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Seasonal variation in crown rot of GF677 and KID I peach rootstocks by *Phytophthora cactorum*, *P. citrophthora* and *P. syringae*

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Summary. Seasonal variation in extent of colonization of GF677 and KID I peach rootstocks by *P. cactorum*, *P. citrophthora*, and *P. syringae* was examined on excised twigs *in vitro*, and by stem inoculations in the experimental field of the Pomology Institute, Naoussa, Greece. Shoot segments of the previous growing season were cut and inoculated in the laboratory in the last ten days of August, 1998, and at monthly intervals thereafter until July 2000. At the same time, rootstock stems were also inoculated directly with mycelium of the pathogens every month. Disease severity was assessed 14 days after inoculation. Both *P. cactorum* and *P. citrophthora* showed two peaks in the extent of colonization, one in April-June, and one in September and October. In contrast, no colonization of plants occurred in July-August or in January-February. *P. syringae* also had two peaks in its extent of colonization, one in November-December and one in March. This fungus was inactive during May-October and January. The maximum and minimum extent of pathogen colonization on plants coincided with maximum and minimum growing temperatures of the fungi. The identification of seasonal variations in the susceptibility of peach trees to *Phytophthora* may facilitate the timing of disease control measures which should coincide with periods when fungal growth is most rapid.

Key words: crown rot, peach rootstock, *Phytophthora*, seasonal variation, temperature.

Introduction

Species of *Phytophthora* recovered from peach trees affected with crown and root rot include *P. cactorum, P. megasperma*, *P. citrophthora, P. syringae, P. cambivora, P. cryptogea* and *P. cinnamomi* (Sarejanni, 1935; Kouyeas, 1971; Kouyeas, 1977; Flores and Hidal, 1983; Kim *et al.,* 1985; Stylianides *et al.*, 1985; Chitzanidis and Stylianides, 1987; Wilcox and Ellis, 1989).

In Greece, there are two types of *Phytophthora* apoplexy of peach trees (Sarejianni, 1935; Kouyeas, 1971; Kouyeas, 1977; Stylianides *et al.,* 1985; Chitzanidis and Stylianides, 1987). One occurs during the hot summer period and is usually caused by *P. cactorum* or *P. citrophthora*, the second type occurs in winter or early spring and is caused by *P. syringae*. Previous studies have demonstrated the existence of seasonal variations in the susceptibility of fruit trees such as apple, citrus and walnut to infection by *Phytophthora* spp. (Mathreron and Mircetich, 1985; Matheron and Matejka, 1989; Browne and Mircetich, 1996). These seasonal variations are thought to be related to repeated episodes of reduced oxygen availability that occur especially during abundant soil flooding (Mircetich and Matheron, 1976; Bernhardt and Grogan, 1982; Wilcox and Mircetich, 1985a; Wilcox and Mircet-

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ich, 1985b; Harris and Tubott, 1986; Wood and Duniway, 1986; Jacobs and Johnson, 1996). The temperature also influences growth and sporulation of *Phytophthora* (Doster and Bostock, 1988; Mizubuti and Fry, 1998).

This study reports the results of a research program carried out at the Pomology Institute, Naoussa, Greece, on seasonal variations in the extent of colonization of GF677 and KID I peach rootstocks by *P. cactorum*. *P. citrophthora* and *P. syringae*.

Materials and methods

Phytophthora isolates

One isolate each of *P. cactorum, P. citrophthora* and *P. syringae* was used in this work. The isolate of *P. cactorum* was recovered from an almond tree showing typical crown rot symptoms in 1997, the *P. citrophthora* and *P. syringae* isolates from citrus trees showing gummosis also in 1997. All isolates had been shown to be pathogenic to peach trees in previous works. Isolates were maintained on cornmeal agar (CMA) at 22°C in the Benaki Phytopathological Collection, Athens, Greece. Fresh cultures were prepared by transferring agar plugs to Petri plates containing fresh CMA.

Excised twig inoculation

Woody segments, 6 cm long and 10 mm in diam, of GF677 and KID I peach rootstock shoots were collected at monthly intervals from August 1998 through July 2000. Shoots were collected from the latest growing season's terminal shoots. Shoots harvested between April and November were carefully stripped of their leaves before inoculation. Shoot collection and inoculation was conducted in the last 10 days of each month.

