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PHYTOPHTHORA SPP. IN POLISH ORNAMENTAL NURSERIES. I. PERENNIAL PLANTS, NEW HOSTS OF *P. CRYPTOGEA*

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Abstract: *Phytophthora cryptogea* was isolated from diseased stem base of *Aquilegia discolor* and rotted leaves of *Saxifraga* and *Sempervivum* spp. Additionally, *Fusarium* species and *Botrytis cinerea* were frequently isolated from diseased parts of plants. Most of *Sempervivum* species and cultivars except *S. soculiferum* were colonized in laboratory conditions by *P. cryptogea*. The isolates from *Alstroemeria aurantiaca, Gerbera jamesonii, Saxifraga arendsii, S. paniculata, Sempervivum arachnoideum* colonised houseleek leaves with the fastest spread of necrosis on plant parts inoculated with cultures from *Gerbera* and *S. arendsii*. The isolate from *S. arendsii* colonized 5 species and cultivars of saxifrage as well as *Iberis sempervivum, Lavendula angustifolia, Sempervivum soboliferum* and *Vinca minor* with the slowest development on periwinkle. In laboratory trials isolate of *P. cryptogea* from houseleek grew on PDA and colonized leaves of that plant at temperature ranging from about 10 to 32.5°C with optimum at 20–30°C.

Key words: *Aquilegia, Saxifraga, Sempervivum*, *Phytophthora cryptogea*, occurrence, colonisation

INTRODUCTION

During the last years perennial crops growing in ornamental nurseries in Poland have suffered from various diseases that were major limiting factors in their production (Orlikowski, unpubl.). A rapid kill of some perennial species may be caused by *Phytophthora* spp., including *P. cinnamomi* Rands (Orlikowski *et al*. 2007), *P. citrophthora* (Smith and Smith) Leonian, *P. parasitica* Breda de Haan var. *nicotianae* (Westcott 1971) and *P. cryptogea* Pethybr. et Laff. (Orlikowski 1978, 2006). In the years 2004–2007 leaf and stem base rot of perennial species were observed in two ornamental nurseries and two gardens. The predominant symptoms on *Sempervivum* spp. and *Saxifraga*

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spp. were brown and later dark brown. Irregularly shaped spots, that appeared first on the youngest, single leaf bases, rapidly expanded upward and to the oldest leaves and all plant. Under moist conditions and temperature above 20°C affected leaves were water – soaked and sometimes covered with white mycelium of *Phytophthora* sp. The disease symptoms occurred on about 5–20% of plants depending on species and cultivars. On *Aquilegia discolor* first symptoms were noticed on leaf blades; they were light red, partly brown and plants wilted and died. The plant bases were dark brown and necrosis spread on leaf petioles. The research reported here established (1) the occurrence of fungi and *Algae* – like *Oomycetes* on *Sempervivum*, *Saxifraga* and *Aquilegia* species and (2) pathogenicity of *Phytophthora cryptogea* isolates toward those and other potential host plants.

MATERIALS AND METHODS

Isolation of *Phytophthora* sp. from diseased plant parts

Diseased plants, taken from perennial nursery situated in south – eastern part of Poland, were collected individually in plastic bags and transferred to the laboratory. On the next day plants were removed from bags, washed under tap water and affected leaves and/or stem bases were separated, rinsed 3 times in distilled water, blotting dried and chosen parts were sterilized over a burner flame. About 5 mm long parts were put on PDA medium. (one – three 90 mm diam Petri dishes/plant in dependence on species and degree of damage). Plates were checked during 4 – day incubation at 24°C in the dark for the presence of *Phytophthora* or other genera. Small parts of colonies, grown around diseased tissues, were transferred into PDA slants. Cultures obtained were grouped on the base of their growth and morphology and selected, representative isolates were identified to genera and species (Szkuta 2004). Confirmation of *Phytophthora* classification to species was performed by DNA analyses using the method described by Orlikowski *et al*. (2006).

