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CHARACTERISATION OF QOI FUNGICIDE SENSITIVITY AND *CYTOCHROME B* GENE OF *PHYLLOSTICTA AMPELICIDA* CAUSAL AGENT OF GRAPE BLACK ROT

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1 INTRODUCTION AND AIMS OF THE STUDY

Black rot of grapes is caused by the fungus *Phyllosticta ampelicida* (Engelm.) Aa (synonym: *Guignardia bidwellii* (Ellis) Viala and Ravaz). The pathogen is native to North America but was detected in Hungary in 1999 (Mikulás and Tomcsányi, 1999). The importance of black rot worldwide depends mainly on the geographical location of the wine region and the weather/climate conditions of the wine region in a given year. In Hungary, the pathogen is persistent in the northern wine regions (e.g. Egri and Tokaj-Hegyalja) and requires constant attention. Under favourable (warm and humid) conditions, *P. ampelicida* is able to infect all green organs of the vine (Molitor and Berkelmann-Loehnertz, 2011). Without adequate protection, infection of young berries can cause severe yield losses ranging from 5% to 100% (Loskill et al., 2009).

The importance of the pathogen has increased in the 21st century, both in Hungary and in other parts of Europe, to the extent that it caused a significant epidemic in Hungary in 2010 and 2014, two years with more than usual summer rainfall (Dula, 2017; Mikulás, 2015). Control of the pathogen relies mainly on plant health measures (e.g. removal of fruit mummies from the plant) and the use of plant protection products. The most effective groups of fungicides for the control of powdery mildew include quinone outside inhibitors (QoIs) and demethylase inhibitors (DMIs), which inhibit mitochondrial respiration, and dithiocarbamates (Hoffman et al., 2002). However, due to health and environmental concerns, the European Union has recently withdrawn the authorisations of a number of active substances, mostly belonging to the DMI and dithiocarbamate groups

(EU Pesticides Database (v.2 .2) Search Active substances, safeners and synergists, 2022). Other trends that may be favorable to the pathogen (e.g. the use of newer grape varieties with powdery mildew resistance and changing weather conditions due to climate change) may overall underline the importance of QoI fungicides in the control of grapevine powdery mildew.

The objectives of this doctoral thesis were:

1. To participate in the establishment of the first large-scale national collection of *P. ampelicida* strains.
2. To evaluate the genetic variability within the *P. ampelicida* strain collection by ISSR genotyping.
3. Verification of phylogenetic relationships between *P. ampelicida* and related *Phyllosticta* species (with particular reference to strain CBS 237.48).
4. To assess the *in vitro* susceptibility to QoI fungicides and the efficacy of alternative respiratory pathways by evaluating mycelial growth and conidial germination of selected *P. ampelicida* isolates.
5. Assessment of fungicide resistance markers in *CYTB* mRNA and
6. to explore the exon/intron structure of the *cytb* gene in isolates/strains of selected *P. ampelicida* and related *Phyllosticta* species.
7. Furthermore, to compare the sequence and exon/intron structure of the *cytb* gene between isolates of *P. ampelicida* collected in different populations/habitats/countries and/or different seasons and strains of other *Phyllosticta* species.

2 MATERIALS AND METHODS

With my collaborating partners in Hungary, we regularly collected grape leaves and berries infected with *P. ampelicida*. From the collected samples, a *P. ampelicida* strain collection (495 isolates) was established by isolating the pathogen. This was supplemented with foreign *P. ampelicida* isolates and *Phyllosticta* strains obtained from international strain collections (13 isolates/strain in total).

The *P. ampelicida* collection of 499 isolates was genotyped using the intermediate simple sequence repeat (ISSR) method. For this purpose, a total of 89 ISSR primers were tested on DNA extracts of eight selected *P. ampelicida* isolates collected from different parts of Europe and/or under different conditions in our country.

Based on this result and other parameters (e.g. place and year of collection, etc.), 48 *P. ampelicida* isolates were selected for mycelial growth inhibition studies. *In vitro* inhibition of mycelial growth by QoI fungicides (azoxystrobin and trifloxystrobin) and an alternative oxidase inhibitor (salicylic hydroxamic acid (SHAM)) was tested with colleagues using the gradient plate technique (Hunt and Sandham, 1969).

To measure the mycelial growth of 48 selected isolates under different treatments, a computer short program (script) was adapted in the MATLAB® R2016b software package (Fuentes et al., 2010). Then, from the results generated by the short program, I calculated the 50% growth rate (50% growth inhibition, EC_{50}) values for each of the four treatments of each fungal isolate, as well as the percentage of mycelial growth inhibition (Ishii et al., 2009).

