

MOLECULAR-GENETIC IDENTIFICATION OF CERTAIN FRUIT TREE MYCOPATHOGENS TRANSMITTED BY APHID VECTORS

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Abstract. This study focuses on the molecular-genetic identification of phytopathogenic fungi transmitted by aphids in fruit orchards. Specimens of *Dysaphis* and *Hyalopterus* aphids were collected from apple and plum trees respectively, and DNA was extracted using the SDS protocol. A two-step PCR method with species-specific primers targeting fungal pathogens (*Monilinia* spp., *Penicillium* spp., and *Botrytis cinerea*) was applied. Amplification products revealed the presence of *P. chrysogenum*, *M. fructigena*, and *B. cinerea* in samples from *Hyalopterus*, while *Dysaphis* samples tested negative for all target fungi. These results confirm that *Hyalopterus* aphids can act as vectors for both generalist and specialist phytopathogens at early stages of the growing season. The findings highlight the complex multi-level interactions between insect vectors, pathogens, and host plants in agroecosystems. Continuous monitoring and early detection of secondary fungal infections are essential for maintaining phytosanitary stability.

Keywords: *phytopathogenic fungi, aphids, vectors, PCR, Monilinia spp., Penicillium spp., B. cinerea.*

INTRODUCTION

The formation of specific pathologies in tree species and their populations—both in natural forest communities and in cultivated orchards, parks, and urban plantings—is largely influenced by biogenic factors. These factors include various living organisms and their associations, encompassing biological systems at different levels of organization. Representatives of different kingdoms, phyla, classes, and numerous lower taxonomic groups are involved: higher and lower plants, fungi, insects, mites, nematodes, protozoa, bacteria, mycoplasmas, phytoplasmas, spiroplasmas, and viruses.

To date, it has been established that most significant infectious diseases of woody plants and mycoses in particular, are in one way or another associated with the activity of insects [10]. Entomochory in the pathology of woody plants is a complex and multifaceted phenomenon that encompasses processes such as

transmission, reservation, reproduction, persistence, and penetration of phytopathogenic infections into plant tissues and organs, including fruits and seeds. The joint colonization of the same plant organs by phytopathogens and dendrophilous insects promotes their interaction and the formation of various forms of contact. These organisms may simultaneously act as both pests and vectors of infection.

Among the dendrophilous insects capable of transmitting phytopathogenic fungi are representatives of various orders: Coleoptera, Lepidoptera, Hymenoptera, Heteroptera, Homoptera, Diptera [8]. Some aphid (order Hemiptera) species, such as the green apple aphid (*Aphis pomi*), are the most widespread pests of apple trees, affecting them throughout the Northern Hemisphere. When aphids multiply en masse, the affected leaves and shoots curl and wilt, leading to a decline in fruit tree productivity. Plum trees are colonized throughout June and sometimes into early July. Under high air temperatures and moderate humidity, due to their high reproductive potential, the number of plum aphid larvae on young plum trees can reach 7–8 thousand per 100 leaves, completely covering the underside of the leaf blades [1]. Additionally, apple aphids are known to play a role in the maintenance and spread of phytopathogenic bacteria such as *Erwinia amylovora*, *E. aphidicola*, *Pseudomonas syringae*, *Dickeya dadantii*, and *Pantoea stewartii* [4]. It is known that microorganisms, including phytopathogens, may enter the aphid body from the surface and internal tissues of host plants. Many of these microbes are capable of adapting to life in the aphid's digestive tract and may even reproduce there. However, scientific researches focusing on aphids (order Hemiptera) as vectors for pathogenic fungi and outbreaks of certain diseases is scarce. Therefore, this topic requires a thorough investigation for its future exploitation in environment-friendly pest control management.

Current work aims to elucidate the role of aphids in transmitting certain pathogenic fungi in plum orchards in the Republic of Moldova. The identification of phytopathogenic fungi transmitted by aphids plays a crucial role in ensuring the phytosanitary stability of agroecosystems. Identifying specific fungal species carried by aphids not only enables effective pest control but also prevents secondary fungal infections. This makes it possible to include not only aphid monitoring and control but also additional fungal infection prevention in the Integrated Pest Management (IPM) system.

