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VIRGINIA APPLE RESEARCH COMMITTEE GRANT REPORTS

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TABLE OF CONTENTS

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2022 ANNUAL REPORT TO VIRGINIA APPLE RESEARCH PROGRAM

Controlling Bitter Pit in 'Honeycrisp' Apples with Abscisic acid (ABA) and Prohexadione Calcium

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Duration of project: One year, March 1, 2022- February 28, 2023.

Background and Justification:

Figure (1): Bitter pit symptoms on 'Honeycrisp' apples.

Bitter pit (BP) is a prevalent physiological disorder in apples that typically occurs during or after cold storage, and sometimes even appears pre-harvest. BP symptoms manifest as dark, sunken lesions on the peel or brown flesh, extending up to 1 cm below the fruit surface (Figure 1). While BP is generally attributed to calcium deficiency in fruit tissue (de Freitas et al., 2010), its incidence does not always correlate with low total calcium content, suggesting more intricate causes (Falchi et al., 2017). In fact, several studies have proposed that BP may involve abnormal partitioning of cellular calcium ions. For instance, increased calcium deposition in vacuoles and/or binding to cell walls can reduce free apoplastic calcium ions (Ca2+), potentially explaining the equal or higher total Ca2+ observed in pitted fruits compared to healthy ones (Saure, 2005; de Freitas et al., 2010). Other nutrients, such as potassium (K+), magnesium (Mg2+), and nitrogen (N), have also been associated with BP development in apples. Elevated K+ and Mg2+ levels have been detected in fruit tissues with calcium deficiency disorders (de Freitas et al., 2010). Both K+ and Mg2+ compete with Ca2+ for binding sites in cell walls and may replace Ca2+, but they cannot fulfill calcium's role in maintaining cell wall integrity (Yermiyahu et al., 1994). Similarly, high N levels can lead to Ca2+ deficiency by promoting vegetative growth, which consequently diverts Ca2+ transport to leaves instead of fruits. As a result, it is widely accepted that K+/Ca2+, N/Ca2+, and Mg2+/Ca2+ ratios are more reliable indicators of BP susceptibility than total calcium content alone (Dris et al., 1998; Lanauskas and Kvikliene, 2006; de Freitas et al., 2010; Marini et al., 2020).

Bitter pit (BP) occurrence is largely influenced by imbalances in fruit mineral composition, which can be attributed to various factors such as soil conditions, vegetative/reproductive growth ratio, tree age, crop load, and rootstocks (Fazio et al., 2020; Valverdi and Kalcsits, 2021). Our 5-year evaluation of 'Honeycrisp' apples on 13 different rootstocks demonstrated significant effects on tree growth, yield, crop load, and more importantly, the incidence of BP at harvest and during storage (3 months after harvest). This finding aligns with other studies where various apple rootstocks (e.g., B.9, M.26, M.9, G.41, and G.890) significantly impacted nutrient composition, partitioning, overall fruit quality, and disorder occurrence in 'Honeycrisp' apples. Notably, fruits on B.9 rootstock exhibited a much lower BP percentage compared to others. Moreover, BP susceptibility has been associated with high potassium (K+) content in fruits, with some rootstocks like G.41 and G.890 contributing to K+ accumulation.

In a recent study, we found that the B.9 rootstock displayed higher endogenous levels of the phytohormone abscisic acid (ABA) than several other rootstocks (Hemeza et al. 2021). Plant tissue calcium supply is closely linked to transpiration, and once deposited in vacuoles, calcium is rarely redistributed, resulting in highly transpiring organs (e.g., leaves) accumulating large amounts of calcium. ABA can reduce stomatal conductance and transpiration rate, potentially enabling greater calcium allocation to fruits rather than leaves, thus reducing BP incidence. Indeed, foliar ABA application during fruit development has been reported to decrease BP severity in 'Super Chief' apples due to increased fruit calcium content, mediated by various calcium partitioning genes (Falchi et al., 2017). However, ABA's potential in controlling BP remains underexplored, and the optimal treatment system is yet to be determined.