For *in vitro* conidial germination fungicide susceptibility testing, two isolates of *P. ampelicida* ('18-3-9' and '19-129' isolates) were selected based on mycelial growth inhibition ranking. From the number of germinating conidia, I calculated the percent inhibition values for the two isolates for different experimental combinations.

The results of the ISSR, mycelium growth inhibition and conidium germination inhibition experiments were subjected to statistical analysis in the statistical computing environment R (RStudio Team: http://www.rstudio.com/).

For the molecular work, DNA was extracted from more than 500 isolates/strains of *Phyllosticta* spp. (based on Edwards et al. 1991). I also performed total RNA extraction from 17 of these isolates and produced cDNA from these extracts. The purified cDNA (fungicide resistance markers) and total DNA (ISSR, phylogenetics and exon/intron structure determination) served as templates for polymerase chain reaction experiments.

The phylogenetic status of *P. ampelicida* and related species was assessed by maximum likelihood (ML) phylogenetic analysis of the ITS locus using 48 native *P. ampelicida* isolates, 3 additional *P. ampelicida* isolates of different geographical origin and two CBS strains (CBS111645 and CBS237.48) isolated from wild grapes.

For the determination of the exon/intron structure of the *cytb* gene, the gene fragments were amplified by PCR from DNA extracts of the selected 13 *Phyllosticta* spp. isolates and their sequence was determined. To verify the fungicide resistance markers in the *CYTB* mRNA sequence, *CYTB* mRNA fragments from cDNA samples were amplified using the self-designed primer pairs GuiCobCdsF (GCCF) and GuiCobCdsR (GCCR).

All PCR products (except ISSR products) were cloned into bacterial vector and sequenced using Sanger sequencing. Finally, the sequences were cut and aligned using CodonCode Aligner software.

3 RESULTS AND THEIR DISCUSSION

3.1. ISSR genotyping

I was able to classify the 499 *P. ampelicida* isolates into a total of eight different clusters/genotypes on the UPGMA dendrogram. The majority of the strains (98.6%) belonged to cluster 1 and 2 (64.8% and 29.7%). For the isolates from the Hungarian wine regions, this proportion was similar, as 61.7% of the strains belonged to cluster 1, while 30.5% belonged to cluster 2. Clusters 4-7 were represented by only one or two isolates from the Egri wine region, while in the Tokaj-Hegyalja wine region only isolates belonging to the two dominant clusters (1 and 2) were found. Of the eight isolates from Moscenicka Draga in Croatia, seven belonged to cluster 1, while the eighth was the sole representative of cluster 3. Of the two isolates from Vinhos Verdes, Portugal, one was the most common and populous member of cluster 1, while the other was the sole representative of cluster 8. Finally, the single isolate from Canneto Pavese in Italy was in cluster 2.

3.2. Phylogenetic analysis of *Phyllosticta* isolates/strains from grapes and wild grapes

The ITS sequence of *Pa. tricuspidata* isolate CBS 237.48 showed a high degree of BlastN similarity (99.28-100%) with sequences of

isolates recently described as *P. partricuspidatae* (Zhou et al., 2015). In addition, our maximum likelihood (ML) analysis showed that it formed a well-supported clade with sequences of *P. partricuspidatae* isolated from *Pa. tricuspidata*. The same sequence showed only ≤97.59% similarity with the ITS sequence of *P. ampelicida* isolates from *V. vinifera*. The result of the ML phylogenetic analysis showed that the eight newly determined sequences of *P. ampelicida* and the sequences stored in GenBank as *P. ampelicida* (or *G. bidwellii*) form a well supported (BS = 97%) clade. A fully supported (BS = 100%) clade was formed by sequences of plant pathogens of the *Vitaceae* family (*P. partricuspidatae* and *P. vitis-rotundifoliae*). The sequence from *P. parthenocissi* strain (CBS 111645) was identical to the sequence from the type strain (GenBank acc. No. NR_147322.1).

3.3. Fungicide susceptibility test

None of the selected isolates showed an EC_{50} value higher than the upper limit of the gradient (0.038 μg ml^{-1}). All isolates with an unmeasurably low EC_{50} value were highly sensitive to the treatments tested (group of bands with EC_{50} category <0.01 μg ml^{-1}).