MATERIALS AND METHODS

At the beginning of the fruit ripening season, specimens of two aphid genera parasitizing apple (*Dysaphis* sp.) and plum (*Hyalopterus* sp.) trees growing in the IGFPP orchard were collected (Fig. 1). DNA extraction from the insects was performed using the ISO 21571 protocol [2].

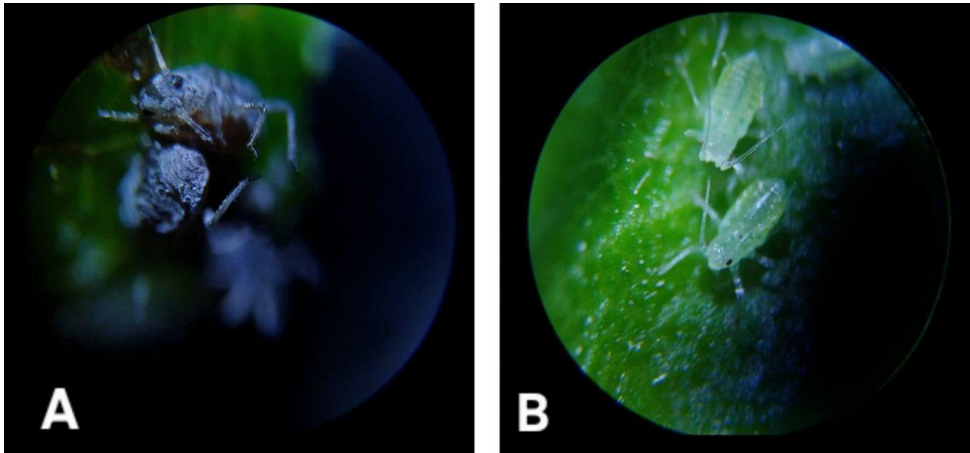


Figure 1. Visualization of aphid specimens collected in the IGFPF fruit orchard under a photon microscope.

A – *Dysaphis*, B – *Hyalopterus*

Table 1. Primer sequences for PCR analysis

Primer	Taxon	Primer orientation	Primer sequence
pchbt1	<i>P. chrysogenum</i>	Forward primer	GTTGCTAATCGATTACAGGCAAAC
pchbt2		Reverse primer	CACCGCTGGGCTAGATTGTGTC
pchbt3		Forward primer	TGATGGGGATTCTGTGTATCA
pchbt4		Reverse primer	CCGCTGCGCTAGATTGTCAA
pex1	<i>P. expansum</i>	Forward primer	CAACCTCCACCCGTGTTT
pex2		Forward primer	ACTCGTCCTGAGAGATTGTGT
pex3		Reverse primer	CGGAATCGGGGGGAGGGG
pex4		Reverse primer	ATTTGGGTTGTATCGGGAGC
mfg1	<i>M. fructigena</i>	Forward primer	CAATTATTGCTGATTGGGTGACA
mfg4		Reverse primer	AGGTGGCGTAATTTTGGAAATTAAGT
mfg2		Forward primer	GATTGGGGTGACAAGTCTATAATCCG
mfg3		Reverse primer	TCTAAAGTCTTCGCTGACCAATC
mla1	<i>M. laxa</i>	Forward primer	AGCAATTACTGCTGACTGGG
mla4		Reverse primer	AGGGCACTTATGCGGAATGTT
mla2		Forward primer	GTTCCATCACTCGCGAAGTCA
mla3		Reverse primer	AAAGTGGCGAAATCTTGAATC
bc1	<i>B. cinerea</i>	Forward primer	TCACCCGCACCTAATTCGTC
bc2		Reverse primer	AGGAGCCTTCTCCCTTGGTT
bc3		Forward primer	TGTGCGAGGTTGAAGTAGTG
bc4		Reverse primer	GCACTCGGCGATCCAGATAA

Molecular identification of pathogens was carried out using a two-step PCR method with primers specific to genomic regions of various fungal pathogens: *Monilinia spp.*, which cause significant spoilage of the harvest, leading to fruit rot;

as well as *Penicillium spp.* and *B. cinerea*, which are producers of mycotoxins. The primers were developed by staff of the Molecular Genetics Laboratory (IGFPP) based on species-specific sequences stored in the GenBank database [7].

