JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 48, No. 4 (2008)

DOI: 10.2478/v10045-008-0058-x

PHYTOPHTHORA CRYPTOGEA AND *P. CITROPHTHORA*; NEW PATHOGENS OF *FORSYTHIA INTERMEDIA* IN POLISH ORNAMENTAL HARDY NURSERY STOCKS

Leszek B. Orlikowski, Magdalena Ptaszek

Research Institute of Pomology and Floriculture
Pomologiczna 18, 96-100 Skierniewice

Received: July 16, 2008
Accepted: November 13, 2008

Abstract: *Phytophthora cryptogea* and *Phytophthora citrophthora* were isolated from rotted stem base of *Forsythia intermedia* cv. Minigold and from substratum, respectively. Additionally *Botrytis cinerea* and *Fusarium* spp. were often isolated from diseased tissues. In the laboratory trials *P. cryptogea* from *F. intermedia* colonised and incited disease of leaf blades and stem parts of all tested cultivars.The species caused necrosis development of leaves and stem of *Ligustrum vulgare*, *Sambucus nigra* and *Syringa vulgaris* after 3 days of inoculation. The isolates from 7 different host plants colonised (except from *Sempervivum arachnoideum*) leaves and stem parts of *F. intermedia* cv. Minigold with the fastest spread of necrosis on plant parts inoculated with isolate from forsythia. *P. citrophthora* from substratum colonised leaves and stem parts of 5 tested cultivars with the quickest spread of necrosis on cv. Goldzauber and Spectabilis.

Key words: *Forsythia* cultivars, *Phytophthora* spp., occurrence, pathogenicity

INTRODUCTION

Cultivars of *Forsythia intermedia* Zab. are often grown as bushes in Polish container nursery stocks. During the last 20 years no problems with the plant health were distinguishable. In Aug. 2007 in one container nursery the symptoms of blighting of cv. Minigold were detected and consequently about 60% of the plants died. First symptoms of disease were observed on leaves which became pale-yellow and pale-red. Infected leaf blades wilted, hung down and turned brown and dark-brown. The symptoms spread out all-over the plant shoots. Dying plants often occurred in

*Corresponding address:
leszek.orlikowski@insad.pl

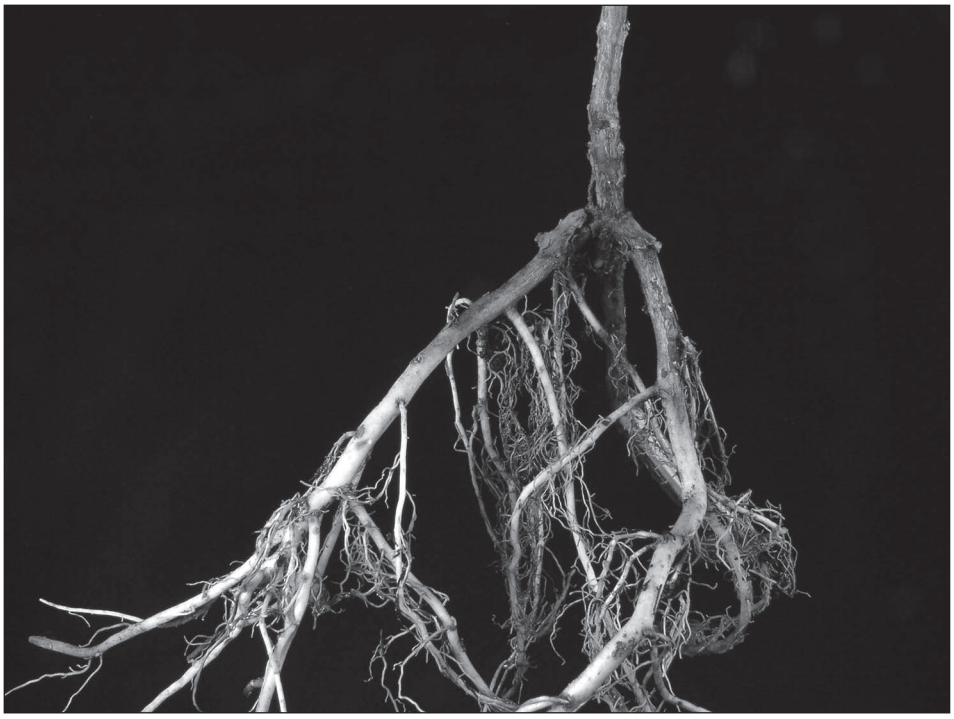

Fig. 1. *Phytophthora* stem base and root rot of *Forsythia intermedia* cv. Minigold

