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Časopis izlazi u elektroničkom izdanju dva puta godišnje, krajem lipnja i prosinca, a predviđena su i dva specijalna izdanja tijekom godine iz biotehničkog područja.
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Riječ gostujućeg urednika

Poštovani čitatelji Glasila Future,

pred Vama je specijalno izdanje časopisa posvećeno profesoru emeritusu Bogdanu Cvjetkoviću. Svojim znanstvenim i stručnim radom, koji traje više od pola stoljeća, prof. Cvjetković ostavio je značajan i neizbrisiv trag ne samo u Hrvatskoj nego i svjetskoj fitopatologiji i fitofarmaciji. Ostavljajući iza sebe brojne generacije diplomiranih inženjera agronomije svojim entuzijazmom i predanošću fitopatologiji uspio je „zaraziti“ te biti predani mentor 18 magistara znanosti te 8 doktora znanosti. Širokog znanja i znanstvenih interesa magistrirao je na Prirodoslovno-matematičkom fakultetu u Zagrebu iz području biljne virologije, a doktorirao na svojem *alma mater* Agronomskom fakultetu u Zagrebu kod profesora Josipa Kišpatića na području biljne mikologije. U želji da ovim brojem djelomično oslikamo široko područje interesa prof. Cvjetkovića, ovo specijalno izdanje obuhvaća tri izvorna znanstvena rada te dva prethodna priopćenja iz područja biljne mikologije, bakteriologije te virologije. Kraj ovog specijalnog izdanja posvećen je crticama iz života dr. Željka Jurjevića, jednog od doktora znanosti koji je doktorirao pod mentorstvom prof. Cvjetkovića, a trenutno s uspješnom karijerom u Sjedinjenim Američkim državama (EMSL Analytical, Inc.). Izrazito mi je drago da su se sudjelovanju u ovom broju odazvali znanstvenici koje se bave fitopatologijom na području Hrvatske, ali i kolege iz inozemstva, dajući svoj značajan doprinos kvaliteti ovog specijalnog izdanja, ali i izražavajući pijetet prof. Cvjetkoviću.

Prvi rad kolegica Dušice Kovačević, Katarine Zečević te Ivane Stanković s Poljoprivrednog fakulteta Univerziteta u Beogradu govori o djelomičnoj molekularnoj karakterizaciji izrazito polifagnog virusa mozaika krastavca izoliranoga iz dvije biljke božura sa simptomima mozaika i klorotičnih prstenova. Nakon potvrde virusa serološkim i molekularnim metodama sekvenciranjem dijela genoma proteinskog omotača utvrđeno je da izolati iz božura pripadaju u podgrupu IA. Autorice skreću pozornost da bi božur kao trajnica mogao imati značajnu epidemiološku ulogu u kontekstu značajnog izvora ovog virusa.

Rad kolega Kirila Bahcevandzieva te Antónia A. Monteira (Research Centre for Natural Resources, Environment and Society - CERNAS, Portugal) vodi nas u područje fenotipskih i genotipskih interakcija između različitih kupusnjača te ekonomski značajnog uzročnika plamenjača kupusnjača (*Hyaloperonospora brassicae*). Kroz istraživanje je utvrđeno da izolati navedenog patogena iz različitih područja Europe pokazuju različite stupnjeve patogenosti. Analizirani model gen-za-gen otvara nove mogućnosti istraživanja rezistentnosti kod različitih kupusnjača te gena za patogenost uzročnika plamenjače.

Da su na gljivične patogene osjetljive i invazivne biljne vrste govori rad autora Darija Ivića i Adrijane Novak (Hrvatska agencija za poljoprivredu i hranu). Analizom stabala pajasena sa simptomima

sušenja i propadanja na području Nacionalnog parka Krka utvrđena je prisutnost 15 različitih vrsta polifagnih gljiva iz rodova *Diaporthe*, *Diplodia*, *Dothiorella*, *Fomitiporia*, *Fusarium*, *Paraconiothyrium*, *Peroneutypa*, *Rosellinia*, *Schizophyllum* te *Verticillium*. Autori ističu da je ulogu utvrđenih gljiva u sušenju i propadanju ove invazivne vrste potrebno utvrditi testovima patogenosti.

Prethodno priopćenje doktorice znanosti Katarine Martinko i studentice Ivone Novaković sa Sveučilišta u Zagrebu Agronomskog fakulteta donosi preliminarne rezultate *in vitro* istraživanja protugljivičnog djelovanja esencijalnih ulja timijana, divljeg mažurana i lovora na uzročnika crne truleži plodova različitih poljoprivrednih kultura (*Aspergillus niger* Tiegh.). Autorice zaključuju da prvenstveno eterična ulja timijana i divljeg mažurana imaju veliki potencijal kao fumiganti u kontroli crne truleži uskladištenih poljoprivrednih proizvoda, te kao takvi predstavljaju svojevrsnu alternativu trenutno često korištenim fungicidima.