Commercially available ABA, sold under the trade name ProTone (Valent BioSciences), is registered in Virginia for fruit thinning in apples and for enhancing color development in red table grapes, indicating its safety during fruit growth and development. Additionally, recent studies have shown that fruit calcium content can be increased through pre-harvest treatments with prohexadione calcium (ProCa) (e.g., Apogee, Kudos), which has demonstrated improvements in BP prevention in 'Braeburn' apples (do Amarante et al., 2020). Collectively, ABA analogs and ProCa are promising plant growth regulators (PGRs) for preventing BP and warrant further investigation on BP-prone cultivars like 'Honeycrisp'.

This study aimed to achieve three main objectives: first, to assess the impact of ABA application, either by itself or combined with ProCa, on the incidence of bitter pit (BP) in 'Honeycrisp' apples at harvest and throughout various storage periods; second, to explore the alterations in fruit mineral nutrient composition (e.g., Ca, K, Mg) as a result of ABA and ProCa treatments; and lastly, to evaluate the effects of the different treatments on fruit quality parameters.

Materials and Methods:

A field trial was conducted to evaluate the effectiveness of ABA and ProCa in preventing bitter pit in Honeycrisp apples at the ASH Jr. AREC in Winchester. Four treatments were applied to the Honeycrisp cultivar (rootstock: MM.111), with each treatment conducted in triplicates (3 trees per treatment) and distributed in the field according to a CRBD design. The treatments included a control (no treatment applied), ABA (Proton SG 20%) applied at

60, 80, and 100 DAFB at a concentration of 400 ppm, ProCa (Kudos) applied at 60, 80, and 100 DAFB at a concentration of 6 fl oz/acre/100 gal, and ABA and ProCa applied together at 60, 80, and 100 DAFB at concentrations of 400 ppm and 6 fl oz/acre/100 gal, respectively. The spray volume for each treatment was adjusted based on 100 gal/acre. Regulaid at 1 pt/100 gal was mixed with all treatments. At harvest, fruits were collected from each tree, divided into three equal groups, and assessed for bitter pit incidence (%), fruit quality, and mineral content in the fruit skins. The remaining two groups were stored at 4 ^{o}C and assessed after 3 months and 6 months of storage. The fruit quality attributes recorded at harvest included weight, diameter, Brix, firmness, and color, and the mineral content was assessed by collecting peels from 5 fruits per tree and drying them at 85 ^{o}C for 24 to 48 hours.

Results:

Impact of Treatment Combinations on BP Progression Over Time

At harvest, the ABA+ProCa treatment had a significantly higher BP (19.2%) compared to the Control (5.0%) (Table 1). The ABA (6.7%) and ProCa (10.8%) treatments had intermediate values with no significant difference from the Control or ABA+ProCa. After 3 months, there was no significant difference in BP among all treatments, with values ranging from 18.8% (ABA) to 33.3% (Control). After 6 months, all treatments showed similar BP percentages, ranging from 24.7% (ABA) to 37.9% (ProCa), with no statistically significant differences between the treatments.

Table 1: Bitter Pit (%) Assessment for Control, ABA, ProCa, and ABA+ProCa Treatments Over Time

	BP (%) at harvest	BP (%) after 3 months	BP (%) after 6 months
Control	5.0 b	33.3 a	36.7 a
ABA	6.7 ab	18.8 a	24.7 a
ProCa	10.8 ab	32.5 a	37.9 a
ABA + ProCa	19.2 a	30.0 a	36.7 a

*Values sharing the same letter within a column are not statistically significant at the 0.05 level

Assessing Fruit Quality Attributes Across Control, ABA, ProCa, and ABA+ProCa Treatments

For firmness, ProCa treatment had a significantly higher value (14.6 lb) compared to ABA (13.7 lb), while Control (14.1 lb) and ABA+ProCa (13.9 lb) treatments showed intermediate values with no significant differences (Table 2). The diameter was statistically similar across all treatments, ranging from 81.3 mm (ABA) to 82.9 mm (ABA+ProCa). The fruit weight was also comparable between treatments, with values between 225.9 g (ABA) and 241.2 g (Control), showing no statistically significant differences. The DA Meter values were consistent at 0.7 for Control and ProCa, and 0.6 for ABA and ABA+ProCa, with no significant differences. Brix values were slightly higher for ProCa and ABA+ProCa treatments (both at 12.7) compared to Control (12.3) and ABA (12.4), but these differences were not statistically significant.