groups of 3–10 bushes. The bases of infected shoots were brown or black- brown and necrosis spread on the roots (Fig. 1).

Leaf chlorosis, lack of shoot growth, wilting, leaf scorch and plant collapse was noticed on *Forsythia viridissima* in Italy and *Phytophthora nicotianae* Breda de Haan was isolated from infected basal stem and roots (Cacciola *et al.* 1994). Hong *et al.* (2005) noticed shoot blight symptoms on *F. intermedia* in the USA and *P. nicotianae* was the causal agent of the disease. The foliage, dead feeder roots, dark streaks up stem wood incited by *P. cinnamomi* Rands was observed on forsythia by Moorman (2007). In the USA *P. cinnamomi* and *P. parasitica* Dastur are considered as the casual agent of root and crown rot and dieback of forsythia (Daughtery and van Broembsen 2001). In the authors' opinion forsythia is not particularly susceptible to *Phytophthora* spp. but some losses may occur when the weather conditions are favourable for pathogen development. In French nurseries *P. cryptogea* Pethybr. et Laff., *P. drechsleri* Tucker and *P. megasperma* Brasier and Griffin caused rot of roots and stem bases on forsythia (Vegh and Bourgeois 1976; Vegh 1987).

The objective of presented studies was to determine a causal agent of forsythias shoot and root rot and colonization of plants by identified pathogens.

MATERIALS AND METHODS

Isolation of *Phytophthora* spp. from the diseased plant parts and substratum

Fourteen plants in containers, showing discoloration of leaf blades, root and stem base rot, collected from the nursery in August 2007, were placed in plastic bags and transferred to the laboratory. The same or next day the plants were washed under tap water and infected stem bases were chosen for mycological analysis. They were rinsed in distilled water, blot dried and sterilized over a burner flame. About 5 mm long parts of stems taken from the border of diseased and healthy tissues were placed on PDA medium in 90 mm Petri dishes (8 fragments/dish) and incubated at 25°C in the dark. During the next 72 hrs colonies grown around the inocula were transferred into PDA slants. The cultures were groupped on the base of their growth, morphology and selected. Only representative isolates were identified to genera and species (Szkuta 2004). Classification of *Phytophthora* to species was performed by DNA analyses using the method described by Orlikowski *et al*. (2006).

Isolation of *Phytophthora* spp. from substratum

About 0,5l of substratum was taken from each container with infected plants of forsythia showing root and stem rot. The samples of soil were placed into trays and flooded with tap water about 1 cm above the soil surface. Young rhododendron leaves of cv. Nova Zembla were transferred into water and trays were covered with foil. After 5-day-incubation at temperature 22–24°C the leaves with dark-green spots were removed, washed in tap water and later distilled water, blot dried and about 5 mm fragments were placed on PDA medium. After 24–48 hrs of incubation the colonies of *Phytophthora* growing around inocula were transferred into slants. Further procedure was similar to the one described the isolation of *Phytophthora* spp. from diseased forsythia. Substratum samples of 6 forsythias were analysed and 8 rhododendron leaves were placed for each tray.

Colonisation of plant parts by *Phytophthora cryptogea* and *P. citrophthora*

***Phytophthora* cultures**. Isolates of *P. cryptogea* from *F. intermedia* and additionally from *Alstroemeria aurantiaca, Aquilegia discolor, Campanula persicifolia, Gerbera jamesonii, Saxifraga paniculata* and *Sempervivum arachnoideum* were used for inoculation of forsythia leaf blades and stem parts. All isolates were taken from the collection of Laboratory of Ornamental Plant Pathology of the Res. Institute of Pomology & Floriculture. Additionally *P. citrophthora* taken from the substratum of infected forsythias was used. Stock cultures were grown on PDA at 24°C in the dark. Three mm diam inocula were taken from the edge of 7-day-old cultures.