Prethodno priopćenje doktorice znanosti Jelene Plavec (Hrvatska agencija za poljoprivredu i hranu) opisuje uzročnika bakterioznog paleža lijeske (*Xanthomonas arboricola* pv. *corylina*) utvrđenog metodom lančane reakcije polimerazom iz rasadnika i komercijalnih nasada lijeske na području Hrvatske. Imajući u vidu sve veću popularnost ove kulture u našoj zemlji autorica skreće pozornost da će u budućnosti biti potrebno povesti više pažnje u praćenju ovog ekonomski značajnog patogena svrstanog na listu reguliranih nekarantenskih štetnika ne samo lijeske, već i drugih vrsta iz roda *Corylus*.

Crtice doktora znanosti Željka Jurjevića sažimlju različite dijelove profesionalnog razvoja prof. Cvjetkovića, ali ujedno predstavljaju i jednu toplu životnu priču protkanu zajedničkim trenucima provedenima s profesorom.

Vežući se na životopisne crtice dr. Jurjevića, i osobno kao jedan od doktoranada, mogu reći da bi se o liku i djelu prof. Cvjetkovića mogla napisati ne jedna, nego više knjiga. Na kraju mogu reći da mi je bila iznimna čast i zadovoljstvo intenzivno surađivati s profesorom sve do njegovog odlaska u mirovinu. I danas, sa životopisnim pričama i neograničenim praktičnim iskustvom, dragi mi je sugovornik na Zavodu za fitopatologiju u čiji razvoj je utkao značajno razdoblje svojega života i kojem je dao svoj neprocjenjivi obol!

Prof. dr. sc. Darko Vončina

Glasilo Future

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SADRŽAJ:

Fungi associated with declining tree of heaven (*Ailanthus altissima*) in Krka National Park

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izvorni znanstveni rad (original scientific paper)

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Abstract

Tree of heaven (*Ailanthus altissima*) is an oriental tree listed as an invasive alien species of Union concern. Its establishment and spread may be especially harmful in protected and ecologically sensitive environments. In 2021, a survey was carried out in Krka National Park to determine the presence of fungi on tree of heaven plants showing a decline, dieback or wilt symptoms. Eighteen symptomatic *A. altissima* plants were found in five locations within Krka National Park. These symptomatic trees were sampled and analysed for the presence of fungi. Fungi isolated were identified by morphology and ITS1/ITS4 sequencing. Fifteen fungal species were identified: *Diaporthe chamaeropis*, *D. eres*, *D. foeniculina*, *D. ravennica*, *Diplodia mutila*, *D. seriata*, *Dothiorella viticola*, *Fomitiporia mediterranea*, *Fusarium oxysporum*, *F. solani*, *Paraconiothyrium brasiliense*, *Peroneutypa scoparia*, *Rosellinia corticium*, *Schizophyllum commune* and *Verticillium dahliae*. The isolated fungi range in their ecological niches from secondary colonisers of the declining tree of heaven wood, to possible endophytes, and plant pathogens.

Key words: invasive species, tree of heaven, mycobiota, plant pathogens, endophytes, saprophytes.

Introduction

Invasive alien species are a major threat to biodiversity (McGeoch et al., 2010; Gentili et al., 2021). Tree of heaven (*Ailanthus altissima* (Mill.) Swingle) is a representative example of an invasive alien plant and is widely recognized as biologically harmful to sensitive ecosystems in Europe and other areas it has invaded (Simberloff et al., 2013; Sladonja et al., 2015). *Ailanthus altissima* is included in the list of invasive alien species of Union concern, as defined by Regulation (EU) 1143/2014 of the European Parliament and of the Council. One of the criteria to be included in this respective list is that the invasive species is likely to cause a 'significant adverse impact on biodiversity or the related

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ecosystem services'. Tree of heaven is a highly adaptable and opportunistic species able to successfully establish itself in new environments (Kowarik and Säumel, 2007; Novak and Kravarščan, 2011). It is native to China and was introduced into Europe during the 18th century as an ornamental plant (Kowarik and Säumel, 2007).

Tree of heaven is present in all Croatian counites (Novak and Novak, 2017). It has been described as especially invasive in the coastal part of the country (Novak and Novak, 2017). Fast spread, competition with indigenous plants and its effects on natural ecosystems make tree of heaven especially harmful to protected areas such as nature parks or national parks. Novak and Novak (2017) noted that tree of heaven has colonized relatively large areas of Krka National Park. The authors state that *A. altissima* within Krka National Park evidently "endangers native species, disturbs the stability of the ecosystem, alters the environment, and defaces the appearance of a landscape".

While the invasion of an alien species may be initially fast, various biotic and abiotic factors may alter such spread over time. In their natural range, plants are exposed to herbivorous vertebrates, insects and pathogens that are coevolutionary adapted to their hosts. Alien plants in new environments often initially lack natural enemies. Kowarik and Säumel (2007) state that "outside of its native range, *Ailanthus altissima* is usually subject to a low herbivore pressure...". While insects damaging to alien plants are usually easier to note and investigate, microorganisms like plant pathogenic fungi, bacteria or phytoplasmas may remain less evident. For *A. altissima*, much attention has been attributed to a wilt disease caused by a highly specialized fungus *Verticillium nonalfalfae* Interbitzin et al. (Kasson et al., 2014; Maschek and Halmschlager, 2018). However, many other fungi have been reported on this plant outside its natural range. Ding et al. (2006) reported 65 fungi found on tree of heaven. Some of them are presumed to be pathogenic, possibly affecting the invasive potential of this plant. This study aimed to check the eventual presence of *V. nonalfalfae* on *A. altissima* in Krka National Park, Croatia. Further, it aimed to identify fungi associated with the dead or declining tree of heaven plants, as the first step to identify the potential pathogens which may impact the spread of this invasive species. Finally, this was the first study on fungi naturally occurring on tree of heaven in Croatia.

