

FIRST NOTICE OF PHYTOPHTHORA CROWN AND ROOT ROT OF *EUPHORBIA PULCHERRIMA* IN POLISH GREENHOUSES

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Abstract: The plant pathogen, *Phytophthora cryptogea*, was recovered from 4/5 of the 75 poinsettias collected from two greenhouse farms. The poinsettias showed stunting and wilting as well as stem base and root rot symptoms. Representative isolates of the species from two poinsettia cultivars, colonized stem parts, leaf petioles, leaf blades and root parts. Stem parts of 6 cultivars were colonized at a slower rate than other poinsettia organs. Isolate from *Euphorbia pulcherrima* also colonized stem parts and leaf blades of *E. amygdaloides*, *E. cyparissias*, and *E. polychroma*. Isolates of *P. cryptogea* from the other 6 plant hosts, colonized the overground parts of poinsettia cv. Allegro. This is the first report describing symptoms and colonization of poinsettia cultivars by *P. cryptogea* in Poland.

Key words: *Euphorbia pulcherrima*, colonization, cultivars, occurrence, *Phytophthora cryptogea*, symptoms

INTRODUCTION

There are several diseases caused by leaf and soil-borne pathogens which affect poinsettia production. Benson *et al.* (2002) mentioned Alternaria blight (*Alternaria euphorbiicola* E.G. Simmons & Engelhard), gray mold (*Botrytis cinerea* Pers.), powdery mildew (*Oidium* sp.), scab (*Sphaceloma poinsettiae* Jenk. and Ruehle), Erwinia blight (*Pectobacterium carotovorum* Jones), Xanthomonas leaf spot (*Xanthomonas campestris* pv. *poinsettiicola* (Patel, Bhatt & Kulkarni), Pythium root rot (*Pythium aphanidermatum* (Edson) Fitzp., *P. irregulare* Buisman), Rhizoctonia stem rot (*Rhizoctonia solani* Kuhn), and black root rot (*Thielaviopsis basicola* Berk. and Broome). Additionally, Orlikowski *et al.* (2007) reported Fusarium stem rot and dieback of poinsettia caused by *F. oxysporum* Schlecht. The occurrence of Phytophthora crown and stem rot of poinsettia caused by *Phytophthora nicotianae* Breda de Haan var. *parasitica* (Dastur) in the USA, was described by Engelhard *et al.* (1979). Six years later, Yoshimura *et al.* (1985) reported the blight of poinsettia leaves, bracts and cyathia caused by the already mentioned species and *P. drechsleri* Tucker. Ann (1992) isolated *P. parasitica* from diseased roots of plants grown in Taiwan, but also mentioned *P. cryptogea* Pethybr. et Laff. In Japan, Kanto (2007) described Phytophthora blight incited by *P. nicotianae* whereas Lamour *et al.* (2003) noticed *P. drechsleri* on diseased poinsettia parts in Florida, USA. De Jensen *et al.* (2006) reported wilt and stem canker of poinsettia (*P. nicotianae*) growing in Puerto Rican greenhouses. In about half of the surveyed farms, the disease severity ranged from 5 to 100%, regardless of whether mefanoxam was used for plant protection or not. Recorded symptoms included stunted plants, thin stems, chlorotic leaves, and

brown-rot bract development. Leaves of affected plants wilted because of stem canker.

The attractiveness and continuing popularity of poinsettia in Poland during the Christmas season creates a high seasonal demand for this crop. At the end of summer and autumn 2010, wilting and dieback was observed on potted poinsettia cuttings and older plants growing in two greenhouses. On a few weeks old cuttings of cvs. Allegro and Saturnus Red, grown separately in two greenhouses, discoloration and wilting of leaf blades were observed. On the base of the stems, brown or dark brown spots were noticed. The spots were first on one side and then extended around the stems. Root tips were turning brown and dying on all plants. During the next 10 days, poinsettias up to 30 cm high, had their single shoots wilt or all of them wilt. Stem base rot extended upwards even to 10 cm, and necrosis spread on leaf petioles and blades. Most of the roots were brown or dark brown. At both farms, the disease severity ranged from about 7 to 10%.

The aim of our study was to isolate, identify, and estimate the pathogenicity of the causal agent of poinsettia dieback.

