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# Entomophthoralean fungi associated with aphids in woody plants in the Arboretum Mlyňany SAS

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**Abstract**
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A survey of entomophthoralean fungi infecting aphids in collections of woody plants in the Arboretum Mlyňany SAS was conducted during vegetation periods 2007 and 2008. Altogether, eight fungal species were identified in the aphid colonies. Of the fungi recorded, *Pandora neoaphidis* (Remaudière et Hennebert) Humber, *Entomophthora planchoniana* Cornu and *Neozygites fresenii* (Nowakowski) Remaudière et Keller were predominating ones. *Zoophthora radicans* (Brefeld) Batko, *Zoophthora aphidis* (Hoffman in Fresenius) Remaudière et Hennebert, *Neozygites cinarae* Keller, *Conidiobolus coronatus* (Costantin) Batko and *Conidiobolus obscurus* (Hall et Dunn) Remaudière et Keller were less important parasitic fungi. The number of species of fungi recorded reflects a great diversity in aphid-pathogenic flora in the arboretum. The entomophthoralean fungi were recorded from 40 aphid species belonging to 4 families (*Aphididae*, *Drepanosiphidae*, *Lachnidae* and *Anoeciidae*). Findings of entomophthoralean infection in *Myzocallis castanicola* Baker, *Myzocallis carpini* (Koch)*, Myzocallis walshii* Monell and *Myzus ligustri* Mosley colonies are considered first reports from these aphid species at all. In Slovakia, *C. coronatus* is recorded from natural aphid colonies for the first time.

**Key words**
Arboretum Mlyňany SAS, entomopathogenic fungi, Entomophthorales, tree aphids

## Introduction

Entomophthoralean fungi have been found to be important mortality factors for aphids all over the world (Latgé and Papierok, 1988). The fungi can cause lethal infections of various aphid species, and they belong to the most effective control agents of natural aphid colonies. At present, 33 entomophthoralean species are known causing mycoses in aphid populations (Barta and Cagáň, 2006a). Intensive studies were realised to contribute to understanding the fungal distribution and epidemiology in insect populations and to the fungal management within the biological control strategies in agricultural crops (Pell et al., 2001). In Slovakia, several studies were realised on entomophthoralean fungi infecting economically important aphids in agriculture (Cagáň and Barta, 2001; Barta and Cagáň, 2002, 2003, 2004), and 15 fungal species have been identified from the infected aphids (Barta and Cagáň, 2006b). Most of the fungal pathogens have been reported from aphids living in various herbs or in field crops (Barta and Cagáň, 2006b). However, the activity of this group of fungi in tree aphid populations has not been documented yet. Different species spectrum of aphids and their pathogens can be expected on trees, therefore investigation of these aphid colonies should reveal additional fungal species in Slovakia.

The Arboretum Mlyňany SAS, a park of exotic woody plants established in southern Slovakia, is focused on planting evergreen woody plants, especially those from the Mediterranean, East-Asian and North American flora. A great richness of dendroflora in the arboretum, with more than 2000 woody plant taxons, provides an opportunity for study the species spectrum of aphids and their fungal pathogens.

We initiated an investigation to determine the species complex of entomopathogenic fungi in aphid colonies associated with the woody plants in the Arboretum

Mlyňany SAS. In this paper, we provide a list of aphid pathogenic fungi from the order Entomophthorales recorded in the aphids.

## Material and methods

We studied aphid pathogens in collections of woody plants in the Arboretum Mlyňany SAS. This botanical garden, with a total area of 67 ha, is situated in southern Slovakia in the village of Vieska nad Žitavou (48°19'12" N, 18°22'09" E) at an altitude of 168–214 m asl. The mean annual temperature is 9.7 °C, and the mean annual precipitation is 558 mm.

The sampling of aphids was carried out at one-week intervals during growing seasons 2007 and 2008. The survey started at the beginning of May and finished at the end of October. All cadavers of aphids (fungus-killed aphids) were collected randomly. If woody plants were found infested with aphids, the aphid colonies were visually examined to find fungus-killed individuals. Cadavers with external symptoms of entomophthoralean infection were carefully removed from the plants and placed into plastic tubes (12 ml). As many as possible aphid cadavers were picked up from aphid colonies at each sampling event. If fungus-killed aphids showed the same external signs of disease in the same colony, they were suspected to be infected by the same fungal pathogen, and they were placed together in one plastic tube. Aphids with apparently different symptoms of disease were placed in separate tubes. The samples of fungus-killed aphids were used for identification of pathogen species with using the keys by Keller (1987, 1991) and Bałazy (1993). Samples of living aphids were also collected from colonies where infected aphids occurred, and the aphids were stored in small polystyrene tubes (5 ml) filled with 70% ethanol. These samples were used for aphid identification. The aphids were identified using the key by Blackman and Eastop (1994).

