

"Assessment of sensitivity to boscalid, fluopyram and tebuconazole in *Monilinia fructicola* isolates obtained from peach orchards in Greece"

S. Testempasis¹, V. Tsintila¹, G. Karaoglanidis¹

¹ Faculty of Agriculture, Forestry and Natural Environment, Laboratory of Plant Pathology, Aristotle Universitity of Thessaloniki. POB 269, 54124, Thessaloniki, Greece.

testempa@agro.auth.gr.

Aim:

The determination of the sensitivity profile of *M. fructicola* isolates obtained from peach orchards in Greece to the SDHI fungicides boscalid and fluopyram and the DMI fungicide tebuconazole.

Introduction:

Brown rot is one of the most important diseases of stone fruit worldwide. In most European countries, the main agents of Brown rot were considered to be *Monilinia laxa* and *M. fructigena*. However, during the last decade *M. fructicola* has been found in high frequencies in most countries around the Mediterranean basin, including Greece. Taking into account that *M. fructicola* is considered to be of higher risk for fungicide resistance development compared to *M. laxa* or *M. fructigena*, this study was initiated aiming to determine the fungicide sensitivity profile of isolates originating from peach orchards in Greece

Materials & Methods:

- M. fructicola isolates (n= 230) were collected from symptomatic peach-fruit during the harvest season from several orchards in the region of Macedonia (northern Greece).
- \circ To determine the resistance profile of the fungal isolates to SDHI fungicides, conidial suspension (40 μ l) of each isolate was spread on Minimal medium (MM) amended with 0.1, 0.5, 1, 2, 5 and 10 μg ml $^{-1}$ boscalid or the fluopyram. After 24 h of incubation at 25 °C in dark, conidia were examined microscopically and the length of germination tube was measured.
- \circ DMI sensitivity assay was performed based on the inhibition of mycelial growth on Potato Dextrose Agar plates amended with 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1 and 3 μg ml $^{-1}$ tebuconazole and inoculated with mycelial plug (5 mm) obtained from 72 h old cultures . After 3 days of incubation at 25 °C in dark, the diameter of mycelia growth was measured.
- The EC₅₀ value (effective concentration that reduces the germ tube or the mycelial growth by 50%) of each isolate was calculated by regressing the relative inhibition of growth against the Log10 fungicide concentration using with GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, California USA).
- o SDHI's subunits B, C, and D were amplified to all resistant isolates to elucidate the molecular mechanisms of resistance in these isolates.

Key Results:

- Among all tested isolates, 53.9% (n=124) were characterized as <u>sensitive</u> to all fungicides.
- The highest frequency of resistant isolate (29.6%) was observed at the DMI fungicide tebuconazole, while the percentage of <u>SDHI's-resistant</u> isolates was 16.5%.
- Amplicon sequencing of SDHI's subunits B, C and D revealed changes in amino acids sequences of SdhC and SdhD subunits.
- Point mutations in SdhC subunit led to an amino acid substitution at codon 73
 (Leucine -> Serine)
- Point mutations in SdhD subunit led to an amino acid substitution at codon 71
 (Asparagine -> Aspartic acid)

<u>Table 1.</u> EC_{50} and resistant factor (RF) values of sensitive, and resistant *M. fructicola* isolates to SDHI fungicides boscalid and fluopyram, and DMI fungicide tebuconazole

Isolate	Boscalid		Fluopyram		Isolate	Tebuconazole	
	EC50	RF	EC50	RF	isolate	EC50	RF
B77-Sens	0.02	1	0.02	1	B18-Sens	0.01	1
B81-Sens	0.04	1	0.04	1	B29-Sens	0.02	1
B12-R	0.39	13	0.54	18	B12-R	0.63	32
B20-R	0.35	12	0.41	14	B21-R	0.53	27
B92-R	0.38	13	0.12	4	B98-R	0.42	21
B101-R	0.32	11	0.3	10	B102-R	0.19	10
B102-R	0.34	11	0.54	18	B93-R	0.29	15
B54-R	0.46	15	0.03	1	B68-R	0.2	10
B58-R	0.41	14	0.11	4	B1-R	0.37	19
B106-R	0.41	14	0.44	15	B31-R	0.41	21

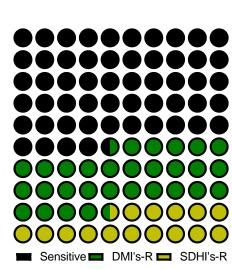
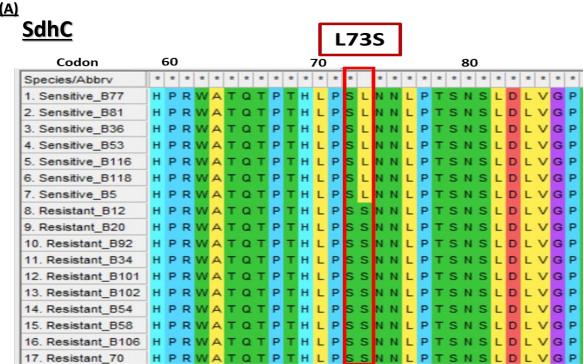
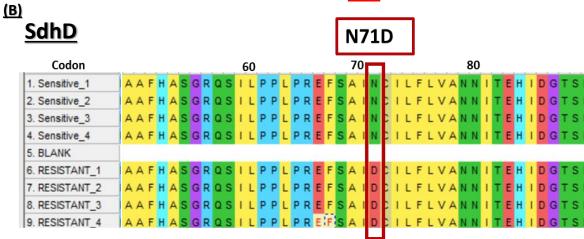


Figure 1. Frequency of Sensitive (■), DMI-Restistant; DMI-R (■) and SDHI-Resistant; SDHI-R (■) isolates collected from diseased peach fruit from Greek orchards





<u>Figure 3.</u> Alignment of deduced amin acids sequences of (A) *SdhC*, and (B) *SdhD* subunits of sensitive and SDHI-resistant isolates of *M. fructicola* isolates. Residues involved in resistance to SDHI's in both subunits are circulated with red color.





Co-financed by Greece and the European Union