Colonisation of plant parts by *Phytophthora cryptogea*

Isolates of the species from *Alstroemeria aurantiaca*, *Aquilegia discolor*, *Gerbera jamesonii, Saxifraga arendsii, S. paniculata*, *Sempervivum arachnoideum* (nurseries D and M) were used for inoculation of *Aquilegia, Saxifraga, Sempervivum*, and other plants in laboratory trials (Tables 2–5) using procedure described by Orlikowski *et al*. (2006). In the greenhouse experiments *Aquilegia, Saxifraga and Sempervivum* growing on the bench were inoculated with isolates of *P. cryptogea* from *S. arachnoideum* and during 12-day incubation at 18–25°C the development of disease symptoms were assessed. Experimental design was completely randomized with 4 replications and 10 leaves and 5 plants in each replication. Trials were repeated twice.

Growth and pathogenicity of *P. cryptogea* in relation to temperature

PDA medium was seeded by isolate of *P. cryptogea* from *S. arachnoideum* using procedure described by Orlikowski *et al.* (2006). Plates (5 for each temperature) were incubated at temperature ranged from 5° to 35°C. Diameter of colonies was measured after 5-day incubation. Pathogenicity of *P. cryptogea* on *Sempervivum* leaves was estimated using the procedure described for laboratory trials at temperature from 5° to 35°C (4 replications x 10 leaves) and experiments were repeated twice.

Table 1. Fungi and *Algae* – like *Oomycetes* isolated from diseased *Sempervivum* spp. and *S. arendsii*; number of settled plants (a) and number of isolates obtained (b)

Genera/species	Isolation time									
	Sempervivum spp. June, 2004 (40 plants)		*Sempervivum* spp. July, 2005 (52 plants)		*S. arendsii* July, 2005 (28 plants)		*Sempervivum* spp. July, 2006 (32 plants)		*S. arendsii* Aug., 2006 (18 plants)	
	a	b	a	b	a	b	a	b	a	b
Alternaria alternata Nees	4	11	12	17	6	15	–	–	11	19
Botrytis cinerea Pers.	8	13	15	24	4	12	6	15	6	10
Cladosporium herbarum Link.	3	5	4	4	1	3	–	–	2	5
Fusarium avenaceum (Fr.) Sacc.	–	–	–	–	5	13	–	–	7	12
Fusarium culmorum (W.G.Sm.) Sacc.	7	12	15	22	–	–	10	18	2	4
Fusarium equiseti (Cda) Sacc.	2	3	7	12	3	5	4	6	–	–
Fusarium solani (Mart.) Sny et Hans	1	4	4	7	2	3	–	–	5	8
Mucor spp.	11	28	26	34	7	11	18	27	4	7
Penicillium spp.	9	14	19	27	4	7	11	18	2	5
Phytophthora cryptogea Pethybr. et Laff.	34	102	40	93	23	74	24	75	16	61
Trichoderma spp.	2	5	7	11	9	17	9	20	5	11

RESULTS

Isolation of *Phytophthora* and other microorganisms from diseased tissues

Ten genera and species were recovered from rotted leaves of *Sempervivum* but *Phytophthora cryptogea* was isolated the most often. The species was present in surveyed nursery during 3-year study. In the first 2 years the species was isolated from about 4/5 of analyzed plants whereas in the third year from 3/4 (Table 1). Among fungi known as plant pathogens 3 *Fusarium* species were isolated and additionally *Botrytis cinerea*. On *S. arendsii* rotted leaves *P. cryptogea* was found in the years 2005–2006 and the species was isolated from 6/7 and 8/9 of analyzed plants, respectively. Four *Fusarium* species and *B. cinerea* were isolated rarely or sporadically (Table 1). On 18 diseased plants of *Aquilegia discolor,* found in the nursery in 2006, *P. cryptogea* occurred on 5/6 of them. Other species, including *B. cinerea* and *Fusarium solani*, were isolated only from a few analyzed plants.

