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Field efficacy and baseline sensitivity of *Septoria steviae* to fungicides used for managing Septoria leaf spot of stevia

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ABSTRACT

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Stevia (*Stevia rebaudiana*) is an herbaceous perennial emerging as a new crop in the southeastern US. Septoria leaf spot caused by *Septoria steviae* is present across all production areas in North Carolina, causing leaf lesions that expand and result in total defoliation when left unmanaged. Fungicide efficacy trials for management of Septoria leaf spot were conducted over 2 years at two field sites. Seven fungicides, single or combination products, reduced disease severity and increased yield compared to non-fungicide treated controls. Azoxystrobin, chlorothalonil, fluopyram, fluxapyroxad, pyraclostrobin, and tebuconazole were screened using an in vitro assay to establish sensitivity profiles for 10 isolates of *S. steviae* that had received 0 or 1 year of fungicide exposure. All *S. steviae* isolates were sensitive to all fungicides evaluated. Successful management of Septoria leaf spot is critical for long term establishment of stevia as a crop in the southeast US.

1. Introduction

Stevia rebaudiana (Bertoni) Bertoni is an herbaceous perennial in the Asteraceae that is rapidly emerging as a new crop in the United States. Stevia leaves contain multiple diterpene glycosides extracted for use as natural, non-caloric sweeteners (Kinghorn, 2003). Commercial use of stevia began in Paraguay, where stevia is native, and entered Japanese markets in the 1970s (Katayama et al., 1976; Carakostas et al., 2008). The USDA approved stevia for use as a non-nutritive sweetener in December 2008 (FDA GRAS Notice GRN 000253 and GRN 000252). As commercial products containing stevia glycosides continue to increase, there has been interest in establishing commercial production of stevia in the US.

North Carolina has been a leading state to evaluate the feasibility of commercial stevia production in the US. High levels of natural rainfall, favorable climate, and existing infrastructure favor stevia production in the southeast US. The first commercial plantings of stevia in NC were established in 2011. Currently, stevia production begins with seeds sown in greenhouse float trays. Seedlings are transplanted to the field eight to ten weeks after germination, typically from late April through May. There is one harvest in September or October at the end of the first growing season. At the time of first frost, the upper portion of the plant dies back, but roots may remain alive through winter allowing for perennial production. However, extended periods of below freezing temperatures can limit overwintering survival (Koehler, unpublished). If plants successfully overwinter, abundant new shoots begin to emerge in March (Koehler and Shew, 2017) allowing for up to two harvests per growing season in the 2nd and 3rd years of production.

In 2015, olive-gray foliar lesions with chlorotic halos that rapidly coalesced and turned necrotic were observed on greenhouse seedlings and in emerging 2nd year plants. Throughout the growing season, the disease progressed upward in the plant leading to total defoliation by the end of the season. Koch's postulates coupled with morphological and DNA-based multilocus sequence analyses of the fungus, identified *Septoria steviae* as the causal agent of Septoria leaf spot of stevia in NC (Koehler, 2018). This disease was first reported in Japan (Ishiba et al., 1982) and later in Canada (Lovering and Reeleder, 1996).

There are currently no fungicides or biological control agents labeled for use on stevia in the US, and efficacious and economically feasible approaches to manage Septoria leaf spot of stevia will be needed. *S. stevia* produces abundant asexual conidia, and under favorable environmental conditions can result in total defoliation of plants and complete crop failure. Additionally, the pathogen overwinters in leaf debris and readily infects newly emerging leaves on stems that emerge after overwintering. This is a major concern for perennial production of the crop. As part of an integrated approach to managing disease, foliar fungicides may reduce late season defoliation due to Septoria leaf spot and accumulation of overwintering inoculum.

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The objectives of this project were to 1) identify efficacious fungicides for management of Septoria leaf spot of stevia and 2) document the baseline sensitivity profiles of *S. steviae* isolates from stevia in North Carolina to selected fungicides.

2. Materials and methods

2.1. Field trial sites and experimental design

Field trials were conducted at the Caswell Research Farm in Kinston, NC and the Upper Coastal Plain Research Station in Rocky Mount, NC in 2016 and 2017. *Stevia rebaudiana* planted in 2015 at both field sites had high levels of Septoria leaf spot disease. At the Kinston site, 10-week-old stevia seedlings were planted at a density of 60,000 plants ha^{-1} with a row spacing of 0.76 m on flat rows on June 9, 2016 and May 26, 2017. Treatments were arranged in a randomized complete block design with four 7.62 m rows per replicate and five replicates per treatment. At the Rocky Mount site 10-week-old stevia seedlings were planted at a density of 54,000 plants ha^{-1} in bedded rows with a spacing of 0.91 m on June 10, 2016 and 31,000 plants ha^{-1} on June 1, 2017. Treatments were arranged in a randomized complete block design with four 12.2 m rows per replicate and five replicates per treatment. Due to the high inoculum levels of *S. steviae* present in overwintering debris, no additional inoculum was added to either experimental test site.

