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OCCURRENCE OF *PHYTOPHTHORA* SPECIES IN RIVERS, CANALS AND WATER RESERVOIRS IN RELATION TO ITS LOCATION, SEASONAL ANALYSIS AND FUNGICIDE RESIDUES

WYSTĘPOWANIE GATUNKÓW *PHYTOPHTHORA* W RZEKACH, KANAŁACH I ZBIORNIKACH WODNYCH W ZALEŻNOŚCI OD ICH LOKALIZACJI, OKRESU DETEKCJI I POZOSTAŁOŚCI FUNGICYDÓW

Abstract: *Phytophthora* spp. were detected from 3 rivers, 2 nursery canals and 2 reservoirs during all year. Number of *Phytophthora* necrotic spots on rhododendron leaves, used as baits, in March, July and October indicated on the lack of significant differences in that group of pathogens in relation to water sources, their location as well as during observation period. Only in one water reservoir *Phytophthora* density was about 2–3 times higher than in 6 other sources. *In vitro* trials showed different reaction of *P. cinnamomi*, *P. citricola* and *P. citrophthora*, often detected in water, on fenamidon and metalaxyl. *P. cinnamomi* was the most sensitive whereas *P. citrophthora* the most resistant. Amendment of river water contaminated with *P. cinnamomi* with metalaxyl at dose 1.6 μg/cm^3 reduced at least twice pathogen density in water. In case of fenamidon such effect was obtained when water was amendment with 8 μg of that compound /cm^3.

Keywords: *Phytophthora* spp. rhododendron baits, detection, fenamidon, metalaxyl, inhibition

Most of *Phytophthora* species, classified by European *Phytophthora* Group as *Algae* like *Oomycetes* (kingdom *Chromista*, class *Oomycetes*, family *Pythiacae*), are the most dangerous soil-borne pathogens of many plants, including hardy nursery stocks. Polish production of ornamental plants, including bushes, trees and perennials, increased every year about 15 %. Most of plants are grown outdoors in containers stands. The surplus water used for plant irrigation or sprinkling and rain run off through drains or open canals to local streams or rivers but is also stored in reservoirs situated in the lowest part of nurseries. About 35 % of such water run back to nursery ponds and is used again for

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plant irrigation [1]. Parts of substratum, rest of fertilizers and plant protection products including metalaxyl, dimetomorph, fenamidon, mancozeb and others often occur in surplus water and they can influence on plant pathogens. Hong and Moorman [2] reported, that some pathogenic bacteria, fungi and viruses can be spread with water. The authors concluded that contaminated water is a primary, if not the sole, source of inoculum for *Phytophthora* diseases of numerous nursery, fruit, and vegetable crops. *Phytophthora* species are one of the most imported plant pathogens [1] causing root and/or stem rot and twig blight of many plants [3–5]. Number of *Phytophthora* propagules in water depending on many factors. The objective of this study was to determine (1) the occurrence of *Phytophthora* spp. in relation to source of water, (2) the seasonal occurrence and frequency of that group of pathogens (3) influence of some protection products on *Phytophthora cinnamomi* Rands detection from river water.

Material and methods

Running water and reservoirs. Three rivers, one swims through forests, and 2 other through horticultural area, 2 canals in 2 hardy ornamental nursery stocks and 2 water reservoirs situated in the same nurseries were chosen for detection of *Phytophthora* spp. and estimation of their population densities in relation to water source and period of baiting.

Detection of *Phytophthora* spp. Top parts of rhododendron leaves cv. Nova Zembla were used according to procedure described by Orlikowski et al [6]. Leaves were held in water about 2 m from the banks from April till October at month intervals. After 5 days baits were put into plastic bags and transported to laboratory. After washing of leaves under tap water and drying number of necrotic spots on each leaf were counted. After desinfection over the burner flame about 5 mm necrotic parts of inocula were put on PDA medium in 90 mm Petri dishes [7]. Within 48 hrs of plates incubation at 25 ^{o}C number of colonies growing around inocula were counted and some of them were transferred into PDA slants. After segregation representative cultures were clean and identified to species on the base of their morphological features [6].

