# Fungicide Sensitivity of *Colletotrichum fioriniae C*ausing Anthracnose on Pistachios in California

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## INTRODUCTION

In 2016, pistachios (*Pistacia vera*) of cv. Red Aleppo showing severe anthracnose symptoms (black, sunken and circular lesions) in fruit, leaves and rachises were observed in Glenn County, CA. By harvest time in September, lesions on fruit and rachises became pink due to mature conidia oozing from the acervuli of the pathogen. The isolates were identified as *Colletotrichum fioriniae*. In the same year, pathogenicity tests were performed, revealing their capacity to reproduce the symptoms observed in the field on fruits and leaves of Sirora, Joley, Golden Hills and Kerman cultivars. To develop appropriate control strategies for this disease, the sensitivity of *C. fioriniae* isolates were assessed for different fungicide groups already registered for controlling the major diseases (Botryosphaeria panicle and shoot blight and Alternaria late blight) of pistachios in California.

In total, 39 isolates of *C. fioriniae* were used to determine their sensitivity to the following commercial fungicides: Abound®, Syngenta (a.i. azoxystrobin), Gem®500SC, Bayer USA LLC (a.i. trifloxystrobin), Quash®, Valent Chemical Com (a.i. metconazole), Tilt®, Syngenta (a.i. propiconazole), Luna® Privilege, Bayer Crop Science (a.i. fluopyram), Fontelis®, Du Pont (a.i. penthiopyrad), Vangard®WG, Syngenta (a.i. cyprodinil), and Scala®SC (a.i. pyrimethanil). Sensitivity assays were performed to determine the effective fungicide concentration that inhibits mycelial growth by 50 percent in relation to their growth on potato dextrose agar without fungicide. This value is known as $EC_{50}$, and is used to monitor pathogen resistance to fungicide. Molecular mutations associated with resistance to quinone outside inhibitor (QoI) fungicide (azoxystrobin and trifloxystrobin) were identified by sequencing the 516-bp of the cytochrome *b* (cyt *b*) gene and making comparisons with sequences retrieved from the GenBank (NCBI).

## RESULTS

Among the surveyed fungicide groups, the demethylation inhibitors (DMI) consistently inhibited mycelial growth at low dosages such as 0.37 µg/ml (ranging from 0.10 to 0.71 µg/ml) to metconazole (a.i. of Quash) and 0.79 µg/ml (ranging from 0.34 to 1.41 µg/ml) to propiconazole (a.i. of Tilt) (Fig. 1A). The succinate dehydrogenase inhibitors (SDHI) had the greatest variation between the two tested products, where penthiopyrad (a.i. of Fontelis) had a mean $EC_{50}$ value of 0.09 µg/ml (ranging from 0.04 to 0.28 µg/ml) (Fig. 1B), and fluopyram (a.i. of Luna Privilege) did not inhibit mycelial growth (data not shown). As observed with Luna Privilege, the two anilino-pyrimidine (AP) fungicides cyprodinil (a.i. of Vangard) and pyrimethanil (a.i. of Scala) were also not able to inhibit growth (data not shown). The fungicide sensitivity of Quinone outside inhibitor (QoI) was tested by using the highest stock solution dosage, and as a result, mean $EC_{50}$ values for azoxystrobin (a.i. of Abound) and trifloxystrobin (a.i. of Gem) were 647.82 µg/ml (ranging from 83.81 to 1,000.88 µg/ml) and 609.13 µg/ml (ranging from 108.35 to 956.39 µg/ml), respectively (Fig. 1C). Based on the QoI results, the sequencing analysis of the cyt *b*

gene revealed that all 37 isolates had the mutation G143A, corresponding to high QoI resistance levels (data not shown).