All inoculations were performed using the excised twig assay of Jeffers *et al*., (1981). CMA (70 ml) amended with pimaricin (10 ppm) was placed in pyrex jars. Agar plugs bearing fungal mycelium were transferred to the jars, and incubated at 23°C for *P. cactorum* and *P. citrophthora*, and at 16°C for *P. syringae* until the fungal mycelium nearly covered the agar surface.

Shoot segments were disinfected in 10% domestic chloride (4.8%) for 3 min and washed three times in distilled water. The basal end of each twig segment was then pared by making tangential cuts 10 mm long and 1–2 mm deep on opposite sides, exposing the cambium region. Ten of these pared twig segments were inserted vertically, distal end up, into the agar medium of each jar at the periphery of the fungal colony. The jars were sealed with parafilm and incubated at ambient conditions in the dark for four days. The twig segments were then removed from the jars and stripped of their periderms with a scalpel. The length of necrosis on each twig and the depth of agar in each jar were measured. Four jars were used for each *Phytophthora* species, two jars for each peach rootstock.

Stem inoculation

In the winter of 1996, GF677 and KID I peach plants were planted in a high-density plantation. The plants were obtained from a tissue culture station (Vitro Hellas, Alexandria, Imathia). In the last ten days of August in 1998, and every month thereafter until July 2000, twenty plants were inoculated on the trunk (10 cm above the soil surface) by removing a 6 mm strip of bark, exposing the cambium, and transferring to each wound so made an agar plug bearing fungal mycelium. The wound was then covered with petroleum jelly and sealed with adhesive tape. There were twenty plants for each *Phytophthora* species, ten for each peach rootstock.

Results were recorded 14 days after each inoculation by scraping the bark and measuring the detected canker. A selective medium developed by Jeffers and Martin (1986) was used for recovering the fungi (corn meal agar amended with pimaricin, 10 mg; ampicillin, 250 mg; rifampicin, 10 mg).

To detect a possible correlation between air temperature and lesion development, the temperature was recorded by a meteorological station located at the Pomology Institute, Naoussa.

Data analysis

The results were subjected to analysis of variance. Duncan's multiple range test was used to compare the average length of cankers obtained with each *Phytophtora* species.

Results

Excised twig inoculation

Lesions caused by the fungi inoculated on shoot segments of peach rootstocks GF677 and KID I

always showed the distinctive orange-brown discoloration of the phloem tissues and gummosis around the necrosis. Necrosis appeared as a light discoloration of the outer bark, and lesion lengths were easily measured without removing the periderm. *P. cactorum* and *P. citrophthora* showed two peaks in extent of colonization achieved on both rootstocks, one in April-June and one in September-October (Table 1, Fig. 1A and B). Segments inoculated with *P. cactorum* or *P. citrophthora* during tree dormancy (December-March) and in July-August showed practically no necrosis. Colonization of segments by those same two pathogens was almost inhibited in November.

When shoot segments were inoculated with *P. syringae* (Table 1, Fig. 1A and B), on the other hand, lesions were detected in November and December, and in February, March and April. With this fungus, the greatest amounts of fungal growth were in November and December on GF677, and in March and December on KID I. There was no colonization with either rootstock in January or in the months from May to October (Table 1, Fig. 1A and B).

Stem inoculation

Results from field experiments generally agreed with those in the laboratory. Artificially infected plants showed typical crown rot symptoms. Necro-

Table 1. Development of twig cankers on shoot segments of peach rootstocks GF677 (A) and KID I (B) inoculated with *Phytophthora cactorum*, *P. citrophthora* and *P. syringae*.

Rootstock	Month	Length of twig canker (cm)[a]		
		P. cactorum	*P. citrophthora*	*P. syringae*
GF677	January	0.0 e[b]	0.0 e	0.0 e
	February	0.0 e	0.0 e	0.8 cd
	March	0.0 e	0.0 e	1.3 a
	April	1.9 d	1.8 b	0.7 d
	May	2.2 c	1.8 b	0.0 e
	June	2.6 b	2.2 a	0.0 e
	July	0.0 e	0.0 e	0.0 e
	August	0.0 e	0.0 e	0.0 e
	September	3.2 a	2.3 a	0.0 e
	October	2.2 c	1.7 c	0.9 c
	November	0.1 e	0.4 d	1.1 b
	December	0.0 e	0.0 e	1.1 b
KID I	January	0.0 f	0.0 e	0.0 e
	February	0.0 f	0.0 e	1.1 c
	March	0.0 f	0.0 e	1.6 a
	April	2.5 d	2.0 c	0.8 d
	May	3.1 c	2.1 c	0.0 e
	June	3.9 b	3.0 a	0.0 e
	July	0.0 f	0.0 e	0.0 e
	August	0.0 f	0.0 e	0.0 e
	September	4.1 a	2.8 b	0.0 e
	October	3.0 c	2.2 c	1.1 c
	November	0.3 f	0.4 d	1.1 c
	December	0.0 f	0.3 d	1.5 b