Inoculation of plant parts

Leaf blades and about 10 cm long 3-month old stem parts of forsythia cultivars (Table 2) and additionally: *Fraxinus excelsior, Ligustrum vulgare, Sambucus nigra* and *Syringa vulgaris* (Table 3) were placed in a tray covered with moist blotting paper and plastic net. The central parts of leaves and the bases of stem pieces were inoculated with *Phytophthora inoculum* (3 mm in diameter). Trays were covered with plastic foil. Within 3–6 day-period of incubation the size (diameter and length) of necrosis was measured. Experimental design was completely randomized with 4 replication and

10 leaf blades and stem parts in each replication The trials were repeated at least twice.

RESULTS

Mycological analysis of diseased *Forsythia intermedia*

Nine genera and species were isolated from rotted stem bases (Table 1). *Phytophthora cryptogea* dominated among obtained isolates. This species was detected from 6/7 of analysed plant parts. *Botrytis cinerea* was isolated from 2/7 of plants, whereas 2 *Fusarium* species from 13/14 of them. Other fungi were isolated sporadically (Table 1).

Table 1. Fungi and *Algae* like *Oomycetes* isolated from 14 plants of *Forsythia intermedia* cv. MinigoldIsolation: Aug., 2007

Genera/species	Number of	
	infected plants	obtained isolates
Alternaria alternata Nees	1	3
Botrytis cinerea Pers.	4	6
Cladosporium herbarum Link.	6	4
Fusarium oxysporum Schlecht.	5	11
Fusarium solani (Mart.) Sny. et Hans	8	14
Mucor sp.	2	4
Penicillium spp.	3	5
Phytophthora cryptogea Pethybr et Laff.	12	45
Trichoderma spp.	4	11

Detection of *Phytophthora* spp. from substratum

P. citrophthora was isolated from substratum samples using rhododendron leaves as a bait.

Colonisation of plant parts by *Phytophthora cryptogea*

An isolate from *F. intermedia* colonized leaf blades and stem parts of 6 forsythia cultivars (Table 2). The quickest spread of necrotic spots was observed on the leaves of cvs. Minigold and Spectabilis in contrast to the slowest on cv. Coutrasol. Necrosis spread the most quick on stem parts of cvs. Golden times and Goldzauber and the most slowly on cv. Coutrasol (Table 2). The isolate from *F. intermedia* colonised leaves of *Ligustrum vulgare, Sambucus nigra* and *Syringa vulgaris.* Although the largest necrotic spots were observed on leaf blades of *S. nigra* and *F. intermedia* cv. Spectabilis after 5-day-incubation (Table 3). A similar tendency was observed on inoculated stem parts of plants (Table 3).

All tested isolates of *P. cryptogea* from 7 various host plants colonised (except from *S. arachnoideum*) forsythia leaf blades and stem parts, however, the quickest spread of necrosis was observed on plant parts inoculated with the isolate from *F. intermedia* (Table 4).

Table 2. Colonisation of *Forsythia intermedia* leaves and stem parts by *P. cryptogea*

Forsythia intermedia cultivars	Diameter/length [mm] of necrosis after days of inoculation			
	leaves		stem parts	
	4	6	4	6
Coutrasol	6.3 a	11.8 a	8.2 a	12.5 a
Golden times	10.2 c	22.5 c	11.2 b	20.5 c
Goldzauber	10.4 c	19.5 b	12.8 c	19.9 c
Minigold	20,1 e	31,8 d	9,4 ab	18,6 b
Spectabilis	15.0 d	30.6 d	10.7 b	18.8 b
Week-End	9.0 b	23.0 c	8.2 a	18.5 b

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

Table 3. Colonisation of plants species by *Phytophthora cryptogea,* isolated from *F. intermedia*

