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FIRST NOTICE OF *PHYTOPHTHORA STEM* BASE ROT ON *SYRINGA VULGARIS* IN A POLISH FIELD NURSERY

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Abstract: *Phytophthora citrophthora* was isolated from grafted lilac (*Syringa vulgaris*). Showing browning of leaf blade edge and necrosis spreading on all leaves. On the stem bases, beginning from the grafted place, necrosis had even spread 20 cm upward, whereas root stock shoots and roots were healthy. Using rhododendron leaves as the bait, *Phytophthora citrophthora* was isolated from soil where growing plants showed stem base rot symptoms. Isolates of *Phytophthora* which were from the stem base, shoot tip of lilac, and soil, colonized leaves, stem parts and roots of lilac. Necroses spread about twice faster on leaf blades than on stem parts. Three isolates of *Phytophthora* colonized also *Forsythia intermedia* and *Ligustrum vulgare.*

Key words: *Syringa vulgaris*, nursery, base rot, *Phytophthora*, occurrence, colonization

INTRODUCTION

In July 2009 browning was observed on grafted lilac (*Syringa vulgaris* L.). The lilacs were growing in a field nursery in sandy soil. The browning was on the top leaf edges and it spread toward the main veins. Such a discoloration was observed, usually on 1–2 plants in different places in the nurseries. During the next 4 weeks symptoms were noticed on the next leaves which hung down and dropped to the ground. On some diseased plants leaf blades were pale-green or yellow-green and they wilted and dropped to the ground. On the base of these lilacs, from the place where the grafting had been started and upward, usually on one side, brown or dark brown necroses had spread even as high as 20 cm. Root stocks stems and roots, however, were healthy. Lilac is very sensitive to leaf and soilborne pathogens, including *Phytophthora*. Vegh (1987) described 22 fungi pathogenic to lilac and among them *P. cactorum* (Leb. and Cohn) J. Schrot. and *P. syringae* (Kleb.) Kleb., whereas Beals *et al*. (2004), Hall *et al*. (1992), Novotielnova (1974), Schwingle *et al*. (2007) and Vegh and le Berre (1985) described *P. inflata* Carros. and Tucker, *P. palmivora* (E.J. Butler) E.J. Butler, *P. citricola* Sawada, *P. ramorum* Werres, De Cock and Man in'tVelt. In Polish hardy ornamental nursery stock *P. citrophthora* (R.E. Sm & E.H. Sm.) Leonian was detected from rotted stem tips and bases of plants (Orlikowski and Szkuta 2005).

The purpose of this study was (1) to isolate and identify a causal agent of the disease and (2) to evaluate a pathogenicity of isolates obtained toward lilac and other plants.

MATERIALS AND METHODS

Plants

Seven grafted 1-year-old lilacs (*Syringa vulgaris*) with yellowing and browning of leaves and stem base rot symptoms were taken from different points of nursery in the southeastern part of Poland. Plants were cultivated in a field in which *Corylus avellana* had previously been grown for 10 years. Root systems of diseased plants with surrounded soil were put individually into plastic bags and transported to the laboratory.

Mycological analyses

From each diseased plant, soil was collected from its root system and put into separate bags. The stems were cutoff over the root system and bark was removed from brown or dark brown parts, washed under tap and distilled water and blotted dry. Pieces of bark which were about 5 cm long were sterilized over a burner flame and about 3 mm diam inokula were transferred into PDA in 90 mm Petri dishes (10 small parts/plate). Within 24–48 h incubation colonies growing around inocula were transferred into PDA slants. After 10 days, the obtained colonies were grouped by growth pattern and morphology, and chosen isolates were identified to genera and species using monographs and keys. In the case of *Phytophthora,* results were confirmed using RAPD-PCR (Trzewik *et al.* 2006) and PCR with species – specific primers for *P. citrophthora* (R. E. Sm. & E.H. Sm.) Leonian Ctph1/Ctph2 (Ersek *et al.* 1994).

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Detection of *Phytophthora* from substratum

The procedure described by Orlikowski (2006) was used. About 500 g of soil samples from each plant were submerged in tap water in photographic trays. Eight rhododendron leaves from the top of Nova Zembla shoots were put on the surface of emerged samples and trays were covered with foil. After 4 days of incubation at 22–24°C in the dark, rhododendron leaves were removed from the soil, washed under tap water, blotted dry, and the number of necrotic spots on each of them was counted. Part of leaves with necrotic spots were transferred on PDA using the same procedure as with pansy parts (Orlikowski *et al*. 2010).