Table 2: Comparative Analysis of Fruit Quality Parameters Among Control, ABA, ProCa, and ABA+ProCa Treatments

	Firmness (lb)	Diameter (mm)	Weight (g)	DA Meter	Brix
Control	14.1 ab	82.4 a	241.2 a	0.7 a	12.3 a
ABA	13.7 b	81.3 a	225.9 a	0.6 a	12.4 a
ProCa	14.6 a	81.9 a	230.7 a	0.7 a	12.7 a
ABA + ProCa	13.9 ab	82.9 a	233.9 a	0.6 a	12.7 a

*Values sharing the same letter within a column are not statistically significant at the 0.05 level

Comparative Analysis of Nutrient Concentrations and Ratios Among Control, ABA, ProCa, and ABA+ProCa Treatments

The concentrations of K, Ca, and Mg, as well as the Mg/Ca and K/Ca ratios, were found to be statistically similar across all treatments (Table 3). For potassium (K), concentrations ranged from 0.684 (Control) to 0.864 (ProCa). Calcium (Ca) concentrations were also comparable, with values between 0.020 (ProCa) and 0.024 (ABA+ProCa). Magnesium (Mg) concentrations were consistent across treatments, ranging from 0.059 (ProCa) to 0.062 (Control). The Mg/Ca ratio varied slightly from 2.620 (ABA+ProCa) to 2.943 (ProCa), while the K/Ca ratio ranged from 30.424 (Control) to 43.485 (ProCa). Since the values share the same letter, there are no statistically significant differences between the treatments for the measured parameters.

Table 3: Evaluation of Nutrient Concentrations and Ratios in Control, ABA, ProCa, and ABA+ProCa Treatments

	K	Ca	Mg	Mg/Ca	K/Ca
Control	0.684 a	0.022 a	0.062 a	2.741 a	30.424 a
ABA	0.822 a	0.022 a	0.060 a	2.786 a	38.718 a
ProCa	0.864 a	0.020 a	0.059 a	2.943 a	43.485 a
ABA + ProCa	0.737 a	0.024 a	0.060 a	2.620 a	32.145 a

*Values sharing the same letter within a column are not statistically significant at the 0.05 level

Conclusions:

In conclusion, the ABA (Proton SG 20%) treatment initially displayed lower values of bitter pit compared to other treatments. As time progressed, the differences in bitter pit incidence among treatments diminished, and by the end of the study, all treatments, including ABA, exhibited similar percentages. This indicates that the ABA treatment's initial performance concerning bitter pit occurrence was not significantly different from other treatments in the long term. Additionally, the study identified significant differences in fruit firmness across treatments, with ProCa (Kudos) demonstrating the highest value. Nevertheless, other fruit quality parameters, such as fruit diameter, weight, DA Meter values, and Brix values, showed minimal or no significant differences among the treatments. Furthermore, the study found no statistically significant differences in the concentrations of K, Ca, and Mg, as well as the Mg/Ca and K/Ca ratios, across all treatments. This implies that the applied treatments did not substantially affect the nutrient concentrations and ratios in the tested fruits.

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2022 ANNUAL REPORT TO VIRGINIA APPLE RESEARCH PROGRAM

Testing New Fungicide Options for Bitter Rot Control

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Duration of project. Two years, March 1, 2022 – November 30, 2023.

Background and Justification. In the last 10 years, the incidence of apple bitter rot caused by *Colletotrichum* species in the Mid-Atlantic U.S. has risen and changed from minor to prevalent rot problem, leading to severe economic losses. Global warming has caused warmer and wetter summers (Frumhoff et al. 2007; Hayhoe et al. 2007; Aćimović and Meredith 2019) favoring bitter rot outbreaks. Over the summer 2021 and 2022, we visited more than 40 apple orchards across Virginia. Our personal observations and loss ratings along with multiple conversations with apple growers revealed that in poorly protected commercial orchards the damage of bitter rot was from minimum 24% on Enterprise to 83% on Granny Smith. Fruit losses before the harvest were 83% on Granny Smith, 54% on Fuji, 53% on Idared and 47% on Honeycrisp (Aćimović and Khodadadi 2021, *unpublished*). In one organic orchard damage was 33% on Goldrush and 24% on Enterprise (Aćimović and Khodadadi 2021, *unpublished*). In one cider apple orchard damage was close to 98% (Aćimović and Khodadadi 2021, *personal records*). Infected fruit are rejected both for fresh consumption sales and by juice facilities. Damage from bitter rot in the eastern U.S. can also occur post-harvest in storages (Biggs & Miller 2001; Sutton et al. 2014; Rosenberger 2016), leading to 2 – 7% of unmarketable fruit. Recent survey of 9 packing houses in Pennsylvania showed that 6 of them had 2 – 14% incidence of bitter rot on harvested fruit (Peter et al. *unpublished*). In the U.S., bitter rot causes economic losses estimated to $282 million per year (Schrenk and Spaulding 1903; Burrill 1907) expressed in today's dollar value (CPI Inflation Calculator). Our recent survey in the Mid-Atlantic U.S. showed an increase in losses to bitter rot in the last 20 years, with the most susceptible cultivars losing up to 44.5% of the crop (Martin et al. 2021).

