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Role of avian vectors in the spread of *Phytophthora species* in Poland

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Abstract *Phytophthora* is an important plant pathogen that can cause serious damage in a wide range of forest species. Understanding how this pathogen disseminates is fundamental to disease management. There is a continual need for improvement of *Phytophthora* species monitoring in natural ecosystems. However, currently there is little evidence for whether avian vectors may be transporting spores and contributing to the spread of the pathogen. This is the first survey of birds as vectors of *Phytophthora* species. Analysis of swabs from feathers from 112 birds belonging to seven species showed that most of them transmit *P. cactorum* and to a lesser extent *P. plurivora*. Pathogens of black alder − *P. alni* and *P. multiformis* were detected in the investigated area, but their frequency was low. This study showed that avian vectors are important in spreading *Phytophthora*. In addition, analysis of swabs from feathers can be sensitive method for detection of *Phytophthora* presence in ecosystems.

Keywords *Phytophthora* · Black alder · Birds · Ecology · Real time PCR

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Black alder *Alnus glutinosa* (L.) Gaertn. is an important forest−forming species in Poland, comprising more than 5% of the species composition and is introduced into the habitat of boggy coniferous and broadleaved forests. Until recently, black alder was considered in Poland as a species with a very low level of susceptibility to pests and diseases. The situation changed at the turn of the century, when the deterioration of the health status of the alder stands began, which locally led to the mass dieback of the trees. The strongest deterioration of health condition of trees was observed in east of Poland (Piętka and Grzywacz 2018).

Among the plant pathogens potentially implicated in this dieback, *Phytophthora* stands out with a significant number of species proven to be aggressive agents that threaten ecosystems stability and plant productivity (Erwin and Ribeiro 1996; Rizzo et al. 2005). *Phytophthora alni* is especially aggressive to alder, but in Poland, a survey of *Phytophthora* associated with alder decline revealed that besides of *P. alni* some other species *P. cactorum*, *P. cinnamoni*, *P. gonapodyides*,

P. lacustris, *P. megasperma*, *P. plurivora*, *P. pseudosyringae* and *P. syringae* were also isolated (Trzewik et al. 2015)). Among them *P. cactorum* and *P. plurivora* are the most widespread (Erwin and Ribeiro 1996; Schoebel et al. 2014).

Phytophthora spreads mainly through the movement of infested soil, water, and infected plant material (Cahill et al. 2008), although there are species that are transmitted aerially (Goodwin 1997). Avian vectors may also play an important role in transport of *Phytophthora*, however, there have been very few studies carried out to examine these possibilities (Hubálek 1974; Keast and Walsh 1979). The aim of this study is to determine significance of birds in the dispersal of *Phytophthora* species.

Swabs from feathers on the throat and belly area were collected during the bird ringing in east of Poland at the Siemianówka reservoir (52.909710 N, 23.849740 E) and in Gruszki (Browsk Forest District 52,825,370 N, 23,796,180 E) during January–February 2017. The Siemianówka reservoir is an important refuge for breeding birds, as well as the place of their stopping and feeding during seasonal migrations. Gruszki is situated at the border of Białowieża Forest. Genomic DNA was extracted from the samples using PureLink Microbiome DNA Purification Kit (ThermoFisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. Amplification of the internal transcribed spacer 1 region was performed as described previously in *P. cactorum* and *P. plurivora* (Nowakowska et al., 2017) and in *P. multiformis* (Nowakowska et al. 2016). Primers and probe used for identification of *P. alni* was designed at this study: forward primer - ctgtcgatgtcaaagttg, reverse – atgggtttaaaagataaggg, probe - acccaaacgctcgccatgata. As positive controls were used DNA isolated from DNA extracted from mycelial cultures of *Phytophthora* (Nowakowska et al. 2017). Briefly, qPCR reaction was performed in a 20 μl volume containing 1 μM each primer (forward and reverse), 10 μl 2x qPCR probe Master Mix (Sigma-Aldrich, Milwaukee, WI, USA) and 2 μl of genomic DNA as template. Thermocycling conditions consisted of initial denaturation at 95 °C for 3 min, and 40 cycles at 95 °C for 30 s, at 55 °C for 30 s and at 72 °C for 30 s. Amplifications were performed using a RotorGene 6000 (Qiagen, Hilden, Germany) following the manufacturer's instructions. Ct values less than 40 were considered a positive detection response.

