Assessment of QoI and CAA fungicide resistance of *Plasmopara viticola* populations in vineyards of the Great Lakes region in the United States of America

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1 Introduction

The biotrophic oomycete *Plasmopara viticola* (Berk & Curt.) is the causal organism of downy mildew, one of the most destructive grapevine diseases throughout the world. This pathogen can severely infect all green tissues including leaves, stems, and clusters. In a severe epidemic, complete defoliation and 100% yield loss can occur under the ineffective management programme (Kassemeyer et al. 2015). The management strategies of this disease include planting resistance cultivars, proper drainage, good aeration to minimize leaf wetness, pruning the infected shoots. However, the effective management mainly relies on numerous prophylactic fungicide applications. Several multi-site fungicides such as mancozeb, folpet, chlorothalonil, copper compounds and single-site fungicides such as quinone outside inhibitors (QoI) and carboxylic acid amide (CAAs) have been widely used for GDM management throughout the worldwide (Gisi and Sierotzki, 2015). However, frequent use of these single-site fungicides can lead to resistance development in *P. viticola* populations and ultimately leads to infective chemical management (Hermann and Stenzel, 2019) (Table 1).

FRAC Code	Commercial name	Production Company	Active Ingredient
11	Reason	Bayer	Fenamidone
11	Sovran	BASF	Kresoxim-methyl
40	Zampro	BASF	Dimethomorph + Ametoctradin
40	Revus 2SC	Syngenta	Mandipropamid
40 + 3	Revus top 4SC	Syngenta	Mandipropamid + Difenoconazole

Table 1: List of fungicide products with active ingredients listed within FRAC 11 and 40 in the United States as defined by the Fungicide Resistance Action Committee.

The QoI fungicides inhibit mitochondrial respiration by blocking electron transfer at Qo site of the cytochrome *bc1* complex (complex III), leading to disruption of ATP synthesis (Bartlett et al. 2002). In several plant pathogens, there are three mutations at codon 129, 137, and 143 of cytochrome *b* associated with QoI resistance (Gisi et al., 2002; Lucas et al., 2015). However, in *P. viticola* QoI resistant populations, a single nucleotide change from guanine to cytosine at codon 143, resulting in amino acid mutation of glycine to alanine is predominant (Chen et al., 2007) (Fig. 1).

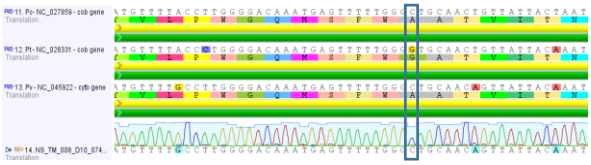

Figure 1: Sequence alignment showing comparison of identified *Cyt b* sequences of *P. viticola* with *Pseudoperonospora cubensis,* and *Peronospora tabacina.*

The CAA fungicides act by inhibiting cellulose biosynthesis in the cell wall. In *P. viticola* populations, the point mutation of glycine to serine at 1105 codon in the cellulose synthase 3 (*CesA3*) predominately confers for CAA resistance (Blum et al. 2010) (Fig. 2).

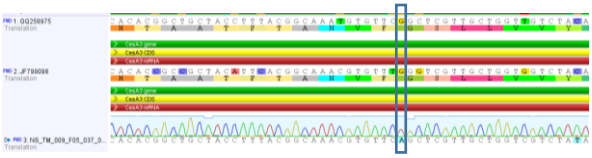

Figure 2: Sequence alignment showing comparison of identified *CesA3* sequences of *P. viticola* (obtained from NCBI) with a resistant isolate collected from NY, USA.

2 Materials and Methods

The objectives of this study were to i) conduct survey to collect GDM samples from vineyards across MI, IN and NY in 2019 and 2020 and test for QoI and CAA resistance using sequencing ii) develop TaqMan assays to detect G1105S mutations responsible for CAA resistance in *P. viticola.*

A survey was conducted in 21 vineyards across three states including Michigan, Indiana, and New York. A total of 130 leaf samples were collected in 2019 and 2020. Leaf discs with *P. viticola* were cut and stored in petri plates at -20ºC. The DNA extraction was conducted using two methods: i) MagMax kit with Kingfisher method ii) crude DNA extraction with chelex extraction. These samples were tested for the presence of QoI and CAA resistance through sanger sequencing.

3 Results

The results showed that higher level of QoI resistance was found from *P. viticola* samples collected from juice and wine

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grapes collected in MI, NY, and IN. However, CAA resistance was only detected from *P. viticola* samples collected from wine grapevines from NY (Fig. 3).

