REDUCING THE RELIANCE ON SYNTHETIC MULTI-SITE FUNGICIDES BY INTEGRATING A BIOPESTICIDE WITH A SINGLE SDHI FUNGICIDE

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ABSTRACT

Venturia inaequalis (Cooke) G Wint is an ascomycete fungus and the causal agent of apple scab, and economically devastating disease of fresh market apples. The disease is characterized by olive-colored, scabby lesions that develop on leaves and fruit, which leads to reduced yield of marketable fruit (Jones and Aldwinkle, 1990; MacHardy, 1996). A lack of resistance among commercially preferred cultivars has mandates the need for more than 10 fungicide applications per season to manage the disease. Due to a history of sequential resistance development shortly after the introduction of single-site fungicides, apple scab management relies heavily on multi-site fungicides such as mancozeb and captan, which protect trees from a wide range of pathogens from bud break through summer (Agnello et al. 2019; Cox, 2015). Currently, multi-site fungicides are used in rotation with single-site SDHI fungicides to manage apple scab. However, there are concerns about the potential off-target impacts of multi-site fungicides, as well as the environmental impact of excessive application. Due to the negative impacts of commonly used multi-site fungicides such as mancozeb and captan on the environment and human health, some countries have considered restrictions and limitations on the use of mancozeb and captan, making it necessary to reduce the use of multi-site fungicide in management programs. In this study we eavaluate a potential apple scab management program that rotates the biopesticide Serenade Opti (a.i. *Bacillus subtilis*) with the single-site fungicide Aprovia (a.i. benzovindiflupyr) and compare the disease control to a conventional management program that rotates the multi-site fungicides Manzate Max and Captec (a.i. mancozeb, captan) with the single-site fungicide Aprovia. The programs were tested on two different application schedules (calendar and a schedule determined by the decision support system NEWA), as well as in two different orchard planting styles (vertical axis and modern super spindle) to compare

the impact on disease control. Over the course of the study, we found that biopesticides are a potentially viable substitute for multi-site fungicides as a rotational partner for single-site fungicides when paired with decision support systems and modern super spindle plantings. There were no significant differences between management programs using biopesticides and programs using multi-site fungicides in either high or low disease pressure scenarios. Our study supports observations made by Boland (1997), that indicates biological controls are most effective when used in environments less conducive to pathogen development. Overall, this study is a proof-of-concept that demonstrates the feasibility of a management program using biopesticides as a rotational partner with single-site SDHI fungicides, and that the program is comparable to industry standard programs that use multi-site fungicides.

BIOGRAPHICAL SKETCH

Emily Fang grew up in Rockville, Maryland, where her passion for plant science was first ignited by summers spent growing cucumbers in her grandmother's back yard. She graduated from the University of Maryland College Park with a Bachelor of Science in plant biology in 2019 and spent the following year as a research intern studying applied plant pathology and resistance development in late season ripe rot of wine grapes at the University of Maryland. In 2020, she pursued a Master of Professional Studies in plant science under the tutelage of Dr. Kerik Cox and explored the impact of fungicide application programs on the development of disease resistance in *Venturia inaequalis* on apples.

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TABLE OF CONTENTS

Introduction

Apple scab, caused by the ascomycete fungus *Venturia inaequalis* (Cooke) G Wint, is an economically devastating disease of fresh market apples. The disease is characterized by olive-colored, scabby lesions that develop on the leaves and fruit, leading to reduced yield of marketable fruit (Jones and Aldwinkle, 1990; MacHardy, 1996). *V. inaequalis* prefers cool and wet environments, and it thrives in temperate climates of the Northeastern United States where season-long chemical management is required. Presently, a lack of resistance in commercially preferred cultivars, mandates the need for more than 10 fungicide applications in a single growing season (Agnello et al., 2019). Moreover, due to a history of sequential resistance shortly following the introduction of each group of single-site fungicides (e.g. demethylation inhibitors, quinone outside inhibitors, and succinate dehydrogenase inhibitors), apple scab management relies heavily on multi-site fungicides, which protect the tree from a wide range of pathogens from bud break through summer (Agnello et al. 2019; Cox, 2015).

Synthetic Multi-site fungicides (e.g. Captan, Mancozeb, and Sulfur) are the cornerstone of chemical management apple scab for apple scab in the eastern United States (Gullino et al. 2010). These fungicides target multiple biochemical processes within fungi, are relatively low cost, and effective for managing selection of resistance to single site-single site fungicides (Runkle et al. 2017). While the multiple modes of action provided by multi-site fungicides allow for resistance management, this broad range of multiple modes of action can make them less effective at managing specific fungal pathogens when compared to a single-site fungicide. In addition, multi-site fungicides must be applied in considerably higher quantities than single-site fungicides and must always be applied before an infection as they only offer pre-infection activity. These characteristics make it difficult for growers to time and limit the number of applications of a multi-site fungicide used per season. The most common multi-site fungicides used in apples are mancozeb and captan (FRAC 2020; Fungicide Resistance Action Committee group M03 and M04), which are preferred for their low cost, multi-site mode of action, and effectiveness against pathogens on a wide range of crops. However, due the broad-spectrum nature of these fungicides, there are also concerns about the off-target effects of these fungicides, as well as concerns about the potential environmental impact of excessive application. For example, mancozeb has been connected to negative impacts on the development and reproductive ability of mammals (Runkle et al. 2017) and is considered an environmental pollutant (Walia et al. 2014), and both fungicides have been listed as potential human carcinogens by the EPA (EPA 1999; EPA 2005). For those reasons, some countries have considered restrictions and limitations on the use of mancozeb and captan, making it necessary to reduce and potentially cease the use of multi-site fungicides entirely (Beckerman et al. 2015b).