Materials and methods

Visual examinations and collection of *A. altissima* samples were carried out in early October 2021. Five localities (Oćestovo, Ivoševci, Ključ, Lozovac and Skradin) within the Krka National Park were surveyed (figure 1). Tree of heaven plants on each locality were searched. Two types of plants were selected for sampling (table 1): 1 – dead plants (plants completely dead, no leaves and/or adventive shoots); 2 – declined plants (part of a crown or individual branches dead, crown partially alive, with or without individual shoots drying). Internal symptoms on selected plants were assessed by cutting branches and checking cross-cuts for the presence of discoloration, necrosis or wood rot. On trunks,

the bark was cut with a knife and internal symptoms inside the cambium and deeper in the wood were inspected. Eighteen plants, dead or showing decline symptoms, were sampled. Samples of wood cuts from trunks and 5-10 cm long fragments of thicker branches were taken from each plant. Plants were marked, designated as PjK and a number (table 1), and their GPS position was recorded.

Samples were analysed in the Laboratory for Mycology, Centre for Plant Protection – CAAF, in the city of Zagreb, Croatia. Each sample was analysed by two parallel procedures. Branch fragments were debarked, rinsed with distilled water, surface sterilized in 70 % ethanol for one min and incubated in moist chambers at 22 °C for 7-10 days. The presence of mycelium, fruiting bodies or other sporulating structures was examined by use of a dissecting microscope. If present, spores were morphologically analysed by light microscopy utilizing a compound microscope at 100x magnification. If possible, preliminary identification to the genus level was carried out following descriptions by Ellis and Ellis (1997) and Kirk et al. (2008). Fungi determined were compared to isolates obtained by the second procedure, described as follows.

Trunk fragments were cut to rectangular 2-4 mm chips, which were rinsed with water and surface sterilized as described above. Five to 15 chips were prepared from each sample. After drying in laminar flow, chips were placed on carrot-piece agar (CPA) and incubated for seven days at 20 °C. Plates were inspected for the development of fungal colonies. To obtain pure isolates, the edge of each colony was transferred to plates with potato-dextrose agar (PDA) and incubated for 10-14 days at 22 °C in darkness.

Fungal colonies developed on PDA were analysed for colony appearance, growth rate, hyphal morphology and the eventual presence of sporulating structures. If present, spores were microscopically examined. After a comparison of isolates with fungi developed on branches incubated in moist chamber, 25 isolates were selected for identification at the species level by molecular methods.

Mycelium was collected from cultures on PDA, frozen at -80 °C for a minute and ground to a fine powder utilizing a manual mortar and pestle. Total DNA extraction was performed by DNeasy® Plant Mini Kit (Qiagen) according to manufacturer instructions. Extracted DNA was quantified by spectrophotometry (Jenway® 7415 Nano). Five µl of extract was used for polymerase chain reaction (PCR) in the mixture of 12,5 µl EmeraldAmp Max® mix (Takara), 5,5 µl of water, and 1 µl of ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers (White et al., 1990). Reaction parameters were modified from White et al. (1990): 95 °C/2 min, 95 °C 20 sec, 55 °C 25 sec, 72 °C 50 sec (35 cycles) and final elongation at 72 °C for 10 min. Reactions were performed in Applied Biosystems® 2720 Thermal Cycler. Products were visualised in 1 % agarose gel, purified by GenElute® PCR-Clean-up Kit (Sigma-Aldrich) and prepared for sequencing. Sequencing was performed in Macrogen Europe®. Species were identified by comparing the similarity of sequences with those retrieved from GenBank® using the BLAST option.

Results

In total 15 fungal species were isolated from dead or declining tree of heaven trees in Krka National Park (Table 1). *Diaporthe ravennica* Thambug., Camporesi & K.D. Hyde was determined in four samples from three locations (Oćestovo, Ivoševci and Skradin). *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero (former name *Eutypella scoparia* (Schwein.) Ellis & Everh.) was identified in three samples from two locations (Oćestovo and Skradin). Species *Rosellinia corticium* (Schwein.) Sacc., *Fomitiporia mediterranea* M. Fisch., *Schizophyllum commune* Fr., *Diaporthe eres* Nitschke and *Fusarium solani* (Mart.) Sacc. were found in two samples. *Diplodia mutila* (Fr.) Fr., *Diplodia seriata* De Not., *Paraconiothyrium brasiliense* Verkley, *Fusarium oxysporum* Schltdl., *Dothiorella viticola* A.J.L. Phillips & J. Luque, *Verticillium dahliae* Kleb., *Diaporthe foeniculina* (Sacc.) Udayanga & Castl. and *Diaporthe chamaeropis* (Cooke) R.R. Gomes, Glienke & Crous were determined in one sample each.

