Baseline Sensitivity to Benzovindiflupyr of *Alternaria alternata* Isolates Exposed to SDHI Fungicides in Pistachio with *Alternaria* Late Blight Disease

Paulo S.F. Lichtemberg, Postdoctoral, Plant Pathology, UC Davis/Kearney
Ryan D. Puckett, Staff Research Associate, UC Davis/Kearney
Daniel G. Felts, Staff Research Associate, UC Davis/Kearney
Herman Doussoulin, Intern Student, UC Davis/Kearney
Themis J. Michailides, Principal Investigator, UC Davis/Kearney

INTRODUCTION

Alternaria late blight (ALB), mainly caused by *Alternaria alternata,* is one of the most devastating diseases in pistachio produced in California's Central San Joaquin Valley. The succinate dehydrogenase inhibitors (SDHI) fungicides represent a broad-spectrum fungicide group with the fastest growth, in terms of new compounds produced and launched into the market. Currently, 20 SDHI active ingredient(s) (a.i.) are listed by the Fungicide Resistance Action Committee (FRAC), with six new a.i. listed in the last five years. In California, ALB can be controlled with the following SDHIs: boscalid (a.i of Pristine), penthiopyrad (a.i. of Fontelis), fluxapyroxad (a.i. of Merivon), and fluopyram (a.i. of Luna Products). However, different resistance levels for the mentioned a.i. were observed to increase, in the last few years. In the Unites States, the a.i. benzovindiflupyr is reported to control many pathogens in wheat, corn, cucurbits, fruit, vegetables, grapevines, peanuts, pome fruit, potato, and soybean. Excellent intrinsic activity of benzovindiflupyr was reported against species of *colletotrichum* and *venturia inaequalis,* with *colletotrichum* exhibiting resistance to the same SDHI used in pistachio.

In this study, 80 isolates of *A. alternata* from California [not exposed (baseline), n=32; exposed, n=48] were used to determine their sensitivity to fluopyram (98.13%, The Bayer Chemical Company) and benzovindiflupyr (Solatenol^{TM} 97%, Syngenta Crop Protection) fungicides throughout the mycelium growth assay (EC_{50}). The baseline isolates were never exposed to SDHIs, while the exposed isolates were collected from orchards where SDHIs have been sprayed for longer than the last three years. The sensitivity density distribution of the isolates tested for fluopyram and benzovindiflupyr was analyzed by plotting the cumulative frequencies of the log-transformed EC_{50} values. Pearson correlation analysis was used to determine cross-resistance between fluopyram and benzovindiflupyr isolate populations. Fungicide efficacy tests were performed on detached pistachio leaves (cv. Kerman) to demonstrate the potential of each a.i. to inhibit six each SDHI-resistant mutants and wild-type isolates.

RESULTS

In this study, the mean EC_{50} value of the baseline isolates of *A. alternata* to benzovindiflupyr in California corresponds to 0.63 µg/ml (min=0.03, CI95%=0.45-0.88, max=7.13 µg/ml, Fig. 1). This was close to the 0.23 µg/ml EC_{50} value reported for fluopyram in previous studies (not statistically analyzed). This information will certainly support the monitoring of *A. alternata* sensitivity for SDHI fungicides in California pistachios and for quantifying changes to EC_{50} values. The sensitivity density distribution analysis revealed similar (*P*-value = 0.21) benzovindiflupyr and fluopyram curves within the SDHI exposed *A. alternata* and no differences (*P*-value = 0.21) between the two isolate populations tested for benzovindiflupyr were found (Fig. 1). Pearson correlation analysis showed that, for all baseline isolates, a significant, positive

and moderate cross-resistance between fluopyram and benzovindiflupyr EC_{50} (*P*-value= 5.3×10^{-6}, r = 0.57) (data not shown). The cross-resistance between the two a.i. tested increased to high (*P*-value = 2×10^{-16}, r = 0.76), when testing all SDHI exposed isolates (data not shown). Similar cross-resistance between fluopyram and benzovindiflupyr were observed in baseline isolates of *Venturia inaequalis* in apple. Similar a.i. intrinsic activity was observed within isolates (data not shown). Similarities between a.i. ingredients suggest that both fungicides are selecting for the same mutation, considering that the isolates carrying the SdhC-H134R mutation were mostly moderate resistant (MR) for both benzovindiflupyr and fluopyram; but SdhB-H277Y/L isolates with high fluopyram EC_{50} value were inhibited to sensitive levels with benzovindiflupyr (data not shown).