Influence of some fungicides on detection of *Phytophthora cinnamomi* from water contaminated with fungicide residues. Linear growth of *P. cinnamomi*, *P. citricola*, *P. citrophthora* on PDA amended with fenamidon and metalaxyl at concentrations 8 and 40 μg of a.i./cm^3 was evaluated after 5-day-incubation at 25 ^{o}C. Relationship between chemicals applied, their concentrations and changes in the population density of *P. cinnamomi* was evaluated in river water contaminated with the pathogen using rhododendron leaf baits [7].

Results and discussion

Detection of *Phytophthora* spp. in water. *Phytophthora* species including *P. cinnamomi* Rands, *P. citricola* Sawada, *P. citrophthora* (R.E Smith and E.H Smith) Leonian and *P. cryptogea* Pethybr. and Laff., were detected from all tested sources of water during all year. Using rhododendron leaves as the bait *Phytophthora* spp. was recovered at almost the same level from rivers, canals and reservoirs (Table 1). Themann et al [1] concluded that even at low winter temperature reservoirs and sediments did not freeze and enable the survival and development of *Phytophthora* species. In addition, the organic materials in sediments and the anaerobic conditions at the base of reservoirs are known to favour survival and development of *Oomycetes* [8, 9]. In the last 2 years checked rivers and reservoirs were froze on the depth about 10–15 cm during the winter time only for a few days so such condition did not significantly effect on *Phytophthora* population. Lack of sudden increase or decrease of *Phytophthora* spots on leaf baits was probably connected with the lack or very low contamination of water by metalaxyl and dimethomorph which may strongly suppressed *Phytophthora* development.Water pH which fluctuated only slightly had no any influence on *Phytophthora* level.

Table 1

Relationship between source of water, detection time and number of *Phytophthora* necrotic spot on rhododendron leaf baits

Source of water	Observation time		
	March	July	October
Korabiewka river	7.8 ab	5.3 a	18.0 cd
Kurowka river	12.3 a–c	8.5 ab	10.0 a–c
Ner river	12.5 a–c	12.8 a–c	9.5 ab
Canal I	13.3 a–c	9.3 ab	13.5 bc
Canal II	11.0 a–c	5.8 ab	12.5 a–c
Pond I	10.8 a–c	11.3 a–c	11.5 a–c
Pond II	6.3 ab	24.0 d	11.0 a–c

Note: Means followed by the same letter do not differ with 5 % of significance; Duncan's multiple range test.

Relationship between fungicides, their concentration and development of *Phytophthora* spp. Amendment of PDA medium with fungicides resulted in different reaction of *Phytophthora* species on active ingredients (Table 2).

Table 2

Relationship between *Phytophtora* species, different fungicides and inhibition [%] of linear growth on potato-dextrose agar amended with chemical at dose 8 (a) and 40 (b) [$\mu g/cm^3$]

Phytophtora species	Fenamidon		Metalaksyl	
	a	b	a	b
P. cinnamomi	100 c	100 b	95 b	95 b
P. citricola	57 b	74 a	83 b	87 ab
P. citrophthora	34 a	64 a	61 a	77 a

Note: see Table 1.

Fenamidon at dose 8 μg of a.i./cm^3 completely inhibited the growth of. *P. cinnamomi* whereas the other 2 species for about 40 %. Increase of fenamidon concentration resulted in the inhibition of *P. citricola* and *P. citrophthora* growth for about 70 %. Metalaxyl already at concentration 8 μg/cm^3 reduced the growth of *P. cinnamomi* in 95 % whereas *P. citrophthora* only 61 % (Table 2). Increase the fungicide concentration 5 times only slightly reduced the growth of *P. citricola* and *P. citrophthora.* Amendment of river water with fenamidon with 1.6 μg of fenamidon/cm^3 did not influence population density of *P. cinnamomi* (Table 3).