To avoid selection of resistance when managing apple scab, single-site fungicides are often used in rotation with multi-site fungicides. While single-site fungicides are more effective at managing apple scab than multi-site fungicides owing to a highly specific mode of action, the single target site mode of action increases risk of resistance development. In the case of apple scab, sequential selection of resistance over the last 50 years has been observed in benzimidazoles, dodine, demethylation inhibitors, and quinone outside inhibitors (Frederick et al. 2014; Koller et al. 1999; Lesniak et al. 2011; Quello et al. 2010; Villani et al. 2016b). Succinate dehydrogenase inhibitors (SDHIs) have only recently been a key component of apple scab management, are considered medium-high risk of resistance development by the FRAC (SDHI; FRAC group 7; FRAC 2020), and therefore must be used in conjunction with other products with different modes of action to prevent the selection of resistance, a role which is presently filled with applications of mancozeb or captan (Agnello et al. 2019).

A possible alternative to the use of synthetic multi-site fungicides for resistance management are biopesticides such as *Bacillus subtilius* and *Bacillus amyloliquefaciens*, which have been gaining popularity as an environmentally sustainable alternative to multi-site fungicides. Like synthetic multi-site fungicides, these biopesticides are composed of antimicrobial metabolites produced during fermentation, which have multiple modes of action affecting many cellular processes, are generally less effective, but have little to no negative environmental impacts (Shafi et al. 2017; Cawoy et al. 2011). While studies have shown biopesticides to have reduced efficacy against apple scab compared to synthetic fungicides (Cox et al. 2017b, Lalancette et al. 2017; Cox and Villani 2015), the formulation and efficacy has been refined over the course of several decades and are now better poised to be used in place of multi-site fungicides.

The goal of the study was to explore the feasibility of implementing a management program for apple scab that uses the biopesticide (Serenade Opti: a.i. *Bacillus subtilis* QST 713) rotated with a highly-effective SDHI fungicide (e,g, Aprovia: a.i. Benzovindiflupyr). This program would be compared to a production standard that utilizes synthetic multi-site fungicides in rotation with an SDHI fungicide. The study will also compare these programs within the context of application timing on a calendar schedule or based on the uses of a decision support system (DSS), which may bolster the efficacy of biopesticide/SDHI rotation program. Similarly, the programs will also be evaluated in two different planting systems (vertical axis and super spindle) to investigate the potential for implementation in modern planting systems.

Materials and methods

Trial design. Trials were performed in two orchards with different training systems. One was a traditional vertical axis planting of 13 year-old 'Galas' grafted onto B.9 rootstocks, while second was a super spindle orchard planting of 3rd leaf 'Galas', grafted to G.935 rootstocks on a 3-wire trellis. The orchards shared local weather patterns and were approximately 600m apart. Each orchard contained a weather sensor (HOBO remote monitoring system RX3000; Onset, Bourne, MA) attached to one of the trees to record daily temperature and relative humidity, which are important for assessing the environmental factor variables predicting infection by *V. inaequalis*. For each orchard and year, the canopy microclimate was evaluated using temperature, average daily relative humidity, and total days with mean % relative humidity at or above 90% (an indicator of leaf wetness).

Treatment programs. To understand the potential for using *Bacillus*-based pesticides in place of synthetic multi-site fungicides for the management of apple scab, the four chemical management plans were implemented as summarized in (Table 1). These programs consisted of an untreated negative control (trt 1) and a positive control in the form of a commercial standard management program of multi-site fungicides Manzate Max (2.4 qt/A, a.i. mancozeb; UPL, King of Prussia, PA) mixed with Captec (2qt/A, a.i. captan; Arysta life Science, Cary, NC) in rotation with the SDHI fungicide product Aprovia (5.0 fl oz/A, a.i. benzovindiflupyr; Syngenta, Greensboro, NC) on a calendar schedule (trt 2). Experimental management programs consisted of a program of Manzate Max mixed with Captec rotated with Aprovia, with application timing based on the apple scab component of the NEWA DSS (http://newa.nrcc.cornell.edu/newaModel/apple_disease) (trt 3), a program of the biopesticide Serenade Opti (20 oz/A, a.i. *B. subtilis* QST 713; Bayer Crop Science, Research Triangle Park, NC) rotated with Aprovia on a calendar schedule (trt 4), and a program of Serenade Opti rotated with Aprovia timed using the DSS (trt 5). Aprovia was used as the experimental SDHI due to its relatively low EC_{50} values against *V. inaequalis in vitro* as well as demonstrated efficacy against apple scab in the field (Ayer et al. 2019a; Cox et al. 2017b; Villani et al. 2016a).