All 48 isolates of *P. ampelicida* were highly sensitive to both azoxystrobin and trifloxystrobin, with mean EC_{50} values (mean ± standard deviation) of 0.029 ± 0.007 and 0.022 ± 0.008 μg ml^{-1}, respectively. The high sensitivity of the *P. ampelicida* isolates to QoIs was also evident from the high growth inhibition percentages, which ranged from 52.23 to 88.64% for azoxystrobin and from 74.80 to 96.15% for trifloxystrobin (Table 8). The percentage inhibition of mycelial growth was significantly lower for azoxystrobin (mean ±

standard deviation: 75.37 ± 7.44%) than for trifloxystrobin (86.78 ± 5.07%).

The addition of SHAM resulted in a decrease in EC_{50} values for azoxystrobin + SHAM and trifloxystrobin + SHAM: 0.024 ± 0.007 and 0.017 ± 0.005 μg ml^{-1}, respectively. On the other hand, mycelial growth inhibition percentages were significantly increased for both azoxystrobin (80.04 ± 6.59%) and trifloxystrobin (90.29 ± 4.52%). The interaction between fungicide and SHAM treatments was not significant ($p = 0.118$).

Inhibition of conidial germination was investigated with two selected *P. ampelicida* isolates. Conidial germination was significantly inhibited by the presence of azoxystrobin and trifloxystrobin. The mean percent inhibition of germination for isolate 18-3-9 (mean ± standard deviation) was 92.45 ± 5.97% and 93.40 ± 4.11% in the presence of azoxystrobin and trifloxystrobin, respectively. For isolates 19-129, these values were very similar in the presence of azoxystrobin (90.24 ± 4.36%) and trifloxystrobin (90.73 ± 5.83%). Similarly, the addition of SHAM resulted in only a slight difference in the effects of the two compounds on germination. However, none of the differences were statistically significant: neither the fungicide, nor the SHAM, nor the isolate effect.

3.4. Molecular *characterization of cytochrome b* gene/mRNA in *Phyllosticta* isolates/strains

A 906 base-pair length fragment of *CYTB* mRNA (cDNA) was amplified, cloned and nucleotide sequenced from different *Phyllosticta* spp. isolates/strains.

The ORF translated from the 906 base-pair mRNA fragment consisted of 302 amino acids and covered the region between the 20th and 320th As positions of the CYTB protein. This section contains two "hot spot" regions of potential point mutations conferring resistance to QoI, located between amino acid positions 120-160 and 250-300. All mRNA fragments examined contained the wild-type codon at positions 129 (TTC → phenylalanine incorporated), 137 (GGT or GGG (in *P. citricarpa*) → glycine incorporated) and 143 (GGT → glycine incorporated). Intraspecific differences were only observed in isolated cases and only in a single clone. Except for these, the clone sequences from eight *P. ampelicida*, three *P. citricarpa* and three *P. capitalensis* strains and their consensus sequences were identical within species. However, interspecific differences were found between sequences from different *Phyllosticta* species. Compared to the consensus sequence of *P. ampelicida* isolates as reference, the number of SNPs varied between 8 and 46. The most similar sequence was found in *P. partricuspidatae*, where eight SNPs were detected along the sequence. Remarkably, I found a larger difference in *P. parthenocissi* with 27 SNPs. The sequences of *P. capitalensis* strains contained 36 SNPs, while the sequences of *P. gaultheriae* contained 37 SNPs. The highest number of SNPs, and thus the greatest divergence from *P. ampelicida*, was found in the sequences of *P. citricarpa* strains (46 SNPs).

I investigated the partial exon/intron structure of the *cytb* gene in eight *P. ampelicida* isolates selected for cDNA sequencing and in five additional *Phyllosticta* spp. strains. In *P. ampelicida*, the 8605 base pair long segment of the *cytb* gene was amplified from eight selected isolates. The exon/intron structure of the *cytb* gene was identical in the eight *P. ampelicida* isolates examined, except for two minor

differences. Only in the isolates Gb30 from Portugal and GbC from Italy I found differences in the sequence of the fourth intron, nucleotide positions 6533 and 6551. RNAweasel identified all these introns as type I self-expressing introns. This 8605 base-pair fragment showed extensive identity, but only 57% overlap with the sequence of the *P. ampelicida cytb* gene available in GenBank during BlastN alignment.

In strains of *P. partricuspidatae* (CBS 237.48) and *P. parthenocissi* (CBS 111645) isolated from *Parthenocissus* spp., I also detected an intron indirectly after codon 143, which resulted in a PCR product of 2.1 and 2.2 kb in length, respectively. RNAweasel identified both introns as type I self-expressing introns.