After obtaining 662 isolates of fungi from rotten apple fruit collected on 36 apple farms throughout the state, we determined that bitter rot in Virginia is caused by *C. fioriniae*, *C. nymphaeae*, *C. fructicola*, *C. chrysophilum*, *C. siamense* and *C. theobromicola* (Khodadadi et al. 2023). This was the first apple bitter rot survey conducted in the state and new reports for the presence of five out of six *Colletotrichum* species on apple in Virginia (all except *C. fructicola*). By the number of isolates, the most dominant species in Virginia were *C. fructicola, C. chrysophilum* and *C. fioriniae*. Since these species differ in the optimal temperature for growth, life cycle, virulence, and fungicide sensitivity, knowing their identity is critical for successful management of bitter rot and the leaf form of this disease called Glomerella leaf spot.

In this project we tested the scope of the efficacy of new and classic fungicides to control bitter rot because an important fungicide group of particular concern for resistance is the Quinine Outside Inhibitors (QoI-s), often referred to as strobilurins or FRAC group 11 fungicides. Plant pathogens are prone to QoI fungicide resistance, which is due to a mutation in the cytochrome *b* gene. This mutation seems stable in plant pathogen populations and does not induce a fitness penalty. Therefore, fungicide resistance will persist in a population once present. Because of the threat of QoI resistance emerging in *Colletotrichum* species, fungicide label requirements limit commercial farms to only four applications per season of any fungicide in the QoI group (Flint Extra, Luna Sensation, Pristine, Merivon). Over the last five years, numerous reports warn that *Colletotrichum* species from apples and other fruit crops around the world are developing resistance to QoI fungicides (Koenig et al. 2012; Forcelini et al. 2016; Kim et al. 2016; Nita & Bly 2016; Munir et al. 2016). In Virginia, QoI fungicides are currently effective against apple bitter rot, but are being overused with 5 to 8 applications per year, in some cases due to use for powdery mildew control early or late in spring.

The goal of this project was to find more fungicides with different modes of action so as to be included in commercial spray programs and alternated with QoI fungicides. Their inclusion would assure that resistance to QoI-s in *Colletotrichum* species from Virginia never becomes a problem leading to failure in bitter rot control. Growers in Virginia are in an excellent position to be proactive in limiting the progression of fungicide resistance among the *Colletotrichum* species populations that exists in our region on grapes, for example, causing ripe rot. Based on our previous research on fungicide for control of apple bitter rot (Aćimović et al. 2020), the concept of alternating the fungicides of different modes of action by using Aprovia (FRAC 7), Omega 500 (FRAC 29), and/or QoI fungicides (FRAC 11), all applied in tank mixes with captan or ziram during June, July or early August will help slow or prevent selection pressure for resistance in *Colletotrichum* species in apple orchards. However, these fungicides needed more testing in Virginia conditions, where complex of *Colletotrichum* species is different.

The key questions we wanted to address was can Regalia plus JMS Stylet Oil, Agri-Fos (now known as Reliant), Prophyt, EcoSwing, Vacciplant, FungOut or Actigard be effective against bitter rot? If any of them were effective, adding them to the overall summer spray program would help implement materials with alternative modes of action from QoI-s to offset the resistance occurrence in *Colletotrichum* species. This project tested these soft fungicides and compared them to synthetic fungicides we tested before (Aćimović et al. 2020). Since the complex of *Colletotrichum* species differs in Virginia than in Pennsylvania and New York, the data from this project can serve as a key guide for growers to select which fungicides to apply to effectively control bitter rot and avoid devastating economic losses. The results from this project can help growers to strategically position and alternate different classes of fungicides (FRAC 7, FRAC 29, FRAC 11, and M03, M04) during the growing season to prevent the development of fungicide resistance in populations of different *Colletotrichum* species to currently overused fungicides in FRAC 11 group. The key aim is to improve control of bitter rot by implementing new fungicides in the spray programs to prevent losses in the following season. Thus, the key economic benefit of this work will be to help reduce and prevent losses of up to 83% of apple fruit in hot and wet years like 2021.

