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FIRST NOTICE OF *PHYTOPHTHORA* AERIAL BLIGHT AND CROWN ROT ON PANSIES IN POLAND[1]

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Abstract: *Phytophthora cactorum* was detected on 9/10 of pansies showing yellowing of leaves and crown rot symptoms and constituted about 90% of isolates obtained. *Botrytis cinerea, Fusarium avenaceum, F. solani* and *Pythium ultimum* were also isolated from diseased tissues. Using rhododendron leaves as the bait, *P. cactorum* was detected in pansy substratum as well as from soil under the mata. Isolates obtained from diseased plants, substratum and soil under mata colonized leaves, stem parts and roots of pansy. Necroses spread faster on organs inoculated with cultures from plants and substratum. Among 25 cultivars inoculated with *P. cactorum*, disease symptoms did not occur on 3 of them, whereas the fastest spread of necrotic spots (3.8 mm/24 hrs) was noticed on 3 cultivars. Isolates of *P. cactorum* from *Begonia semperflorens* and *Malus domestica* colonized leaf petioles of pansy with significantly faster spread when isolates from begonia and pansy were used for inoculation.

Key words: occurrence, *Phytophthora*, isolate, pansy, substratum, soil, isolation, colonization

INTRODUCTION

Pansies (*Viola x wittockiana*) are important ornamentals which have grown in popularity over the last 10 years as plants for gardens and landscapes. When planted in autumn they will grown till early summer. The quality of plants may be strongly decreased by diseases. It was 100 years ago that Wolf (1910) mentioned pathogens on pansies. He mainly mentioned leaf spot pathogens, among them *Colletotrichum vilae-tricoloris* Smith and *Cercospora violae-tricoloris* Smith which are still the most common (Mullen and Hagen 2001). Among soil-borne pathogens *Thielaviopsis basicola* Berk et Broome, *Myrothecium roridum* Link. and *Phytophthora nicotianae* var. *nicotianae* Breda de Haan can cause high losses in pansy production (Leahy 1998; Mullen and Hagen 2001). Also Hwang and Benson (2005) isolated *P. nicotianae* A1 compatibility type from diseased pansy. About 20 years earlier, however, Schubert (1983) classified *Viola* as a resistant plant on *P. nicotianae* var. *parasitica* Dastur. In the autumn of 2009 aerial blight and crown rot symptoms were observed on pansies grown in nursery containers in the southwest part of Poland. In the opinion of the growers, the disease also occurred on plants in previous years with losses of even up to 30%. The purpose of this study was (1) isolation of a causal agent of the disease from affected plants (2) identification to genera and species and (3) evaluation of its pathogencity to pansy cultivars and other plants.

MATERIALS AND METHODS

Plants

Pansies were taken from a horticulture farm which produced about 100.000 plants for autumn planting on November 11th, 2009. Plants were grown in one liter pots on black mata covering sandy-clay soil in nursery containers in the southwest part of Poland. On about 20% of plants were observed; growth inhibition, yellowing of leaves near the base with the disease spreading upwards, dying of single stems and a slowing down of all branching (Fig. 1).

Fig. 1. Development of *Phytophthora* crown rot of pansies

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The color of stem tissue above and sometimes below the substratum line changed to gray-brown and dark-brown, on the length to 5 cm. The stem tissue appear water soaked. On some plants white mycelium covering the diseased parts of stem bases was observed (Fig. 2). Disease symptoms were mainly observed on pansies from the Colossus group and on cv. Delta Premium Yellow with Blotch.

Fig. 2. *Phytophthora* crown rot of pansy

Mycological analyses

Thirty plants with yellowing of leaves and stem base necroses were taken together with substratum, from different farms. They were put individually into plastic bags and transported to the laboratory. Each diseased plant was washed under tap water, dried between two layers of blotting paper and separated into 10 cm parts.

After sterilization over a burner flame, stem or root pieces which were about 5 mm long were put on PDA medium in 90 mm diam Petri dishes (10 parts/plate). Within 24–48 hrs incubation colonies growing around inocula were transferred into PDA slants. After the next 10 days the obtained colonies were grouped on the basis of growth pattern and morphology and chosen isolates were identified by genera and species, using monographs and keys. In the case of *Phytophthora,* confirmation of isolates according to species, was performed by DNA analysis using PCR with species specific primers (Boersma *et al*. 2000; Trzewik *et al*. 2006).