Plant species and cultivars	Diam/length [mm] of necrosis after days			
	3		5	
	leaves	stem parts	leaves	stem parts
Forsythia intermedia Minigold	14.6 f	7.1 d	26.7 e	15.6 c
F. intermedia Spectabilis	11.3 e	9.0 e	21.1 d	20.5 d
Fraxinus excelsior	0 a	0 a	3.1 a	3.1 a
Ligustrum vulgare	2.8 b	4.0 c	4.2 b	8.1 b
Sambucus nigra	7.2 d	10.3 f	40.0 f	23.5 e
Syringa vulgaris	4.9 c	2.2 b	12.3 c	3.8 a

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

Table 4. Colonisation of leaf blades of *Forsythia intermedia* cv. Minigold by different isolates of *P. cryptogea*

Source of isolates	Diam/length [mm] of necrosis after days of inoculation			
	4		6	
	leaves	stem parts	leaves	stem parts
Alstroemeria aurantiaca	2,4b	0a	2,5b	0a
Aquilegia discolor	3,2b	0a	3,2b	3,6
Campanula persicifolia	8,1c	6,8c	12,8c	10,4d
Forsythia intermedia	12,3d	7,1c	17,5d	20,0e
Gerbera jamesonii	0a	5,2b	0a	6,5c
Saxifraga paniculata	0a	0a	3,3b	0a
Sempervivum arachnoideum	0a	0a	0a	0a

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

Colonisation of forsythia cultivars by *Phytophthora citrophthora*

The species caused necrosis on the plant parts of 5 tested cultivars. The quickest spread of disease was detected on leaves of cvs. Goldzauber, Spectabilis and stem parts of cvs. Golden times, Goldzauber and Spectabilis (Table 5).

Table 5. Colonisation of *Forsythia intermedia* cultivars by *P. citrophthora* isolated from substratum. Trial: Sept., 2007

Cultivars	3		5	
	leaves	stem parts	leaves	stem parts
Coutrasol	5.4 b	5.5 a	9.0 a	6.3 a
Golden Times	3.5 a	9.0 c	12.7 b	17.4 e
Goldzauber	7.1 c	6.8 b	15.3 c	15.9 d
Spectabilis	7.7 c	7.1 b	14.6 c	12.9 c
Week-End	4.9 b	7.1 b	9.3 a	11.9 b

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

DISCUSSION

P. cryptogea has been known in Polish horticulture since 1964, first of all as the causal agent of *Gerbera jamesonii* foot and stem base rot of some ornamental pot plants (Orlikowski 1978, 1996). In 1993 this species was isolated together with *P. cinnamomi* and *P. citricola* from diseased stem parts of *Abies alba, Pinus mugho* var. *pumilo* and *P. nigra* (Orlikowski et al. 1995). In the beginning of XXI century the species was also detected from diseased *Sempervivum* and *Saxifraga* species (Orlikowski and Ptaszek 2007) as well as from nursery ponds water (Orlikowski, unpubl.). In surveyed nursery stocks the pathogen was found for the first time on infected forsythia plants. It is possible that *P. cryptogea* was transferred on imported, rooted cutting of forsythia or with water used for plant sprinkling.

The data obtained revealed that plants from the family *Oleaceae* were also colonized by *P. cryptogea* but the quickest spread of necrosis was observed on *Sambucus nigra* plant parts. Inoculation of forsythia with isolates of *P. cryptogea* from some diseased greenhouse plants and *Campanula persicifolia* caused colonization of leaves and/or stem parts of *F. intermedia*. It indicates that plants, belonging to the same family, different forsythia cultivars in nursery stocks and some residues of perennial and greenhouse plants may be considered as a potential source of the studied pathogen.

P. citrophthora is not a new pathogen of ornamental plants. The species has been already isolated from diseased *Syringa vulgaris* and *Podocarpus alpinus* rotted shoots (Orlikowski and Szkuta; Szkuta 2004). Moreover the pathogen was detected from nursery ponds and river water (Orlikowski , unpubl.). In the surveyed nursery pond water used for plant sprinkling was the most possible source of that species.

