Survival and Resistance Stability of SDHI Fungicides on Different *Alternaria alternata* Mutants Causing Alternaria Late Blight in Pistachios

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INTRODUCTION

Alternaria late blight (ALB), mainly caused by *Alternaria alternata,* is an important disease of the California pistachio, and its control relies on multiple fungicide applications, including the succinate dehydrogenase inhibitors (SDHI; FRAC 7) group. Increased ALB resistance levels were observed just a few years after the registration of Pristine (a.i. boscalid), accompanied with moderate to high cross-resistance with other registered SDHI fungicides. Molecular studies of resistant isolates of *A. alternata* revealed the presence of several single-point mutations in different locations of the succinate dehydrogenase gene, contributing to different levels of SDHI resistance. For effective resistance management, it is necessary to understand the stability of fungicide resistance and to predict the survival in the orchard of individual mutants in California pistachio. If resistant mutants, in a fungal population, have lower survival than the wild-type, their frequency will decline in the absence of the fungicide pressure. The objectives of this study were (i) to assess the resistance stability of *A. alternata* mutants to SDHI fungicides *in vitro*, (ii) to evaluate the resistance stability of *A. alternata* populations in the field by removing the SDHI fungicides from the spray program, and (iii) to evaluate the survival of *A. alternata* mutants after successive transfers *in vitro*.

In our study, fungicide resistance levels were determined throughout the effective concentration that inhibit mycelial growth by 50 percent (EC_{50}) in comparison to growth in the absence of fungicide. In total, four SDHI fungicides were tested: the boscalid (bo, a.i. of Pristine, BASF), fluopyram (fp, a.i. of Luna Experience, Sensation, and Privilege, Bayer), fluxapyroxad, (fd, a.i. of Merivon, BASF) and penthiopyrad (pe, a.i. of Fontelis, DuPont). The stability of fungicide resistance and mutant survival were evaluated for 15 isolates, three of each belonging to the following mutants: $SdhC^{H134R}$, $SdhB^{H277Y}$, $SdhB^{H277L}$, and $SdhB^{H277R}$ (the superscript indicates the mutation of these isolates), and the nonmutant or wild-type isolate group. These isolates were cultured on potato dextrose agar (PDA) without fungicide, and subjected to 12 successive transfers in PDA media to imitate different pathogen life cycles. The survival components we evaluated were: sporulation, germination rate, and area under mycelial growth curve. Evaluations occurred after the first and twelfth transfer cycles. The results presented here include data from the first and second year experiments.

RESULTS

Resistance instability after 12 successive transfers were observed only in the $SdhB^{H277L}$ mutant, where the resistance levels to boscalid, fluxapyroxad, and penthiopyrad were significantly reduced to ranges corresponding to reduced and low-sensitivity levels (Table 1). The $SdhB^{H277R}$ showed a significant instability to fluxapyroxad but not to boscalid, penthiopyrad and fluopyram (Table 1). Stable EC_{50} values were observed for the $SdhB^{H277Y}$, $SdhC^{H134R}$ and the wild-type isolates (Table 1). These results suggest that the mutants H277L and H277R may increase their sensitivity to SDHI fungicides such as boscalid, penthiopyrad and fluopyram after a certain period in the absence of SDHI fungicides use. But this is not true for the other two resistant mutants, H134R and H277Y, which account for 51.2 percent of the current population (surveyed in 2015). Despite the resistance of mutants H134R and H277Y, which has been shown to be stable, their survival parameters suffered a penalty after 12 successive transfers in PDA media. It was observed in our results that both H134R and H277Y decreased their spore production and germination (only the H134R mutant), while the wild-type had only numerical and not statistical reductions for the same parameters (data not shown). These results suggest that wild-type isolates may have competitive advantages and eventually could displace mutants in an orchard where the use of SDHI fungicides has discontinued. However, specific experiments need to be performed to test this hypothesis.

In our field experiment, the SDHI fungicides were discontinued from the spray program of three commercial pistachio orchards. The resistance levels were observed to decrease at Orchard 1 (first experiment year) for boscalid and fluopyram, but not for fluxapyroxad and penthiopyrad (data not shown). At Orchard 2, the penthiopyrad resistance was affected by the proposed management but not by the other three fungicides. In the second-year experiment, only numerical reductions were observed (data not shown). Additionally, the mutant frequencies from the first population (early 2016) were compared with the latest population (late 2017). The mutant comparison after two successive years without SDHI applications was shown to be affected by the field spray management. In Orchard 1, the mutations such as S135R, H277Y/L/R and D123E, found in 2016, were not observed in 2017. In Orchard 2, the H134R remained stable, and the wild-type frequency was consequently increased (data not shown). In the same Orchard 2, the H134R and H277Y initially observed in 2016 were not found in 2017, but sensitivity values were not changed. Finally, in Orchard 3, we observed an increased frequency of H134R, which resulted in a reduction of wild-type isolates.

CONCLUSION AND APPICATIONS

In conclusion, the experiments presented here demonstrate that *A. alternata* isolates carrying the mutations H134R and H277Y have stable resistance levels when tested under laboratory conditions, but their predicted survival suggests that they may suffer a penalty, which could lead to a competition disadvantage against the wild-type isolates, in the absence of fungicide. The removal of SDHI fungicides from the pistachio spray program showed some effect on the mutant composition of *A. alternata* populations, but these changes did not reflect reduced resistance levels. A third year of SDHI removal is suggested to continue the monitoring of resistance in these three selected orchards.

Table 1. The effect of successive transfer on the sensitivity to SDHIs *in vitro* of *Alternaria alternata* causing late blight of pistachio

Mutants	**Transfer**	**n**	**Boscalid**		**Fluxapyroxad**		**Fluopyram**		**Penthiopyrad**	
			EC_{50}		**EC_{50}**		**EC_{50}**		**EC_{50}**	
$SdhB^{H277L}$	1st	3	122.88	a	110.72	a	13.27	a	8.50	a
	12th	3	8.67	b	6.50	b	6.96	a	2.36	b
$SdhB^{H277R}$	1st	3	99.29	a	0.95	a	0.21	a	0.69	a

	12th	3	53.18	a	0.10	b	0.11	a	0.44	a
$SdhB^{H277Y}$	1st	3	122.88	a	6.49	a	0.52	a	9.39	a
	12th	3	84.51	a	1.26	a	0.46	a	5.58	a
$SdhC^{H134R}$	1st	3	122.88	a	57.59	a	4.42	a	30.74	a
	12th	3	109.16	a	68.94	a	7.73	a	9.54	a
wild-type	1st	3	0.24	a	0.02	a	0.45	a	0.49	a
	12th	3	0.57	a	0.10	a	0.54	a	0.26	a