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The survey of some factors affecting bark lesion development caused by *Phytophthora cactorum* on common beech and other broadleaved trees

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ABSTRACT: The three experiments relating to the pathogenicity of *Phytophthora cactorum* to beech and other forest tree species were carried out. The experiments were aimed to confirm pathogenicity of the pathogen, to compare its pathogenicity with the other *Phytophthora* species isolated from woody plants in the Czech Republic (*P. gonapodyides*, *P. cambivora*, *P. citricola* s.l., *P. cinnamomi*, *P. citrophthora*), to confirm its substrate specificity and diverse pathogenicity to common forest tree species (common beech, pedunculate oak, sycamore, small-leaved lime, black alder, common ash) and to determine the influence of excessive watering on the stem canker development. We found out that the tested isolate of *P. cactorum* was more effective to the host than isolates of *P. gonapodyides* and *P. cambivora*. The isolates of *P. cinnamomi* and *P. citrophthora* caused the largest necroses. It emerged that all tested tree species were susceptible to *P. cactorum*. The most susceptible tree species were sycamore and common beech. The most resistant tree species were common ash and pedunculate oak. The existence of substrate specificity of the pathogen was unequivocally confirmed. It was found out that the water stress could play an important role in the bark lesion development. We found out important differences in lesion development in different periods during growing season (June, September).

Keywords: artificial infection; bark lesion; broadleaved trees pathogenicity; common beech; *Fagus sylvatica*; *Phytophthora cactorum*; substrate specificity; water stress

Phytophthora cactorum (Lebert & Cohn) J. Schröt is the dangerous pathogen of some broadleaved tree species belonging to genera *Acer, Aesculus, Castanea, Fagus, Fraxinus, Juglans, Prunus, Pyrus, Quercus, Salix, Ulmus* etc. and many ornamentals including *Rhododendron* spp. (Erwin, Ribeiro 1996). Especially, *P. cactorum* has been known as a cause of damping-off disease in beech seedlings in several European countries (Erwin, Ribeiro 1996). The small-scale nursery survey in Germany revealed that beech fields are regularly infested with *P. cactorum* (Jung et al. 2005). On the other hand, the pathogen causes collar and stem lesions of beech and other woody plants. The disease severity has arised in some European countries recently (e.g. Jung et al. 2005; Brasier, Jung 2006).

The damping-off disease in beech seedlings was repeatedly mentioned in the Czech Republic (e.g. Jančařík 2003 and many others). The diseases of ornamentals caused by *P. cactorum* were reported in the area as well (e.g. Nicklová-Navrátilová 1949; Cejp 1961). However no extensive investigation of *Phytophthora* species (including *P. cactorum*) on forest tree species has been carried out in the Czech Republic yet. We have found neither precise

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pathogen description in Czech contemporary phytopathological literature nor an isolate deposited in any Czech culture collection yet. The morphological similarity of many *Phytophthora* species (including *P. cactorum*) is well-known. Thus some Czech reports of *P. cactorum* without clear confirmation of the pathogen identity should be regarded with some caution.

The first authentic isolates of *Phytophthora cactorum* were acquired during the contemporary investigation of phytophthora diseases of forest and ornamental woody plants from beech, white poplar, horse chestnut and rhododendron (Mrázková et al. 2008; Cerny et al. 2009). Although the investigations leading to definition of the host spectrum and to decription of the pathogen variability in the Czech Republic have still been in progress, we started the experiments concerned with the pathogenicity of *P. cactorum* to forest tree species. These experiments were aimed to compare pathogenicity of *P. cactorum* with another *Phytophthora* species isolated from woody plants in the Czech Republic (*P. gonapodyides*, *P. cambivora*, *P. citricola* s.l., *P. cinnamomi*, *P. citrophthora*), to confirm the pathogenicity of *P. cactorum* to common forest tree species (common beech, pedunculate oak, sycamore, small-leaved lime, black alder, common ash), to detect potential substrate specificity within *P. cactorum*, and to verify the effect of water stress on the stem lesion development caused by *P. cactorum*. The article deals with the outcomes of these first infection experiments.