The primers I designed amplified a 3.2 kb and a 1.5 kb long product from *P. citricarpa* (CBS 828.97) and *P. capitalensis* (CBS 119720) mtDNA templates. Both strains carried a type I self-expressing intron after either codon 143 (*P. citricarpa*) or codon 163 (*P. capitalensis*). A 3.2 kb fragment of *P. citricarpa* showed high identity to *P. citricarpa* and moderate identity to *P. ampelicida* GenBank sequences. While the *P. capitalensis* sequence showed high similarity with both *P. ampelicida* and *P. citricarpa* GenBank-i sequences. The sequence of the *cytb* gene sequence from the *P. gaultheriae* strain was the only one in which no intron was found near codon 143. Instead, primer pairs amplified an exon 282 base pairs long encoding the sequence between amino acid positions 134 and 229, and BlastN alignment showed that this was most similar to the mRNA sequence of *P. ampelicida*.

4 CONCLUSIONS AND PROPOSALS

Black rot of grapes has been a growing problem in some areas of Europe since the beginning of the 21st century. Although the importance of the pathogen may continue to increase in these areas in the future, a comprehensive study of *P. ampelicida* isolates for QoI resistance has not yet been carried out. The present work describes the in vitro baseline susceptibility of 48 *P. ampelicida* isolates from Hungary (selected by ISSR) to the QoI agents azoxystrobin and trifloxystrobin, as well as the characteristics of the *cytochrome b* gene and mRNA of 8 selected *P. ampelicida* isolates/strains and some other *Phyllosticta* species from different geographical origins.

Isolates of *P. ampelicida* of the same geographical origin were in several cases placed in different clusters in the post-genotyping analysis, and therefore geographical origin did not fully explain the differences seen in the ISSR samples. The relatively low number of domestic nSSR and ISSR clusters/genotypes may indicate that the pathogen was introduced to Hungary by a single introduction or that it has low sexual reproductive activity in Hungary. The idea of a single introduction of *P. ampelicida* into Hungary was previously suggested by Mikulás and Tomcsányi (1999) and seems to be supported by both Rinaldi et al. (2017) and the present results.

Our phylogenetic analysis with ITS squirrels confirmed the results of Zhang et al. (2013) and Zhou et al. (2015) that isolates from different host plants previously classified uniformly as *P. ampelicida* can in fact be grouped into distinct clades or species. This is important not only for the ease of separating the three species (*P. ampelicida*, *P. partricuspidatae*, *P. parthenocissi*), but also provides further

evidence to support our understanding of the differences in the cytochrome b sequences (mRNA and mtDNA) of these species.

In my studies, trifloxystrobin was found to be more effective than azoxystrobin in inhibiting mycelial growth in *P. ampelicida* isolates, which may be due to the minimal difference in chemical properties of the compounds (Bartlett et al., 2002). All 48 *P. ampelicida* isolates were highly sensitive to both azoxystrobin and trifloxystrobin for mycelial growth. However, even higher than the average percentage inhibition of mycelial growth (76.2 and 87.3% for azoxystrobin and trifloxystrobin, respectively), they exhibited a higher percentage of inhibition against *P. ampelicida* conidia. These results indicate that these two active substances may still be effective in the control of black rot.

Elevated levels of mycelial growth and conidial germination inhibition have been reported in the presence of an alternative oxidase inhibitor (SHAM) in some plant fungal pathogens. Similar data were obtained for inhibition of mycelial growth and conidial germination in *P. ampelicida* isolates, where the addition of SHAM significantly increased the percentage inhibition of mycelial/germ tube growth and correspondingly decreased EC_{50} values. These data suggest that in *P. ampelicida*, it is possible that the alternative oxidase pathway does not adequately replace mitochondrial respiration during conidial germination, and therefore QoI fungicides effectively inhibit this process. These results, together with some plant antioxidants (e.g. flavones; Avila-Adame et al., 2003), which are thought to play a similar role to SHAM in vitro under field conditions, suggest that the partially inhibited growth and thus survival of *P. ampelicida* using the alternative-oxidase pathway under field conditions seems unlikely.