Colonisation of plant parts by *P. cryptogea*

The species colonized leaves of 8 *Sempervivum* species but did not cause any symptoms on *S. soboliferum* (Table 2).The data obtained indicated different reaction of tested *Sempervivum* species and cultivars to the pathogen. *S. arachnoideum*, *S. spinosum* and 3 cultivars (Alladyn, Mohogany, Lipar) were classified to the 4th group of the most susceptible plants. On their leaves necrosis spread about 7 mm/24 hrs whereas on leaf blades from the second group about 3 mm/24 hrs (Table 2).

Table 2. Susceptibility of *Sempervivum* species and cultivars to *P. cryptogea* from *S. arachnoideum*; length of necrosis (mm) on leaves 3 days after inoculation

Groups	*Sempervivum* species and cultivars
I. 0 a	*S. soculiferum*
II. 5.3 b – 10.3 d	*S. arachnoideum* Red Papaver, *S. ciliosum*, *S. ciliosum* Borisii, *Sempervivum* x *funeckii*, *S. tectorum* Purpurem
III. 12.5 e – 15.7 g	*Sempervivum* x *hybridum* Odditum, *Sempervivum* sp. Noir, *Sempervivum* sp. Fidel, *Sempervivum* sp. Pelcolor, *Sempervivum* sp. Commader Hay, *Sempervivum* sp. Othello
IV. 17.5 h – 20.1 j	*Sempervivum arachnoideum* Rubin, *Sempervivum* sp. Alladyn, *Sempervivum* sp. Mohogany, *Sempervivum* sp. Lipar, *Sempervivum spinosum*

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

Isolates of *P. cryptogea* from the greenhouse growing plants (*Alstroemeria, Gerbera*) as well as from field plants colonized *Aquilegia* and *Sempervivum* leaf blades with significant differences for particular cultures (Table 3). In both studies, after 3 and 5-day incubation the quickest spread of necrosis was observed on *Aquilegia* inoculated with culture from *A. discolor, G. jamesonii* and *S. paniculata* whereas the slowest on leaves treated with culture from *S. arachnoideum* D. Inoculation of *Sempervivum* with isolates of *P. cryptogea* from plants growing under covering and from *S. paniculata* and *S. arachnoideum* (Table 3) showed that all of them caused necrosis of leaves. Significant differences were especially seen after 5-day incubation. The quickest disease

spread was noticed when isolates from *G. jamesonii* and *S. arachnoideum* M. were used for inoculation of leaves whereas the slowest on leaf blades affected with *Alstremeria* isolate (Table 3).

Table 3. Colonisation of *A. discolor* (A) and *S. arachnoideum* (S) leaves by *P. cryptogea* in relation to isolate used for inoculation

Source of isolates	Length of necrosis [mm] after days of inoculation			
	3		5	
	A	S	A	S
Alstroemeria aurantiaca	4.1 b	12.3 b	10 c	18.5 a
Aquilegia discolor	5.2 de	11.2 ab	11.3 d	24.2 cd
Gerbera jamesonii	5.2 de	11.4 ab	13.3 e	26.6 e
Saxifraga arendsii	4.8 cd	12.0 b	8.8 b	25.5 c–e
Saxifraga paniculata	5.4 e	11.2 ab	10.9 d	23.5 bc
Sempervivum arachnoideum D	3.2 a	9.8 a	6.6 a	21.9 b
Sempervivum arachnoideum M	4.8 bc	11.9 b	9.6 c	26.1 de

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

The species colonized leaves of 3 cultivars of *S. arendsii*, *S. cotyledons* and *S. paniculata*. Analyses of necrosis length 6 days after leaf blades inoculation showed the quickest spread of the disease on *S. arendsii* Rot whereas the slowest on *S. arendsii* Peter and Aureovariegata (Table 4).