[a] Average of 20 replicates per month representing data from two consecutive years.
[b] Within each a column, numbers followed by same letters are not significantly different ($P>0.05$) according to Duncan's multiple range test.

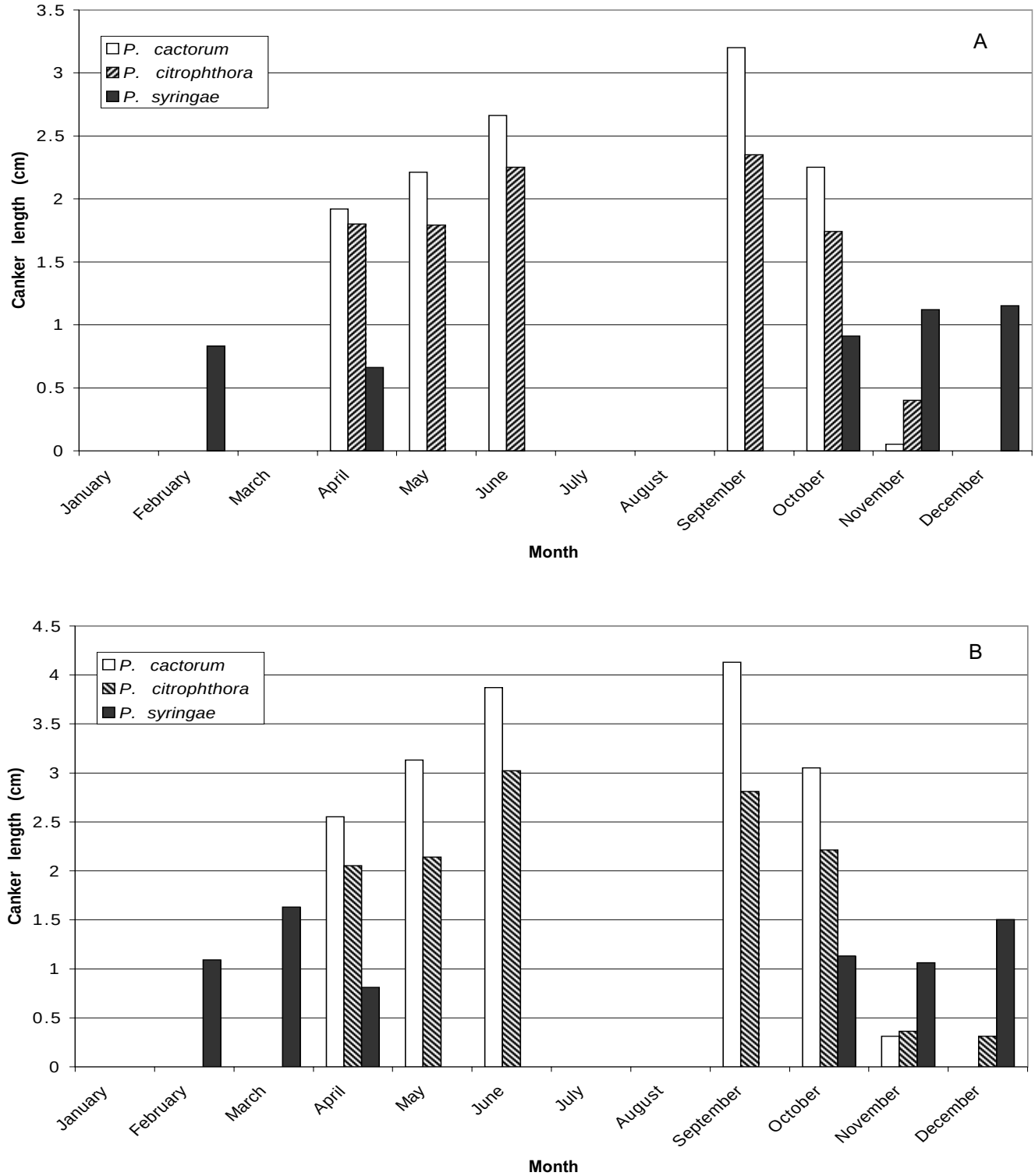


Fig. 1 Graphical representation of the data for shoot segments of GF677 (A) and KID I (B) peach rootstocks shown in Table 1.

