

SHORT COMMUNICATIONS

The August Cieszkowski Agricultural University, Poznań, Poland

LABORATORY STUDY ON PATHOGENICITY OF STRAWBERRY ISOLATES OF *PHYTOPHTHORA CACTORUM* TO SOME ORNAMENTAL AND FRUIT-BEARING PLANTS

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Introduction

A soil borne pathogen hosted by a wide range of plants, *Phytophthora cactorum*, is known to cause two strawberry diseases: crown rot and leather rot of fruit (Paulus 1990, "Compendium..." 1992, Duncan 2002). Crown rot of strawberry caused by *P. cactorum* was detected for the first time in Poland on 'Elsanta' cultivar in 1994 (Bielenin 2002). Symptoms of leather rot on strawberry fruits were found two years later (Gołębniak and Wachowiak 1999, Bielenin 2002). Some authors (van der Scheer 1971, Seemüller and Schmidle 1979, Harris and Stickels 1981, Bielenin 2002) believe that crown rot is caused by a specific pathotype that only affects strawberry. The crown rot pathotype was first detected in Germany (Deutschmann 1954). Others (Hantula et al. 1997, 2000) claim the two diseases are not caused by different *P. cactorum* isolates.

This paper reports a research conducted in order to clear up this discrepancy of opinion, and to fill in the shortage of data due to the relatively recent occurrence of the two diseases in Poland. The aim of the study was to test the ability of *P. cactorum* isolates obtained from strawberry to affect other species of fruit-bearing plants, as well as some ornamental shrubs.

Materials and methods

The studied material comprised shoots of fruit-bearing plants and ornamental shrubs including common peach tree (*Persica vulgaris*) – cultivars 'Reliance' and

'Siewka Jerzykowska', red raspberry (*Rubus idaeus*) – cultivars 'Canby' and 'Polana', blackberry (*R. fruticosus*) – cultivars 'Orkan' and 'Gazda', and grapevine (*Vitis vinifera*) – cultivars 'Michigan' and 'Schulyer', *Forsythia* × *intermedia*, *Ligustrum vulgare* and *Euonymus fortunei* – cultivars 'Emerald Gaiety' and 'Emerald'n Gold'.

Four isolates of *P. cactorum* were obtained from strawberries in western Poland. K-1 and K-2 had been isolated from strawberry crown with rot symptoms, whereas O-3 and O-5 came from strawberry fruit with symptoms of leather rot. Cultures were grown on Merck PDA, in darkness, at 23–24°C, approximately.

Segments of soft-wood shoots, approximately 15-cm-long, devoid of leaves were inoculated with 5-mm-diameter PDA disks overgrown by 14-day-old *P. cactorum* cultures. The disks were inserted into incisions, approximately 1-cm-long and a few millimetres deep, made with scalpel in the middle part of each shoot.

The employed method of inoculation is similar to that described by Borecki and Millikan (1969) and developed for studying *P. cactorum* pathogenicity to apple stems under laboratory conditions.

The inoculated shoots were placed in plastic bags and incubated under room conditions at 22–24°C, to be examined for the length of the necrosis developed three and six days after inoculation. Sterile PDA disks were used in the control combination. Experimental design was completely randomised with four replications and 10 shoot parts in each replication. The experiments were performed twice.

The results were processed statistically by means of computer program STAT, and a t-Student test was performed to compare mean values at significance level $\alpha = 0.05$.

After the assessment of shoot infestation, samples of necrotic tissues were examined under a Jenamed 2 light microscope for the presence of *P. cactorum* hyphae and oospores.

Results

Pathogenicity of *Phytophthora cactorum* strawberry isolates to fruit-bearing plants

All the inoculated shoots of peach, raspberry and grapevine were affected by the strawberry isolates of *P. cactorum*, while the shoots of blackberry remained unaffected.

The necrosis on the affected shoots varied between plant species and isolates used. The length of necrosis on 'Reliance' peach shoots inoculated with different *P. cactorum* isolates ranged from 13.7 to 23.9 mm three days after inoculation, and from 48.6 to 77.5 mm six days after inoculation (Table 1). The necroses caused by K-2 were significantly smaller than those caused by the other isolates. Also on 'Siewka Jerzykowska' shoots the extent of necrosis three and six days after inoculation was found to vary between isolates.

Table 1

Colonization of shoot segments by *Phytophthora cactorum* isolates from strawberry; length of necrosis (mm)

Isolate	Three days after incubation	Six days after incubation	Three days after incubation	Six days after incubation
	Peach			
	‘Reliance’		‘Siewka Jerzykowska’	
K-1	19.3 b	77.5 b	26.7 b	104.6 a
K-2	13.7 a	48.6 a	32.4 c	129.2 b
O-3	18.8 b	67.9 b	23.8 a	101.2 a
O-5	23.9 b	76.3 b	54.9 d	128.4 b
	Raspberry			
	‘Canby’		‘Polana’	
K-1	2.1 a	23.7 a	10.2 b	30.7 b
K-2	8.5 c	32.5 b	10.5 b	37.9 bc
O-3	14.9 d	42.7 c	16.3 c	44.6 c
O-5	4.7 b	36.9 bc	0.0 a	17.1 a
	Grapevine			
	‘Michigan’		‘Schulyer’	
K-1	0.5 a	21.7 ab	9.4 d	22.4 c
K-2	0.5 a	17.0 a	6.1 b	12.8 a
O-3	0.9 a	23.5 ab	7.1 c	16.0 ab
O-5	0.2 a	28.7 b	0.0 a	18.4 bc
	<i>Euonymus fortunei</i>			
	‘Emerald Gaiety’		‘Emerald’n Gold’	
K-1	12.4 a	40.1 a	7.9 d	11.9 a
K-2	11.5 a	37.0 a	6.7 c	12.4 a
O-3	10.7 a	35.3 a	4.9 b	10.1 a
O-5	11.5 a	60.4 b	1.0 a	61.4 b

Within each plant species values in columns marked by the same letters do not differ at 5% level of significance.