REFERENCES

Cacciola S.O., Belisario A., Pane A., Magnano di San Lio G. 1994. *Forsythia*: A new host of *Phytophthora nicotianae* in Italy. Plant Dis. 78: 525–528.

Daughtery M.L., von Broembsen S. 2001. *Forsythia* diseases. p. 168–170. In "Diseases of Woody Ornamentals and Trees in Nurseries (R.K. Jones, D.M. Benson). APS Press, St. Paul, Minnesota.

Hong C.X., Richardson P.A., Kong P. 2005. Shoot blight of *Forsythia intermedia* in Virginia nurseries caused by *Phytophthora nicotianae*. Plant Dis. 89, p. 430.

Moorman G. 2007. *Phytophthora* root rot. Online. http://www.ppath.cas.psu.edu/EXTENSION/plant_disease/pdf%20Misc/Phytophthora%20Root%20Rot%20on%20Woody%20Ornamentals.pdf

Orlikowski L.B. 1978: The occurrence of *Phytophthora cryptogea* Pethybr. et Laff. in gerbera (*Gerbera jamesonii* Bolus) growing sites. Bull. Pol. Ascad. Sci. 26: 495–498.

Orlikowski L.B. 1996. *Phytophthora* stem rot of *Pelargonium*. Phytopathol. Pol. 12: 79–86.

Orlikowski L.B., Gabarkiewicz R., Skrzypczak Cz. 1995. *Phytophthora* species in Polish ornamental nurseries. I. Isolation and identification of *Phytophthora* species. Phytopathol. Pol. 9: 73–79.

Orlikowski L.B., Ptaszek M. 2007. *Phytophthora* spp. in Polish ornamental nurseries.I. perennial plants, new hosts of *P. cryptogea*. J. Plant Prot. Res. 47(4): 401–408.

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

Orlikowski L.B., Trzewik A., Wiejacha K. 2006: *Phytophthora tropicalis,* a new pathogen of ornamental plants in Poland. J. Plant Prot. Res. 46: 103–109.

Szkuta G. 2004. Występowanie, izolacja identyfikacja i szkodliwość gatunków z rodzaju *Phytophthora* w szkółkach roślin iglastych. Praca doktorska, AR Kraków, 191 pp.

Vegh I. 1987. Champignons des arbustes et arbustes d'ornament. INRA, Paris, p. 26.

Vegh I., Bourgeois M. 1976. Le deperissement des arbustes d'ornament provoque par des *Phytophthora*, principalement *P. cinnamomi*. J. d'etude sur les cultures en conteneurs, Paris, 8.01.1976: 35–62.

POLISH SUMMARY

PHYTOPHTHORA CRYPTOGEA I *P. CITROPHTHORA*; NOWE PATOGENY *FORSYTHIA INTERMEDIA* W POLSKICH SZKÓŁKACH ROŚLIN OZDOBNYCH

Phytophthora cryptogea izolowano z porażonej podstawy pędów *Forsythia intermedia* odm. Minigold, a *P. citrophthora* z podłoża spod roślin z objawami chorobowymi. Dodatkowo, z tkanek roślin izolowano *Botritis cinerea* oraz *Fusarium* spp. W warunkach laboratoryjnych *P. cryptogea* kolonizował liście i fragmenty pędów wszystkich testowanych odmian forsycji. Omawiany gatunek powodował nekrozę liści i pędów *L. vulgare*, *S, nigra* i *S. vulgaris* już po 3 dniach od inokulacji. Izolaty z 7 różnych roślin żywicielskiach (z wyjątkiem izolatu z *S. arachnoideum*) kolonizowały liście i pędy *F. intermedia* odm. Minigold, przy czym nekroza rozwijała się najszybciej na fragmentach roślin zainokulowanych *P. cryptogea* z forsycji. Gatunek *P. citrophthora* wyizolowany z podłoża kolonizował liście i fragmenty pędów 5 testowanych odmian forsycji, przy czym najszybszy rozwój nekrozy obserwowano na odm. Goldzauber i Spectabilis.