Detection of *Phytophthora* from substratum

The procedure described by Orlikowski (2006) was used. Substratum or soil samples were collected from 3 pansy farms (marked as K1, K2 and K3) in the south-west part of Poland. Substratum taken from 4 pots, in which growing pansies showed crown rot symptoms, were put into a tray, mixed together and 500 g of such a mixture was submerged with tap water. Substratum samples were collected from different parts of farms. Soil samples from under the mata were collected from 4 different places and mixed together. They were analyzed for the presence of *Phytophthora*. Eight rhododendron leaves from the top of cv. Nova Zembla shoots were put on the surface of emerged samples and ashes, and covered with foil. After 4 days of incubation in the dark, at 22–24°C, rhododendron leaves were removed from trays, washed under tap of water, blot dried and the number of necrotic spots on each of them was counted. Part of the leaves with necrotic spots were transferred on PDA using the same procedure as with the pansy plant parts.

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

Stock cultures were grown on PDA medium in the dark, at 24°C. After 7 days, pieces of medium which were 3 mm in diameter were transferred onto the middle of leaves or stem bases and root parts in trays with sterile moist blotting paper covered with plastic net. Within 6 days, the diameter of spots or the length of necroses was measured. Additionally, colonization of pansy leaf petioles by *P. cactorum* from begonia and diseased apple trees was estimated using the same procedure as in previous trials.

Experimental design was completely randomized with 4 replications and 5 plant parts in each replication. Trials were repeated at least twice at 2 week intervals.

RESULTS AND DISCUSSION

Fungi isolated from diseased pansies

P. cactorum (Lebert et Cohn) J. Schrot. dominated among 5 species isolated from diseased stem bases. The species were obtained from 27 analyzed plants whereas *Botrytis cinerea* Pers., *Fusarium avenaceum* (Fr) Sacc., *F. solani* (Mart.) Sny et Hans., *Penicillium* spp. and *Pythium ultimum* Trow were recovered from 7 pansies. Isolates of *P. cactorum* constituted about 90% of all obtained culture.

Detection of *P. cactorum* from substratum and soil

P. cactorum was detected from all analyzed samples of pansy substratum (Fig. 3) as well as from under the

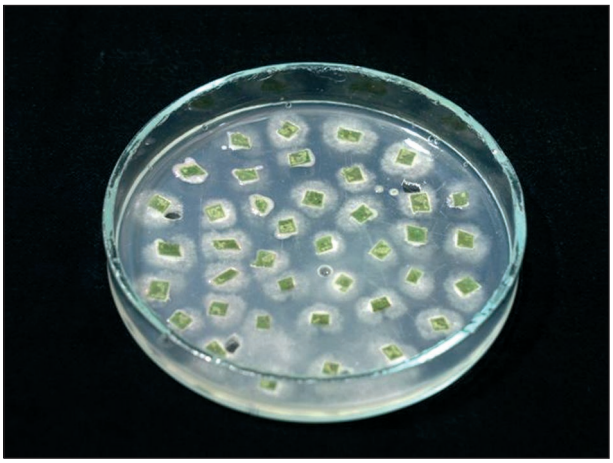


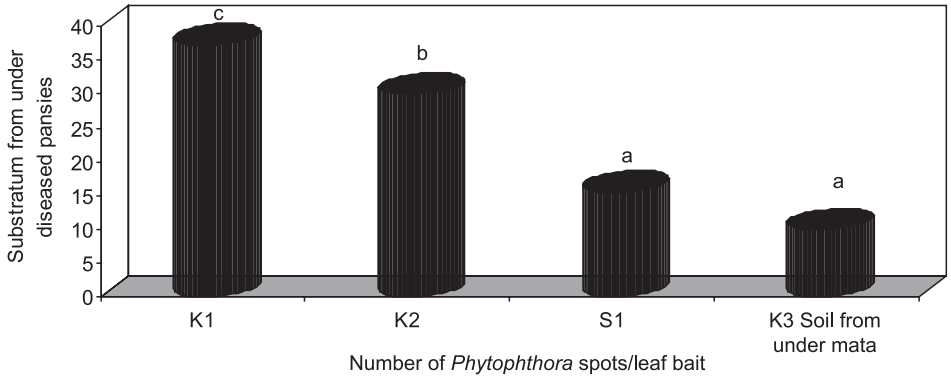
Fig. 3. Colonies of *Phytophthora* growing around pieces of leaf bait used for isolation of. *P. cactorum* from pansy substratum

mata (Fig. 4). Analyses of spot number on baiting rhododendron leaves showed significant differences between *P. cactorum* density. The population density of that species was the highest on K1 farm; it was the lowest on S1 farm and in soil K3 under the mata (Fig. 4).