In the *P. ampelicida* and other *Phyllosticta* spp. isolates/strains studied here, the most frequently tested point mutations in *CYTB* mRNA were not present, which is in agreement with previous sequence results from *P. ampelicida*, *P. citricarpa* and *P. capitalensis* isolates (Miessner et al, 2011; Hincapie et al., 2013; Stammler et al., 2013). The scattered SNPs in this fragment of *CYTB* mRNA (not found in all clones) were not associated with small, albeit detectable, differences in EC_{50} values between *P. ampelicida* isolates. However, these results are in line with our expectations, because such variability in EC_{50} variability is generally detectable even in populations with baseline susceptibility (Avila-Adame et al., 2003; Di et al., 2016; Hincapie et al, Furthermore, amino acid substitutions due to SNPs in the *cytb* gene coding regions can lead to significantly elevated EC_{50} values, as has been shown for example in *Botrytis cinerea* and *Alternaria alternata* (Veloukas et al., 2014; Vega and Dewdney, 2014). Comparing the corresponding fragment of the consensus *CYTB* cDNA sequence of the eight selected *P. ampelicida* isolates with the sequence published by Miessner et al. (2011; GenBank identifier: JF785546) (from *P. ampelicida* and *P. partricuspidatae*, identical), I found a high degree of similarity: it differed from my own consensus sequence in only two SNPs. However, the sequence of Miessner et al. (2011) was identical to the corresponding fragment of the *CYTB* cDNA sequence of *P. partricuspidatae* (CBS 237.48), the strain they had also studied, and thus they published its sequence instead of the nucleotide sequence of *P. ampelicida*.

Examination of the sequence of the *cytb* gene revealed that in four of the six *Phyllosticta* species examined (*P. ampelicida, P. citricarpa, P. parthenocissi* and *P. partricuspidatae*) a type I intron was detected immediately after codon 143. According to the "intron hypothesis",

this means that the "persistence" of G143A amino acid substitution in these species is unlikely. Exceptional exon/intron structure was only found in the strain *P. gaultheriae*: no intron was identified after codon 143, suggesting that the G143A point mutation may occur in this species. However, its spread is highly unlikely, as no chemical control is used against this pathogen, being a natural enemy of *Gaultheria shallon*, an invasive shrub that reduces local plant diversity (Prescott et al., 1993).

Overall, it can be concluded that the *P. ampelicida* isolates tested are susceptible to QoI fungicides *in vitro*, and in addition, according to the intron theory, the presence of a type I self-expressing intron in the *cytb* gene after codon 143 indicates a low risk of QoI resistance in *P. ampelicida*. Thus, this group of compounds may be useful in the future for the control of *P. ampelicida*.

5 NEW SCIENTIFIC FINDINGS

1. I contributed with more than 70 isolates to the establishment and maintenance of the first *P. ampelicida* strain collection in Hungary, consisting of several hundred isolates.
2. I genotyped 499 mainly indigenous *P. ampelicida* isolates by the ISSR method using three primers, resulting in the classification of isolates into eight clusters/genotypes. More than 98 % of the isolates belonged to two genotypes.
3. Phylogenetic relationships between *P. ampelicida*, *P. partricuspidatae* and *P. parthenocissi* species were checked by ITS-based maximum likelihood analysis, which resulted in the conclusion that strain CBS 237.48 was presumably not classified in *P. ampelicida* but in *P. partricuspidatae*.

4. Determined the *in vitro* baseline susceptibility of 48 *P. ampelicida* isolates to two QoI fungicides, azoxystrobin and trifloxystrobin. I found that trifloxystrobin was more effective than azoxystrobin *in vitro*, showing significantly lower EC50 and higher inhibition percentages.
5. I have shown that when the *cytochrome c* oxidase pathway is inhibited, the alternative oxidase pathway is detectable, although it does not play a significant role in *P. ampelicida* respiration.
6. I found that the germination of conidia of the two selected *P. ampelicida* isolates *in vitro* was inhibited to a very high extent (90-95 %) by the presence of both azoxystrobin and trifloxystrobin (100 μg ml^{-1}). The alternative respiratory route had no detectable effect on conidial germination over the period/conditions studied.
7. The sequence of a 906 base-pair fragment of *CYTB* mRNA was determined in 8 *P. ampelicida* isolates and one strain each of *P. partricuspidatae*, *P. parthenocissi*, *P. citricarpa*, *P. gaultheriae* and *P. capitalensis* species. I found that the codons of the three most frequently occurring fungicide resistance markers (F129L, G137R and G143A) encode wild-type amino acids in all isolates/strains I examined.
8. I determined the sequence of the *cytb* gene segment surrounding codon 143 in 8 *P. ampelicida* isolates and in one strain each of *P. partricuspidatae*, *P. parthenocissi*, *P. citricarpa*, *P. gaultheriae* and *P. capitalensis* (for *P. partricuspidatae*, *P. citricarpa* and *P. capitalensis* I only confirmed previous literature results). I found that all isolates/strains of all species studied, with the exception of *P. gaultheriae* and *P. capitalensis*, contain a type I self-expressing intron immediately after codon 143. This,

according to the "intron theory", prevents the spread of the G143A point mutation in populations of these species.