Table 2. Development of stem cankers on plants of peach rootstocks GF677 and KID I with *P. cactorum*, *P. citrophthora* and *P. syringae*.

Rootstock	Month	Length of stem canker (cm)[a]		
		P. cactorum	*P. citrophthora*	*P. syringae*
GF677	January	0.0 g[b]	0.0 e	0.0 e
	February	0.0 g	0.0 e	2.2 c
	March	3.1 e	2.5 d	5.3 a
	April	6.5 c	3.7 cd	1.7 d
	May	8.6 b	6.3 b	0.0 e
	June	9.1 b	4.4 bc	0.0 e
	July	0.0 g	0.0 e	0.0 e
	August	0.0 g	0.0 e	0.0 e
	September	15.1 a	11.4 a	0.0 e
	October	7.0 c	5.3 bc	0.0 e
	November	4.2 d	2.6 d	3.5 b
	December	2.8 f	2.3 d	3.5 b
KID I	January	0.0 f	0.0 g	0.0 e
	February	0.0 f	0.0 g	3.6 c
	March	2.0 e	3.5 e	6.4 a
	April	15.0 c	6.0 d	2.1 d
	May	15.8 bc	7.0 d	0.0 e
	June	16.4 b	15.2 b	0.0 e
	July	0.0 f	0.0 g	0.0 e
	August	0.0 f	0.0 g	0.0 e
	September	20.6 a	16.6 a	0.0 e
	October	14.6 c	9.6 c	0.0 e
	November	5.3 d	4.2 e	5.4 b
	December	1.9 e	1.9 f	5.6 b

[a] Average of ten replicates per month representing data from two consecutive years.
[b] Within each a column, numbers followed by same letters are not significantly different (*P>0.05)* according to Duncan's multiple range test.

sis showed the distinctive orange-brown coloration, and gummosis was also observed around the necrotic area.

Over a 2-year-period, colonization of GF677 plants by *P. cactorum* or *P. citrophthora* was greatest in September-October and in April-June (Table 2, Fig. 2A). The most extensive necrosis appeared in September. In contrast, pathogens did not grow in January-February or in July-August. Colonization also occurred in November, December and March, but was less than in the months of peak colonization.