The fungicide efficacy test performed, at the recommended label rate of each Luna Privilege (a.i. fluopyram), Aprovia (a.i. benzovindiflupyr), and a second Aprovia treatment at same a.i. concentration as Luna Privilege, were performed in detached pistachio leaves (cv. Kerman). The results showed best lesion control achieved by protective applications of Luna Privilege for either H134R mutant and wild-type genotypes (data not shown). No differences between the two Aprovia (a.i. benzovindiflupyr) dosages were observed in the experiment. The results obtained in this study revealed that benzovindiflupyr and fluopyram present similar intrinsic activity *in vitro*, but benzovindiflupyr does not inhibit mutant and wild-type isolates as effectively as the fluopyram in the detached leaves assay. Differences may be associated with different binding capacities and arrangements of the SDHI molecule in the binding pocket.

CONCLUSION AND APPLICATIONS

The benzovindiflupyr active ingredient could not properly control SdhC-H134R mutants (commonly selected by the usage of fluopyram), but high intrinsic values were observed in the inhibition of all other SDHI mutant genotypes found in *A. alternata* from California pistachio. Benzovindiflupyr may be effective for control of ALB, when rotating or mixing with appropriate fungicides. Sensitivity studies of other SDHI a.i. listed by FRAC are suggested in order to find a fungicide capable of inhibiting those isolate mutants that are mostly selected for by the use of boscalid and fluopyram. Aprovia fungicide is not yet registered for use in pistachio.

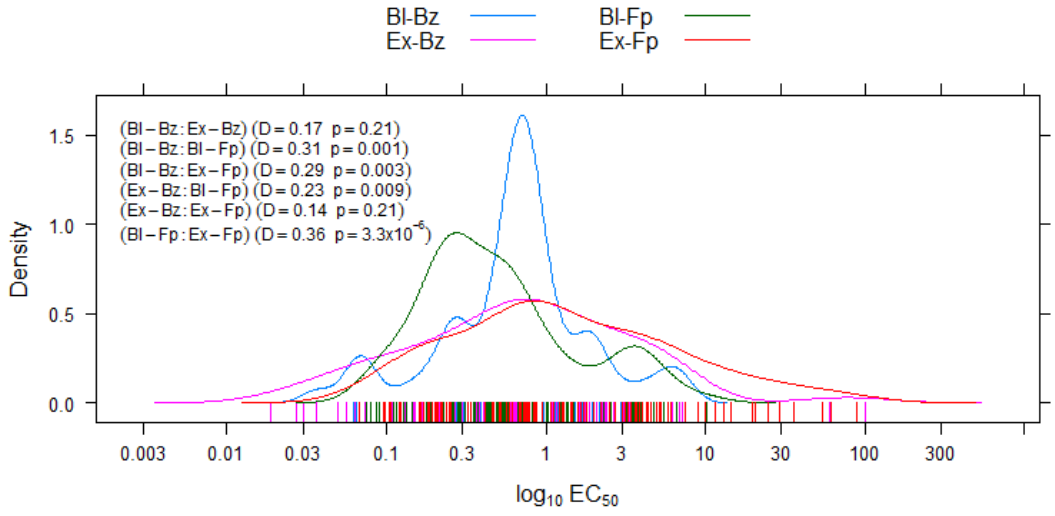

Figure 1. Sensitivity density distribution between benzovindiflupyr (bz) and fluopyram (fp) tested for not exposed (baseline, bl) and SDHI-exposed (ex) isolates of *Alternaria alternata.*