Project objective. Expand options for bitter rot control during summer with different modes of action and thus evaluate efficacy of single-fungicide full season spray programs in Table 1 in management of bitter rot. Our project has potential to yield alternative and organic materials for bitter rot control and aims to expand options to fungicide active ingredients with different modes of action to FRAC 11 fungicides (Table 1), and by their use offset potential risks for FRAC 11 group resistance.

Cultivars. We used 22-year-old apple trees, which included the cultivars ‘Idared’ and ‘Golden Delicious’ on M.111 rootstock, with 8 ft between trees, 14 ft between trees in a panel (set); 28ft between tree plots, 30’ between rows. Treatments were replicated on four trees of each cultivar using a randomized complete block design (RCBD). Each replicate plot consisted of both cultivars stated above.

Orchard fruit inoculation. We prepared *C. fructicola* inoculum for this trial by inoculating immature apple fruit of ‘Golden Delicious’ in the laboratory with *C. fructicola* mycelial plugs and incubating the fruit at 77°F in the dark for 15 days or until bitter rot lesions yielded fungal spores on the fruit surface. Once sporulation was detected on all fruit the inoculated fruit were placed in meshed (onion) bags and then hung as inoculum on 7 June 2022 in the middle top of the canopy of each ‘Idared’ and ‘Golden Delicious’ trial tree in treatments #1 to #19 in Table 1 (growth stage: fruit size ~25 mm).

