

## RESISTANCE OF *VENTURIA INAEQUALIS* TO HEXACONAZOLE, TRIFLOXISTROBIN AND CAPTAN

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### ABSTRACT - RESISTANCE of *Venturia inaequalis* to HEXACONAZOLE, TRIFLOXISTROBIN and CAPTAN

4.3% of 2.2 million tons of apple produced in Turkey annually is obtained from Çanakkale. Apple scab caused by *Venturia inaequalis* (Cooke.) Wint. is the most important problem observed in the apples growing in the province. Especially if the disease pressure is high, it cannot be controlled and thus it causes a significant loss in yield and quality despite the intensive spraying against the disease.

The objective of this study was to investigate whether this problem originated from fungicide sensitivity. Sensitivities of *V. inaequalis* isolates to hexaconazole (DMI), trifloxystrobin (Strobilurin) and Captan (Trichloromethylthiocarboxamide) were determined for twenty isolates by measuring the germination rate of conidiospore obtained from single scab lesions. According to ED<sub>50</sub> value, the isolates treated with captan and hexaconazole varied from 0.01 to 0.3 µg/ml. The sensitivity of the isolates to both fungicides was found less than the sensitivity to trifloxystrobin. The isolates treated with trifloxystrobin were divided in two groups (0.01-0.03 and 0.03-0.1 µg/ml). On the other hand, according to minimum inhibition concentration, the isolates at captan and trifloxystrobin were in three groups (0.3-1; 1-3 and 3-10 µg/ml), however for hexaconazole they were in two groups (1-3 and 3-10 µg/ml).

*In vitro* studies showed that the sensitivity of *V. inaequalis* to Demethylation Inhibitor fungicide hexaconazole and Trichloromethylthiocarboxamide fungicide captan was reduced.

**Keywords:** *Venturia inaequalis*, sensitivity, fungicide.

## INTRODUCTION

In Turkey *Venturia inaequalis* is one of the most important apple diseases and causes serious losses of yield and quality (TÜRKOĞLU, 1956; BENLIOĞLU AND KILIÇ, 1995). The producers apply 14-15 sprayings in a vegetation period if climatic conditions are favorable for the disease. Nevertheless, *Venturia inaequalis* cannot be controlled in some gardens despite intensive spraying. The failure in the combat against apple *Venturia inaequalis* has been associated with the decrease in the sensitivity of *V. inaequalis* as a consequence of the use of DMIs for long years against the disease (SMITH ET AL., 1991; SHOLBERG AND HAAG, 1993; ROBERTS AND CRUT, 1994; PALANI AND LALITAKUMARI, 1999; KÖLLER AND WILCOX, 2001). Hexaconazole and captan have long been used in Turkey against apple *Venturia inaequalis*. Floxystrobin, on the other hand, began to be used only recently compared to the other two fungicides. BENLIOĞLU AND KILIÇ (1995) found that the sensitivity of the isolates they collected from the province of Eğirdir in Isparta (Turkey) to hexaconazole and flusilazole had decreased. Although *V. inaequalis* conidia with reduced sensitivity to Qol inhibitors were obtained in another study from the garden treated with trifloxystrobin, it was determined that the performance of the fungicides in the group was not reduced, yet they had resistance risks (KÜNG FÄRBER ET AL., 2000). On the other hand,

KÖLLER ET AL. (2004) reported that the isolates obtained from the gardens treated with Qol had high sensitivity to kresoxim-methyl and trifloxystrobin and that this finding was characterized with G143A cytochrome *b* mutants.

In this study, the sensitivity of *V. inaequalis* isolates obtained from the apple gardens in Çanakkale to hexaconazole, floxystrobin and captan was investigated.