Colonization of pansy parts by *P. cactorum* isolates

Three isolates obtained from the plant base, infested substratum and from under mata, colonized leaf blades, petioles, stem parts and roots of pansy (Table 1). The slowest development of necroses was noticed on roots and there were no significant differences between isolate used and length of colonized tissues. Significant differences were found in the length of necroses on leaf petioles and stem parts or diameter of spots on leaves. These values were similar in the case of inoculation of the mentioned parts of pansy, by isolates from plant base and substratum. Culture from soil under the mata was less pathogenic (Table 1).

In the next trial, colonization of 4 cultivars of pansy by isolate of *P. cactorum* from substratum K1 was estimated (Fig. 5). Measurement of necrotic spots on leaves 4 and 6 days after their inoculation, showed that the disease spread significantly faster (about 4,5 mm/24 hrs) on cvs



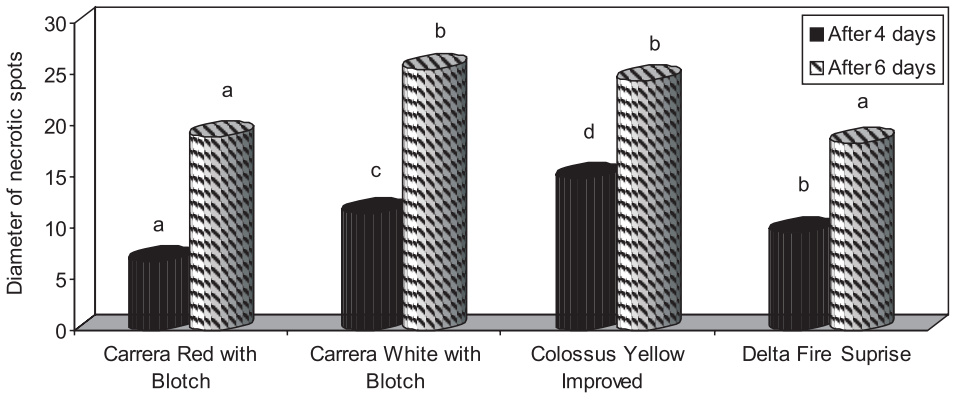
Values in columns with the same letters, do not differ with 5% of significance according Duncan's multiple range test

Fig. 4. Number of necrotic spots on leaf baits of rhododendron used for detection of *P. cactorum* from naturally infested substrata and from soil under mata

Table 1. Relationship between source of *P. cactorum* isolates, and development of necroses on pansy organs; diam/length of necroses in mm, 5 days after inoculation

Part of pansy	Source of isolates		
	B1 from the plant base	P2 from naturally infested substratum	M3 from the soil under the mata
Leaf blades	21.1 b	22.2 b	17.4 c
Leaf petioles	17.2 b	9.7 a	7.9 a
Stem bases	35.0 c	32.0 c	12.5 b
Roots	9.5 a	7.0 a	8.5 a

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



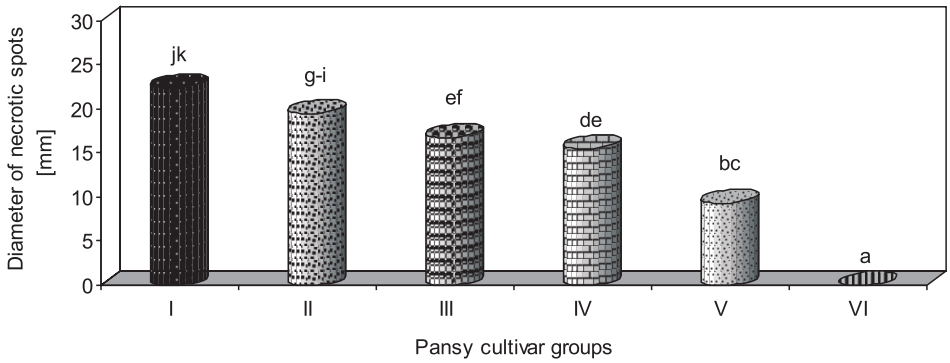
Values in columns with the same letters, do not differ with 5% of significance according Duncan's multiple range test

Fig. 5. Colonization of 4 pansy cultivars by isolate of *P. cactorum* from substratum

Table 2. Colonization of leaf petioles of pansy by isolates of *P. cactorum* from different host plants

Source of *P. cactorum*	Length of necroses in mm after days of incubation	
	5	7
B. semperflorens – stem base	6.7 b	12.7 b
M. domestica – stem base	4.0 a	6.4 a
Viola x *wittrockiana* – rotted shoot	6.3 b	14.9 b

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



Values in columns with the same letters, do not differ with 5% of significance according Duncan's multiple range test