In addition, two ancillary programs were added for 2020 and 2021, which only consisted of applications of Serenade Opti timed using the NEWA DSS (trt A), and a program that only consisted of applications of Aprovia timed using NEWA DSS (trt B). While neither of the ancillary programs are commercially feasible, they were used to illustrate the impact of biopesticide and SDHI fungicide individually.

Table 1. Treatment programs implemented in the super spindle and vertical axis orchards in 2019, 2020, and 2021.

Treatment	Programs	Active Ingredients	Application timing
1	Untreated	-	-
2	Manzate Max (2.4 qts/A) and Captec (2qt/A) rotated with Aprovia (5.0 fl oz/A)	mancozeb, captan, benzovindiflupyr	Calendar (every 7/10 days)
3	Manzate Max (2.4 qts/A) and Captec (2qt/A) rotated with Aprovia (5.0 fl oz/A)	mancozeb, captan, benzovindiflupyr	DSS
4	Serenade Opti (20.0 oz/A) rotated with Aprovia (5.0 fl oz/A)	*B. subtilis* QST 713, benzovindiflupyr	Calendar (every 7/10 days)
5	Serenade Opti (20.0 oz/A) rotated with Aprovia (5.0 fl oz/A)	*B. subtilis* QST 713, benzovindiflupyr	DSS
Ancillary Programs			
A	Serenade Opti (20.0 oz/A) only	*B. subtilis QST 713*	DSS
B	Aprovia (5.0 fl oz/A) only	benzovindiflupyr	DSS

* Application rates for fungicide products are listed in amount per acre for apples according to their Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) section 3 labels

All treatment programs began on the same day, when the trees were at the phenological stage of tight cluster (25 April in 2019, 2 May 2020, 17 April in 2021), and continued until the 8th application at 4th cover, which was at often end of June or beginning of July depending on the program and year.

Programs using Aprovia followed application use guidelines for the fungicide listed by the Section 3 label under United States Federal Insecticide, Fungicide, and Rodenticide Act (FIFIRA). The FIFIFA guidelines state that no more than 2 consecutive applications can be applied in the program, and no more than four applications can be used throughout the season. Ancillary trt B did not follow these guidelines because it consisted of only Aprovia applications for the entire season. All applications were made with a Solo 435 piston backpack sprayer (Solo, Newport News, VA) in the super spindle orchard to avoid fungicide drift within treated panels, while a Solo 475-B gas-powered mist blower (Solo, Newport News, VA) was used in the vertical axis planting, where the spacing between rows and plot was wider. Fungicides were applied dilute to runoff in both orchards, and all products were applied at the highest labeled commercial rate.

For calendar-based programs, fungicides were applied on a 7–10-day schedule until petal fall. After petal fall, the interval between applications was extended to 10-14 days. Rotational programs on calendar schedules were conducted so that Aprovia applications were applied after two consecutive applications of either a mix of Captec and Manzate Max (trt 2) or Serenade Opti (trt 4). For treatments 3 and 5, Aprovia applications were timed based on apple scab infection periods as predicted by the NEWA DSS (NEWA.cornell.edu) using a threshold of at least 15% predicted daily ascospore release with more than 35h leaf wetness. For these treatments, if there was no infection predicted to meet the treatment threshold for the week, Serenade Opti (trt 5) or

Manzate Max and Captec (trt 3) was applied instead to maintain coverage between infection events.

Apple scab assessment. Apple scab assessments began in each orchard once the first lesions appeared in any of the untreated plots (negative control; trt 1). Orchards were rated for apple scab 5-7 times every 10-20 days to track disease progress. During each assessment, the incidence of apple scab symptoms was determined on both terminal leaves and fruit. Apple scab incidence on terminal leaves was expressed as the number of terminal leaves with apple scab lesions out of eight fully developed leaves from the tip of the shoot. Incidence on fruit was expressed as the number of fruit with apple scab lesions out of the total fruit in each cluster. For each four replicate plots, a total 20 shoots or clusters were assessed. At each assessment, the incidence data was expressed as a percentage, and was used to calculate the area under the disease progress curve (AUDPC) at the end of the season.

For each orchard and year, the effect of the management program on the mean AUDPC of apple scab on terminal leaves and fruit was determined using generalized linear mixed models, using the PROC GLIMMIX procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). For models with significant effects, differences between the treatments were determined using the LSMEANS procedure in SAS 9.4, where a=0.05 level of significance, adjusted for Tukey's HSD to control for family-wise error.

SDHI Fungicide sensitivity Assessment To investigate concerns of the proposed treatment programs selecting for fungicide resistance, SDHI sensitivity assays were performed for each program in each orchard and year. Approximately 10 days after the final application of SDHI fungicide, a minimum of 5 leaves with sporulating lesions were collected from each tree, for a total of 15-30 lesions per management program, per orchard. Individual leaf lesions represent single ascospore infections and were subjected to a relative growth assay on a discriminatory dose of 0.02 μg ml-1 of benzovindiflupyr (Aprovia), selected based on 10x the baseline EC50 value for *V. inaequalis*. Conidia were extracted using the procedure described from Ayer et al. (2019a) and Villani et al. (2016a). In short, individual excised lesions were vortexed for 30 sec at 3200 RPM in 1ml dH_2O and resulting conidia ($1x10^4$ conidia/mL) were suspended in and spread on potato dextrose agar (PDA) containing the discriminatory dose of technical grade benzovindiflupyr (Aprovia; Syngenta; Greensboro, NC), as well as the antibiotics streptomycin sulfate and chloramphenicol at 50 μg ml-1 to reduce bacterial contamination (PDA++). Isolate growth on fungicide amended medium was compared to growth of conidia from the same lesion on a control plate of PDA++ without benzovindiflupyr (Aprovia). The plates were incubated for a week at room temperature, then conidial germination and secondary hyphal growth was measured using a SPOT Idea digital camera and SPOT Basic Software (Diagnostic Instruments Inc., Sterling Heights, MI) that was attached to an Olympus SZX12 stereoscope (Olympus America Inc., Center Valley, PA). Percent relative growth (%RG) for each treatment was calculated by comparing the isolate growth on benzovindiflupyr (Aprovia) amended media to that on the control (PDA++) medium.