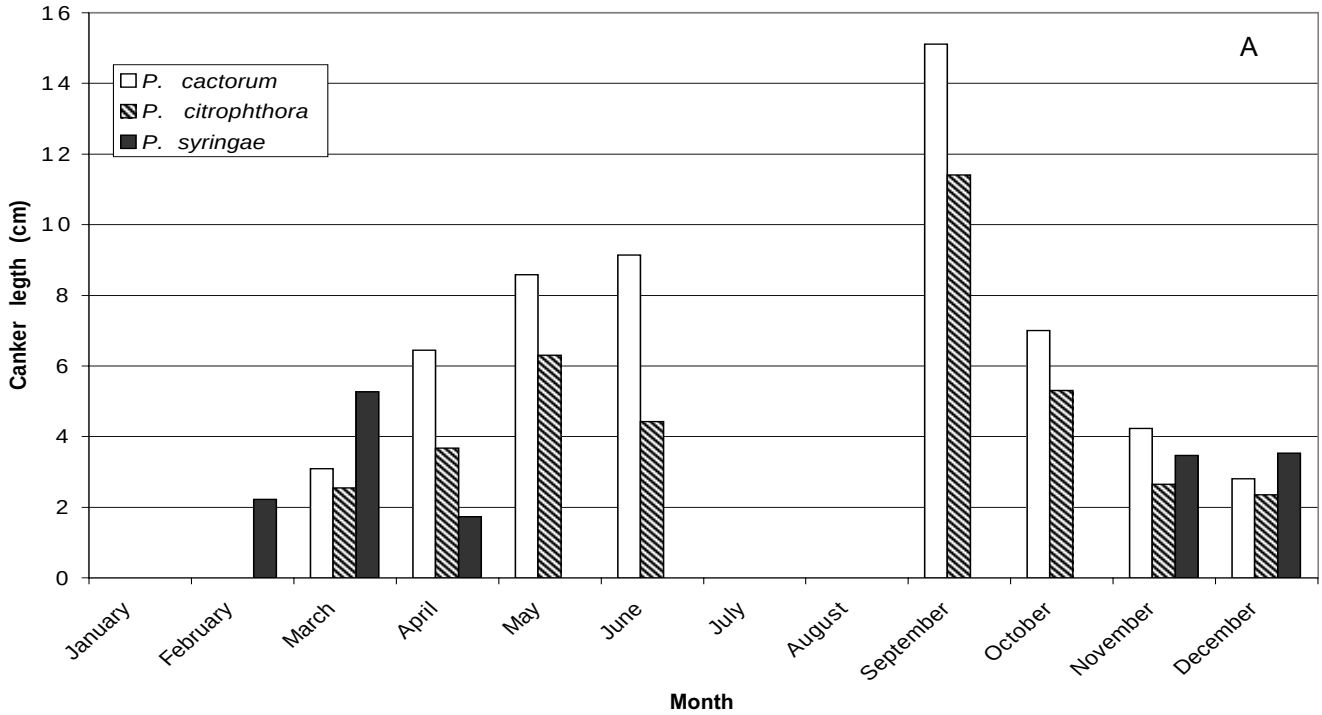
In the GF677 plants inoculated with *P. syringae* (Table 2, Fig. 2A and B) maximum lesion development occurred in March and in November-December. The most extensive fungal growth occurred in March. Fungal growth was significantly reduced in February and April compared with periods of maximum colonization. This fungus was not active from May to October or in January.

Inoculation on shoot segments of KID I produced results similar to those on GF677 (Table 2, Fig. 2B)

Fungi were isolated from necrotic crown tissues in all months except those when the pathogens were not active.