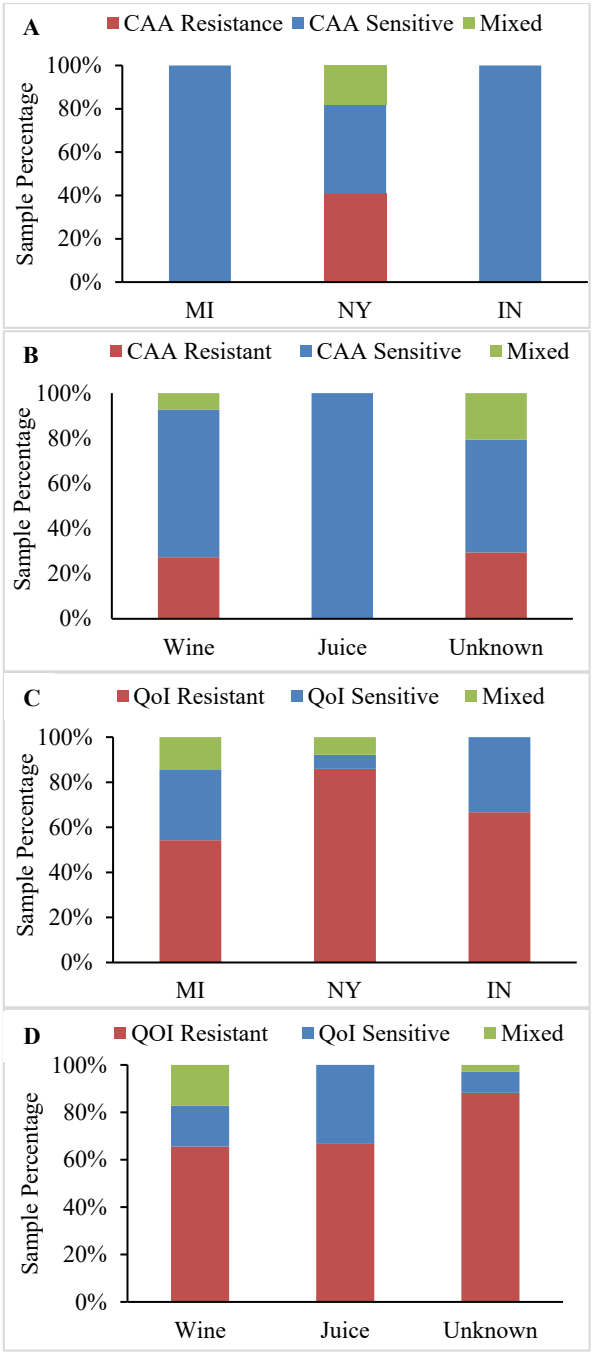

Figure 3: Percentage of *P. viticola* samples resistant to CAA, and QoI fungicides in MI, IN, and NY during 2019 and 2020 sampling. A) Percentage of CAA resistance categorized by A) State; B) grape type including Juice, and wine grapes. C) Percentage of QoI resistance categorized by C) States ; D) grape type. States; includes MI, NY, and IN. Juice grape samples; includes Niagra, Concord. Wine grape samples; includes Chardonnel, Chancellor, Chardonnay, Cabernet Franc, Pinot Noir, Reisling, Vignoles and Dorn Felder

A TaqMan probe-based qPCR assay was developed in this study in order to detect CAA resistance in *P. viticola*. This assay was conducted on Biorad qPCR CFX 96 machine.

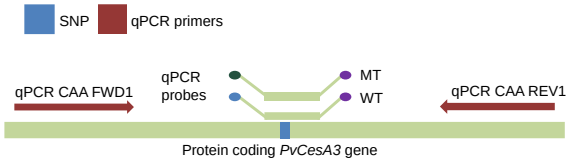

Figure 4: The graphical representation of TaqMan assay detecting CAA resistance in *P. viticola* : The *P. viticola* fungicide-resistant MT allele; qPCR primers and probes binding locations in the *PvCesA3* gene. Fluorophores used for developed probes for detection of G-1105 wildtype (WT) and S-1105 mutant (MT) alleles were FAM (blue) and HEX (green), respectively.

To evaluate the limit of detection of the assay, purified DNA of CAA- resistant and CAA-sensitive *P. viticola* isolates were serially diluted ranging from 1 ng to 100 ag and loaded separately into a reaction. Three biological replicate reactions were used to construct the standard curve plots of the log of concentration and cycle of threshold. The assay showed sensitivity upto 0.01ng of DNA with R^2 value = 0.946, and 0.993 for wild-type allele and mutatnt allele, respectively (Figs. 4 and 5).