MATERIAL AND METHODS

Phytophthora isolates used in the study

The *Phytophthora* isolates used in the study were acquired in 2006 and 2007 from different regions in Bohemia. The isolates of *P. cactorum* were acquired from stem lesions of white poplar, common beech and horse chestnut. The identity of the isolates was verified by morphological analysis as well as by the analysis of the ITS regions (Cerny et al. 2009). *P. cactorum* isolates are deposited in the Silva Tarouca Research Institute for Landscape and Ornamental Gardening (RILOG) culture collection, Culture Collection of Fungi (Prague) and their sequencies in GenBank. The *P. cactorum* isolates used in the study: P066.07 (isolated from white poplar, CCF Acc. No. 3757, GenBank Acc. No. EU562207), P078.07 (common beech, CCF 3768, GenBank EU638290) and P100.07 (horse chestnut, CCF 3762, GenBank EU562209). The short morphological description of the species was given in Cerny et al. (2009). The isolates of the other *Phytophthora* species used in the experiments are deposited in RILOG culture collection and four of them in Culture Collection of Fungi (CCF), Prague. Their identity was confirmed by morphological analysis as well as by the analysis of the ITS regions (Mrazkova et al. 2007, Mrázková et al. 2008; Černý et al. 2008). The isolates used in the study are *P. gonapodyides* isolate No. P002.06 (isolated from stem lesion of red oak, CCF Acc. No. 3681, GenBank Acc. No. EF194774), *P. cambivora* P020.06 (stem lesion of sweet chestnut, CCF 3682, GenBank EF194777), *P. citricola* s.l. P159.07 (root rot of pedunculate oak), *P. citrophthora* P081.07 (leaf spot of rhododendron, CCF 3768, GenBank EU638290), and *P. cinnamomi* P107.07 (collar rot of rhododendron, CCF 3763, GenBank EU562211). Jung and Burgess (2009) revealed this year that *P. citricola* composed from group of very close taxa (*P. plurivora*, *P. multivora*, *P. citricola* group I, and *P. citricola* s.s.). Because of the unclear identity of the Czech population of this pathogen, we use in this article the name *P. citricola* s.l.

Plant material

In the experiment we used the 2/3-year old saplings (height 40–60 cm) of common beech, pedunculate oak, sycamore, small-leaved lime, black alder, and common ash. The saplings were potted at the end of March 2007 into 18 × 18 cm plastic containers filled with sterile peat substrate (pH 5). Then the saplings were cultivated in hotbed until they came into leaf. During the course of own experiments the saplings were cultivated in greenhouse at temperature ca 23–25°C, air humidity 40–60% and watered with tap water if needed. All the three infection experiments took 4 weeks; the plants were randomized.

Infection experiments

Infection experiment I. Comparison of *Phytophthora* spp. pathogenicity to common beech.

The tested *Phytophthora* species were: *P. cactorum* (isolate No. P078.07), *P. gonapodyides* (P002.06), *P. cambivora* (P020.06), *P. citricola* s.l. (P159.07), *P. citrophthora* (P081.07), and *P. cinnamomi* (P107.07). There were used 2-year old saplings of common beech in the experiment. The stems of all saplings were surface sterilized with 95% ethanol. There were made injuries with a cork borer (5 mm diameter) about 5 cm above the collar. The agar plugs (5 mm diameter) from actively growing colony margin were placed in the

Table 1. Length of bark lesions caused by different *Phytophthora* species on common beech saplings after 4 weeks. Values marked by the same letter ($^{a, b, c}$) are not statistically different

Species	Mean (± SE)
P. gonapodyides	13.17 (± 0.71)^{a}
P. cambivora	16.17 (± 2.21)^{a}
P. cactorum	25.67 (± 2.70)^{b}
P. citricola s.l.	28.00 (± 1.40)^{bc}
P. citrophthora	37.25 (± 7.31)^{bc}
P. cinnamomi	40.67 (± 4.41)^{c}

SE – standard error

injuries and sealed with Parafilm. The control plants were treated in the same manner with sterile agar plugs. There were 20 plants in each infection treatment and in the control group, too. The length of all lesions was measured at the end of the experiment. The experiment was carried out in June 2008.