## Results and discussion

Eight species of entomopathogenic fungi belonging to three entomophthoralean families were identified from aphid colonies in the course of the survey: *Pandora neoaphidis* (Remaudière et Hennebert) Humber, *Entomophthora planchoniana* Cornu, *Zoophthora radicans* (Brefeld) Batko and *Zoophthora aphidis* (Hoffman in Fresenius) Remaudière et Hennebert from the family Entomophthoraceae, *Neozygites fresenii* (Nowakowski) Remaudière et Keller, *Neozygites cinarae* Keller from then family *Neozygitaceae* and *Conidiobolus obscurus* (Hall et Dunn) Remaudière et Keller and *Conidiobolus coronatus* (Costantin) Batko from the family Ancylistaceae. The eight species of entomopathogenic fungi recorded in the arboretum reflect a great diversity in the aphid-pathogenic mycoflora. Of the Entomophthorales recorded, seven species have already been reported from Slovakia (Barta and Cagáň, 2006b), but *C. coronatus* is considered as the first record from natural aphid colonies in Slovakia. The fungal pathogens were identified altogether from 40 aphid species. A complete list of aphids, their host trees and fungal pathogens is in Table 1. Majority of the aphid species belonged to the family Aphididae (28 species) and the remaining species were from the families Drepanosiphidae (10 species), Lachnidae (one species), and Anoecidae (one species).

Table 1. List of aphid species and their fungal pathogens observed on woody plants in Arboretum Mlyňany SAS in 2007 and 2008

| Host woody plant | Aphid species | Aphid pathogens |
|---|---|---|
| *Acer buergerianum* Miq. | *Periphyllus testudinaceus* (Fernie) | *Neozygites fresenii* |
| *Acer campestre* L. | *Periphyllus testudinaceus* (Fernie) | *Pandora neoaphidis* |
| *Acer platanoides* L. | *Drepanosiphum platanoidis* (Schrank) | *Entomophthora planchoniana* |
| | | *Conidiobolus coronatus* |
| *Acer pseudoplatanus* L. | *Periphyllus testudinaceus* (Fernie) | *Neozygites fresenii* |
| *Betula pendula* Roth | *Callipterinella calliptera* (Hartig) | *Pandora neoaphidis* |
| | *Euceraphis betulae* Koch | *Neozygites fresenii* |
| | | *Entomophthora planchoniana* |
| *Betula platyphylla* Sukatschev | *Callipterinella calliptera* (Hartig) | *Pandora neoaphidis* |
| | *Euceraphis betulae* Koch | *Neozygites fresenii* |
| | | *Entomophthora planchoniana* |
| *Castanea sativa* Mill. | *Myzocallis castanicola* Baker | *Entomophthora planchoniana* |