Table 3

Relationship between 2 fungicides, their concentrations and occurrence of *Phytophtora cinnamomi* in river water; number of spots/leaf bait

Concentration [μg/cm^3]	Fenamidon	Metalaksyl
0.0	23 c	23 c
1.6	22 c	9.5 b
8.0	9.5 b	5.3 a

Note: see Table 1.

Increase of the fungicide level to 8 μg/cm^3 resulted in the decrease of propagule numbers at least twice. Activity of metalaxyl at 8 μg/cm^3 was about twice higher than fenamidon (Table 3). Results obtained indicated on lack or minimal amount of fenamidon and metalaxyl in analysed water sources and lack of influence on *Phytophthora* spp. (Table 1).

Themann et al [1] reported a dramatic increase in *Phytophthora* isolates in recycled nursery irrigation water from autumn to spring because of reservoirs are refill very rare so there are no dilution of water as well as application of fungicide specific to *Oomycetes* during that period. Studies of Oudemans [10] indicated that application of 2 kg of metalaxyl/ha to manage *Phytophthora* root rot of cranberry, caused by *P. cinnamomi,* had no significant, long-term effect on the recovery of that species from drainage canals.

Conclusions

1. Our study showed the occurrence of *Phytophthora* spp. in rivers, nursery canals and reservoirs

2. Location of water source, implements of surrounding of rivers and detection period had no significant differences on the number of *Phytophthora* necrotic spots on rhododendron leaf baits

3. Fenamidon and metalaxyl, specific fungicides against *Oomycytes,* already at concentration of 8 μg/cm^3 almost completely inhibited the development of *P. cinnamomi* whereas *P. citrophthora* slightly reacted on that compounds

4. Amendment of river water with metalaxyl at dose 1.6 μg/cm^3 decreased *P. cinnamomi* density at least twice. Such effect was also observed when fenamidon was added to water at concentration 8 μg/cm^3.

5. Results obtained indicated on lack or minimal amount of tested fungicides in water sources because of only slightly differences in *Phytophthora* levels during analysed periods.

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WYSTĘPOWANIE *PHYTOPHTHORA* SPP. W RZEKACH, KANAŁACH I ZBIORNIKACH WODNYCH W ZALEŻNOŚCI OD ICH LOKALIZACJI, OKRESU DETEKCJI I POZOSTAŁOŚCI PESTYCYDÓW

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Abstrakt: Stosując wierzchołkowe liście różanecznika odm. Nova Zembla, wykrywano *Phytophthora* spp. przez cały rok w 3 rzekach, 2 kanałach i 2 zbiornikach wodnych. Liczba nekrotycznych plam, stwierdzanych na liściach pułapkowych w marcu, lipcu i październiku wykazała brak znaczących różnic w liczebności tej grupy patogenów w badanych źródłach wody niezależnie od miejsca ich usytuowania, zagospodarowania oraz czasu detekcji. Tylko w jednym zbiorniku wodnym w szkółce liczebność *Phytophthora* spp. była około 3-krotnie wyższa aniżeli w pozostałych 6 źródłach wody. Doświadczenie *in vitro* wykazało zróżnicowaną reakcję *P. cinnamomi, P. citricola* i *P. citrophthora* na fenamidon i metalaksyl. Gatunek *P. cinnamomi* okazał się najwrażliwszy na te środki, podczas gdy najodporniejszym. okazał się *P. citrophthora.* Wprowadzenie metalaksylu w stężeniu 1,6 $\mu g/cm^3$ do wody rzecznej, skażonej przez *P. cinnamomi*, spowodowało redukcję liczebności patogenu na liściach pułapkowych co najmniej dwukrotnie. W przypadku fenamidonu taki efekt uzyskano, gdy środek dodano do wody w stężeniu 8 $\mu g/cm^3$.

Słowa kluczowe: *Phytophthora* spp., pułapki różanecznikowe, wykrywanie, fenamidon, metalaksyl, hamowanie