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Plant Health Brief

First Report on Quinone Outside Inhibitor Resistance of *Alternaria alternata* Causing Alternaria Brown Spot in Tangerines in São Paulo, Brazil

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Alternaria brown spot, caused by *Alternaria alternata* (Fr.:Fr.) Keissler, is the most important disease of tangerines (*Citrus reticulata* Blanco) and tangors (*Citrus sinensis* L. Osbeck × *Citrus reticulata* Blanco) in Brazil. Since being first reported in São Paulo, Brazil, in 2003, the disease has been intensively controlled using quinone outside inhibitor (QoI) fungicides. Isolates resistant to QoI fungicides have been detected in Florida (Vega et al. 2012). In 2017, growers reported failures in the control of Alternaria brown spot with QoI fungicides in several citrus-growing areas of São Paulo state.

Twenty-eight diseased fruit from three municipalities of São Paulo state were collected to verify the presence of isolates resistant to QoI fungicides and the possible mutations responsible for the loss of the fungus sensitivity to these fungicides. Twenty-eight monosporic isolates of *A. alternata* were obtained from fruit samples. Four monosporic isolates of *A. alternata* obtained from tangerines not treated with QoI fungicides in São Paulo state, collected in 2003 and 2004, were used to determine the baseline sensitivity to the QoI fungicides azoxystrobin and pyraclostrobin. *A. alternata* conidia from 7- to 9-day-old cultures grown in V8 medium were collected for the preparation of conidial suspensions (10^4 spores/ml). Conidia germination tests were performed on 1.5% agar medium containing the fungicides azoxystrobin (Vantigo, 500 g of active ingredient [a.i.]/kg) or pyraclostrobin (Comet, 250 g of a.i./liter) at 0, 0.001, 0.01, 0.1, 1, 10, and 100 µg/ml. Because salicylhydroxamic acid (SHAM) had no effect on the sensitivity of *A. alternata* conidial germination to QoI fungicides (Mondal et al. 2005), 100 µg of SHAM/ml was added to all concentrations to suppress the alternative oxidase pathway (Ma et al. 2003). Three aliquots of 30 µl of conidial suspension were placed in a Petri dish and incubated at 27°C for 12 h in the dark. After the incubation period, a 50-µl aliquot of lactoglycerol was added over the conidial suspension to stop conidia germination. Spores were considered germinated when they had a germ tube greater than or equal to the conidium length. Three replicates were used for each isolate at each fungicide concentration, and 100 conidia were assessed by replication. The experiments were performed twice. The percentage of germination on each fungicide concentration were used to estimate the effective concentration to inhibit 50% of conidia germination (EC_{50}) by linear regression. DNA extraction, polymerase chain reaction (PCR), and sequencing were performed on two isolates collected in 2003 and two collected in 2017 to verify the presence of possible mutations that may cause loss of sensitivity to QoI fungicides. Primers cytb2f and cytb3r were used to amplify the cytochrome *b* (*cyt b*) "hot spot" region containing codons 129, 137, and 143 that are responsible for loss in sensitivity to QoI fungicides (Grasso et al. 2006).

The EC_{50} values for baseline sensitivity of *A. alternata* collected in 2003 and 2004 ranged from 0.0009 to 0.0270 µg/ml for pyraclostrobin and from 0.0160 to 0.1300 µg/ml for azoxystrobin. These results are similar to those observed in Florida, where baseline sensitivity to pyraclostrobin and azoxystrobin ranged, respectively, from 0.004 to 0.036 µg/ml and from 0.060 to 0.254 µg/ml (Vega et al. 2012; Vega and Dewdney 2014). The isolates collected in São Paulo from the 2017 season had EC_{50} values ranging from 1.45 to over 100 µg/ml for pyraclostrobin, and all isolates had values over 100 µg/ml for azoxystrobin. These isolates were considered resistant to both fungicides based on the criteria adopted by Vega and Dewdney (2014), which classified as resistant the isolates with EC_{50} values higher than 0.5 µg/ml for pyraclostrobin and 5 µg/ml for azoxystrobin. Sequencing of the purified PCR products of the two selected resistant isolates with EC_{50} over 100 µg/ml showed the point mutation G143A, whereas the sensitive isolates showed no mutation when compared with the sensitive reference isolate CPI-ORI-2S (accession number JQ437357) (Vega et al. 2012). This is the first report of QoI resistance in *A. alternata* causing Alternaria brown spot associated with a G143A point mutation in Brazil. Further monitoring is required to ascertain the geographic extent for antiresistance measures to be taken.

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The author(s) declare no conflict of interest.

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