## MATERIAL AND METHOD

### *Venturia inaequalis* isolation and production

The leaves infected with *Venturia inaequalis* in 2010 vegetation period were collected from the apple gardens in four different locations of Çanakkale, and they were placed into polyethylene bags and transferred to the laboratory in cooling containers. Sterile pure water (25 µl/ml) with Tween 85 was dropped on the leaf lesions inside the sterile container with the help of a micropipette, and conidiospores permeated into the water. Conidial suspension was inoculated into the water agar inside the petri dishes. The conidiospores germinated in the water agar medium were taken and inoculated into PDA (Potato Dextrose Agar) medium, and single conidium cultures were obtained by incubating at 20 °C for 18 days. *V. inaequalis* cultures developed in PDA medium were transferred into petri dishes and tubes with sterile PDYA (Potato Dextrose Yeast Extract Agar) medium and incubated at 20 °C for 30 days. The cultures developed in PDA medium were then transferred to VE medium (Apple Juice-Malt Extract Amino Acid Solution) and left to incubation at 20 °C for 25 days.

### Determination of the sensitivity of isolates to fungicides

Conidia obtained from the cultures developed in VE medium were taken and spore suspension was prepared in the density of  $10^5$  conidium/ml. Spore suspension (25 µl) was inoculated into petri dishes with PDA to which trifloxystrobin, hexaconazole and captan were added (0,001; 0,003; 0,01; 0,03; 0,1; 0,3; 1;3 and 10 µg effective material/ml) with the help of a micropipette. Only sterile pure water was placed into the control petri dishes. The petri dishes were inhibited in the dark with 7% formalin at 20 °C for 48 hours after inoculation. After inhibition, the conidia inside the petri dishes (100 conidia/petri) germinated, atrophied, and were considered ungerminated. Conidia with a tube twice longer than conidium in the counts were considered as germinated (Fig. 1).

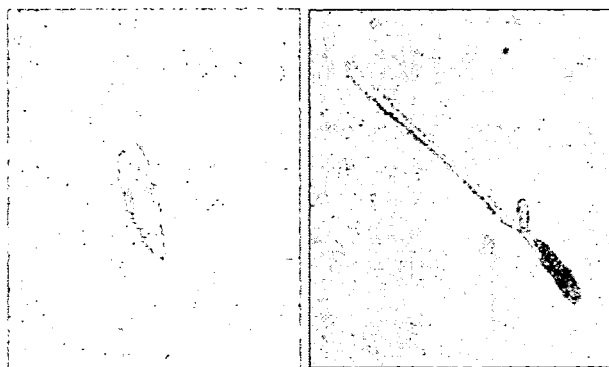


Figure 1. Germinated and ungerminated conidia (Original).

Germination rate was determined by proportioning the germinated conidia to the total conidia number. The data were evaluated by finding ED<sub>50</sub> and MIC (Minimum Inhibition Concentration) values. ED<sub>50</sub> values of fungicides were determined by using logarithmic charts. The trials were designed as four replications in random block trial design.

## RESULTS AND DISCUSSION

The sensitivity of isolates to fungicides in *in vitro* trials according to ED<sub>50</sub> values is presented in Table 1.

**Table 1. Distribution of *V. inaequalis* isolates according to ED<sub>50</sub> values (µg / ml).**

Fungicide	Number of isolates	<0,001	0,001-0,003	0,003-0,01	0,01-0,03	0,03-0,1	0,1-0,3	0,3-1	1-3	3-10
Captan	20	0	0	0	7	12	1	0	0	0
Hexaconazole	20	0	0	0	6	13	1	0	0	0
Trifloxystrobin	20	0	0	0	12	8	0	0	0	0

A decrease was observed in the sensitivity of *V. inaequalis* to captan and hexaconazole according to ED<sub>50</sub> values. *V. inaequalis* isolates were gathered in three groups in captan and hexaconazole according to ED<sub>50</sub> values (0,01-0,03 µg/ml, 0,03-0,1 µg/ml and 0,1-0,3 µg/ml), while they were gathered in two groups in trifloxystrobin (0,01-0,03 µg/ml and 0,03-0,1 µg/ml) (Table 1). It was determined according to ED<sub>50</sub> values that the sensitivity of isolates to captan and hexaconazole intensively used in apple gardens in the region was lower than their sensitivity to trifloxystrobin. It was especially considered remarkable that the same isolate has a similar sensitivity to both fungicides despite the absence of cross-resistance between them. While the previous studies had not reveal any reduction in the sensitivity of *V. inaequalis* to captan; it was found that different fungi, such as *Botrytis cinerea*, had decreased sensitivity to fungicides and that this decrease was permanent (DELEN ET AL., 1999; 2000). Some *V. inaequalis* isolates had higher concentrations according to ED<sub>50</sub> values (0,03-0,1 µg/ml and 0,1-0,3 µg/ml), and this finding was associated with the possibility that the pathogen had developed resistance against captan used in the region for long years (Table 1). This decrease in sensitivity indicates that there may be individuals in the fungal population which have decreased sensitivity also to hexaconazole, a sterol biosynthesis inhibitor (SBI). In the study conducted with isolates obtained from Eğirdir in the city of Isparta, it was determined that the pathogen's sensitivity to flusilazole and hexaconazole in Triazole group decreased (BENLIOĞLU AND KILIÇ, 1995).