6 PUBLICATIONS LIST

Basic requirements

Impact factor articles:

Áron N. Horváth, Orsolya Molnár, Márk Z. Németh, Alexandra Pintye, Tamás Dankó, Zsolt Spitzmüller, Zsuzsanna Váczy, Kálmán Z. Váczy, Giovanni Onesti, Pedro Reis, Cecilia Rego, Zsolt Bereczky, Levente Kiss and Gábor M. Kovács (2024). Revisiting the intron hypothesis of QoI resistance in *Phyllosticta ampelicida*, the causal agent of grape black rot, and other *Phyllosticta* species. *Plant Pathology* **In Press**

Horváth, Á. N., Németh, L., Vörös, L., Stirk, W. A., van Staden, J., & Ördög, V. (2023). Cataloguing microalgae and Cyanobacteria strains from the Mosonmagyaróvár Algal Culture Collection with *in vitro* antagonistic activity against phytopathogenic fungi and oomycetes. *Phytoparasitica*, 1-16. https://doi.org/10.1007/s12600-023-01045-2

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Vági, P., Knapp, D. G., Kósa, A., Seress, D., **Horváth, Á. N**., & Kovács, G. M. (2014). Simultaneous specific *in planta* visualization of root-colonizing fungi using fluorescence in situ hybridization (FISH). *Mycorrhiza*, *24*(4): 259-266. https://doi.org/10.1007/s00572-013-0533-8

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Nemeth, Z M, Pintye, A, **Horvath, Á N,** Vagi, P, Kovacs, G M, Gorfer, M, Kiss, L (2019). Green Fluorescent Protein transformation sheds more light on a widespread mycoparasitic interaction. *Phytopathology* 109(8): 1404-1416. https://doi.org/10.1094/PHYTO-01-19-0013-R

Pintye, A., Németh, M. Z., Molnár, O., **Horváth, Á. N.**, Matolcsi, F., Bókony, V., ... & Kovács, G. M. (2023). Comprehensive analyses of the occurrence of a fungicide resistance marker and the genetic structure in *Erysiphe necator* populations. *Scientific Reports*, 13(1): 15172. https://doi.org/10.1038/s41598-023-41454-1

Other scientific articles

Molnár, Orsolya; Németh, Z. Márk; Horváth, N. Áron; Matolcsi, Fruzsina; Kovács, M. Gábor; Pintye, Alexandra (2019). A növénykórokozó gombák DMI-fungicidekkel szembeni rezisztenciájának molekuláris biológiai háttere. Növényvédelem 80 (N.S. 55): 11 pp. 480-492.

Conference abstract

Horváth N. Á., Kiss L., Váczy K. Z., Váczy Zs. És Bereczky Zs.: A szőlő feketerothadását okozó *Guignardia bidwellii* (anamorf: *Phyllosticta ampelicida*) és néhány közeli rokon faj strobilurin-rezisztenciája (**Előadás**: 63. Növényvédelmi Tudományos Napok, Budapest, 2017.02.21.)

Á. N. Horváth, L. Kiss, K.. Z. Váczy, Zs. Váczy, G. Onesti, M. Z. Németh, G.. M. Kovács, A. Pintye, Zs. Bereczky: Resistance to QoI fungicides in the grape black rot pathogen, *Guignardia bidwellii*, and related species, in the light of the *CYTB* gene structure: preliminary results (**Előadás**: Molecular Biology of Plant Pathogens, Durham, 2017.03.29.)

Németh Z. M., **Horváth N. Á.**, Knapp G. D., Gorfer M., Kovács M. G.: Fluoreszcensen világító gyökérkolonizáló endofiton gomba létrehozása genetikai transzformációval. VI. Magyar Mikológiai Konferencia, 2017. július 3-5., Szeged

Á. N. Horváth, L. Kiss, K.. Z. Váczy, Zs. Váczy, G. Onesti, M. Z. Németh, G.. M. Kovács, A. Pintye, Zs. Bereczky: The cytb gene structure and resistance to QoI fungicides in the grape black rot pathogen, Guignardia bidwellii and related species. Science Protecting Plant Health 2017, 26-28 September 2017, Brisbane, Australia.

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