I. Carrera Yellow White Blotch, Carrera Red With Blotch, Delta Yellow With Blotch
II. Carrera Deep Blue, Carrera Blue With Blotch, Carrera Yellow, Carrera White with Blotch, Carrera Rose With Blotch, Colossos Tricolor, Colossus Lovcuder Soprise, Delta Pure Light
III. Colossos White Purple Ring, Delta Pure Old Yellow, Delta White With Blotch
IV. Colossos White, Collosos Pure Rose, Colossos Rose Surprise, Delta Yellow White Red Wing
V. Colossos Yellow Imp., Delta Fire Surprise, Delta Persien Surprise, Delta Beacons Field
VI. Delta Violet Old White, Delta True Blue, Delta Yellow With Purple Wing

Fig. 6. Colonization of pansy cultivar leaves by *P. cactorum*, isolate from diseased stem base; diam of necrotic spots in mm 6 days after inoculation

Carrera White with Blotch and Colossus Yellowed Improved than on Carrera Red with Blotch and Delta Fire Surprise (Table 2).

Pansy growers indicated a different susceptibility of cultivars to *P. cactorum*. In laboratory trials colonization of leaves taken from 25 cultivars growing in the same conditions and during the same time, was estimated. The obtained results showed different reactions of tested cultivars to the *P. cactorum* isolate from diseased stem (Fig. 6). Disease symptoms did not develop on 3 cultivars. On the others, necrotic spots spread on leaves from about 1.5 to 3.8 mm/24 hrs with the fastest development on Carrera Yellow with Blotch, Carrera Red with Blotch and Delta Yellow with Blotch (Fig. 6).

Inoculation of pansy leaf petioles by isolates from begonia and apple tree resulted in colonization of tissues with significantly slower necroses spread, when the culture from *Malus domestica* was used for inoculation (Table 2).

Our studies indicate that pansy is the new host of *P. cactorum* in Polish horticulture. The species was previously found as causal agent of crown rot of *Sorbus aucuparia, Pelargonium grandiflorum* (Orlikowski *et al.* 2004, 2010), *M. domestica* and *Begonia semperflorens* (Orlikowski *et al.*, unpubl.). Mullen and Hagan (2001) noticed that foliar blight and crown rot symptoms developed rapidly at a temperature of 28°C or higher. The author found that excessive application of fertilizer will result in increased susceptibility of plants to *Phytophthora*. In our studies, among 25 tested cultivars a significant reaction of them to *P. cactorum* was found. The results obtained indicated that Carrera Yellow White Blotch, Carrera Red With Blotch and Delta Yellow with Blotch are very susceptible to *Phytophthora* crown rot. Growing of pansies in nursery containers, usually situated on small slops, favours the development and spread of the pathogen. The formation of zoosporangia on diseased stem was caused by a higher temperature in pots than in soil, watering of plants by sprinkling, and splash which released zoospores into the water. Zoospores are then carried to other plants spreading the pathogen in the nursery. Detection of *P. cactorum* in soil under mata indicated the presence of the pathogen and that means the possibility of its survival for at least 3–5 years. Besides infested soil in containers in nurseries, imported young seedlings may be even sporadically invaded by the pathogen. Occurrence of that species in new pansy farms confirmed this hypothesis. Additionally, other ornamentals, including begonia and pelargonia could also be the pathogen source.

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POLISH SUMMARY

PIERWSZE DONIESIENIE O WYSTĘPOWANIU ZARAZY PĘDÓW I ZGNILIZNY PODSTAWY BRATKÓW W POLSCE

Gatunek *Phytophthora cactorum* uzyskano z 9/10 analizowanych bratków wykazujących żółknięcie i zamieranie liści i zgniliznę podstawy. Stanowił on około 90% wszystkich uzyskanych izolatów. *Botrytis cinerea, Fusarium avenaceum, F. solani* i *Pythium ultimum* izolowano tylko sporadycznie. Stosując liście różanecznika jako pułapki, *P. cactorum* wykryto w podłożu spod porażonych roślin oraz w glebie pod matą na kontenerowni. Izolaty *P. cactorum* z porażonych roślin, podłoża i z gleby kolonizowały liście, części łodyg i korzenie bratków, przy czym zgnilizna rozwijała się szybciej na organach zainokulowanych izolatami z porażonej rośliny i podłoża. Spośród 25 odmian bratków zainokulowanych *P. cactorum*, zgnilizna nie rozwijała się na 3 z nich, natomiast najszybszy rozwój choroby (3,8 mm/dobę) stwierdzono na 3 innych odmianach. Izolaty *P. cactorum* z porażonej begonii i jabłoni kolonizowały ogonki liściowe bratków przy istotnie szybszym rozwojem nekrozy w przypadku ich zakażenia przez kultury z begonii.