Infection experiment II. Confirmation of substrate specificity in *P. cactorum*.

There were tested three *P. cactorum* isolates Nos P066.07, P078.07, P100.07 we had acquired from different hosts in different locations in the Czech Republic. There were used 3-year old saplings of common beech, pedunculate oak, sycamore, small-leaved lime, black alder, and common ash (15 plants in each isolate/host combination and in control groups). The inoculation process was the same as described before. The experiment was carried out in June, too.

Infection experiment III. Confirmation of water stress effect on lesion development.

The *P. cactorum* isolate No. P078.07 and 2-year old saplings of common beech were used in the experiment. The inoculation process was the same as described above. The first group (15 saplings) was artificially infected by the isolate of *P. cactorum*. The second one (15 saplings) was inoculated and waterlogged and put in trays. The stable water level in containers was kept ca 3 cm above the bottoms. The experiment was carried out in September.

The application of the identical isolate (P078.08) in all three experiments and the same inoculation and cultivation technique allowed us to compare the infection development between two periods during the growing season: June (the first and second experiments) and September (non-waterlogged treatment in the third experiment).

Statistical evaluation

The length of stem necroses in all three experiments was measured after 4 weeks. Statistical evaluation was done by means of the statistical package STATISTICA 8.0 (StatSoft Inc.). The variability in measured data was too high, so we transformed them by common logarithm. Then the assumptions of normality and homogeneity were tested. The assumption of normality was fullfilled in all three experiments. The Levene's tests of homogeneity of variances remained positive ($P < 0.01$), however the share of maximal and minimal standard deviations (SD) in length of lesions in particular groups of plants was relatively low ($max_{SDi}/min_{SDi} < 3$) and enabled the processing of the first and second

Fig. 1. Bark lesions caused by different *Phytophthora* species on common beech saplings after four weeks. From left to right: control, *P. gonapodyides*, *P. cambivora*, *P. cactorum*, *P. citricola* s.l., *P. citrophthora*, *P. cinnamomi*

experiments by means of ANOVA (Hendl 2006). The third experiment was assessed with use of *t*-test with separate variance estimates. The differences in lesion length between June and September was assessed with non-parametric Mann-Whitney *U* test.

RESULTS

Infection experiment I. Comparison of *Phytophthora* spp. pathogenicity to common beech.

The analysis of variance showed, that the common logarithm of lesion length was statistically influenced by factor *Phytophthora* species (SS = 2.18, df = 5, MS = 0.44, $F = 17.53$, $P << 0.01$). The post-hoc comparisons (Tukey's test) showed important differences among studied *Phytophthora* isolates (Table 1). Isolate of *P. gonapodyides* was the least aggressive (mean of lesion length was 13.17 mm), the most aggressive was the *P. cinnamomi* isolate (mean 40.67 mm) (Fig. 1). *P. cactorum* isolate (mean 25.67 mm) was moderately pathogenic. The length of lesions caused by *P. cactorum* was statistically different from those caused by *P. gonapodyides* and *P. cambivora* isolates on one hand and from the most aggressive *P. cinnamomi* isolate on the other hand (Table 1).

Infection experiment II. Confirmation of substrate specificity in *P. cactorum*.

The analysis showed, that the lesion length in the experiment was influenced by host species and by interaction of host species and isolate identity, too. The effect of the host species and the interaction was statistically highly conclusive ($P < 0.000$). The effect of isolate *per se* was not proved (Table 2).