Table 1. Continued

| Host woody plant | Aphid species | Aphid pathogens |
|---|---|---|
| *Carpinus betulus* L. | *Myzocallis carpini* (Koch) | *Entomophthora planchoniana* |
| *Cerasus avium* (L.) Moench | *Myzus cerasi* (Fabricius) | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| | | *Zoophthora aphidis* |
| | | *Pandora neoaphidis* |
| | | *Conidiobolus coronatus* |
| *Cornus mas* L. | *Anoecia corni* (Fabricius) | *Zoophthora aphidis* |
| *Cornus sanguinea* L. | | *Entomophthora planchoniana* |
| *Crataegus monogyna* Jacq. | *Dysaphis crataegi* (Kaltenbach) | *Pandora neoaphidis* |
| *Corylus avellana* L. | *Myzocallis coryli* (Goeze) | *Entomophthora planchoniana* |
| *Corylus maxima* Mill. 'Purpurea' | *Myzocallis coryli* (Goeze) | *Entomophthora planchoniana* |
| *Elaeagnus angustifolia* L. | *Capitophorus elaeagni* | *Neozygites fresenii* |
| *Elaeagnus multiflora* Thunb. | (del Guercio) | *Entomophthora planchoniana* |
| *Elaeagnus umbellata* Thunb. | | |
| *Eucommia ulmoides* Oliv. | *Myzus persicae* (Sulzer) | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| | | *Conidiobolus coronatus* |
| *Euonymus alatus* (Thunb.) Sieb. | *Aphis fabae* Scopoli | *Neozygites fresenii* |
| *Euonymus europaeus* L. | *Aphis fabae* Scopoli | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| *Euonymus grandiflorus* Wall. ex Roxb. | *Aphis fabae* Scopoli | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| *Euonymus hamiltonianus* Wall. | *Aphis fabae* Scopoli | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| *Euonymus hamiltonianus* Wall. var. maackii | *Aphis fabae* Scopoli | *Pandora neoaphidis* |
| | | *Conidiobolus obscurus* |
| *Euonymus yedoensis* Koehne. | *Aphis fabae* Scopoli | *Pandora neoaphidis* |
| *Fagus silvatica* L. | *Phyllaphis fagi* (L.) | *Entomophthora planchoniana* |
| *Fagus silvatica* L. 'Rohanii' | *Phyllaphis fagi* (L.) | *Entomophthora planchoniana* |
| *Hibiscus syriacus* L. | *Aphis fabae* Scopoli | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| | *Aphis gossypii* Glover | *Neozygites fresenii* |
| *Hippophae rhamnoides* L. | *Capitophorus elaeagni* | *Neozygites fresenii* |
| | (del Guercio) | *Entomophthora planchoniana* |
| *Laburnum anagyroides* Med. | *Aphis craccivora* Koch | *Neozygites fresenii* |
| *Ligustrum vulgare* L. | *Myzus ligustri* Mosley | *Entomophthora planchoniana* |
| *Malus sikkemensis* (Wenzig) Koehne | *Aphis pomi* De Geer | *Pandora neoaphidis* |
| | | *Neozygites fresenii* |
| *Malus* sp. | *Aphis pomi* De Geer | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| | | *Conidiobolus obscurus* |
| | | *Zoophthora aphidis* |

Table 1. Continued

| Host woody plant | Aphid species | Aphid pathogens |
|---|---|---|
| | *Dysaphis plantaginea* (Passerini) | *Entomophthora planchoniana* |
| *Padus avium* Mill. | *Rhopalosiphum padi* (L.) | *Pandora neoaphidis* |
| | | *Neozygites fresenii* |
| | | *Conidiobolus obscurus* |
| | | *Zoophthora aphidis* |
| | | *Zoophthora radicans* |
| *Picea abies* Karst. | *Cinara pilicornis* (Hartig) | *Conidiobolus obscurus* |
| | | *Neozygites cinarae* |
| *Populus nigra* L. | *Chaitophorus leucomelas* Koch | *Zoophthora radicans* |
| *Prunus x amygdalo-persica* (West.) Rheder | *Myzus persicae* Sulzer | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| *Prunus domestica* L. | *Hyalopterus pruni* (Goeffroy) | *Neozygites fresenii* |
| *Prunus spinosa* L. | *Hyalopterus pruni* (Goeffroy) | *Entomophthora planchoniana* |
| *Prunus persica* (L.) Batsch | *Myzus persicae* Sulzer | *Conidiobolus obscurus* |
| | | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| | | *Zoophthora radicans* |
| *Quercus rubra* L. | *Myzocallis walshii* Monell | *Entomophthora planchoniana* |
| *Rhamnus catharticus* L. | *Aphis nasturtii* Kaltenbach | *Neozygites fresenii* |
| *Ribes alpinum* L. | *Cryptomyzus gaelopsidis* (Kaltenbach) | *Pandora neoaphidis* |
| | | *Neozygites fresenii* |
| | *Cryptomyzus korschelti* Börner | *Entomophthora planchoniana* |
| | *Cryptomyzus ribis* (L.) | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| *Robinia pseudoacacia* L. | *Aphis craccivora* Koch | *Neozygites fresenii* |
| *Rosa canina* L. | *Macrosiphum euphorbiae* (Thomas) | *Entomophthora planchoniana* |
| | *Macrosiphum rosae* (L.) | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| | | *Neozygites fresenii* |
| | *Metopolophium dirhodum* (Walker) | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| *Rosa x hybrida* | *Macrosiphum rosae* (L.) | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| | *Metopolophium dirhodum* (Walker) | *Entomophthora planchoniana* |
| *Rubus* sp. | *Aphis idaei* van der Goot | *Neozygites fresenii* |
| | *Macrosiphum funestum* (Macchiati) | *Pandora neoaphidis* |
| | | *Neozygites fresenii* |
| | *Macrosiphum rosae* (L.) | *Pandora neoaphidis* |
| | *Sitobion fragariae* (Walker) | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| *Salix caprea* L. | *Cavariella pastinacae* (L.) | *Entomophthora planchoniana* |
| | *Cavariella theobaldi* (Gillette et Bragg) | *Entomophthora planchoniana* |
| *Sambucus nigra* L. | *Aphis sambuci* L. | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| | | *Conidiobolus obscurus* |