Table 4. Colonisation of *Saxifraga* spp. by isolate of *P. cryptogea* from *S. arendsii*

Saxifraga species	Length of necrosis [mm] after days of incubation	
	3	6
S. arendsii Rot	8.3 b	29.5 c
S. arendsii Peter Pan	5.3 a	13.2 a
S. arendsii Aureovariegata	6.3 a	12.0 a
S. cotyledons Pyramidalis	5.3 a	18.7 b
S. paniculata Bis	6.0 a	21.2 b

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

The isolate of the pathogen from *S. arendsii* colonized leaves of *Iberis sempervirens*, *Lavendula angustifolia*, *Sempervivum soboliferum* and *Vinca minor* with the quickest spread of necrosis on *S. soboliferum* (Table 5).

In the greenhouse trial isolate of *P. cryptogea* from *S. arachnoideum* caused leaf and petiole rot of *A. discolor*, *S. arendsii* and *S. arachnoideum* (Table 6). After 6 and 12-day

incubation quicker spread of *Phytophthora* rot was observed on *A. discolor* than on *S. arendsii* and *S. arachnoideum*.

Tabela 5. Colonisation of different plant species by isolate of *P. cryptogea* from *S. arendsii*

Plant species	Length/diameter of necrosis [mm] after days of incubation	
	3	6
Iberis sempervivum	6.3 b	15.1 c
Lavendula anqustifolia	5.3 b	10.0 b
Sempervivum soboliferum	5.8 b	21.0 d
Vinca minor	2.8 a	4.3 a

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

Table 6. Development of *Phytophthora* leaf rot on perennials inoculated with *P. cryptogea* from *S. arachnoideum,* in the greenhouse trial

Plant species	Length of necrosis [mm] after days of incubation	
	6	12
Aquilegia discolor	36.3 b	62.8 b
Saxifraga arendsii	14.5 a	27.5 a
Sempervivum arachnoideum	11.8 a	24.3 c

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

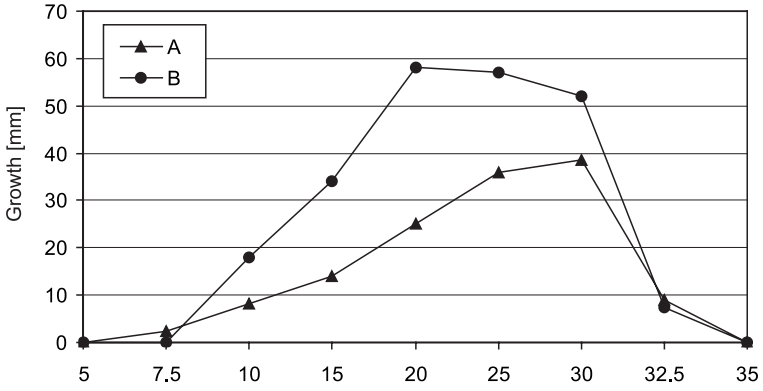

Fig. 1. Growth of *P. cryptogea* on PDA and colonisation of Sempervivum leaves; diam of colony (mm) after 5 days (A) and length of necrosis (mm) after 6 days of incubation (B)

Growth and pathogenicity of *P. cryptogea* in relation to temperature

Data presented in Figure 1 indicated the quickest development of the pathogen at 25–30°C with cardinal temperature between 5 and 7.5°C and 32.5–35°C. The fastest colonization of *Sempervivum* leaves was observed at temperature ranged from 20° to 30°C. Necrosis development was not observed on leaves incubated at 7.5°C and at 35°C (Fig. 1).