Results

Impact of Seasonal Weather on the development of Apple Scab. While the weather in 2019 was exceptionally conducive for the development of apple scab, there was little rainfall during the peak risk period during the 2020 and 2021 seasons. From May to June there was 4.42" of rainfall and 264 hours of leaf wetness in 2019, in contrast to the 1.30" and 1.86" of rainfall and 140 and 121 hours of leaf wetness during the same period in 2020 and 2021, respectively. In some years, there were observable differences in microclimate between vertical axis and super spindle orchards. For example in 2019, there was an average of 77.25% relative humidity vertical axis plantings compared to 75.36% in super spindle plantings. The cumulative differences in relative humidity were also observed. The canopies in vertical axis orchard canopies had 21 days where mean relative humidity was >90% for more than a hour, while super spindle canopies had only 18 days. In 2020, there was less than 1.5" of rain from May to June, and there were no notable differences in the mean relative humidity or number of days where the mean daily relative humidity was over 90% between the canopies of the two plantings. In 2021, there was 1.86" rain during this period, and there were 22 days where relative humidity was over 90% in vertical axis planting and 18 days in super spindle planting.

At the end of the season each treatment program had a total of 8 fungicide applications, with the number of each type of fungicide application varying depending on the application conditions of each program (Table 2). For programs following a calendar schedule, there were 6 applications of Captec and Manzate Max or Serenade Opti, and 2 applications of Aprovia in all 3 years. For the programs where applications were timed based on apple scab infection events as timed by NEWA DSS, the number of applications of each fungicide varied from year to year based on the predicted infection periods. In 2019 and 2021, NEWA DSS predicted 4 infection periods with over the threshold of 15% predicted daily ascospore release or 35 hours of leaf wetness, while only 3 infection periods were predicted in 2020.

Table 2. The number of applications for each fungicide varied based on the program and weather conditions that year.

Treatment	Program	Serenade Opti	Manzate Max and Captec	Aprovia
1	Untreated control	-	-	-

2019				
2	Mazate Max, Captec, Aprovia (calendar)	-	6	2
3	Mazate Max, Captec, Aprovia (DSS)	-	4	4
4	Serenade Opti, Aprovia (calendar)	6	-	2
5	Serenade Opti, Aprovia (DSS)	4	-	4
2020				
2	Mazate Max, Captec, Aprovia (calendar)	-	6	2
3	Mazate Max, Captec, Aprovia (DSS)	-	5	3
4	Serenade Opti, Aprovia (calendar)	6	-	2
5	Serenade Opti, Aprovia (DSS)	5	-	3
2021				
2	Mazate Max, Captec, Aprovia (calendar)	-	6	2
3	Mazate Max, Captec, Aprovia (DSS)	-	4	4
4	Serenade Opti, Aprovia (calendar)	6	-	2
5	Serenade Opti, Aprovia (DSS)	4	-	4

Impact of season weather and management of programs on the development of apple scab. Disease assessments began during the last week of May or the first week of June (June 3rd 2019, June 2nd 2020, May 20th 2021) when symptoms developed in the untreated plots (negative control) and continued through fruit maturation. Overall, the development of scab differed between each site and years was dependent on seasonal weather. For example, in 2019 the mean AUDPC in the vertical axis on untreated fruit was 5190.83 (100% disease incidence), while untreated plots in super spindle plantings had a lower mean AUDPC of 4123.1 (79.38% incidence). Similarly in terminal leaves, the mean AUDPC in 2019 was 3320.81 (28.13% incidence) and 4011.69 (48.13% incidence) in the vertical axis and super spindle orchards, respectively. In 2020, the AUDPC of fruit in untreated plots was 44.58 (3.85% incidence) in the vertical axis orchard and 9.38 (1.43% incidence) for the super spindle orchard, which was likely due to a lack of rainfall compared to 2019. Likewise, terminal leaves had comparably lower mean AUDPCs in 2020, with vertical axis orchards having a mean AUDPC of 26.88 and the super spindle orchards having a 31.79, both representing 1% disease incidence at the end of the season for 2020. Compared to the 2020 season, the increased rainfall through June and July in

2021 led to slightly higher incidence of apple scab on the terminal leaves and fruit in the untreated plots. Terminal leaves in 2021 had a mean AUDPC of 278.806 (2.9 % incidence) in the vertical axis orchard and a 560.358 (24.3 % incidence) in the super spindle orchard. Similarly, the mean AUDPC of symptoms on fruit was 460.146 (19.0 % incidence) and 424.572 (48.0 % incidence) in vertical axis and super spindle orchards, respectively.