MATERIALS AND METHODS

Laboratory analysis of diseased plants

Seventy-five plants at different stages of development were collected from two greenhouses. In the first object (ST), poinsettias, with 'Allegro' as the main cultivar, were grown on about 20 000 m², whereas in the second (RS) on an area of 7000 m² with cv. Saturnus Red as the main cultivar (about 60% of growing plants). Plant samples to-

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gether with peat substratum were transferred in plastic bags to the laboratory. The procedure described by Orlikowski and Szkuta (2002) was used to isolate microorganisms from the diseased poinsettia tissues. Plants stem parts and roots were washed under running tap water and later with distilled water. Next, they were blotted dry and sterilized over a burner flame. Plant parts, which were about 5 mm in diameter were taken from the border of healthy and diseased stems tissues and transferred into Petri dishes (90-mm diameter) containing PDA (Potato Dextrose Agar). After 48 h of incubation at 25°C in the dark, small actively growing plugs of *Phytophthora* mycelium were transferred onto PDA slants. The slants were stored in the dark at 25°C, in the laboratory incubator.

Identification of isolates

Representative isolates were identified by morphological and cultural characteristics (Erwin and Ribeiro 1996). Identification was confirmed by PCR, with species-specific primers (Boersma *et al.* 2000; Minerdi *et al.* 2008).

Analysis of substratum for the presence of *Phytophthora* sp.

Substratum taken from poinsettia pots with disease symptoms, were also sampled for the occurrence of *Phytophthora* spp. by soil baiting technique using rhododendron leaves cv. Nova Zembla (Orlikowski and Ptaszek 2010). Leaves were laid in 10 mm of standing water, on the surface of the flooded substratum, in photographic tray (30 × 25 × 5 cm) at 22–24°C. After a 4 day-incubation, the leaves were removed, rinsed in tap water, and distilled water, blotted dry, and sterilized over a burner flame. The parts, which were about 5 mm in diameter, were transferred onto PDA. The rest of the procedure was similar to that done with the isolation and identification of *Phytophthora* spp. from diseased poinsettias.

Pathogenicity tests

Cultures

Isolates of *Phytophthora cryptogea* from diseased stem-bases of cvs. Allegro and Saturnus Red, and additionally from 6 other plant species growing under cover and in the field were chosen for further studies. Cultures were maintained on PDA at 25°C in the dark. For inoculation of plant parts, 3 mm in diameter plugs of medium which were overgrown by *Phytophthora* isolates and taken from the edge of 7-day-old cultures, were used.

Plant species and cultivars

Euphorbia amygdaloides, *E. cyparissias*, *E. polychroma*, *E. pulcherrima* and cultivars Allegro, Cristalo, Ice Punch, Peter Star Red, Poinsettia Pink, and Saturnus Red were used for the inoculation trials with *P. cryptogea* to estimate them as potential source of the pathogen.

Inoculation

About 5 cm long stem parts, leaf petioles, leaf blades and root parts were transferred to trays having sterile, moist, blotting paper covered with thin plastic net to keep plants over the blotting paper. *Phytophthora* inocula were

put on the bases of stem parts and roots as well as on the centre part of leaf blades. Then the trays were covered with thin foil. After a 5–7 day incubation, the length and diameter of necrosis were measured.

Greenhouse trial

Phytophthora cryptogea isolates obtained from the affected stem base of poinsettia, and substratum from the diseased plants were used for peat infestation. Cultures were grown on PDA medium in 90 mm Petri dishes at 25°C. Five mm in diameter discs overgrown by *P. cryptogea*, were taken from the edge of 7-day-old cultures and transferred onto Petri dish plates with rolled oats (Orlikowski 1999). After a 2-week-incubation at 25°C, cultures were blended with 150 ml of sterilised water in a Waring Blender to form a thick slurry. The homogenates were mixed with peat at a ratio of one plate per 1 dm³ of peat. After infested peat was stored in plastic bags for 10 days, the population density of *P. cryptogea* was measured using the method of Flowers and Hendrix (1969). By dilution of infested peat with uninfested substratum, the population density was assayed on the level of 350 colony-forming units/g of air dry substratum. Peat infested with both isolates of *P. cryptogea* and uninfested substratum (the control), were poured into 1 dm³ pots, and rooted poinsettia cuttings (cv. Allegro) were planted. Plants were cultivated on greenhouse benches at temperatures which ranged from 17 to 26°C for 10 weeks. At 2 week-intervals, number of plants showing disease symptoms were noted.

Statistical analyses

The experimental design was completely randomized with 4 replications and 5 plant parts and 10 cuttings in each replication. Trials were repeated twice, at 2 week intervals. The obtained results were elaborated using an analysis of variance. Significant treatment differences were evaluated by Duncan's multiple range test ($p = 0.05$).