The pressure of the fungicide sprays these isolates have been exposed to, over the years, helps explain the results obtained for QoI fungicides (a.i. azoxystrobin and trifloxystrobin). In the orchard from where samples were collected, the grower reported up to seven fungicide applications annually, including five QoIs alone or in mixed formulations to control Botryosphaeria panicle and shoot blight. The number of spray applications performed is justified by the need to control Botryosphaeria panicle and shoot blight caused by *Botryosphaeria dothidea* because this disease can be severe in this orchard, annually. However, the overuse of these chemical groups has affected the sensitivity of *Colletotrichum* populations that cause anthracnose in pistachio. The sensitivity variability within the SDHI fungicides is in accordance with other *Colletotrichum* studies performed in different crops. Penthiopyrad (a.i. of Fontelis) and fluopyram (a.i. of Luna Privilege) belong to different SDHI chemical groups, and this may cause the difference of inhibition performance between products. The UC IPM website also reports limited anthracnose control in almonds when using Luna Privilege, and no data is available for AP fungicides such as Scala and Vanguard. The highest *C. fioriniae* sensitivity levels were obtained for the DMI fungicides, and no DMI resistant isolates were observed. To reduce the risk of a pistachio anthracnose outbreak, as well as to manage the resistance build-up of *Alternaria* populations in Glenn and other counties, it is important to reduce the number of fungicide applications by following models that predict the most efficient fungicide usage. These models incorporate wetness duration and temperature, and are readily available and proven to reduce the number of sprays. However, a weather logger is needed in the orchard to monitor the critical weather parameters.

## CONCLUSION AND APPLICATIONS

The current pistachio registered fungicides Fontelis (a.i. penthiopyrad, SDHI), Quash (a.i. metconazole, DMI), and Tilt (a.i. propiconazole, DMI) exhibit the highest inhibition capacity of *C. fioriniae* mycelial growth *in vitro*. However, recommendation for controlling pistachio anthracnose will depend on fungicide efficacy trials in commercial orchards. The G143A mutation conferring resistance to Gem (a.i. trifloxystrobin, QoI) and Abound (a.i. azoxystrobin, QoI) was present in all 37 sequenced isolates, and its overuse should be avoided in order to prevent field failure in controlling anthracnose. Determining the predicted (isolate growth and reproduction characteristics) and realized fitness, the mechanism of resistance to multiple fungicides are suggested for further studies in order to propose anti-resistance strategies for this pistachio pathogen.

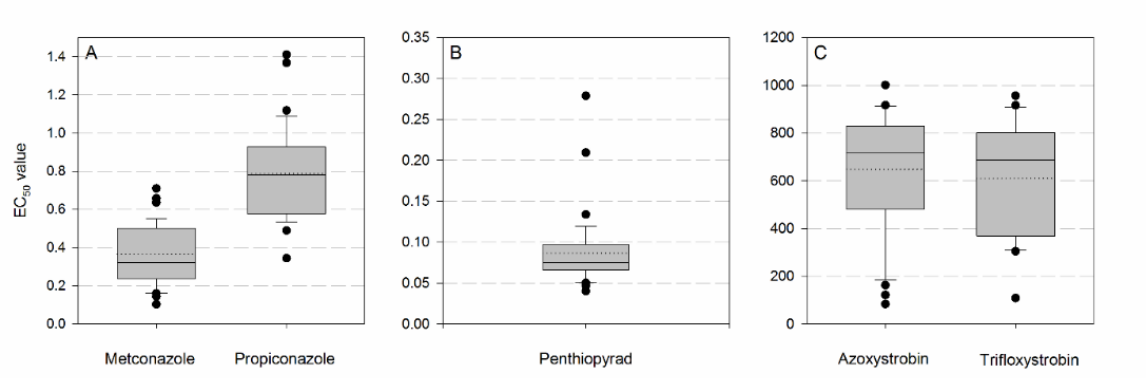


**Figure 1**. Fungicide sensitivity values of *Colletotrichum fioriniae* to DMI fungicides (A), SDHI fungicide (B) and QoI fungicides (C).