Colonization of plant parts by isolates of *P. cactorum*

Leaves and stem parts from grafted lilac, and rootstocks of *S. vulgaris* were used. Additionally, colonization of 4 plant species from the Oleacae family was assessed. Stock cultures of. *P. citrophthora* were grown on PDA medium at 24°C in the dark. After 7 days, 3 mm diam pieces of medium overgrown with the species were transferred onto the middle of leaves or stem bases in trays with sterile moist blotting paper covered with plastic net. Within 6 days the diameter of spots or length of necroses was measured. Experimental design was completely randomized with 4 replications and 5 plant parts in each rep. Trials were repeated at least twice, at 2 week intervals.

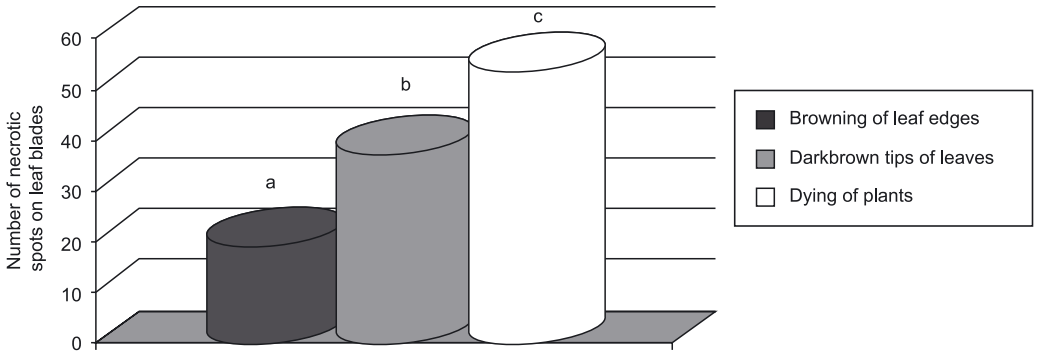
RESULTS

Isolation of *Phytophthora* sp. from stem base of lilac and from soil

P. citrophthora was isolated from 7 stem parts with browning of tissues on the grafted place and spreading upwards. Additionally *Botrytis cinerea* Pers., *Fusarium solani* (Mart.). Sny. et Hans., *Penicillium* spp. and *Trichoderma* spp. were isolated occasionally from diseased tisssues. Colonies of *P. citrophthora* consisted of about 2/3 of the all cultures obtained. *P. citrophthora* was also isolated from soil taken from the root zones of plants with differentiated disease symptoms (Fig. 1). Statistical analysis showed about 3 times less necrotic spots on bait leaves taken from soil under plants with the first disease symptoms than from soil under dying lilac. On bait leaves taken from soil under lilacs with browning of leaves and in which the spread of symptoms was downward, the number of necrotic spots was 2 times higher than from under lilac with the first leaf discolouration (Fig. 1).

Colonization of leaves and stem parts by *P. citrophthora*

Necrotic spots on leaves of grafted lilac and its rootstock were significantly the smallest when culture obtained from diseased tip of lilac was used for their inoculation (Table 1). The disease spread the quickest on leaf blades inoculated by isolate from lilac stem base. Isolate from soil caused the quickest spread of necroses



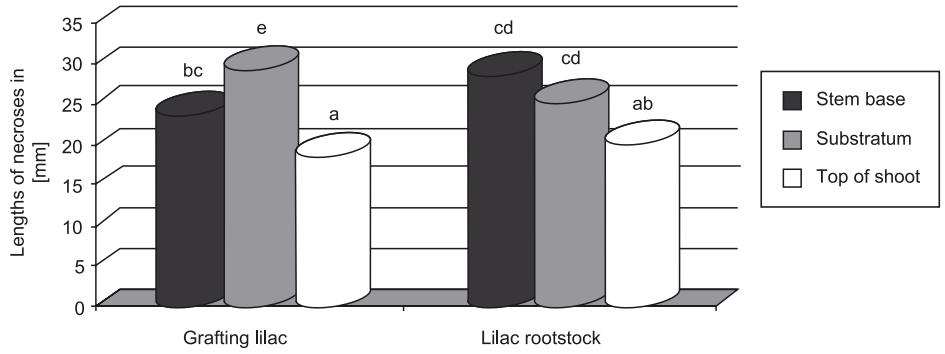
Means on columns in figure followed by the same letter, do not differ with 5% of significance (Duncan's multiple range test)