Big statistically significant differences in length of necrosis caused by different isolates were also observed on ‘Canby’ raspberry shoots three days after inoculation (Table 1). The shortest necroses were found on shoots inoculated with K-1, and the longest ones on those inoculated with O-3. Also on ‘Polana’ shoots the longest necroses were caused by O-3 isolate (Phot. 1).

On ‘Michigan’ grapevine shoots three days after inoculation the necroses were small with no statistically significant differences in length between isolates (Table 1). Three days later, however, some differences were found to occur. The response of ‘Schulyer’ shoots varied between isolates, significant differences being observed



Phot. 1. Necrosis on the shoots of raspberry 'Polana' affected by strawberry isolate of *Phytophthora cactorum* (six days after inoculation); on the left – shoot from control combination (photo by A. Gołębnik)

as soon as three days after inoculation. Both three and six days after inoculation the longest necroses were found on shoots inoculated with K-1.

None of the fruit-bearing plant species developed necrosis in the control combination with sterile PDA disks inserted into incisions.

Pathogenicity of *Phytophthora cactorum* strawberry isolates to ornamental shrubs

All the inoculated *E. fortunei* shoots were affected, while the shoots of *F. × intermedia* and *L. vulgare* remained unaffected by the pathogen. No statistically significant differences in length of necroses caused by different isolates were found on 'Emerald Gaiety' shoots three days after inoculation; six days after inoculation, however, the necroses on shoots inoculated with O-5, an isolate from strawberry fruit, were longer than those caused by the other isolates. Also in 'Emerald'n Gold' the longest necroses were found on shoots inoculated with O-5 (Table 1).

None of the ornamental shrub shoots developed necrosis around the incisions in the control combination.

Results of microscopic studies

Microscopic examination of sections obtained from affected raspberry shoots of both tested cultivars revealed spherical yellow brown *P. cactorum* oospores of diameter ranging from 25 to 30 μm . Oospores were found also in affected shoots of 'Schulyer' grapevine. In shoots of peach, as well as those of 'Michigan' grapevine and *E. fortunei*, only *P. cactorum* hyphae were observed.

Discussion

The study results suggest that *P. cactorum* isolates from strawberry crown or fruit can affect other fruit-bearing plant species, as well as some ornamental shrubs. In the infection experiments performed, shoots of peach, raspberry, grapevine and *E. fortunei* were found affected to different extent by *P. cactorum* isolates from strawberry. Assumed as a measure of pathogenicity, the extent of necrosis caused by the pathogen on the tested shoots indicates that the isolates from strawberry fruit are not more pathogenic than those obtained from strawberry crown. The presence of *P. cactorum* oospores in tissues of raspberry and grapevine stems inoculated with isolates from strawberry crown or fruit gives reasons to believe that *P. cactorum* from strawberry can possibly affect other plant species as well.

Seemüller and Schmidle (1979), Harris and Stickels (1981) and Bielenin (2002) claim that strawberry crown rot is caused by a new *P. cactorum* pathotype, different from isolates obtained from apple tree. Also Duncan (2002) suggests a specific *P. cactorum* pathotype as the cause of strawberry crown rot. In her comparative study of *P. cactorum* isolates obtained from strawberry crown and from apple tree, Bielenin (2002) found isolates from strawberry to cause only minor necrosis on apple stocks, and strawberry cuttings inoculated with isolates from apple to remain unaffected. The same author (Bielenin 2002) used a technique of native protein separation based on electrophoresis in polyacrylamide gel to compare *P. cactorum* isolates obtained from strawberry crown to those coming from apple. No differences between the studied isolates were revealed, despite the fact that isolates coming from strawberry were remarkably more pathogenic to strawberry than to apple stems.

In a study by Hantula et al. (2000), using the RAMS technique for studying *P. cactorum* isolates from different hosts, including strawberry and apple, no clear genetic differences were found between the studied isolates. The authors believe leather rot of strawberry fruit and strawberry crown rot not to be caused by different *P. cactorum* isolates. Isolates from strawberry crown and those from strawberry fruit with symptoms of leather rot had previously been proven not to differ genetically (Hantula et al. 1997). The same authors showed the European population of *P. cactorum* to differ significantly from the population of the pathogen in North America (Hantula et al. 2000). Lilja et al. (1998), in their comparative study using the RAPD technique, investigated *P. cactorum* isolates from birch and from strawberry, to find them different and classify in separate groups. Wound inoculation

with isolates from strawberry caused necrosis in *Betula pendula*, while no disease symptoms were found on strawberry plants inoculated with isolates from birch (Hantula et al. 1997).

The cited data and the results of this study do not provide sufficient ground to determine with certainty whether strawberry crown rot, which has recently become a problem in strawberry plantations in many countries, is caused by a specific *P. cactorum* pathotype. Further research is necessary to give answer to this question. Pot or field experiments using selected fruit-bearing plant and ornamental shrub species should be performed to confirm the results of laboratory studies, as the results obtained in laboratory experiments can differ from results of field investigations.

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