The differences in susceptibility to the pathogen among host species were evident from the first view (Table 3, Fig. 2). The most susceptible host species to the pathogen inoculation was sycamore (mean of lesion 48.16 mm; $P < 0.05$) and the second one was beech (mean of lesion 22.07 mm). The differences among lesion extent on beech, alder and lime were distinct, but not statistically significant. The statistically ($P < 0.05$) most resistant hosts were ash (mean of lesion 5.07 mm) and oak (4.53 mm). When the effect of interaction host and isolate was evaluated (i.e. host specificity), it showed that the isolate P066.07 was significantly more aggressive in oak (mean of lesion 9.87 mm) than the other two isolates (2.47 and 1.27 mm). The post-hoc test showed significant differences among lesions caused by different isolates in hosts and potentially complicated pattern of the substrate specificity (Table 3). All the three tested isolates were aggressive towards sycamore, beech and alder in similar pattern (the most aggressive was the P078.08 isolate acquired from beech, the least agressive one was the P066.07 from poplar). The susceptibility of lime to particular isolates was nearly equal (Table 3, Fig. 2). The pattern of aggressivity in ash was rather different – the more aggressive was the isolate P100.08 compared to P066.08 ($P < 0.05$). The most aggressive isolate in oak (in comparison to both others) was P066.07 ($P < 0.05$).

Infection experiment III. Confirmation of water stress effect on lesion development.

The experiment showed unequivocal change in lesion length in the water-stressed treatment. The stem necroses on plants subjected to water stress were more extended than those on non-stressed ones ($P < 0.01$). The mean of lesion length was 6.67 mm in the non-stressed group and 18.67 mm in the stressed one (Fig. 3).

Comparison of lesion development in two different periods during growing season

The test (Mann-Whitney *U* test) showed, that the lesion length was importantly different ($P << 0.01$) between June and September (Fig. 4). The average length of stem lesion in June was 26.88, and in September 6.73 mm only.

Table 2. The effect of factors (host, isolate, interaction) on lesion length

Source of variation	SS	df	MS	*F*	*P*	η^2
Host species	51.27	5	10.25	109.74	< 0.000	0.69
Isolate	0.27	2	0.14	1.45	0.24	0.01
Isolate – host species interaction	6.83	10	0.68	7.31	< 0.000	0.23
Error	23.17	248	0.09			

SS – sum of squares. df – degrees of freedom. MS – mean square. *F* – *F* ratio. *P* – significance level. η^2 – ratio of explained variability

DISCUSSION

The variability in lesion length on particular hosts and treatments in our experiments was relatively high. This phenomenon occurred in other *Phytophthora* infection experiments (i.e. Jung et al. 2005) and it seemed to be common. The cause of the variation could be ascribed to the physiological status of the host tissues, which could have a profound influence on the apparent susceptibility of the plant material to *Phytophthora* colonization as had suggested Matheron et al. (1988). The variation and its negative effect on evaluation could be limited with use of sufficient amount of saplings in experiments. Furthermore it is necessary to use physiologically uniform material and to make experiment precisely.

The differences in *Phytophthora* species aggressiveness found out in our experiment resembled in general features the differences which had been detected in other experiments. *Phytophthora gonapodyides* is soil species usually causing rot of root hair. The short extent of stem lesion in the experiment was not surprising – similar outcomes were obtained by Jung et al. (2005). *Phytophthora cambivora* was regarded as an aggressive species in other trials (Thomidis et al. 2003; Jung et al. 2005) which caused more extent lesions. The cause of difference could be ascribed to the partial loss of pathogenic potential of our isolate during its cultivation (it was acquired in 2006) or to the variation in substrate specificity in *P. cambivora*. The detected pathogenicity of *P. cactorum* resembled the outcomes of Jung et al. (2005), Thomidis et al. (2003, 2008) etc. We concluded that *P. cactorum* and *P. citricola* s.l. could be very dangerous to common beech and other woody plants in our nurseries, parks and forests because they have commonly been isolated in the Czech Republic recently (Mrazkova et al. 2007; Mrázková et al. 2008; Cerny et al. 2008). *Phytophthora citrophthora* and *P. cinnamomi* are alien polyphagous invasive species from tropical zone (Erwin, Ribeiro 1996). These two species caused

Table 3. The extent of bark lesion caused by *P. cactorum* in host spectrum. Lesions in hosts followed by the same letter ([a, b] second column) were not significantly different (two-way ANOVA, effect of host evaluated only, Tukey's test; $P > 0.05$). The lesion lengths caused by particular isolates in identical host (substrate specificity) followed by the same character ([*, ×] fourth column) were statistically different (Duncan's test; $P \leq 0.05$)