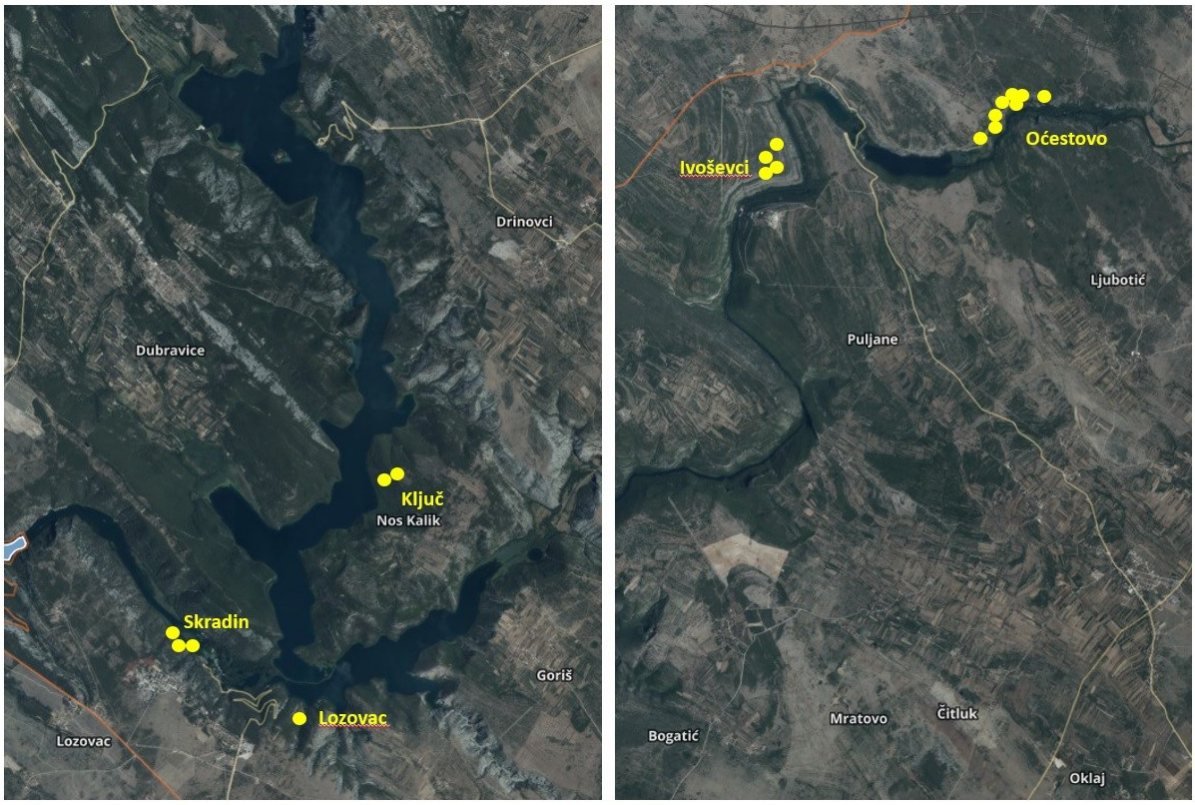

Figure 1. *Locations surveyed (yellow dots) for dead or declined tree of heaven (*Ailanthus altissima*) in Krka National Park.*

Table 1. *Tree of heaven (*Ailanthus altissima*) sample identification label, locations and fungal species identified on dead or symptomatic plants.*

Sample (plant)	Location	Category	Fungal species found
PjK-1	Oćestovo	dead plant	*Fusarium solani*
PjK-3	Oćestovo	declined plant	*Diplodia mutila* (= *Botryosphaeria stevensii*)
PjK-5	Oćestovo	dead plant	*Rosellinia corticium* *Diaporthe eres*
PjK-7	Oćestovo	dead plant	*Eutypella scoparia* (= *Peroneutypa scoparia*)
PjK-9	Oćestovo	dead plant	*Eutypella scoparia* (= *Peroneutypa scoparia*)
PjK-10	Oćestovo	dead plant	*Rosellinia corticium* *Paraconiothyrium brasiliense*
PjK-11	Oćestovo	declined plant	*Fusarium oxysporum*
PjK-12	Oćestovo	declined plant	*Diaporthe ravennica*
PjK-14	Ivoševci	declined plant	*Dothiorella viticola* *Diaporthe eres*
PjK-15	Ivoševci	dead plant	*Diaporthe foeniculina* *Schizophyllum commune*
PjK-16	Ivoševci	declined plant	*Diaporthe ravennica* *Fusarium solani*
PjK-17	Ivoševci	declined plant	*Diaporthe ravennica*
PjK-21	Ključ	declined plant	*Diplodia seriata*
PjK-22	Ključ	dead plant	*Schizophyllum commune*
PjK-24	Lozovac	declined plant	*Fomitiporia mediterranea* *Diaporthe chamaeropis*
PjK-26	Skradin	declined plant	*Diaporthe ravennica*
PjK-27	Skradin	dead plant	*Eutypella scoparia* (= *Peroneutypa scoparia*)
PjK-28	Skradin	dead plant	*Verticillium dahliae* *Fomitiporia mediterranea*

