 10 cm above soil using a flamed knife to remove 6 mm phloem tissue from the trunk to expose the cambium. Inoculation was a previously described with *P. cactorum* or *P. citrophthora*. Wet cotton was placed on the wound which were wrapped with adhesive tape to avoid desiccation. Twenty days later, periderm was removed by scrapping and the length of necrosis was recorded. This experiment was conducted in May 1999 and repeated in September. There were 100 plants for each rootstock. Fifty for each *Phytophthora* species, 10 for each treatment. Non-wounded plants were used as control.

2.8. Statistical analysis

The experimental design used throughout the experiments was completely randomized. Data were analyzed by one-way analyses of variance and treatment means were separated by the Duncan's multiple range test ($P = 0.05$). All experiments were conducted twice, results were similar so data were combined.

3. Results

3.1. Experiment 1

The highest rates of mycelial growth (diameter) for *P. cactorum* occurred between 20 and 25 °C. No growth was observed outside the temperature range 15–30 °C. Maximum mycelial growth (diameter) of *P. citrophthora* occurred between 20 and 25 °C with no growth outside the range 10–30 °C (Table 1).

Table 1
Radial growth (mm per day) of *P. cactorum* and *P. citrophthora* on CMA after 7-day period of incubation at various temperatures[a]

Temperatures (°C)	Radial growth (mm per day)[b]	
	P. cactorum	*P. citrophthora*
0	0 d	0 c
5	0 d	0 c
10	0 d	0 c
15	2.5 b	2.1 b
20	3.8 a	3.8 a
25	3.6 a	3.7 a
30	1.7 c	3.5 a
35	0 d	0 c

[a] Values followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] Each value represents the mean of two experiments, each with six replicates.

3.2. Experiment 2

Maximum development of lesions on excised segments inoculated with *P. cactorum* and *P. citrophthora* was recorded between 20 and 25 °C (Table 2). The total length lesion was recorded between 15 and 30 °C on excised segments inoculated with *P. cactorum* and *P. citrophthora* (Table 2). Excised segments inoculated with *P. cactorum* or *P. citrophthora* and incubated at 20 and 25 °C showed symptoms of gummosis around necrosis.

Table 2
Pathogenicity of *P. cactorum* and *P. citrophthora* on excised stem and trunks of GF677 trees at various temperatures[a]

Temperatures (°C)	Radial growth (mm per day)[b]			
	Excised stem segments		Trunk inoculation	
	P. cactorum	*P. citrophthora*	*P. cactorum*	*P. citrophthora*
0	0 d	0 d	0.13 d	0.15 e
5	0 d	0 d	0.26 d	0.19 de
10	0 d	0 d	0.30 d	0.27 cd
15	1.10 c	1.90 a	0.92 c	0.34 bc
20	2.75 a	1.68 b	1.87 a	0.77 a
25	2.91 a	1.67 b	1.19 b	0.40 b
30	1.68 b	1.41 c	0.77 c	0.40 b
35	0 d	0 d	0 e	0 f

[a] Values followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] Each value represents the mean of two experiments, each with 20 replicates.

3.3. Experiment 3

Peach plants inoculated with *P. cactorum* and *P. citrophthora* showed the typical crown rot symptoms at 0–35 °C. Maximum development of lesions on stems of peach plants inoculated with *P. cactorum* and *P. citrophthora* occurred at 20 °C (Table 2). *P. cactorum* and *P. citrophthora* could be re-isolated only from plants incubated at 20 and 25 °C.

3.4. Experiment 4

The ability of both pathogens to infect the segments depends on wounding date. Segments inoculated with *P. cactorum* or *P. citrophthora* 40 and 30 days after wounding were not infected. However, both pathogens infected segments up to 20 days after wounding. No significant difference was observed in length of necrosis on segments inoculated with either pathogen 20, 10 or 1 day after wounding (Table 3). No necrosis was observed on control segments.

3.5. Experiment 5

The ability of *P. cactorum* and *P. citrophthora* to infect peach trees with bark-injuries was evaluated (Table 4). Both *Phytophthora* species could not infect trees that had been wounded 40 and 30 days before inoculation. However, crown rot symptoms were observed on peach trees inoculated with *P. cactorum* or *P. citrophthora* 20, 10 and 1 day after wounding. Canker development on plants inoculated with either pathogen 20, 10 and 1 day after wounding was similar (Table 4). *P. cactorum* and *P. citrophthora* could be re-isolated only from GF677 and KID I rootstocks inoculated 1, 10 and 20 days after wounding. Control plants did not show any crown rot symptom.