In 2019 all management programs (trt 2-5) had significantly lower ($P < 0.05$) mean AUDPCs for apple scab when compared to untreated controls on terminal leaves and fruit. The end of season incidence on both fruit and leaves for these programs was always less than 30% in vertical axis plantings and 20% in super spindle plantings.

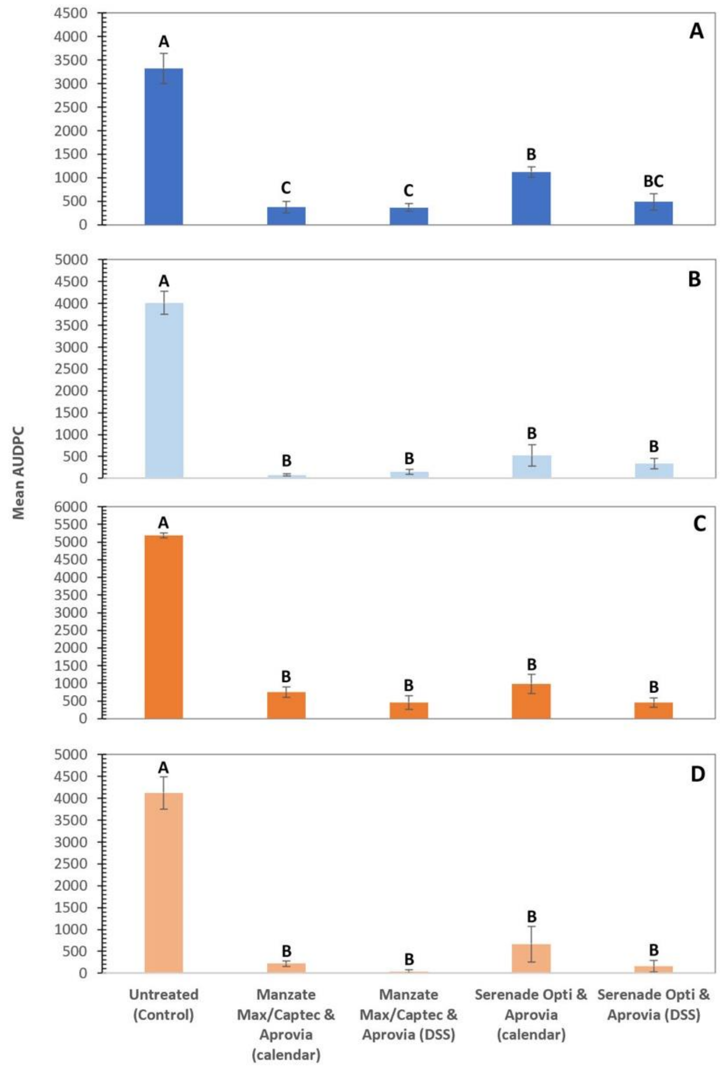

Figure 1. Mean area under disease progress curve (AUDPC; ± the standard error) apple scab symptoms on terminal leaves (A, B) and fruit (C, D) for each program in 2019, with the darker colored bars representing vertical axis (A, C) plantings and lighter colored bars representing super spindle (B, D) plantings. In each graph, the letters above the bars indicate significant differences based on Tukey HSD test ($p < 0.05$)

In 2020, all programs (trt 2-5) had complete disease control (0% incidence) on fruit and leaves, and only the untreated plots in both orchards developed apple scab (3.85% incidence in vertical axis, 1.43% in super spindle). However, there were no significant ($P > 0.05$) differences in the mean AUDPC between any programs for apple scab on fruit. The ancillary program plots (trt A and B) had a low incidences of apple scab in the vertical axis orchard (<0.5% incidence) and no apple scab developed in the super spindle orchard. Similar to the untreated controls, these ancillary programs were not significantly different ($P > 0.05$) from any programs in either orchard. Likewise, development of symptoms on terminal leaves in 2020 in both orchards was similarly low, but there were some significant differences ($P > 0.05$) between programs in the vertical axis planting. In the vertical axis orchard, only the management programs that timed SDHI applications using NEWA DSS (trt 3 and 5) were significantly different ($P <0.05$) from the untreated control. Both programs provided complete control during the season (mean AUDPC=0, 0% disease incidence). In the super spindle orchard, all management programs, including the ancillary programs (trt 2-5, A, B) were significantly different ($P <0.05$) from the untreated control ($P < 0.05$). An additional late season rating was performed on 24 August 2020 due to late season rainfall, which corresponded with an observed increase in apple scab lesions on terminal leaves. At this time, untreated controls reached about 5% disease incidence in both orchards. While disease incidence for all proposed management programs remained significantly different ($P <0.05$) from the untreated controls, among the ancillary programs, only the treatment using Serenade Opti alone (trt A) was not significantly different ($P > 0.05$) from the untreated control, with an incidence of 2.34% in vertical axis orchards and 3.91% incidence in super spindle orchards ($P > 0.05$).