2.2. Chemical treatments

Treatments included seven fungicides or fungicide combinations and a non-treated control (Table 1). Fungicides were banded over rows using a TeeJet TP8006 flat fan nozzle on a CO_2 sprayer calibrated to deliver product at a rate of 280.6 L H_2O ha^{-1}. Fungicide treatments included a demethylation inhibitor (DMI) (TebuStar, Agri Star, Ankeny, IA), a combination fungicide with DMI and quinone outside inhibitors (QOI) (Quadris Top SBX, Syngenta, Greensboro, NC), combination fungicides with QoI and succinate dehydrogenase inhibitors (SDHI) (Elatus, Syngenta, Greensboro, NC; and Priaxor, BASF, Research Triangle Park, NC), combination fungicides with DMI and SDHIs (Luna Experience, Bayer, Research Triangle Park, NC; and Aprovia Top, Syngenta, Greensboro, NC), and a combination fungicide with a QoI and multi-site inhibitor (Quadris Opti, Syngenta, Greensboro, NC). Two end-of-season fungicide applications were made in each trial beginning when plants reached three to five percent of leaf area covered by Septoria leaf spot (Table 1). All treatments at the Kinston site, including the control, also received three early season cover sprays of 229 g a.i. ha^{-1} tebuconazole (TebuStar, Agri Star, Ankeny, IA) to control *Sclerotium rolfsii* in 2016 and 2017.

2.3. Disease scoring, yield measurements, and statistical analysis

Disease was allowed to develop naturally at each site and disease severity was assessed visually as percent leaf area damage due to Septoria leaf spot lesions. The center two rows of each four-row plot were rated in each of the trials. In 2016, subplot harvests were conducted in each trial by harvesting 3-m-long sections of the center two rows of each plot and weighing total stem and leaf biomass. First harvests were conducted on September 23 at Rocky Mount and September 30, 2016 at Kinston. Yield data was calculated by converting harvest weights to kg stem and leaf weight per hectare. A second harvest, which simulated a delayed or late harvest, was conducted on October 26, 2016 at the Rocky Mount site. In 2017, subplot harvests were conducted in each trial by harvesting from two 0.6-m-long sections of the center two rows of each treatment and stripping leaves from stems to obtain only leaf biomass. First harvests were conducted on September 22 at Rocky Mount and September 29 at Kinston. A late season harvest was conducted on October 13, 2017 at the Rocky Mount site. Yield data were calculated by converting subplot leaf harvest weights to kg leaf weight per ha.

Due to differences in harvesting method, trials from 2016 to 2017 were analyzed separately. Within each year, first harvest disease severity ratings and first harvest yield data were pooled across location. Second harvest disease severity ratings and yield data from the Rocky Mount site were analyzed separately for each year. Data were subjected to mixed-model analysis of variance (ANOVA) using PROC GLIMMIX in SAS (version 9.4). For combined trials with no significant interaction between trial and treatment, data from each trial were combined for analysis. If there were significant interactions, trials were analyzed separately. For combined trials, treatment was a fixed effect and trial, replication, and the overall error term were random effects. For individual trials, treatment was a fixed effect and replication and the overall error term were random effects. Fixed effects were tested for significance at $\alpha = 0.05$ and significant differences among treatment means were separated using paired t-tests.

2.4. In-vitro fungicide sensitivity screening

The sensitivity profiles of 10 single-spore isolates of *S. steviae* were assessed by point inoculation of conidia onto fungicide-amended media (Pappas et al., 2010). Concentrations and fungicides screened included: 0.001, 0.01.0.1, 1, and 10 mg L^{-1} a.i. for azoxystrobin (Heritage TL, Syngenta Crop Protection Inc., Greensboro, NC), chlorothalonil (Daconil, Syngenta Crop Protection Inc., Greensboro, NC), fluopyram (Indemnify, Bayer Crop Science, Research Triangle Park, NC), fluxapyroxad (Xzemplar, BASF, Research Triangle Park, NC), and tebuconazole (TebuStar, Agri Star, Ankeny, IA); and 0.00001, 0.0001, 0.001, 0.01, and 0.1 for pyraclostrobin (Headline, BASF, Research Triangle Park, NC). Azoxystrobin and pyraclostrobin belong to the quinone outside inhibitor (QoI) group [fungicide resistance action committee (FRAC)

Table 1
Fungicides evaluated for management of Septoria leaf spot of stevia caused by *Septoria steviae*.

Treatment	Active ingredients	FRAC group	Product rate (a.i. g ha^{-1})	Application timings	
				2016[a]	2017[b]
Non-Treated Control	–	–	–	–	–
TebuStar	tebuconazole	3	227	1, 3	1, 2
Quadris Top SBX	azoxystrobin + difenoconazole	11 + 3	115 + 115	1, 3	1, 2
Elatus	azoxystrobin + benzovindiflupyr	11 + 7	205 + 99	2, 3	1, 2
Priaxor	pyraclostrobin + fluxapyroxad	11 + 7	195 + 97	2, 3	1, 2
Luna Experience	tebuconazole + fluopyram	3 + 7	124 + 124	1, 3	1, 2
Aprovia Top	difenoconazole + benzovindiflupyr	3 + 7	115 + 77	1, 3	1, 2
Quadris Opti	azoxystrobin + chlorothalonil	11 + M	112 + 1121	2, 3	1, 2

[a] 2016 Application timings at Kinston, NC were (1) 24 Aug and 7 Sept or (2) 24 Aug and 14 Sept. Application timings at Rocky Mount, NC were (3) 18 Aug and 9 Sept.
[b] 2017 Application timings at Kinston, NC were (1) 23 Aug and 8 Sept. Applications timings at Rocky Mount, NC were (2) 18 Aug and 8 Sept.