On the other hand, the dose range of 8 isolates in trifloxystrobin was between 0,03 and 0,1 µg/ml according to ED<sub>50</sub> values, and this finding indicates a resistance risk although the fungicide began to be used only recently in the region. KÖLLER ET AL. (2004) reported that cytochrome bound to Qol center in the fungal cell where trifloxystrobin and kresoxim-methyl was found and thus there was no resistance against the fungicides inhibiting respiration, yet the isolates of *V. inaequalis* obtained from the gardens where Qol's were intensively used has high resistance against both fungicides and these were the mutants of G143A cytochrome b. In the study conducted by OLAYA AND KÖLLER (1999) in 5 leasing apple growing regions in North America, they detected 25 different *V. inaequalis* populations against kresoxym-methyl which is in the same group as trifloxystrobin (QoI), and their baseline sensitivity values was found to be 0,35 µg/ml as ED<sub>50</sub> value.

These previous studies indicate that resistant individuals may be found also in the apple gardens in Çanakkale region although *V. inaequalis* isolates have not developed resistance yet against trifloxystrobin in this location. The sensitivity of isolates to fungicides according to MIC (minimum inhibitory concentration) values is given in Table 2.

**Table 2. Distribution of *Venturia inaequalis* isolates according to MIC values ( $\mu\text{g} / \text{ml}$ )**

Fungicide	Number of isolates	<0,0 - 01	0,001-0,03	0,003-0,01	0,01-0,03	0,03-0,1	0,1-0,3	0,3-1	1-3	3-10
Captan	20	-	-	-	-	-	-	5	9	6
Hexaconazole	20	-	-	-	-	-	-	-	5	15
Trifloxystrobin	20	-	-	-	-	-	-	8	10	2

The isolates demonstrated a different distribution according to MIC (Minimum Inhibitory Concentration) values. The isolates formed 3 groups in captan and trifloxystrobin according to MIC values (0,3-1  $\mu\text{g}/\text{ml}$ ; 1-3  $\mu\text{g}/\text{ml}$ ; 3-10  $\mu\text{g}/\text{ml}$ ), and 2 groups in hexaconazole (1-3  $\mu\text{g}/\text{ml}$ ; 3-10  $\mu\text{g}/\text{ml}$ ) (Table 2). While 6 isolates were completely inhibited against captan in the concentration rate of 3-10  $\mu\text{g} / \text{ml}$ , 15 isolates in hexaconazole and 2 isolates in trifloxystrobin were inhibited in this dose range. A significant part of the isolates were found to have low sensitivities to hexaconazole in the SBI group according to MIC values. A similar decrease in sensitivity according to MIC values was observed against captan with 6 isolates, which is in the group of Trichloromethylthiocarboxamids and does not have a specific mode of action, and against trifloxystrobin in Qol group with only 2 isolates (Table 2).

## CONCLUSIONS

The sensitivity of isolates to fungicides were observed to be higher when  $\text{ED}_{50}$  values were considered, but it was found to be lower according to MIC values, indicating that these isolates have different sensitivities to fungicides. This study also revealed that the isolates collected from Çanakkale region had similar sensitivities to captan in the group of Trichloromethylthiocarboxamids, hexaconazole in Triazole group, and trifloxystrobin in Stroblurin group.

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