Table 1. Continued

| Host woody plant | Aphid species | Aphid pathogens |
|---|---|---|
| *Sambucus nigra* L. 'Laciniata' | *Aphis sambuci* L. | *Pandora neoaphidis* |
| | | *Entomophthora planchoniana* |
| *Spirea thunbergii* Siebold ex Blume | *Aphis spiraecola* Patch | *Neozygites fresenii* |
| *Tilia cordata* Mill. | *Eucallipterus tiliae* (L.) | *Entomophthora planchoniana* |
| *Tilia platyphyllos* Scop. | *Eucallipterus tiliae* (L.) | *Entomophthora planchoniana* |
| *T. platyphyllos Scop. 'Laciniata'* | *Eucallipterus tiliae* (L.) | *Entomophthora planchoniana* |
| *Tilia petiolaris* DC. | *Eucallipterus tiliae* (L.) | *Entomophthora planchoniana* |
| *Viburnum opulus* L. | *Aphis fabae* Scopoli | *Neozygites fresenii* |
| *Viburnum dilatatum* Thunb. | *Aphis viburni* Scopoli | *Entomophthora planchoniana* |
| *Viburnum trilobum* Marshall | *Aphis fabae* Scopoli | *Neozygites fresenii* |

Findings of Entomophthorales from tree aphid hosts were usually made in spring and late autumn, with isolated recordings of *E. planchoniana*, *N. fresenii* and *P. neoaphidis* during summer (from July through September). These three entomophthoralean species were also the most commonly recorded ones in the aphid colonies in the arboretum. Although they were the most frequently encountered in the aphid colonies, no epizootics due to these fungal pathogens were observed.

*E. planchoniana* was the predominant and the most prevalent aphid-pathogen in aphid colonies. It was identified from 28 aphid species and recorded in almost 84% aphid colonies of all mycosis-positive colonies. This fungus is known worldwide (Keller, 1987; Bałazy, 1993), and it is frequently reported as a causal agent for epizootics in pestiferous aphids in field crops (eg Milner et al., 1980; Feng and Nowierski, 1991). Keller (1987) noticed that the fungus preferred relatively dry habitats and did not occur in dense humid crops.

*N. fresenii* was the second most frequent aphid pathogen in the arboretum. It was identified from 18 aphid species and recorded in 49% mycosis-positive colonies. *N. fresenii* is known from nearly all continents including the South Pacific region (Keller, 1997). It is considered to be the best adapted to tropical conditions (Steinkraus et al., 1991; Keller, 1997), although it is effective in the subpolar region, as well (Nielsen et al., 2001). It was observed that *E. planchoniana* and *N. fresenii* prefer different habitats. Whereas *E. planchoniana* infects aphids living in relatively dryer microclimate at upper levels of vegetation, *N. fresenii* is frequent aphid pathogen in dense colonies in humid habitats in dense ground vegetation (Barta and Cagáň, 2006b).

*P. neoaphidis* was recorded from 14 aphid species in the arboretum. This fungus is usually considered to be the most important and most prevalent pathogen on aphids infesting field crops (Keller, 1991; Pell, et al. 2001; Barta and Cagáň, 2006b). In Slovakia, it is considered to be the most significant pathogen in natural aphid colonies (Barta and Cagáň, 2006b). The species has a worldwide distribution, and it has been reported from almost all continents (Keller, 1991; Bałazy, 1993). It is characteristic with an inborn tendency to develop epizootic disease, and it showed high effective in natural control of various aphid species. Its potential for epizootic development in aphid populations has also been documented in a number of studies (eg Dean and Wilding, 1971; Feng and Nowierski, 1991; Barta and Cagáň, 2003). Even if this fungus was found the most prevalent in aphid populations in our previous study (Barta and Cagáň, 2006b), its natural occurrence in the tree aphid colonies was of lower importance in the arboretum.