Host	Total mean of lesion (± SE)	Isolate	Mean of lesion (± SE)
A. pseudoplatanus	48.16 (± 5.38)	P066.07	33.53 (± 6.53)*
		P078.07	58.93 (± 8.75)*
		P100.07	52.00 (± 11.35)
F. sylvatica	22.07 (± 1.47)[a]	P066.07	16.13 (± 2.24)*
		P078.07	26.87 (± 2.27)*
		P100.07	23.20 (± 2.46)
A. glutinosa	16.00 (± 1.07)[a]	P066.07	17.00 (± 1.13)
		P078.07	20.00 (± 2.28)*
		P100.07	11.00 (± 1.18)*
T. cordata	13.90 (± 0.53)[a]	P066.07	13.21 (± 1.00)
		P078.07	14.69 (± 0.77)
		P100.07	13.86 (± 0.99)
F. excelsior	5.07 (± 1.02)[b]	P066.07	2.53 (± 0.96)*
		P078.07	4.80 (± 1.59)
		P100.07	7.87 (± 2.29)*
Q. robur	4.53 (± 0.91)[b]	P066.07	9.87 (± 1.80)*×
		P078.07	2.47 (± 1.13)*
		P100.07	1.27 (± 0.33)×

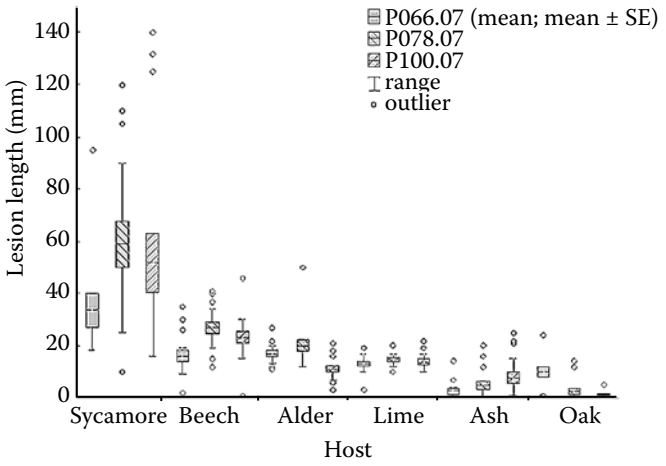

Fig. 2. Extent of bark lesions caused by *P. cactorum* isolates Nos P066.07, P078.07 and P100.07 on sycamore, beech, alder, lime, ash and oak saplings

much more damage in our experiment than the others. This finding is in agreement with the other infection experiments (Brasier, Jung 2003; Thomidis et al. 2008). The outcomes and extremely broad host spectrum of the both species (Erwin, Ribeiro 1996; Farr, Rossmann 2009) indicate, that *Phytophthora citrophthora* and *P. cinnamomi* potentially pose a high risk to our broadleaved forest trees.

The outcomes of our second experiment confirmed different sensitivity of the host species to *P. cactorum* as well as the substrate specificity in *P. cactorum*. Our outcomes are in agreement with other authors. Pathogenicity experiments proved by Hantula et al. (2000) showed that *P. cactorum* strains had a tendency towards host specialization. The host specialization in *P. cactorum* was found by Thomidis (2003) and Bhat et al. (2006), too. The difference in *P. cactorum* pathogenicity was found in soil population (Darmono et al. 1991) and in apple trees population (Boughalleb et al. 2006).

Our third experiment briefly confirmed the causality between waterlogging stress and more intensive stem lesion development. The reports of this relation have not been published so far, but its confirmation should be very important, because dozens of *Phytophthora* disease events occurred in water stress conditions or in environment with high soil humidity. This result is in accordance with general finding that stress of the host accelerates disease development. *Phytophthora* diseases can be accelerated by several stress factors – for instance root and collar rot by water stress (waterlogging as well as drought), wounding, low light intensity, high temperatures, nitrogen content, soil compaction and aeration, other diseases etc. (e.g. Erwin, Ribeiro 1996; Balci, Halmschlager 2003; Fonseca et al. 2004), irrigation regime and technology (Utkhede 1999), environmental factors – microbial status of substrate, pH of substrate, ground cover (Erwin, Ribeiro 1996), manuring practice, soil tillage and human mobility (Fonseca et al. 2004; Martins et al. 2007) etc. Therefore it is not surprising that the stem lesion development can be accelerated by waterlogging stress.