Swabs from feathers on the throat and belly area collected from 112 birds belonged to seven species: *Chloris chloris* ($n = 22$), *Carduelis spinus* ($n = 18$), *Parus ater* ($n = 6$), *Parus caeruleus* ($n = 31$), *Parus major* ($n = 28$), *Parus montanus* ($n = 7$) and *Parus palustris* ($n = 3$). Pathogens of black alder were detected in investigated area at low frequency. *P. alni* was found in two samples from *Parus major* and one from *Parus caeruleus*; *P. multiformis* in one sample from *Parus major* and one from *Parus caeruleus*. Frequency of *Phytophthora* transmission by different bird species are presented in Fig. 1.

In contrast species known to be pathogenic on alder, *P. cactorum* and *P. plurivora* were frequently detected. Out of 112 analyzed specimens 95 (84.8%) carried *P. cactorum* and 35 (31.2%) *P. plurivora*. The highest transmission rate was detected in Eurasian siskin (*Carduelis spinus),* European greenfinch (*Chloris chloris)* and blue tit *(Parus caeruleus).* 90.9% of *Ch. chloris*, 94.4% of *C. spinus* and 90.3% of *Parus caeruleus* transferred *P. cactorum*. The lowest frequency of transmission of *P. cactorum* was observed in *Parus ater* (50%) and *Parus montanus* (57.1%). The frequency of *P. plurivora* transferring by *Parus ater* and *Parus montanus* was similar to *P. cactorum,* while the transfer by other bird species was 3–4 fold lower.

Our experiment clearly demonstrated that birds can transmit *Phytophthora* and that frequency of transmission was dependent on bird species. The European greenfinch is found in woodland edges, farmland hedges and gardens. The Eurasian siskin is found in forested areas, both coniferous and mixed woodland, where it feeds on seeds of all kinds. The Eurasian siskin has an unusual migration pattern, as every few years in winter it migrates southwards in large numbers. Alder and spruce seeds are their natural food supplies through the winter and when this food diminish through the winter, siskins make their way into gardens to find food (Clement 1999). Being birds feeding on predominantly seed goldfinches do need to drink more than most other species so the availability of water is essential to their life success.

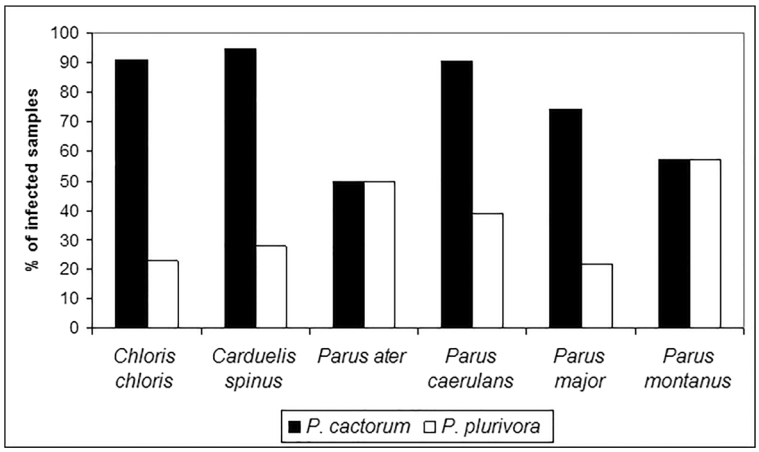

Fig. 1 Frequency of *P. cactorum* and *P. plurivora* transmission by different bird species

This may explain very high transmission rate of *Phytophthora* by these bird species. The coal tit (*P. ater)* and the willow tit (*P. montanus)* are confined to coniferous forest, which is their preferred habitat (Ekman 1979). The coal and willow tits are all−year residents making only local movements in response to particularly severe weather. The great tit (*P. major)*, the marsh tit (*P. palustris*) and the blue tit (*P. caeruleus*) are bound to deciduous forests. In coniferous forests *P. montanus* forages in the inner canopy, while *P. ater* forages in the outer parts of tree crowns. Because of their different habitat requirements, there is little interference between the two groups of species. These species are separated ecologically mainly by their different foraging zones in trees (Alatalo et al. 1985; Alatalo and Moreno 1987). At present time we do not know why *Phytophthora plurivora*, which is the most common species in Poland, was generally found 2-fold less frequently than *Phytophthora cactorum.* Two bird species were revealed as an exception from this rule: *P. ater* and *P. montanus,* sharing equal frequency of aforementioned pathogenic species.

Detection of DNA does not determine if the pathogen is able to cause infection or not. Chlamydospores of *Phytophthora cinnamomi* can survive within the intestinal tract of golden whistler (*Pachycephala pectoralis*) and a rufus whistler (*P. rufiventris*) (Keast and Walsh 1979), however there are no reports about survival of other *Phytophthora* species. Investigation of viability of the spores will be important step in investigation of Phytophthora transmission by birds.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

Human and animals rights No human and/or animal participants were involved in this research.

Informed consent All authors consent to this submission.

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