Correlation of temperature and lesion development

There was a direct relationship between tem-



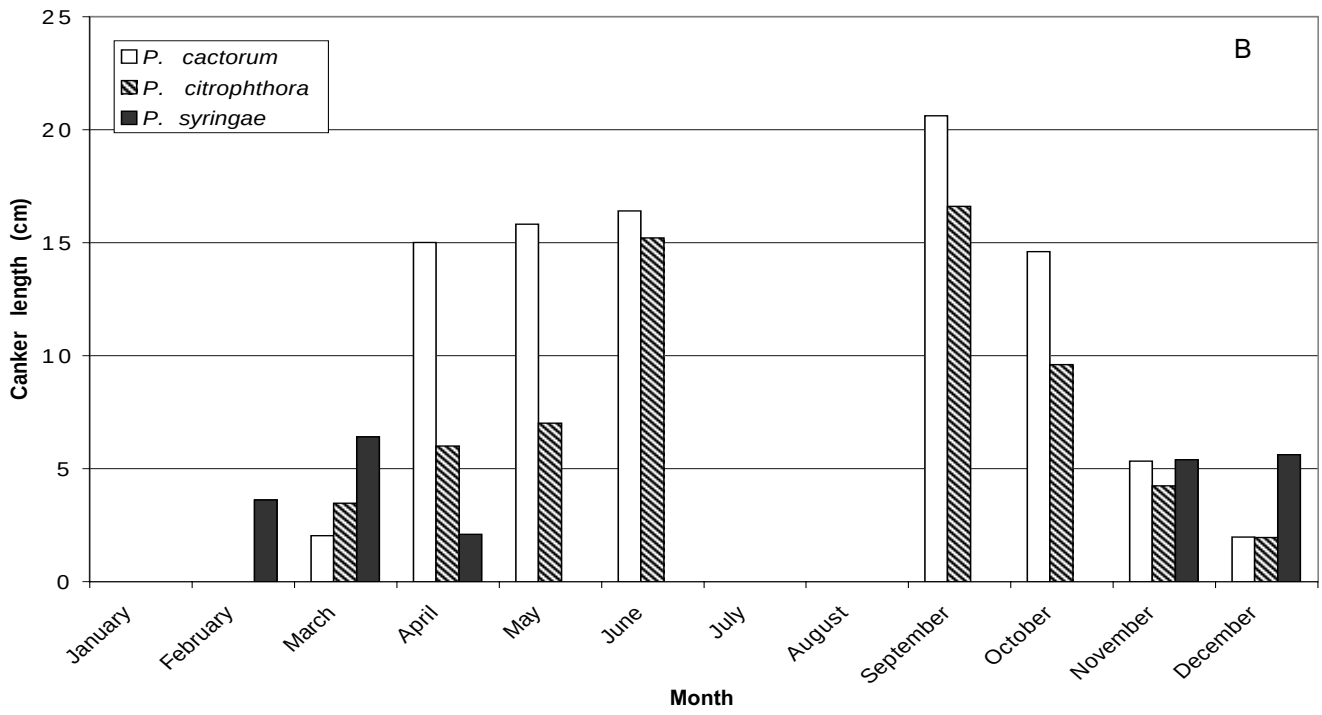


Fig. 2 Graphical representation of the data for planta of GF677 (A) and KID I (B) peach rootstocks shown in Table 2.

Table 3. Mean air temperatures for the years 1998-1999 and 1999-2000.

Month	Mean air temperatures (°C)	
	1998-1999	1999-2000
January	1.4	2
February	6.8	5
March	9.7	9.2
April	17	16
May	21.5	21.1
June	24	23.8
July	26.8	26.3
August	26.9	26.8
September	21.8	20.3
October	17.7	18.1
November	10.1	9.8
December	7.1	7.6

perature and canker development (Table 3). The minimum growing temperature for *P. cactorum* and *P. citrophthora* is around 5 °C, the optimum 20–25 °C and the maximum 30 °C. *P. syringae* does not grow above 26 °C or below 5 °C. The optimum temperature for growth of this pathogen is 15–20 °C (Erwin and Ribeiro, 1996). The minimum growth of *P. cactorum* and *P. citrophthora* coincided with air temperatures least suitable for their growth, and maximum growth colonization occurred when temperatures were optimal for fungal growth.

Discussion

The study detected seasonal variation in the colonization rate of peach trees by *P. cactorum*, *P. citrophthora* and *P. syringae*. Similar seasonal variations of *Phytophthora* species have also been reported on other hosts. Matheron and Matejka (1989) found seasonal variations in the colonization rate of citrus rootstock and scion tissues by *P. citrophthora* and *P. parasitica*. Similarly, Matheron and Mircetich (1985), reported seasonal variation in the susceptibility of *Juglans hindsii* and *Paradoy* rootstocks of English walnut to *Phytophthora citricola*. Browne and Mircetich (1996) investigated the effect of the month of inoculation on the severity of disease caused by *P. cactorum* and *P. cambivora* on apple trees using the excised shoot method, and Jeffers and Aldwinckle (1986), examined seasonal variation in colonization of two apple rootstocks by five *Phytophthora* species and found that these species fell into two groups depending on the time of their peak colonization on the two rootstocks.

As stated above, there are two types of apoplexy in peach, one occurring in summer and caused by *P. cactorum* and *P. citrophthora,* and one in winter, caused by *P. syringae*. Seasonal variation in the susceptibility of peach trees may be the cause of this. In April-June and September-October, when *P. cactorum* and *P. citrophthora* are most active, trees show the first type of apoplexy, and in November, December and March, when colonization by *P. syringae* is greatest, trees show symptoms of the second type.

A relationship was also noted between temperature and canker development. Here too, maximum invasion coincided with the optimum growing temperature for both pathogens.

The seasonal variation in susceptibility of peach trees to *Phytophthora* spp. has important implications for the proper timing of measures to control *Phytophthora* spp. Matheron and Matejka (1993), stated that seasonal variations in the susceptibility of citrus rootstocks to *Phytophthora* made it possible to time disease control measures so as to coincide with those periods when disease development was most intense. More recently, Matheron and Porchas (1996), reported that the more efficient use of fungicides to control *Phytophthora* root rot of citrus was possible by limiting fungicide application to times when soil temperature favored disease development. The results of this study show that with peach too it is possible to time protection measures such as fungicides so as to exploit seasonal variations in the capacity of *Phytophthora* species to colonize host tissue.

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