Fig. 1. Relationship between degree of lilac infection in the field nursery and number of *Phytophthora* leaf spots on rhododendron bait as a measure of *P. citrophthora* density in soil

Table 1. Colonization of *Syringa vulgaris* leaf blades by *P. citrophthora* isolates; diameter of leaf spots 7 days after inoculation

Testing plants	Source of isolates		
	S_1 stem base	S_2 soil	S_3 top of shoot
S. vulgaris grafting shoots	44.2 c	37.7 a	32.7 a
S. vulgaris rootstock	51.0 e	47.6 d	32 a

Means in columns, followed by the same letter, do not differ with 5% of significance (Duncan's multiple range test)



Means on columns in Figure followed by the same letter, do not differ with 5% of significance (Duncan's multiple range test)

Fig. 2. Relationship between source of *P. citrophthora* isolates, plants and development of necroses on stem parts

Table 2. Colonization of leaf blades (a) and stem parts (b) of plants from the Oleaceae family by *P. citrophthora* isolates from lilac and soil; diameter/length of necroses in mm 7 days after inoculation

Tested plant species	Source of *P. citrophthora* isolates					
	S_1 stem base		S_2 soil		S_3 top of shoot	
	a	b	a	b	a	b
C. avellana	6.1 b–d	10.4 b	7.0 d	9.6 b	3.7 ab	11.5 bc
F. intermedia	21.2 e	28.5 f	7.6 d	19.1 d	6.3 cd	13.1 c
L. vulgare	19.6 e	23.4 e	4.1 a–c	9.6 b	6.6 d	9.8 b
P. coronarius	4.0 a–c	2.8 a	3.3 a	2.2 a	2.3 a	2.4 a
S. vulgaris root stock	51.0 h	28.5 f	47.6 g	25.6 e	32.0 f	20.8 d

Means in columns, followed by the same letter, do not differ with 5% of significance (Duncan's multiple range test)

on stem parts of grafted plants (Table 1). Rootstocks stem fragments were colonized similarly, both by isolates of *P. citrophthora* from stem base and soil (Fig. 2).

Analysis of colonization of leaves and stem parts of 4 plant species from the Oleacae family indicated slower spread of necroses on *P. coronarius* (Table 2). In the colonization of other plant species parts, source of the isolate used had significant influence on necroses development. The quickest development of necroses was observed on leaves and stem parts of lilac rootstock. The spread of necroses was, however, slower when isolate from tip of lilac shoot was used for inoculation. Lilacs leaves were colonized quicker than stem parts. Isolate from lilac stem base, colonized parts of stems of *F. intermedia* and *L. vulgare* more quickly than it colonized leaves (Table 2).

DISSCUSION

Grafted cultivars of lilac are planted more often in Polish gardens and landscape than 10 years ago. They are also exported to western Europe. These factors cause that production of plants, especially in field nurseries, increase every year. *S. vulgaris* rootstocks are also used as hedge plants. The statement of *P. citrophthora* on grafted plants is the first report of *Phytophthora* crown rot of lilac in Poland. Also, from *Forsythia intermedia* (Orlikowski and Ptaszek 2007) and *Euonymus* spp. (Orlikowski *et al*. unpubl.) *P. citrophthora* was detected only on diseased plants grown in containers from imported materials. Disease symptoms observed on invaded lilacs, include yellowing and browning of leaf edges, indicating the toxin production by the pathogen. Mycological analyses of invaded plants showed the domination of *P. citrophthora* among other isolated species. The lack of disease symptoms on rootstocks but their occurrence on stem base rot of grafted plants indicates 2 possible sources of the pathogen. The most probable are lilac stem parts used for plant grafting. The occurrence of the disease at different points in the nursery confirm this hypothesis. *Coryllus avellana* plants growing for 10 years as the mother plants in the field before lilac production, were colonized by *P. citrophhtora* very slowly and this exclude this plant as the pathogen host. Detection of *P. citrophthora* taken from the soil under affected lilacs in different stages of disease development indicates that pathogen may survive in the plant environment and spread with water and on machines.