Discussion

All fungal species isolated from the dead or declining tree of heaven plants have been described as plant pathogens in some plant host systems such as *Fomitiporia mediterranea* on grape (*Vitis* spp.) (Fischer, 2002). However, many of them are also widely considered as endophytes, secondary parasites or saprophytic species developing on dead plant tissues (Leslie and Summerell, 2006; Gomes et al., 2013; Petrini et al., 2013; De Errasti et al., 2014). Since pathogenicity tests were not performed, it cannot be presumed to which extent particular fungal species contributed to the decline or dieback of *A. altissima* plants. The role of the fungi in a decline of trees is often complex. Generally, only a relatively small number of fungal species are severe pathogens able to kill an adult tree. Abiotic and biotic factors more often show cumulative and interconnected effect, contributing jointly to tree death or decline. Symptomatic tree of heaven plants may have been stressed or damaged by shocks like freezing or drought (Kowarik and Säumel (2007), making them vulnerable to fungal infections. Fungi from genera like *Diplodia* and *Diaporthe*, as well as wood-rotting species like *F. mediterranea* or *S. commune* are sometimes described as pathogenic to plants damaged by stress (Slippers and Wingfield, 2007; Gomes et al., 2013; Moretti et al., 2021).

All fungal species found on tree of heaven are polyphagous, described on numerous woody plants. Among them, *Fusarium solani* and *F. oxysporum* are probably the most ubiquitous (Leslie and Summerell, 2006). These *Fusarium* species are frequently isolated from numerous annual and perennial plants (Leslie and Summerell, 2006). Depending on the strain and the host they are infecting, they may be destructive pathogens, weak secondary invaders or only saprophytes (Leslie and Summerell, 2006). Similarly to *Fusarium* species, the genus *Diaporthe* also comprises severe plant pathogens, weakly pathogenic species, endophytes and saprobes (Gomes et al., 2013; Udayanga et al., 2014). It may be noted that pycnidia of all *Diaporthe* species found in the present study developed abundantly on debarked and discoloured wood of *A. altissima* samples in the laboratory. *Diaporthe* species are found on many woody plants, but taxonomic uncertainties arising from their morphological similarity have brought confusion on a host range of particular species (Udayanga et al., 2014). Certain *Diaporthe* species have been described both as endophytes and pathogens. For example, *D. eres*, found in one sample, has been described as a common endophyte on a number of plant hosts (Udayanga et al., 2014). However, it has also been found as a pathogen, e.g., on blackberry (Vrandečić et al., 2011) or hazelnut (Arciuolo et al., 2021). Three other *Diaporthe* species were found in the present study, *Diaporthe ravennica*, *D. foeniculina* and *D. chamaropis*. They may form a complex of closely related *Diaporthe* species on *A. altissima*, as it is known in the case of other woody species (Dissanayake et al., 2017; Chen et al., 2014; Udayanga et al., 2014; Gomes et al., 2013). *Diaporthe ambigua* has been recorded as a pathogen on kiwi (Auger et al., 2013) and grapevine (van Niekerk et al., 2005). *Diaporthe foeniculina* has been described as a pathogen of chestnut (Annesi et al., 2016), avocado (Guarnaccia et al., 2016) or eucalyptus (Deidda et al., 2016). This species has been found on *A. altissima* in Italy (Dissanayake et al., 2017), but without an assessment of its potential pathogenicity.

Two widespread wood-rotting basidiomycetes were found in this study, *S. commune* and *F. mediterranea*. *Schizophyllum commune* is one of the most common secondary colonizer of declining or dead woody plants (Ohm et al., 2010), and it could be presumed that it has a similar role on tree of heaven. *Fomitiporia mediterranea* is known as a much more potent pathogen (Moretti et al., 2021), reported as the most important causal agent of grapevine esca disease (Fischer, 2002; Moretti et al., 2021). Basidiocarps of *F. mediterranea* were noted on the trunks of both *A. altissima* plants from which the fungus was isolated.

Species *Rosellinia corticium* and *Peroneutypa scoparia* have been found in samples of *A. altissima* with distinctive internal symptoms. Plants were dead, and black discoloration was visible under the bark. Both fungal species have been described as endophytes, secondary colonizers and saprophytes on numerous woody plants (Acero et al., 2004; Petrini, 2013; de Errasti et al., 2014; Ćelepirović et al., 2020). Relatively recently, *P. scoparia* has been confirmed as pathogenic to kiwifruit (Castilla-Cayuman et al., 2018).

Fungi which are most likely to be pathogenic on tree of heaven in this research are Botryosphaeriaceae species (*Diplodia* and *Dothiorella* genera) and *Verticillium dahliae*. *Diplodia mutila* is known as a frequent causal agent of cankers and dieback of woody plants (Sutton, 1980; Slippers and Wingfield, 2007). It has been associated with the decline of oak (Ragazzi and Mesturino, 1978), ash (Kraj et al., 2013) and pines (Mohali and Encinas, 2001). In agriculture, *D. mutila* was reported as one of the causal agents of grapevine Botryosphaeria dieback (Kaliterna et al., 2011), as well as a pathogen of stone fruits, pome fruits and walnuts (Slippers et al., 2006; Chen et al., 2014; Diaz et al., 2018). *Diplodia seriata* has been reported on more than 200 woody hosts (Farr and Rossman, 2013). It is well-known as a grapevine pathogen (Úrbez-Torres, 2011), but its pathogenicity has also been confirmed on many other plant species (Phillips et al., 2007). *Dothiorella viticola* has been described in 2005 (Luque et al., 2005). It has been reported as a causal agent of canker disease on some crops, such as citrus (Adesemoye et al., 2014) or grapevine (Luque et al., 2005).