In 2021, the incidence of apple scab symptoms on untreated fruit and leaves at the end of season incidence was only slightly higher than what was observed in 2020. The incidence of symptoms on fruit and leaves for untreated checks was 2.9 % and 19.0 % in vertical axis plantings and 24.3 to 48% in super spindle plantings. Mean AUDPCs for all management programs (trt 2-5) fruit were all significantly lower (P > 0.05) for apple scab symptoms on terminal leaves and fruit, except for the program where Serenade Opti and Aprovia was applied on a calendar schedule on fruit in the vertical axis orchard, (P < 0.05). The end of season incidence on both fruit and leaves for all management programs was always less than 6 % in vertical axis plantings and 8% in super spindle plantings.

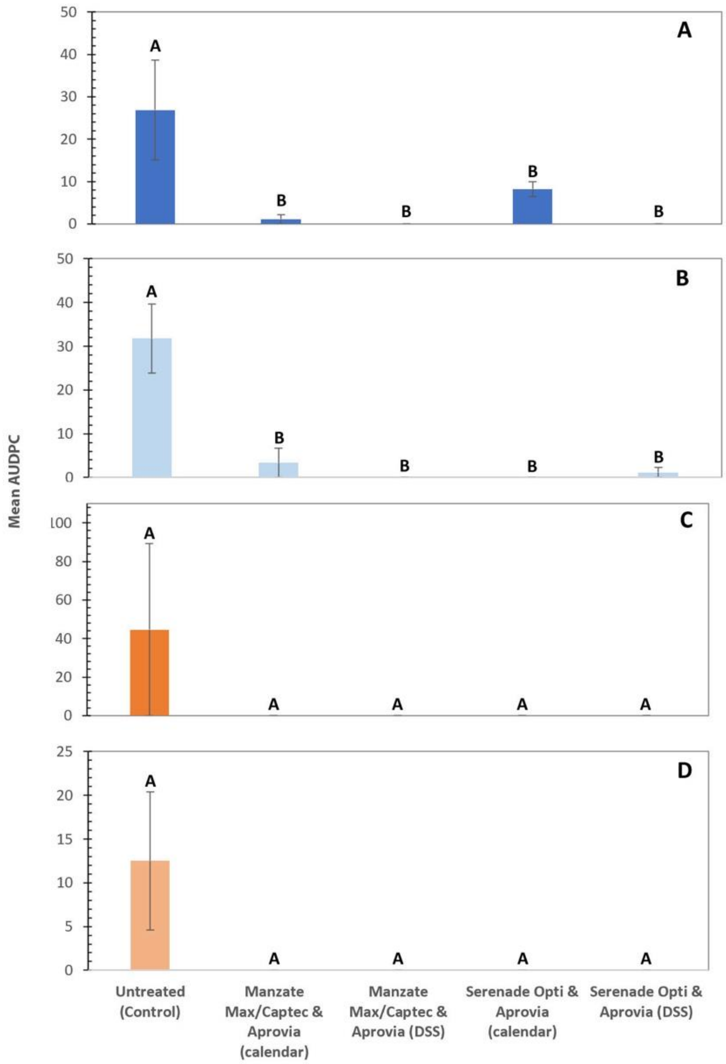

Figure 2. Mean area under disease progress curve (AUDPC; ± the standard error) apple scab symptoms on terminal leaves (A, B) and fruit (C, D) for each program in 2020, with the darker colored bars representing vertical axis (A, C) plantings and lighter colored bars representing super spindle (B, D)

plantings. In each graph, the letters above the bars indicate significant differences based on Tukey HSD test (p <0.05)