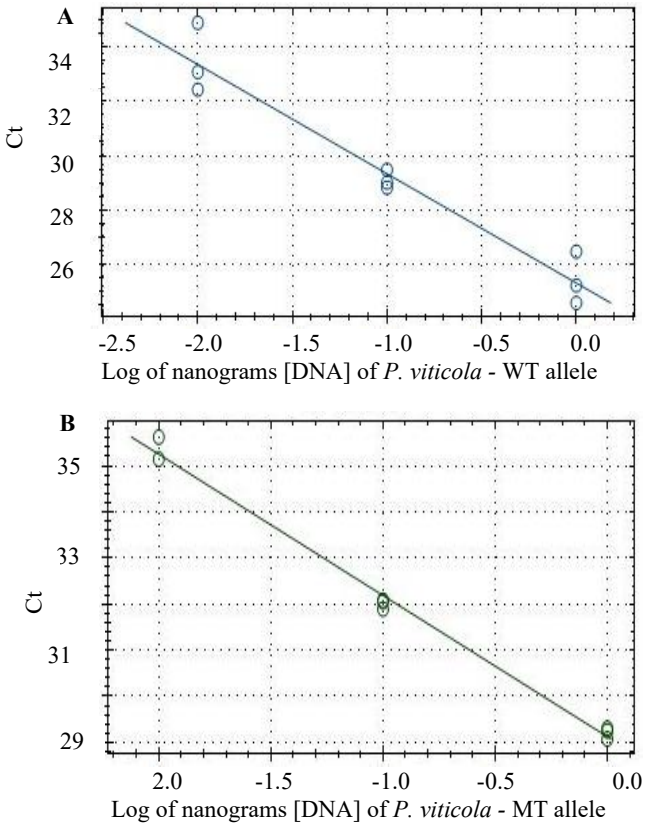

Figure 5: A) Standard curve plot of DNA of *P. viticola* isolate that contained G-1105, a wild type allele. B) Standard

curveplot of DNA of *P. viticola* isolate that contained S-1105, a mutant type allele.

To test the specificity of this assay, a total of sixteen *P. viticola* isolates were tested using Biorad qPCR machine. The accuracy of the assay was confirmed by sanger sequencing. The results showed 100% accuracy in distinguishing both type of alleles (Table 2).

Isolate	Genotype by sequencing	[a]TaqMan assay FAM [b]CT (WT)	HEX [b]CT (MT)
N-149	MT	-	25.82
AN-R2	MT	-	24.79
AN-R1	MT	-	27.19
N-193	MT	-	33.79
N-189	MT	--	25.17
N-194	MT	-	27.02
N-202	MT	-	25.54
N-199	MT	-	25.26
CLARKS	WT	24.18	-
DM-FEN2	WT	22.49	-
HW-TRANS-4	WT	23.67	-
N-169	WT	23.49	-
N-166	WT	35.50	-
N-173	WT	29.64	-
N-174	WT	28.25	-
M-128	WT	35.30	-

Table 2: Specificity of the TaqMan-probe based assay using crude and purified DNA samples collected from the Great Lakes region (United States) by comparing the outcome with Sanger sequencing.
[a]Flourophores used in TaqMan probe-based assay are fluorescein (FAM) and hexachlorofluorescein (HEX) dyes.
[b] CT= cycle threshold

4 Conclusions

Recent survey of many states in the United States showed that widespread resistance of QoI/ Frac 11 in *P. viticola* populations was present, however, high level of CAA resistance was detected in NY. Both juice and wine grapes had high resistance to QoIs but CAA resistance was only found on wine grapes.

The TaqMan-probe based assay developed in this study showed accurate and consistent detection of G1105S mutation in *P. viticola.* This assay will be tested for specificity using a downy mildew species on various hosts. The validation of the assay will be performed on samples collected by using different techniques such as spore traps, cotton swabs, and Toughspots. This assay can be a promising tool for rapid detection of CAA resistance by diagnostic laboratories that could help in timely and efficient disease management of grape downy mildew

References

1. Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M. and Parr-Dobrzanski, B. (2002) The strobilurin fungicides. *Pest Management Science*, 58, 649– 662.
2. Blum, M., Waldner, M. and Gisi, U. (2010) A single point mutation in the novel PvCesA3 gene confers resistance to the carboxylic acid amide fungicide mandipropamid in *Plasmopara viticola*. Fungal Genetics and Biology, 47, 499-510.
3. Chen, W.J., Delmotte, F., Richard-Cervera, S., Douence, L., Greif, C. and Corio-Costet, M.F. (2007) At least two origins of fungicide resistance in grapevine downy mildew populations. Applied and Environmental Microbiology, 73, 5162– 5172.
4. Gisi, U., Sierotzki, H., Cook, A. and McCaffery, A. (2002) Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Management Science, 58, 859– 867.
5. Gisi, U. and Sierotzki, H. (2015) Oomycete fungicides: phenylamides, quinone outside inhibitors, and carboxylic acid amides. In: H. Ishii and D.W. Hollomon (Eds.) Fungicide Resistance in Plant Pathogens. Tokyo, Japan: Springer, 145– 174.
6. Hermann, D. and Stenzel, K. (2019) FRAC mode-of-action classification and resistance risk of fungicides. In: P. Jeschke, M. Witschel, W. Kräme and U. Schirmer (Eds.) *Modern Crop Protection Compounds*. Weinheim, Germany: Wiley, 589– 608.
7. Kassemeyer, H. H., D. M. Gadourym, G. Hill, and W. F. Wilcox. (2015) Compendium of grape diseases, disorders, and pests. *Plant Biology*, 46-52.
8. Lucas, J.A., Hawkins, N.J. and Fraaije, B.A. (2015) The evolution of fungicide resistance. Advances in Applied Microbiology, 90, 29– 92.