Verticillium dahliae is a cosmopolitan pathogen causing wilt disease in many plant hosts (Inderbitzin et al., 2011; Inderbitzin and Subbarao, 2014). The fungus has been reported to cause progressive wilt of tree of heaven in Toscana, Italy (Pisuttu et al., 2020). The pathogenicity of *V. dahliae* on *A. altissima* has been proven (Pisuttu et al., 2020). The authors are stating that *V. dahliae* has been probably found as a causal agent of *A. altissima* wilt in Italy long ago, as they found in description of Goidanich (1935). Another *Verticillium* species, *V. nonalfalfae*, is causing wilt of tree of heaven and has a perspective as a biocontrol agent against this invasive plant. The efficacy and host specificity of *V. alfalfae* was proven in studies conducted in the USA (Kasson et al., 2014) and Austria (Maschek and Halmschlager, 2018; Lechner et al., 2023).

All fungi found in this study are reported on various woody hosts, many of them present in the natural habitats of Krka National Park. Other plants probably serve as an inoculum for tree of heaven infection or colonization, whether as pathogens, weak pathogens, endophytes or saprophytes. Plant pathogenic fungi may naturally influence the spread of *A. altissima* and its ability to invade new areas. Although it is not realistic to expect that the fungi described could significantly contain the spread of *A. altissima*, the results show how invasive alien plants are exposed to various microorganisms in newly invaded areas.

Conclusion

In newly invaded areas, tree of heaven (*Ailanthus altissima*) is exposed to various naturally present fungi. Many fungal species living as pathogens, endophytes or saprophytes on other wood plants can attack or colonize tree of heaven. Fifteen fungi were found on dead tree of heaven plants, or plants showing decline symptoms in Krka National Park. Their role cannot be known without pathogenicity tests.

References

Acero, F.J., González, V., Sánchez-Ballesteros, J., Rubio, V., Checa, J., Bills, G.F., Salazar, O., Platas, G., Peláez, F. (2004). Molecular phylogenetic studies on the Diatrypaceae based on rDNA-ITS sequences. *Mycologia 96*, 249-259. http://dx.doi.org/10.2307/3762061

Adesemoye, A.O., Mayorquin, J.S., Wang, D.H., Twizeyimana, M., Lynch, S.C., Eskalen, A. (2014). Identification of species of Botryosphaeriaceae causing bot gummosis in citrus in California. *Plant Disease 98*, 55-61. https://doi.org/10.1094/pdis-05-13-0492-re

Annesi, T., Luongo, L., Vitale, S., Galli, M., Belisario, A. (2016). Characterization and pathogenicity of *Phomopsis theicola* anamorph of *Diaporthe foeniculina* causing stem and shoot cankers on sweet chestnut in Italy. *Journal of Phytopathology 164*, 412-416. https://doi.org/10.1111/jph.12426

Arciuolo, R., Leggieri, M.C., Chiusa, G., Castello, G., Genova, G., Spigolon, N., Battilani, P. (2021). Ecology of *Diaporthe eres*, the causal agent of hazelnut defects. *PLoS One 16(3)*, https://doi.org/10.1371/journal.pone.0247563

Auger, J., Perez, I., Estero, M. (2013). *Diaporthe ambigua* associated with post-harvest fruit rot of kiwifruit in Chile. *Plant Disease 97*, https://doi.org/10.1094%2FPDIS-10-12-0990-PDN

Castilla-Cayuman, A., Lolas, M., Diaz, G.A. (2018). First report of *Peroneutypa scoparia* causing cane dieback in kiwifruit in Chile. *Plant Disease 103*, https://doi.org/10.1094/PDIS-06-18-1092-PDN

Chen, S.F., Morgan, D.P., Hasey, J.K., Anderson, K., Michailides, T.M. (2014). Phylogeny, morphology, distribution, and pathogenicity of Botryosphaeriaceae and Diaporthaceae from English walnut in California. *Plant Disease 95*, https://doi.org/10.1094/PDIS-07-13-0706-RE

Ćelepirović, N., Novak Agbaba, S.N., Vlahović, M.K. (2020). DNA barcoding of fungi in the forest ecosystem of the Psunj and Papuk mountains in Croatia. *South-East European Forestry 11*, https://doi.org/10.15177/seefor.20-17

De Errasti, A., Novas, M.V., Carmarán, C. (2014). Plant-fungal association in trees: Insights into changes in ecological strategies of *Peroneutypa scoparia* (Diatrypaceae). *Flora 209*, 704-710. https://doi.org/10.1016/j.flora.2014.07.006

Deidda, A., Buffa, F., Linaldeddu, B.T., Pinna, C., Scanu B. (2016). Emerging pests and diseases threaten *Eucalyptus camaldulensis* plantations in Sardinia, Italy. *IForest-Biogeosciences and Forestry 9*, 883-891. https://doi.org/10.3832/ifor1805-009