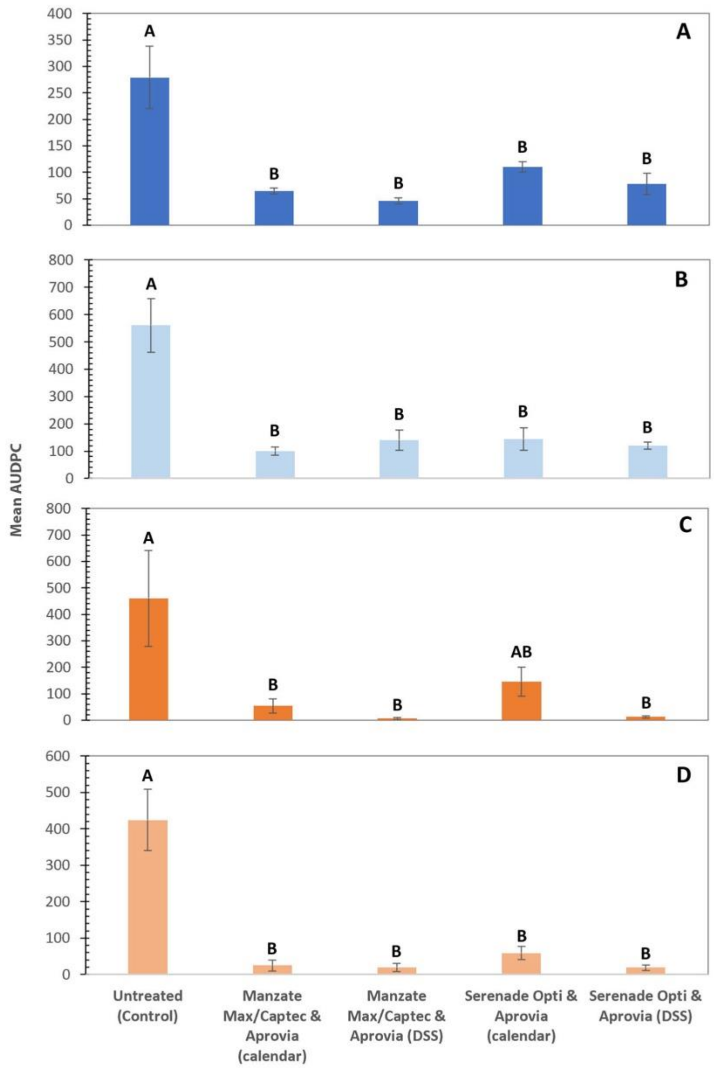

Figure 3. Mean area under disease progress curve (AUDPC; ± the standard error) apple scab symptoms on terminal leaves (A, B) and fruit (C, D) for each program in 2021, with the darker colored bars representing vertical axis (A, C) plantings and lighter colored bars representing super spindle (B, D) plantings. In each graph, the letters above the bars indicate significant differences based on Tukey HSD test (p <0.05)

SDHI Fungicide Sensitivity Assessment. Isolates from 2019 and 2021 were cultured for all management programs with 8-32 lesions per treatment, with the differences in the number of isolates reflecting the success of conidial isolation, as well as overall disease incidence. In 2020, the low incidence of apple scab (<3%) prevented us from collecting enough leaf lesions to perform SDHI sensitivity assays. In fact, there were no leaf lesions observed in many of the management plots in both orchards in 2020. For the 2019 and 2021 isolates, no treatments had any isolates with mean %RG > 50% on the 10 x baseline EC50 discriminatory dose, which would indicate the potential development of quantitative fungicide resistance. Across all programs and years, the mean %RG for benzovindiflupyr was between 20 and 40%, suggesting that all isolates were still sensitive.

Discussion

Due to the history of rapid resistance development to single-site fungicides of *V. inaequalis* (Frederick et al. 2014; Koller et al. 1999; Lesniak et al. 2011; Quello et al. 2010; Villani et al. 2016b), multi-site fungicides like mancozeb and captan have become an essential component of commercial management programs (Agnello et al. 2019; Villani et al. 2019; Jurick II and Cox 2017). Increasing concerns about the environmental impact of these multi-site fungicides mean that despite their effectiveness, they may not remain sustainable in future commercial markets (Beckermann et al. 2015b). This study has established paradigms by which biopesticides could be used in place of synthetic multi-site protectant fungicides while still effectively managing apple scab and reducing selection for fungicide resistance. We aimed to identify conditions that would enhance the efficacy of the biopesticide Serenade Opti as a rotational partner for the single-site SDHI fungicide Aprovia without compromising disease control or selecting for fungicide resistance. The proposed solution would take advantage of disease forecasting and planting systems practices that reduce disease pressure by shortening drying time of fruit and foliage, increase fungicide application precision, and improve the coverage of fungicides.

Over the course of the study, we found that biological controls are a potentially viable substitute for multi-site fungicides as a rotational partner with SDHI fungicides in modern super spindle plantings when applications are timed using disease forecasting systems. Over three years the program was tested under radically different management scenarios in terms of disease pressure resulting from seasonal precipitation patterns. While the 2020 season represented a light pressure season, the 2019 and 2021 seasons represented high and moderate disease pressure. Regardless, no significant differences between management programs were observed on leaves and fruit in either orchard. While there were slight observed differences in the AUDPC between programs where Serenade Opti was used in place of Manzate Max and Captec, especially in the vertical axis orchard in 2019 and 2021, the differences were not statistically significant ($P > 0.05$), and likely neither commercially nor biologically relevant. Similarly, management programs using Serenade Opti had the lowest observed levels of disease when applied to the super spindle orchard and when timed using the NEWA DSS. The observations are supported by Boland (1997), who found biological controls have higher efficacy when used in environments that are less conducive to pathogen development. Indeed, canopy pruning is also a recommended cultural practice to reduce the humidity in the canopy therefore reducing the development of apple scab. In both 2019 and 2021, the higher levels of rainfall led to observable microclimate differences between super spindle and vertical axis orchards, supporting the idea that training for reduced canopy density may be beneficial for managing disease in temperate apple production regions of seasons where there is considerable precipitation in the spring. Interestingly, despite the higher relative humidity and leaf wetness hours in the vertical axis orchard, the incidence of apple scab was higher in the super spindle orchard. One possibility for this discrepancy might be the size of the barren herbicide strip in each orchard. In the vertical axes, the herbicide strip is nearly 8 ft wide and covers the entire width of the canopy and might allow for reduced overwintering despite the size of the trees. By comparison, in the super spindle orchard the herbicide strip is barely 2ft wide and beyond is thick grass and weeds that would allow for greater success with overwintering. It may be that the increasing size of the herbicide strip could lead to reduced overwintering inoculum and might be an effective means of enhancing management of apple scab.