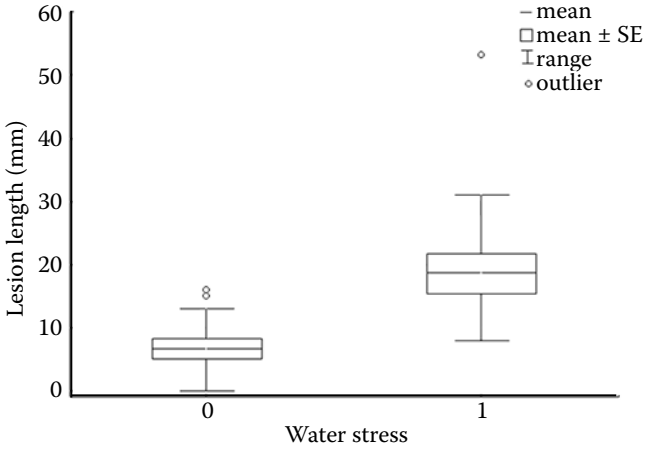

Fig. 3. Extent of bark lesions caused by *P. cactorum* isolate P078.07 on beech saplings without water stress (0) and with water stress (1)

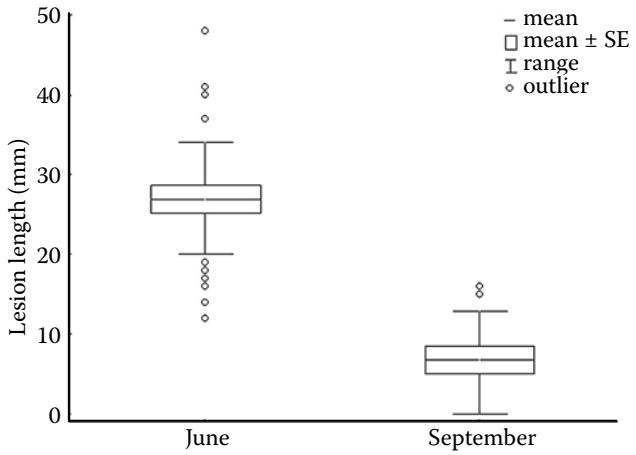

Fig. 4. Extent of bark lesions caused by *P. cactorum* isolate P078.07 on beech saplings in June and September

The seasonal variation in the host (apple tree) susceptibility to *P. cactorum* was demonstrated by Zondo et al. (2007), who found that the host susceptibility generally cycled to a low level during dormancy in winter and to a high level during active growth in summer. Similar pattern was found in other studies in *Phytophthora* (e.g. Robin et al. 1994; Brasier, Kirk 2001). Possibly, the identified decrease in stem lesion length in September could be connected with incoming period of host dormancy.

Our results (Mrazkova 2007; Cerny et al. 2008, 2009; Černý et al. 2008; Mrázková 2008; this study) confirmed that *Phytophthora* spp. causing diseases of woody plants should be taken more seriously in the Czech Republic. The attention has to be given not only to *P. cactorum* (relatively frequently cited in the Czech Republic, see above) and to quarantine species (*P. ramorum*, *P. kernoviae*) but also to the other highly pathogenic species contemporary spreading in Europe (*P. alni*, *P. cambivora*, *P. cinnamomi*, *P. citricola* s.l., *P. citrophthora* etc.). Spreading of these *Phytophthora* spp. in the Czech Republic represents high risk to our indigenous broadleaved forest trees. Better familiarity with *Phytophthora* species, appropriate cultivation of plant material and sanitary practice are of great importance to prevent potential substantial losses in the future.

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