RESULTS

Pathogens occurring on poinsettia plants

From 40 of the analysed, diseased plant parts, collected from farm ST, 32 of them yielded *P. cryptogea*. Additionally, *Alternaria alternata* Ness, *Botrytis cinerea* Pers., *Cladosporium herbarum* Link., *Mucor* spp., *Penicillium* spp. were detected from diseased tissues.

Among 35 poinsettias showing stem base rot and wilt symptoms, collected from greenhouse RS, *P. cryptogea* was recovered from 28 of cv. Saturnus Red. Besides the already mentioned species, *Fusarium avenaceum* (Cda) Sacc., *Pythium ultimum* Trow and *Trichoderma* spp. were isolated.

Pathogenicity of *P. cryptogea* isolates

The first laboratory inoculation trial was done with 4 poinsettia (*E. pulcherrima*) cultivars from the ST farm using isolate from cv. Allegro (Table 1). The culture colonised all parts of the poinsettias. After a 7-day-incubation, necrosis spread much faster on leaf blades (33.1–49.5 mm) and roots (32.1–44.5 mm) than on stem parts (21.9–34.6 mm).

Table 1. Relationship between poinsettia cultivars and colonization of plant parts by *P. cryptogea* isolate from cv. Allegro; length/diameter of necrosis (mm) 7 days after inoculation

Cultivars	Stem parts	Roots	Leaf blades	Leaf petioles
Allegro	33.9 b	34.9 a	49.5 b	39.0 b
Cristalo	21.9 a	32.1 a	33.1 a	31.1 a
Ice Punch	34.6 b	44.5 b	44.7 b	36.7 b
Peter Star Red	29.8 b	43.0 b	44.0 b	44.6 c

Means in columns, followed by the same letter, do not differ significantly at the 5% level according to Duncan's multiple range test

The largest differences in the rate of necrosis development were observed on leaf petioles. The spread of necrosis was from 31.1 to 44.6 mm/7 days. Cultivar Cristalo was significantly less susceptible than other tested cultivars (Table 1).

Inoculation of 6 cultivars of poinsettia organs by two isolates of *P. cryptogea* from cvs. Allegro and Saturnus Red, resulted in the colonization of all of them (Table 2). Stem parts were more resistant to infection than leaf petioles and leaf blades, probably due to degree of tissue suberisation. Additionally, isolate from cv. Allegro was more pathogenic toward stem parts than those from cv. Saturnus Red (Table 2). The obtained results indicated that different poinsettia cultivars are very susceptible to *P. cryptogea*, and also showed that all commercially growing cultivars may be the source of the pathogen.

In the root inoculation trials, two tested *P. cryptogea* isolates caused necrosis on abscised roots. The analysis of the colonization of root parts of cvs. Peter Star Red and Saturnus Red by the tested isolates, indicated that there was a small difference or a lack of differences in tissues colonization. Necrosis development was about 8 mm/24 h (Fig. 1).

Inoculation of 4 *Euphorbia* species, including *E. pulcherrima*, by *P. cryptogea* isolate from cv. Allegro, resulted in the development of necrosis, on stem parts and leaf blades (Table 3). The quickest spread of disease symptoms was observed on stem parts of *E. amygdaloides*, whereas the slowest was observed on *E. cyparissias*. Furthermore, the leaf blades of *E. pulcherrima* were the most susceptible to the tested isolate (Table 3). These results indicated that different plant species belonging to the Euphorbiaceae family are highly susceptible to *P. cryptogea*, and they could also be a source of that pathogen.

In pathogenicity trials with *P. cryptogea* isolates from 7 different host plants, all tested cultures were able to infect (Table 4) overground parts of poinsettia cv. Allegro. Isolates from *Gerbera jamesonii* and *Lycopersicon esculentum* were weaker colonizers of poinsettia organs than others. The quickest spread of disease was observed after inoculation of tissues by isolates from *E. pulcherrima*, *Saxifraga paniculata*, and *Sempervivum arachnoideum*. In general, stem parts were colonized more slowly than the other tested poinsettia organs. After 7 days of incubation, the length of necrosis was between 12.4–34.1 mm, whilst on leaf petioles 18.5–42.5 mm, and on leaf blades 24.4–53.5 mm (Table 4).