The remaining five fungal pathogens were identified from one to six aphid species (*C. obscurus* (6 aphid species), *Z. aphidis* (4 species), *Z. radicans* (3 species), *C. coronatus* (3 species), *N. cinarae* (1 species)). From these five fungi, *N. cinarae* and *Z. aphidis* are host-specific parasites in aphids. The first one is monophagous, infecting only *Cinara pilicornis* (Hartig) (Keller, 1997) and the second one is oligophagous pathogen with limited host spectrum (Barta and Cagáň, 2006a). In the previous studies from Slovakia, *Z. aphidis* was only recorded from aphids inhabiting trees (Barta and Cagáň, 2006a, b) and in our observations, *Myzus persicae* Sulzer is a new host for this fungal species. *C. obscurus* is a common aphid pathogen with a broader range of hosts (Keller, 1987). It was only sporadically observed in arboricolous aphid colonies during our survey which corresponds with the previous results from Slovakia (Barta and Cagáň, 2006b). *C. coronatus* has been recorded from natural aphid colonies in Slovakia for the first time. Usually it is considered as a widespread soil saprophyte utilising a variety of substrates. It has been recorded from detritus, living plants, various dead arthropods and occasionally from mammals (Bałazy, 1993). It can successfully infect insect from different orders, but it does not play a significant role in controlling aphid populations (Remaudière et al., 1981; Papierok, 1985; Hatting et al., 1999).

The results presented in this study confirm activity of entomophthoralean fungi in populations of arboricolous aphids. However, prevalence of fungal diseases and fungal species spectrum reached a lower level compared to the previous studies on the occurrence of aphid entomopathogenic fungi in Slovakia (Barta and Cagáň, 2006b). This may be explained with the fact that less aphid species were collected during our surveys and different aphid species spectrum was studied. Several new data are presented in this paper. *Myzocallis castanicola* Baker, *Myzocallis carpini* (Koch), *Myzocallis walshii* Monell and *Myzus ligustri* Mosley were found killed by entomophthoralean disease for the first time. These four aphid species were infected by *E. planchoniana*. *C. coronatus* has been recorded from natural aphid colonies in Slovakia for the first time.

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# Huby z radu Entomophthorales parazitujúce vošky na drevinách v Arboréte Mlyňany SAV

## Súhrn

V rokoch 2007 a 2008 sme sledovali výskyt entomopatogénnych húb z radu Entomophthorales v kolóniách vošiek na zbierkových drevinách Arboréta Mlyňany SAV. Spolu sme v kolóniách vošiek identifikovali 8 druhov entomopatogénnych húb. Dominantný výskyt sme zaznamenali pri druhoch *Pandora neoaphidis* (Remaudière et Hennebert) Humber, *Entomophthora planchoniana* Cornu a *Neozygites fresenii* (Nowakowski) Remaudière et Keller. Druhy *Zoophthora radicans* (Brefeld) Batko, *Zoophthora aphidis* (Hoffman in Fresenius) Remaudière et Hennebert, *Neozygites cinarae* Keller, *Conidiobolus coronatus* (Costantin) Batko a *Conidiobolus obscurus* (Hall et Dunn) Remaudière et Keller boli menej významné z pohľadu prevalencie. Počet zaznamenaných druhov húb poukazuje na významnú druhovú diverzitu entomopatogénnej flóry vošiek v arboréte. Entomopatogénne huby boli identifikované zo 40 druhov vošiek patriacich do 4 čeľadí (Aphididae, Drepanosiphidae, Lachnidae a Anoeciidae). Na voškách *Myzocallis castanicola* Baker, *Myzocallis carpini* (Koch)*, Myzocallis walshii* Monell a *Myzus ligustri* Mosley bola infekcia entomopatogénnymi hubami z radu Entomophthorales zaznamenaná po prvýkrát. *C. coronatus* bol zistený v prirodzených kolóniách vošiek po prvýkrát na Slovensku.

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