Diaz, G.A., Latorre, B.A., Ferrada, E., Gutiérrez, M., Bravo, F., Lolas, M. (2018). First report of *Diplodia mutila* causing branch dieback of English walnut cv. Chandler in the Maule Region, Chile. *Plant Disease 102*, https://doi.org/10.1094/PDIS-11-17-1860-PDN

Ding, J.Q., Wu, Y., Zheng, H., Fu, W.D., Reardon, R., Liu, M., 2006. Assessing potential biological control of the invasive plant, tree-of-heaven, *Ailanthus altissima*. *Biocontrol Science and Technology 16*, 547-566, https://doi.org/10.1080/09583150500531909

Dissanayake, A.J., Camporesi, E., Hyde, K.D., Zhang W., Yan, J.Y., Li, X.H. (2017). Molecular phylogenetic analysis reveals seven new *Diaporthe* species from Italy. *Mycosphaere 8*, 853-877. http://dx.doi.org/10.5943/mycosphere/8/5/4

Ellis, B.M., Ellis, J.P. (1997). Microfungi on Land Plants - An Identification Handbook. The Richmond Publishing, Slough, UK.

Farr, D.F., Rossman, A.Y., (2013). Fungal databases. Systematic Mycology and Microbiology Laboratory, ARS, USDA. [http://nt.ars-grin.gov/fungaldatabases/]

Fischer, M. (2002). A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycological Progress 1*, 315-324. http://dx.doi.org/10.1007/s11557-006-0029-4

Gentili, R., Schaffner, U., Martinoli, A., & Citterio, S. (2021). Invasive alien species and biodiversity: impacts and management. *Biodiversity 22*, 1-3. https://doi.org/10.1080/14888386.2021.1929484

Goidanich, G. (1935). Novi casi di tracheomicosi da 'Verticillium' in Italia. *Bollettino della Stazione di Patologia Vegetale, Roma, 15*, 548-554.

Gomes, R.R., Glienke, C., Videira, S.I., Lombard, L., Groenewald, J.Z., Crous, P.W. (2013). *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia 31*, 1-41. https://doi.org/10.3767%2F003158513X666844

Guarnaccia, V., Vitale, A., Cirvilleri, G., Aiello, D., Susca, A (2016). Characterization and pathogenicity of fungal species associated with branch cankers and stem-end rot of avocado in Italy. *European Journal of Plant Pathology 146*, 963-976. https://doi.org/10.1007/s10658-016-0973-z

Inderbitzin, P., Bostock, R.M., Davis, R.M., Usami, T., Platt, H.W., Subbarao, K.V. (2011). Phylogenetics and taxonomy of the fungal vascular wilt pathogen *Verticillium*, with the descriptions of five new species. *PLoS One 6(12)*, https://doi.org/10.1371/journal.pone.0028341

Inderbitzin, P., Subbarao, K.V. (2014). *Verticillium* systematics and evolution: how confusion impedes Verticillium wilt management and how to resolve it. *Phytopathology 104*, 564-574. https://doi.org/10.1094/phyto-11-13-0315-ia

Kaliterna, J., Miličević, T., Cvjetković, B. (2011). Bolesti vinove loze uzrokovane fitopatogenim gljivama iz porodice Botryosphaeriaceae. *Glasilo biljne zaštite 6*, 424-430.

Kasson, M.T., Short, D.P.G., O'Neal, E.S., Subbarao, K.V., Davis, D.D. (2014). Comparative pathogenicity, biocontrol efficacy, and multilocus sequence typing of *Verticillium nonalfalfae* from the invasive *Ailanthus altissima* and other hosts. *Phytopathology 104*, 282-292. https://doi.org/10.1094/phyto-06-13-0148-r

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A. (2008). Dictionary of the Fungi. CAB International, UK.

Kowarik I, Säumel I. (2007). Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. *Perspectives in Plant Ecology, Evolution and Systematics 8*, 207-237. https://doi.org/10.1016/j.ppees.2007.03.002

Kraj, W., Kowalski, T., Zarek, M. (2013). Structure and genetic variation of *Diplodia mutila* on declining ashes (*Fraxinus excelsior*) in Poland. *Journal of Plant Pathology 95*, 499-507. http://dx.doi.org/10.4454/JPP.V95I3.015

Lechner, Y., Maschek, O., Kirisits, T, Halmschlager, E. (2023). Further pathogenicity testing of *Verticillium nonalfalfae*, a biocontrol agent against the invasive Tree of Heaven (*Ailanthus altissima*), on non-target species in Europe. *Phytoparasitica* 51, 113-130. https://doi.org/10.1007/s12600-022-01032-z

Leslie, J.F., Summerell, B.A. (2006). The Fusarium laboratory manual. Oxford: Blackwell Publishing, UK.