Table 2. Relationship between source of *P. cryptogea* isolates, *E. pulcherrima* cultivars, and colonization of plant parts; length/diameter of necrosis (mm) 7 days after inoculation

Cultivars	Isolate from cv. Allegro			Isolate from cv. Saturnus Red		
	stem parts	leaf petioles	leaf blades	stem parts	leaf petioles	leaf blades
Allegro	31.1 d-f	43.0 c	45.8 cd	21.9 a-c	43.5 c	39.8 a-c
Cristalo	24.7 c	43.5 c	44.5 cd	17.7 a	44.5 c	35.5 a
Ice Punch	33.5 ef	41.9 bc	43.5 b-d	19.3 ab	39.9 bc	42.4 bc
Peter Star Red	26.8 cd	46.0 c	49.0 d	23.2 a-c	45.4 c	37.6 ab
Poinsettia Pink	34.8 f	30.8 a	45.7 cd	32.6 ef	35.8 ab	42.0 bc
Saturnus Red	33.8 e-g	32.8 a	43.5 b-d	30.9 de	44.2 cd	49.3 d

Means in columns, followed by the same letter, do not differ significantly at the 5% level according to Duncan's multiple range test

Table 3. Colonisation of *Euphorbia* spp. parts by *P. cryptogea* isolate from *E. pulcherrima* cv. Allegro; length/diameter of necrosis (mm) 7 days after inoculation

<i>Euphorbia</i> species	Stem part	Leaf blades
<i>E. amygdaloides</i>	22.2 c	20.0 b
<i>E. cyparissias</i>	7.1 a	13.3 a
<i>E. polychroma</i>	13.9 b	16.3 a
<i>E. pulcherrima</i>	14.4 b	29.9 c

Means in columns, followed by the same letter, do not differ significantly at the 5% level according to Duncan's multiple range test

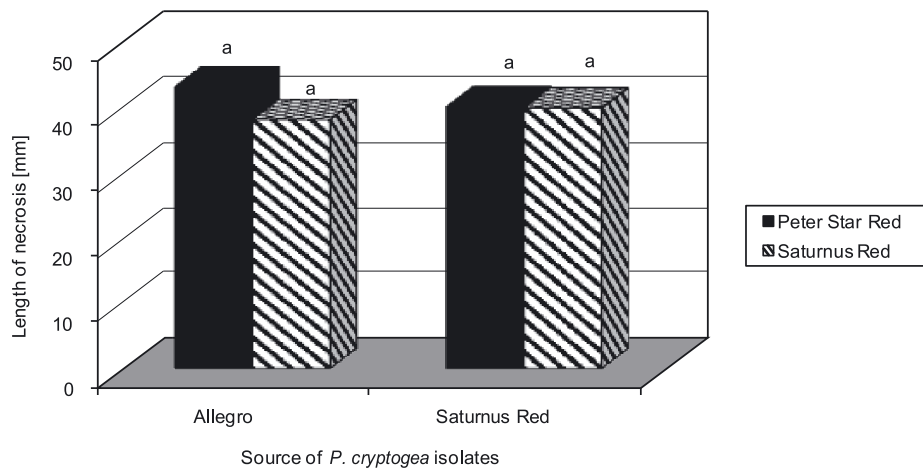


Fig. 1. Relationship between source of *P. cryptogea* isolates, poinsettia cultivars, and colonisation of roots 5 days after inoculation. Means in columns, followed by the same letter, do not differ significantly at the 5% level according to Duncan's multiple range test

Table 4. Relationship between source of *P. cryptogea* and colonisation of poinsettia parts by isolates from different host plants; length/diameter of necrosis (mm) 7 days after inoculation

Source of isolates	Stem parts	Leaf petioles	Leaf blades
<i>Alstroemeria × hybrida</i>	17.9 b	34.2 bc	23.4 a
<i>Euphorbia pulcherrima</i>	34.1 d	36.7 cd	53.5 d
<i>Forsythia intermedia</i>	14.9 ab	29.8 b	36.4 b
<i>Gerbera jamesonii</i>	14.1 ab	18.5 a	24.4 a
<i>Lycopersicum esculentum</i>	12.4 a	30.7 b	25.8 a
<i>Saxifraga paniculata</i>	32.8 d	40.5 de	47.3 c
<i>Sempervivum arachnoideum</i>	28.4 c	42.5 e	52.6 d

Means in columns, followed by the same letter, do not differ significantly at the 5% level according to Duncan's multiple range test

Greenhouse trial

Transplanting of rooting poinsettia cuttings into peat infested with *P. cryptogea* from the diseased plant, resulted in the sporadic occurrence of stem base rot symptoms after a 2-week-growth period (Fig. 2). Within the next 8 weeks, wilting of plants and development of stem ne-

erosis on the base was observed on poinsettia grown in peat infested with both isolates. After a 10 week-growth period, about 7/10 plants were affected by *P. cryptogea*. Statistical analysis showed the lack of significant differences between the pathogenicity of tested isolates in all observation periods (Fig. 2).