Probation of searching of potential plant hosts from Oleacae family for the detected pathogen, showed on the already mentioned *F. intermedia* and additionally *Ligustrum vulgare*. Stem parts of both species were colonized by *P. citrophthora* from diseased stem base about 4 mm/24 h, but also from other sources. It is possible that in field production, where individual plants are not checked as often as they are in container nurseries, *Phytophthora* stem base

rot may occur on 2 mentioned species and probably others and then spread onto lilac.

This study showed that *P. citrophthora*, identified the first time on citrus trees in California (Smith and Smith 1906), may occur not only in the subtropical but also in the temperate zone including not only ornamental nurseries but also Polish forests (Oszako and Orlikowski 2004).

REFERENCES

Beales P.A., Schlenzig A., Inman A.J. 2004. First report of ramorum bud rot and leaf blight (*Phytophthora ramorum*) on *Syringa vulgaris* in the UK. Plant Pathol. 53 (4), p. 525.

Ersek T., Schoelz J.F., English J.T. 1994. PCR amplification of species-specific DNA sequences can distinguish among *Phytophthora* species. Appl. Environ. Microbiol. 60 (7): 2616–2621.

Hall G., Dobson S., Nicholls C. 1992. First record of *Phytophthora inflata* in the United Kingdom. Plant Pathol. 41 (1): 95–97.

Novotielnowa N.S. 1974. Fitoftorowyje Griby. Nauka, Leningrad: 85–86, 91–92.

Orlikowski L.B., Szkuta G. 2005. Occurrence of *Phytophthora citrophthora* on *Syringa vulgaris* in Poland. Acta Mycol. 40 (2): 175–180.

Oszako T., Orlikowski L.B. 2004. The first noting of *Phytophthora citrophthora* on *Picea abies* in a forest stand. Phytopathol. Pol. 34: 81–85.

Orlikowski L.B. 2006. Relationship between source of water used for plant sprinkling and occurrence of *Phytophthora* shoot rot and tip blight in container-ornamental nurseries. J. Plant Protection Res. 46 (2): 163–168.

Orlikowski L.B., Ptaszek M. 2008. *Phytophthora cryptogea* and *P. citrophthora*; new pathogens of *Forsythia intermedia* in Polish ornamental hardy nursery stocks. J. Plant Protection Res. 48 (4): 511–517.

Orlikowski L.B., Ptaszek M., Wojdyła A., Skrzypczak Cz. 2010. First notice of *Phytophthora* aerial blight and crown rot of pansies in Poland. J. Plant Protection Res. 50 (2): 233–237.

Schwingle B.W., Smith A.J., Pansy Blanchette R.A. 2007. *Phytophthora* species associated with diseased woody ornamentals in Minnesota nurseries. Plant Dis. 91 (1): 97–102.

Smith R.E., Smith E.H. 1906. A new fungus of economic importance. Bot. Gaz. 42: 215–221.

Trzewik A., Wiejacha K., Orlikowski L.B., Orlikowska T. 2006. Identification of five *Phytophthora* species , causing agents of diseses of nuresy perennials, trees and shrubs on the base of DNA markers amplified with non-specific primers. Phytopathol. Pol. 41: 27–37.

Vegh I. 1987. Champignons des Arbustes et Arbustes D'ornament. INRA, Paris, 26 pp.

Vegh I., le Berre A. 1985. Quelques maladies nouvelles a *Phytophthora* des arbustes d'ornament en France. Rev. Horticole 258, 35: 37–39.

POLISH SUMMARY

PIERWSZA INFORMACJA O WYSTĘPOWANIU ZGNILIZNY PODSTAWY PĘDU *SYRINGA VULGARIS* W POLSKIEJ SZKÓŁCE POLOWEJ

Żółknięcie i brązowienie liści oraz zgniliznę podstawy pędu okulizowanych lilaków (*Syringa vulgaris*), nawet do 20 cm od miejsca uszlachetnienia, stwierdzono w szkółce polowej w południowo-wschodniej Polsce. Z porażonej podstawy pędów oraz z gleby pobranej spod porażonych roślin, izolowano głównie *Phytophthora citrophthora*. Izolaty tego gatunku z porażonej podstawy pędu, wierzchołka oraz gleby kolonizowały liście i części łodyg odmiany szlachetnej oraz podkładki. Zgnilizna rozwijała się około 2-krotnie szybciej na blaszkach liściowych aniżeli na częściach łodyg. Badane izolaty kolonizowały również tkanki *Forsythia intermedia* i *Ligustrum vulgare,* natomiast zgnilizna rozwijała się bardzo wolno na *Philadelphus coronarius*.