Table 3
Necrosis development at four wounding dates in peach rootstock segments (GF677, KID I) infected with *P. cactorum* and *P. citrophthora*[a]

Days after wounding	Radial growth (mm per day)[b]			
	P. cactorum		*P. citrophthora*	
	GF677	KID I	GF677	KID I
Unwounded	0.00 b	0.00 b	0.00 b	0.00 b
1	1.60 a	2.55 a	1.46 a	2.00 a
10	1.75 a	2.65 a	1.42 a	1.93 a
20	1.62 a	2.41 a	1.56 a	1.83 a
30	0.00 b	0.00 b	0.00 b	0.00 b
40	0.00 b	0.00 b	0.00 b	0.00 b

[a] Values followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

[b] Each value represents the mean of two experiments, each with 20 replicates.

Table 4
Necrosis development at four wounding dates in peach rootstocks (GF677, KID I) infected with *P. cactorum* and *P. citrophthora*[a]

Days after wounding	Radial growth (mm per day)[b]			
	P. cactorum		*P. citrophthora*	
	GF677	KID I	GF677	KID I
Unwounded	0.00 b	0.00 b	0.00 b	0.00 b
1	1.60 a	2.01 a	1.58 a	1.63 a
10	1.63 a	2.06 a	1.48 a	1.62 a
20	1.68 a	2.10 a	1.57 a	1.63 a
30	0.00 b	0.00 b	0.00 b	0.00 b
40	0.00 b	0.00 b	0.00 b	0.00 b

[a] Values followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] Each value represents the mean of two experiments, each with 10 replicates.

4. Discussion

Temperature has a large influence on growth and sporulation of *Phytophthora* (Doster and Bostock, 1988; Horner and Wilcox, 1996; MacDonald and Duniway, 1978a,b). Matheron and Mateika (1992) reported that temperatures affected lesion development on stems of rough lemon inoculated with *P. citrophthora* or *P. parasitica*. According to Grant and Byrt (1984) even in a susceptible species of plants, the degree to which root tissue is colonized is temperature dependent.

Season variation in the susceptibility of plants to invasion by *Phytophthora* was strongly dependent on temperature (Matheron and Mateika, 1993). Apple seedlings infected with *P. cactorum* died more rapidly at 25 °C than 18 °C (Harris and Tobutt, 1986). In this work, it was found that the fastest growth of *P. cactorum* and *P. citrophthora* took place in the range 15–30 °C (Table 2). Because the fungus was not recovered from lesions exposed to the extremes of this temperature range, there is no way of knowing for certain that small lesions developing at 0 or 35 °C were a result of infection with *Phytophthora*. The cardinal temperatures for mycelial growth of *P. cactorum* and *P. citrophthora* in culture vary as follows: minimum 3–6 °C, optimum 25–27 °C and maximum 30–34 °C (Braun and Krober, 1958; Ershad, 1971; Erwin and Ribeiro, 1996; Sewell and Wilson, 1964). As temperature range for growth of pathogens on artificial media as well as lesion development on infected excised shoot segments does not correspond with temperature range for necrosis development on intact trees, laboratory data cannot be extended directly to the field.

Bark injuries also influence the susceptibility of plants and the virulence of pathogens. Bostock and Doster (1985) found that canker on almond trees with profuse gumming associated with pruning wounds was caused by *Phytophthora syringae*. In this study, it was found that both *P. cactorum* and *P. citrophthora* can infect injured peach trees up to 20 days after wounding.

It is very important to know the optimum temperatures for growth and sporulation of a pathogen because it could be used to time fungicide application or other disease

control measures so that they coincide with periods when the threat of sporulation by the pathogen and subsequent disease development is high (Matheron and Mateika, 1992). Infection of peach trees with *P. cactorum* and *P. citrophthora* in orchard possibly occurs when the soil temperatures fluctuate within 15–30 °C. It is possible, therefore, that the occurring of two types of *Phytophthora* crown rot on stone fruits mentioned in the introduction depend on environmental temperature. Also, injured plants need extra protection by applying chemicals or other cultural measures for, at least, 20–30 days.

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