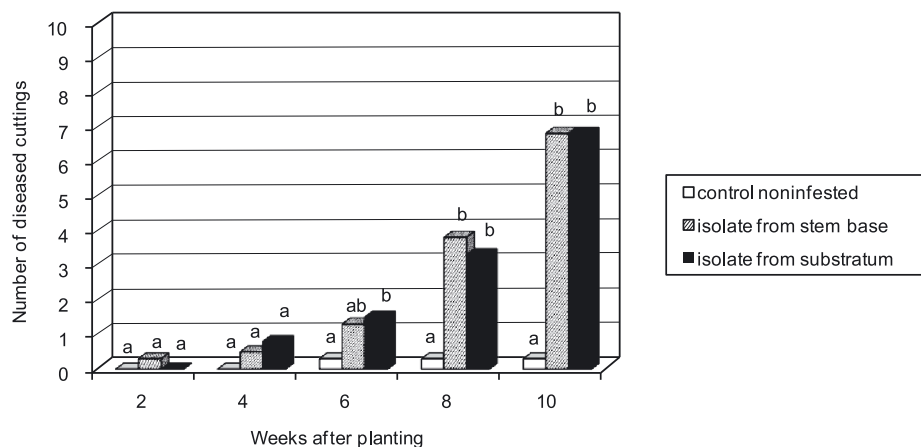


Fig. 2. Relationship between source of *P. cryptogea* isolates, growing period, and healthiness of poinsettia cuttings cv. Allegro. Means in columns, followed by the same letter, do not differ significantly at the 5% level according to Duncan's multiple range test

DISCUSSION

Results obtained during this study showed that besides *F. oxysporum* (Orlikowski *et al.* 2007), *P. cryptogea* presents a serious threat for poinsettia growers as well. Further studies which deal with the possibility of its control are necessary. *Phytophthora cryptogea* dominated among 9 genera and species isolated from 75 diseased stem bases of *E. pulcherrima*. The species settled 4/5 of poinsettias in both greenhouses whereas other fungal species occurred rarely or sporadically. It is possible that *B. cinerea* and *F. avenaceum*, known as plant pathogens, colonized weakened tissues of poinsettia infected already by *P. cryptogea*. In both greenhouse farms, transplanting of young rooted poinsettias to new pots and fresh peat substratum, resulted in the occurrence of disease symptoms. Stem base rot and wilting symptoms, mainly on cv. Allegro in one farm and cv. Saturnus Red in the other, indicated that these cultivars were the primary sources of *P. cryptogea* brought to greenhouse farms. Other cultivars grown at both farms, did not show any disease symptoms. The indication was that *P. cryptogea* was brought in on rooting cuttings.

Symptoms observed on affected poinsettias were similar to those described for diseases caused by *P. cryptogea* on other ornamental plants (Daughtrey *et al.* 1995). The symptoms include stunt, wilt, chlorosis, stem base and root necrosis. Ann (1992) detected this species from diseased poinsettia roots in Taiwan, but did not study its pathogenicity. Our results from pathogenicity trials of the mentioned species to 6 poinsettia cultivars indicated that, till now, there are no resistant varieties on the market. For this reason, the data may be an important indication for poinsettia breeders to include in a breeding programme new cultivars, which are resistant to *P. cryptogea* and other species from that genera.

Preliminary tests demonstrated that *P. cryptogea* isolates recovered from 7 different plant species are not only restricted to their hosts but exhibited a wide range of activity. All of them colonized poinsettia and also other species, but significant differences in their pathogenicity was noticed on particular plant parts. Studies of Orlikowski and Ptaszek (2007; 2008; 2010) and Ptaszek and Orlikowski (2010) showed the colonization of new host plants, including bushes and perennials, by that species. A wide plant range of *P. cryptogea* was described earlier by Erwin and Ribeiro (1996), but during the last 20 years the number of hosts increased by at least 30%. These new hosts could be the source of *P. cryptogea* for horticultural plants, including *E. pulcherrima*. This study indicated *Euphorbia* spp. as the next hosts for *P. cryptogea*. This is the first report describing the disease caused by that pathogen on *E. pulcherrima* in Polish greenhouses and probably in other European countries.

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