Luque, J., Martos, S., Phillips, A.J.L. (2005). *Botryosphaeria viticola* sp. nov. on grapevines: a new species with a *Dothiorella* anamorph. *Mycologia 97*, 1111-1121. https://doi.org/10.1080/15572536.2006.11832759

Maschek, O., Halmschlager, E. (2018). Effects of *Verticillium nonalfalfae* on *Ailanthus altissima* and associated indigenous and invasive tree species in eastern Austria. *European Journal of Forest Research 137*, 197-209. https://doi.org/10.1007/s10342-018-1099-y

McGeoch, M.A., Butchart, S.H.M., Spear, D., Marais, E., Kleynhans, E.J., Symes, A., Hoffmann, M. (2010). Global indicators of biological invasion: Species numbers, biodiversity impact and policy responses. *Diversity and Distributions 16*, 95-108. https://doi.org/10.1111/j.1472-4642.2009.00633.x

Mohali, S.R., Encinas, O. (2001). Association of *Diplodia mutila* with blue stain of Caribbean pine in Venezuela. *Forest Pathology 31*, 187-189. http://dx.doi.org/10.1046/j.1439-0329.2001.00234.x

Moretti, S., Pacetti, A., Pierron, R., Kassemeyer, H.-H., Fischer, M., Péros, J.-P., Perez-Gonzalez, G., Bieler, E., Schilling, M., Di Marco, S., Gelhaye, E., Mugnai, L., Bertsch, C., Farine, S. (2021). *Fomitiporia mediterranea* M. Fisch., the historical Esca agent: a comprehensive review on the main grapevine wood rot agent in Europe. *Phytopathologia Mediterranea 60*, 351-379. https://doi.org/10.36253/phyto-13021

Novak, N., Kravarščan, M. (2011). Invazivne strane korovne vrste u Republici Hrvatskoj. Hrvatski centar za poljoprivredu, hranu i selo, Zagreb.

Novak, M., Novak, N. (2017). Rasprostranjenost invazivne strane vrste pajasena [*Ailanthus altissima* (Mill.) Swingle] po županijama Republike Hrvatske. *Glasilo biljne zaštite 3*, 329-337.

Ohm, R.A., De Jong, J.F., Lugones, L.G., Aerts, A., Kothe, E., Stajich, J.E., De Vries, R.P., Record, E. (2010). Genome sequence of the model mushroom *Schizophyllum commune*. *Nature Biotechnology 28*, 957-963. https://doi.org/10.1038/nbt.1643

Petrini, L.E. (2013). *Rosellinia* – A world monograph. Bibliotheca Mycologica 205, Stuttgart, Germany.

Phillips, A.J.L., Crous, P.W., Alves, A. (2007). *Diplodia seriata*, the anamorph of *Botryosphaeria obtusa*. *Fungal Diversity 25*, 141-155.

Pisuttu, C., Marchica, A., Bernardi, R., Calzone, A., Cotrozzi, L., Nali, C., Pellegrini, E., Lorenzini, G. (2020). Verticillium wilt of *Ailanthus altissima* in Italy caused by *V. dahliae*: new outbreaks from Tuscany. *iForest – Biogeosciences and Forestry 13*, 238-245. https://doi.org/10.3832/ifor3238-013

Ragazzi, A., Mesturino, L. (1987). *Diplodia mutila* in Italia: associata al 'deperimento della quercia'? *Italia Forestale e Montana 42*, 264-274.

Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A,, Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., Vilà, M. (2013). Impacts of biological invasions: what's what and the way forward. *Trends in Ecology and Evolution 28*, 58-66. https://doi.org/10.1016/j.tree.2012.07.013

Sladonja, B., Sušek, M., Guillermic, J. (2015). Review on invasive tree of heaven (*Ailanthus altissima* (Mill) Swingle) conflicting values: assessment of its ecosystem services and potential biological threat. *Environmental Management 56*, 1009-1034. https://doi.org/10.1007/s00267-015-0546-5

Slippers, B., Smit, W.A., Crous, P.W., Coutinho, T.A., Wingfield, B.D., Wingfield, M.J. (2006). Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruit trees in South Africa and other regions of the world. *Plant Pathology 56*, 128-139. https://doi.org/10.1111/j.1365-3059.2006.01486.x

Slippers B., Wingfield, M. J. (2007). Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews 21*, 90-106. https://doi.org/10.1016/j.fbr.2007.06.002

Sutton, B.C. (1980). The Coelomycetes. Commonwealth Mycological Institute, Kew, UK.

Udayanga, D., Castelbury, L.A., Rossman, A.Y., Chukeatirote, E., Hyde, K.D. (2014). Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. *Fungal Diversity 67*, 203-229. https://doi.org/10.1007/s13225-014-0297-2

Úrbez-Torres, J.R. (2011). The status of Botryosphaeriaceae species infecting grapevines. *Phytopathologia Mediterranea 50*, 5-45. https://doi.org/10.14601/Phytopathol_Mediterr-9316

Van Niekerk, J.M., Groenewald, J.Z., Farr, D.F., Fourie, P.H., Halleen, F., Crous, P.W. (2005). Reassessment of *Phomopsis* species on grapevines. *Australasian Plant Pathology 34*, 27-39. https://doi.org/10.1071/AP04072

Vrandečić, K., Jurković, D., Ćosić, D., Postić, J., Riccioni, L. (2011). First report of cane blight on blackberry caused by *Diaporthe eres* in Croatia. *Plant Disease 95*, 612. https://doi.org/10.1094/pdis-11-10-0860

White, T.J., Bruns, T., Lee, S., Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: A guide to methods and applications (M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, eds.). New York, Academic Press Inc., pp. 315-322.

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