DISCUSSION

The data obtained indicated *P. cryptogea* as the predominant agent isolated from rotted leaves and stem bases of *Aquilegia, Saxifraga* and *Sempervivum* species. The pathogen was earlier found in ornamental hardy nursery stocks. Orlikowski *et al*. (1995) isolated it from rotted bases of *Abies alba*, *Pinus mugho* var. *pumilo* and *P. nigra*, whereas Szkuta (2004) from diseased *Abies glauca* and *Chamaecyparis lawsoniana*. The species was also recovered from rhizosphere of *C. lawsoniana* and *A. glauca* (Orlikowski and Ptaszek, unpubl.). In the studies of Themann *et al.* (2002) *P. cryptogea* was detected in water and sediments of German hardy ornamental nursery reservoirs. Till 1990 in Polish horticulture the mentioned species has been known only as the causal agent of *Phytophthora* foot rot of gerbera and stem rot of pachypodium and pelargonium (Orlikowski 1978, 1996, 2003). It is possible, that in the lack of closed recirculation system in greenhouses and plastic tunnels, the pathogen easily spread with surplus water from such objects to local streams, lakes or rivers and from those sources also to ornamental nurseries. In surveyed nursery only water from a well was used for plant sprinkling. It suggests that imported new species or cultivars of perennials could be the source of *P. cryptogea*. During sprinkling of plants, the pathogen present on diseased plant parts may be transferred with water drops on neighbouring perennials or its zoospores are transported in surplus water. Vegetative propagation of *Sempervivum* and *Saxifraga* spp. by their fragmentation favours also the pathogen expansion. The results obtained indicate the lack of drastic differences in colonization of plants by *P. cryptogea* isolates obtained from both greenhouse and field growing plants. It indicates that one or a few hosts may be the source of that species or points to the lack of specialization specific for host plant and the pathogen.

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REFERENCES

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

Orlikowski L.B. 2003. *Phytophthora* stem rot of *Pachypodium lameri* and its control. Phytopathol. Pol. 5: 17–21.

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

Orlikowski L.B. 2006. Gatunki rodzaju *Phytophthora* jako czynniki ograniczające plon i jakość roślin ozdobnych. Zesz. Probl. Post. Nauk Rol. 510: 407–411.

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

Orlikowski L.B., Valjuskaite A. 2007. New record of *Phytophthora* root and stem rot of *Lavendula angustifolia*. Acta Mycol. 42: 193–198.

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

Themann K., Werres S., Luttmann R., Dienner H.A. 2002. Observations of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock. Eur. J. Plant Pathol. 108: 337–343.

Westcott C. 1971. Plant Disease Handbook. 3rd ed. Van Norstrand Reinhold Co., New York, Cincinnati, Toronto, London, Mebourne, 709 pp.

POLISH SUMMARY

PHYTOPHTHORA SPP. W POLSKICH SZKÓŁKACH ROŚLIN OZDOBNYCH. I. BYLINY, NOWE ROLINY ŻYWICIELSKIE DLA *P. CRYPTOGEA*

Phytophthora cryptogea izolowano z porażonej podstawy pędów i liści *Aquilegia discolor, Saxifraga arendsii* i *Sempervivum* spp. Dodatkowo, z tkanek niektórych roślin izolowano gatunki rodzaju *Fusarium* i *Botrytis cinerea.* Większość gatunków i odmian rojnika, poza *S. soculiferum*, było kolonizowanych w warunkach laboratoryjnych przez *P. cryptogea*. Izolaty tego gatunku z *Alstroemeria aurantiaca, Gerbera jamesonii, Saxifraga arendsii, S. paniculata* i *Sempervivum arachnoideum*, kolonizowały liście rojnika, przy czym nekroza rozwijała się najszybciej na blaszkach liściowych zainokulowanych kulturami z *G. jamesonii* i *S. arendsii*. Izolat z *S. arendsii* kolonizował liście 5 gatunków i odmian skalnicy oraz *Iberis sempervivum, Lavendula angustifolia, Sempervivum sobuliferum* i *Vinca minor* przy czym nekroza rozwijała się najwolniej na barwinku. W badaniach laboratoryjnych izolat *P. cryptogea* z rojnika rozwijał się na pożywce PDA oraz kolonizował liście *S. arachnoideum* w zakresie temperatury od około 10 do 32,5°C przy optimum 20–30°C.