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Plant Health Brief

Colletotrichum Species Isolated from Massachusetts Cranberries Differ in Response to the Fungicide Azoxystrobin

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Fruit rot is the most prevalent disease of cranberry (*Vaccinium macrocarpon*). It involves as many as 15 fungal species; incidence and distribution of any given species vary with year and geographic location (Oudemans et al. 1998). *Colletotrichum* species (*C. acutatum* and *C. gloeosporioides*) have been identified as cranberry fruit rot pathogens (Shear et al. 1931). Overwintering pathogens and repetitive use of fungicides with similar modes of action raise concern for development of resistance. Resistance to azoxystrobin by *Colletotrichum* has been documented in other fruit crops (Forcelini and Seijo 2016; Hu et al. 2005). Azoxystrobin, a quinone-outside inhibitor fungicide, was approved for use in cranberry in 2003, and a single application delivers between 78 and 202 ppm under typical chemigation conditions. Twenty-nine isolates, visually identified by morphological characteristics to be within the *C. acutatum* species complex, were collected from diseased fruit in commercial Massachusetts cranberry farms. Six isolates were collected prior to registration of azoxystrobin (unexposed), and 22 isolates were from locations where the fungicide had been applied (exposed). We created a third category, "sensitive", represented by one additional isolate, which exhibited high mycelial inhibition to azoxystrobin. We hypothesized that mycelial growth would differ for isolates based on prior fungicide exposure.

We conducted this experiment in 2016. Based on prior experience, 0.25-strength potato dextrose agar and V8 agar were used to generate cultures. Azoxystrobin was added to clarified V8 agar plates at 1, 3, 5, and 10 ppm. Although not necessary for *Colletotrichum* species (Forcelini and Seijo 2016), 100 ppm of salicylhydroxamic acid (SHAM) was added to the fungicide solution. Treatments, including an untreated control and SHAM alone, were each replicated at least six times. Single-spore isolates of *Colletotrichum* were plated onto fungicide-amended media; three 4-mm-diameter pieces were transferred from single-spore plates to each fungicide-amended plate. Radial mycelial growth was measured after 7 days (from the center in three directions and averaged) and used as the response in analysis of variance (ANOVA), followed by Tukey's honestly significant difference test ($P \leq 0.05$). Predictors were isolate group (exposed, unexposed, and sensitive), fungicide concentration, and their interaction. Percentage growth relative to the untreated control was calculated for the 29 isolates. Because inhibition is a common parameter measured to indicate resistance, three inhibition groups were calculated: <50%, 50 to 60%, and >60% inhibition. More than 50% inhibition of mycelial growth is generally considered significant inhibition (i.e., sensitivity to the fungicide). We describe patterns of inhibition at different fungicide concentrations but did not conduct formal statistical analyses on this parameter.

Radial mycelial growth for the sensitive isolate decreased more than exposed and unexposed isolates as fungicide concentration increased (ANOVA; interaction $P < 0.001$; Fig. 1). Contrary to our hypothesis, mycelial growth of exposed and unexposed isolates did not differ with fungicide concentration ($P > 0.05$ for the interaction including exposed and unexposed groups only).

No test isolate showed <40% inhibition of mycelial growth (i.e., indicative of resistance), compared with the untreated control, at any tested dose. The sensitive isolate had >60% inhibition of mycelial growth at all tested doses (Fig. 2). Three exposed and two unexposed isolates had 50 to 60% inhibition of mycelial growth at 1 ppm. The number of isolates that exhibited sensitivity to fungicide increased as concentration increased; there were 23 isolates with <50% growth inhibition at 1 ppm, but this declined to 2, 1, and 0 isolates at 2, 5, and 10 ppm of azoxystrobin, respectively (Fig. 2).

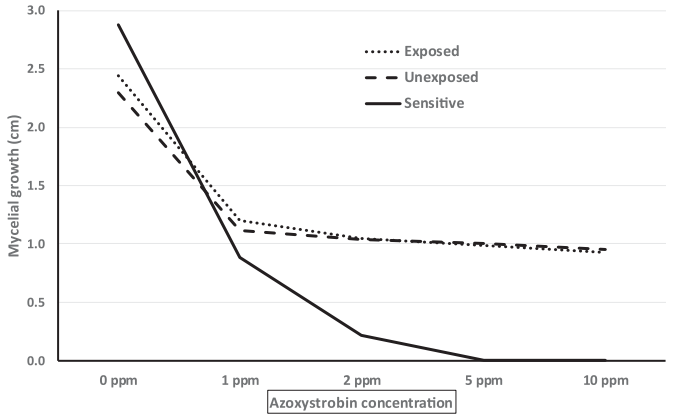

FIGURE 1

Response of radial mycelial growth of *Colletotrichum* isolates collected from Massachusetts cranberry fruit to increasing concentrations of azoxystrobin. Isolates were categorized as follows: collection prior to azoxystrobin registration (unexposed, $n = 6$), collection from fruit treated with azoxystrobin (exposed, $n = 22$), and high sensitivity to azoxystrobin (sensitive, $n = 1$).

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The author(s) declare no conflict of interest.

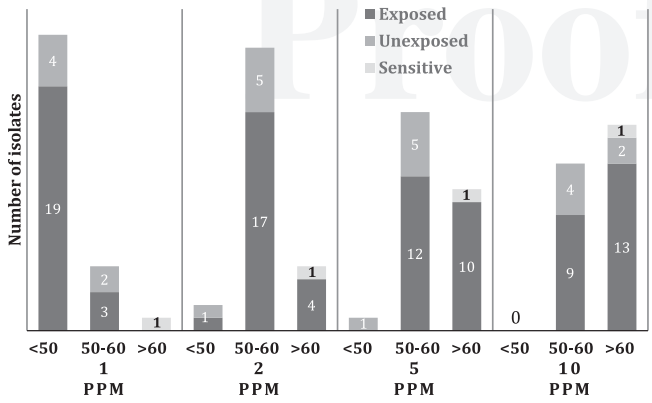

FIGURE 2

Number of *Colletotrichum* spp. isolates (n = 29), collected from Massachusetts cranberries, which fell into three groupings of mycelial growth inhibition (<50%, 50 to 60%, and >60%) at four concentration levels of the fungicide azoxystrobin. Isolates were categorized as follows: collection prior to azoxystrobin registration (unexposed, n = 6), collection from fruit treated with azoxystrobin (exposed, n = 22), and high sensitivity to azoxystrobin (sensitive, n = 1). Values greater than 50% exceed the conventional threshold for mycelial inhibition (i.e., are considered sensitive to the fungicide at this concentration). Numbers in bars represent the number of isolates.

We hypothesized that the unexposed isolates would be more sensitive to azoxystrobin than previously exposed isolates, but unexposed and exposed isolates had a similar dose response (Fig. 1). Although interpretation of our results is limited by the absence of sequencing data or in vivo assay, our study is the first report of the relative sensitivity of exposed and unexposed field-acquired cultures for the cranberry fruit rot pathogen, *Colletotrichum*, to azoxystrobin. Although the current study did not identify fungicide-resistant isolates, azoxystrobin is known to have a high risk of resistance development (Gisi et. al. 2002), and so incorporating fungicides with different modes of action for cranberry fruit rot control is still a prudent management strategy.

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AUTHOR QUERIES

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