Amplification included two consecutive rounds of 30 cycles each in a 25 μL reaction mixture containing: 66 mM Tris-HCl (pH 8.4), 16 mM $(\text{NH}_4)_2\text{SO}_4$, 2.5 mM MgCl_2 , 0.1% Tween 20, 7% glycerol, 100 μL^{-1} BSA, 0.2 mM of each dNTP, 1.25 units of Taq DNA polymerase (Thermo Fisher Scientific), 5 pM of each forward and reverse primer, and 10 ng of DNA template.

The first round began with a 3-minute initial denaturation at 95°C, followed by 30 seconds of denaturation at 95°C, 30 seconds of annealing at 60°C, and 30 seconds of elongation at 72°C. The second round was conducted without initial denaturation. Both rounds concluded with a final elongation step of 7 minutes at 72°C. Amplification products were separated by electrophoresis in a 1.5% agarose gel using 1×TBE buffer at 6 V/cm for one hour. Visualization was carried out using a transilluminator at a wavelength of 302 nm.

RESULTS AND DISCUSSION

Molecular analysis revealed a marked qualitative difference in fungal species associated with aphid populations of different species. Visualization of amplification products in samples containing DNA extracted from *Hyalopterus* revealed target sequences of *Penicillium chrysogenum*, *M. fructigena*, and *B. cinerea*. In contrast, no target sequences of the reviewed pathogens were detected in *Dysaphis* samples. The overall results of the PCR analysis using the aforementioned primers are summarized in Table 2.

Table 2. Identification of mycopathogens in the analyzed samples*

Pathogen \ Aphid	<i>Hyalopterus</i>	<i>Dysaphis</i>
<i>P. chrysogenum</i>	+	-
<i>P. expansum</i>	-	-
<i>M. fructigena</i>	+	-
<i>M. laxa</i>	-	-
<i>B. cinerea</i>	+	-

* “+” sign indicates presence of the fungi in the sample, “-” sign - absence

The emergence of founding female aphids in the main zone of harmful activity is observed from March to early April, coinciding with the bud scale separation of fruit trees. During the growing season, up to 10 generations typically develop. On the plant organs affected by aphids (leaves, annual shoots, flowers, and fruits), sugary secretions appear, which promote the development of saprophytic fungi by serving as an additional nutrient substrate essential for their activity [8].

Thus, in addition to their direct harmful impact on fruit trees, aphid colonies, by acting as vectors for pathogenic fungi, can contribute to the accumulation of damage in the host plant. As a result, a disruption of water balance and loss of nutrients occurs, which may lead to leaf and fruit drop by mid-summer, while the remaining fruits become deformed and begin to rot.

Based on the obtained results, it can be concluded that aphids of the *Hyalopterus* genus are capable of acting as vectors for both polyphagous fungi and specialized species such as *Botrytis cinerea* and *Monilinia fructigena*, potentially serving as sources of infection at the beginning of the growing season and during early fruit development.

Thus, pathological processes in fruit orchards, initially triggered by a single phytopathogen, tend to evolve over time into systemic, multifactorial pathologies involving numerous other organisms. As a result, several models of multi-level interaction systems emerge within agroecosystem pathogenesis, involving insect vectors, phytopathogens, and host plants.

CONCLUSION

The molecular-genetic analysis demonstrated the ability of early generations of *Hyalopterus* aphids to transmit pathogenic fungi such as *P. chrysogenum*, *Botrytis cinerea*, and *Monilinia fructigena*. A comprehensive assessment of the vector potential of the studied aphid genera requires further investigation and identification of fungal pathogens in fruit trees at different insect developmental stages.

Due to the variability of the mycopathogenic composition in fruit orchards and the pronounced susceptibility of mycopathosystems to abiotic and anthropogenic environmental factors, there is an increasing need for monitoring and timely identification of secondary infection foci to enhance the effectiveness of phytosanitary measures in orchard management.

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