There were no statistically significant differences in apple scab development between the management programs for either orchard in any year, though it was observed that programs where SDHI applications were timed using the NEWA DSS (trt 3, 5) had lower levels of scab on leaves and fruit than programs where SDHIs were applied on a calendar schedule. While these trends were more apparent during the 2019 and 2021 seasons due to higher rainfall, these two treatment programs appeared to offer improved management in 2020 as well, despite the overall low disease incidence. As noted by Shtienberg and Elad (1997), the use of disease forecasting improves the efficacy of biopesticides against *B. cinerea*, and disease forecasting systems can similarly be used to time the application of SDHIs to target the germinating *V. inaequalis* conidia, which are more sensitive to SDHI fungicides than mycelium (Ayer et al. 2019a). By using the NEWA DSS, SDHIs could be applied only prior to severe infection events as defined by the threshold conditions of the current study, where more than 15% ascospore release and more than 35 hours of leaf wetness were predicted. Biopesticides, which may be less effective during times of high disease pressure, can then be applied during the time between infection periods and periods of time with lower levels of predicted leaf wetness to ensure coverage and resistance management.

Fungicide sensitivity was only evaluated in 2019 and 2021 when there was sufficient rain and disease development. During these seasons, isolates from all treatments, including the untreated control, had a mean relative growth below 50% on benzovindiflupyr suggesting that all isolates were moderately sensitive, and no treatment had begun to select for quantitative resistance. Due to a lack of leaf lesion development resulting from the complete control of apple scab by all management programs in 2020, we were unable to evaluate selection for resistance in that season. While we cannot draw any concrete conclusions on the impact of our proposed management programs on the development of resistance in the 2020 season, selection for fungicide resistance is unlikely when the pathogen population is eliminated. Other studies have shown that biological controls can manage fungicide-resistant pathogens such as *Penicillium expansum* on apple (Chand-Goyal and Spotts 1997) and *B. cinerea* (Hovinga and Derpmann 2020) on grape. Due to the multi-site mode of action of biopesticides, it is theorized that they can be a key tool in reducing resistance selection, but presently, a longer-term study investigating the role biological controls can play in *V. inaequalis* management is needed to best validate the concept.

While specific fungicides and biopesticides were used in this study as a proof-of-concept, there are numerous other SDHI fungicides and *Bacillus*-based biopesticides that could potentially be implemented in the management program and be even more effective than the currently proposed program under the same use practices. Barriers to the implementation of the proposed program include hesitancy by growers to replace multi-site fungicides with biological controls given their history of ineffective disease control, as well as the long-established effectiveness and lower cost of synthetic multi-site fungicides compared to biopesticides (Rosenberger et al. 2000; Strickland and Cox 2020; Yoder et al 2007; Yoder et al. 2014b; Yoder et al. 2016). However, as a proof-of-concept, this study demonstrates that rotating biopesticides with SDHIs provides the same levels of control as the industry-standard programs using a rotation of Manzate Max and Captec with SDHIs, even during seasons with considerable rainfall like 2019. One of the questions pondered was the relative contribution of Aprovia applications to that of Serenade Opti or the mix of Captec and Manzate Max. To address this question, ancillary programs were performed in 2020 and 2021 which evaluated the efficacy of only Serenade Opti (trt A) or Aprovia (trt B) only. In both these years neither were significantly different ($P > 0.05$) from one another in mean AUDPC. At the same time, there were no observed differences between programs where Aprovia was rotated with Captec and Manzate Max (trt 2, 3) and the program only applying Aprovia (trt B) in 2020 and 2021. Other than the potential benefit of resistance management, these observations call into question the necessity of Captec, Manzate Max, or Serenade Opti in years or periods of low risk of infection. However, the differences between these treatments may be more noticeable in seasons with higher levels of rainfall and higher disease pressure such as 2019. In orchards composed of large trees in years of heavy rainfall, the level of control afforded by biopesticides may be similar to what has been seen in biopesticide-only programs from earlier studies. (Rosenberger et al. 2000; Strickland and Cox 2020; Yoder et al. 2016).

Potential future refinements to this management concept could include shorter application intervals for biopesticides, or applications of single-site fungicides other than SDHIs, allowing for multiple modes of action and improved fungicide resistance management. In the very least, application of multi-site protectant fungicides as a rotational partner in management programs can be substituted with biopesticides later in the season when there is apple scab pressure due to warmer weather. Additionally, changing climate increases the chance of increased drought periods between heavier rains, which may make a management paradigm like the one proposed more feasible in the future. Apple growers may consider implementing a management program using biopesticides to reduce the use of multi-site protectant fungicides for a multitude of reasons, such as accessing specialty domestic and export markets, which may have fungicide use restrictions, or a more intrinsic motivation by the farmer to move towards a more sustainable production system (Lefebvre et al. 2015). Despite these goals, the low price of products such as Manzate Max and Captec, especially in contrast to the higher price of biopesticides, still pose a hurdle to implementation (Rosenberger 2003). In the future, new policies and regulations may place restrictions on the use of multi-site fungicides, as has occurred in other countries (Pesticide Action Network 2020), making the implementation of alternative programs more critical. In conclusion, this study establishes a framework for replacing synthetic multi-site fungicides with biological controls, with an increased potential for sustainable disease management when combined with modern super-spindle planting systems, disease forecasting using a DSS, and rotations with highly effective single-site fungicides. Through future refinement, the industry can move towards increased sustainability in tree fruit production and the management of fungal diseases.

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