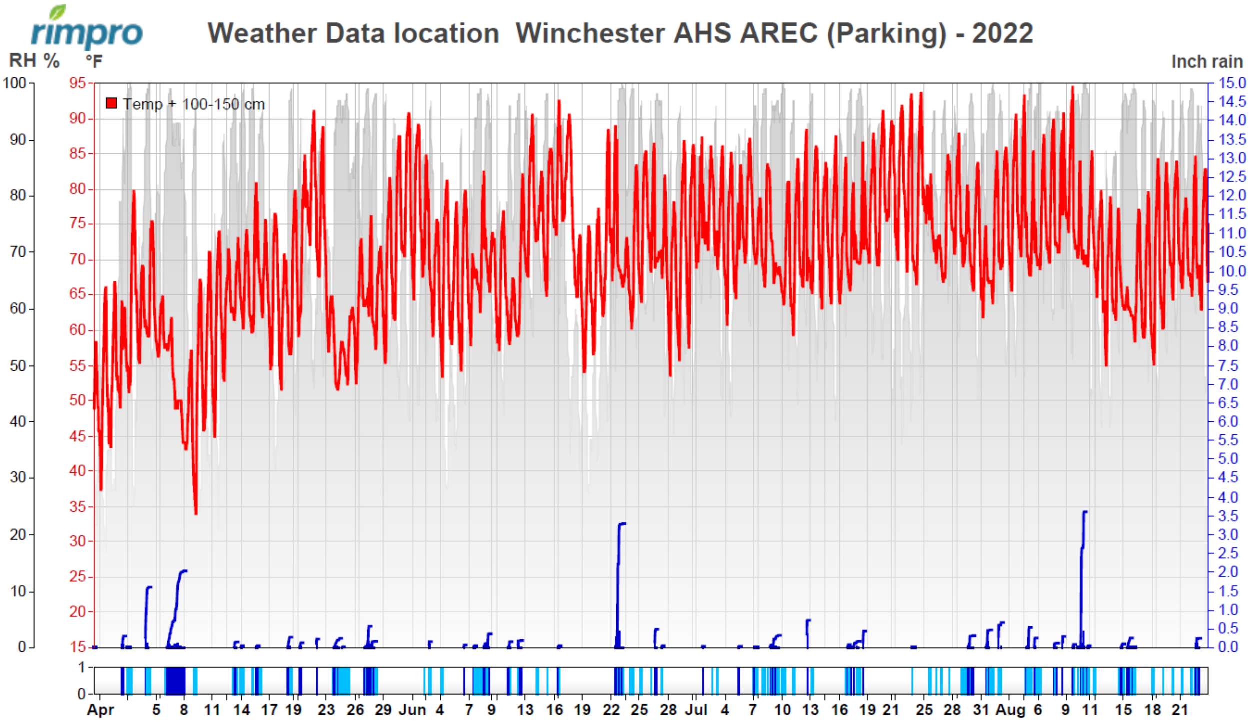

Figure 1. Weather conditions in 2022 during the apple bitter rot trial at Winchester, VA with ample rain events and above average rain amounts, that allowed numerous disease infection periods for apple bitter rot. Source: RIMpro B.V., France, subscription-based service.

Disease rating. The fruit bitter rot incidence was visually rated twice and much before the usual harvest dates for both cultivars because the extremely favorable weather conditions allowed multiple infection periods very early and throughout the summer (Figure 1). Thus, the first rating was performed on 25-26 July 2022, at the time when apple bitter rot symptoms were first visible across the region and the experimental orchard block 30 at AREC. We rated fruit the second time from 1-5 August 2022 as the disease incidence appeared to increase. The mean percent bitter rot incidence on apple fruit was calculated from the number of fruit with bitter rot lesions versus the number of fruit without lesions in a per cluster basis, totaling to 200 fruit for each cultivar and treatment (50 fruit per each tree replicate). Disease incidences on fruit for each treatment were subjected to LSD or Tukey's test (α = 0.05) for a completely randomized design (block as the factor was not significant in both rating data sets).

Table 1. Treatments for control of apple bitter rot evaluated in 2022 allowing to compare natural and alternative fungicides to synthetic fungicides.

#	Spray materials and rate	Active ingredient (FRAC code, Mode of Action)	Application stage/ timing*	Spray interval
1	Regalia 64 fl oz/A + JMS Stylet-Oil 1 gal/100 gal	extract from *Reynoutria sachalinensis* (P05, anthraquinone elicitor)	3rd to 9th cover spray*	14 days or 2 inches of rain, whichever comes first, but if no rain occurred for 14 days, extend spray interval to 21 days, under the condition that we do not get rain during the 7 additional days. If any rain event occurs between 14 and 21 days, apply fungicide before that rain regardless was 21 days reached or not.
2	Regalia 128 fl oz/A + JMS Stylet-Oil 1 gal/100 gal			
3	Actigard 2 oz/A	acibenzolar-*S*-methyl (P01, SAR activator)		
4	Reliant 2.5 quarts/A	(P07, phosphonates)		
5	Prophyt 64 fl oz/A			
6	EcoSwing 0.5 Gal/A	extract of *Swinglea glutinosa* (BM01, affects fungal spores and germ tubes, induced plant defense)		
7	Vacciplant 60 fl oz/A	laminarin (P04, polysaccharide elicitor)		
8	FungOUT 3.75 gal/A	1.07% citric acid (NA**)		
9	Flint Extra 2.9 fl oz/A	trifloxystrobin (11, QoI)		
10	Sovran 6.4 oz/A	kresoxim-methyl (11, QoI)		
11	Cabrio 11.84 oz/A	pyraclostrobin (11, QoI)		
12	Aprovia 5.5 fl oz/A	benzovindiflupyr (7, SDHI)		
13	Omega 500 13.8 fl oz	fluazinam (29, UOPP)		
14	Omega 500 6.9 fl oz	fluazinam (29, UOPP)		
15	Ziram 6 lb/A	ziram (M03, multisite)		
16	Captan 80 WDG 3 lb/A	captan (M04, multisite)		
17	Ferbam Granuflo 4.6 lbs/A)	ferbam (M03, multisite)		
18	**Grower Standard** • Inspire Super 12 fl oz/A + Captan 80 WDG 2.5 LB/A • Topsin M 1 lb + Captan 80 WDG 2.5 lb • Topsin M 1 lb + Captan 80 WDG 2.5 lb • Prophyt 64 fl oz + Captan 80 WDG 2.5 lb • Flint Extra 2.9 oz + Captan 80 WDG 2.5 lb • Flint Extra 2.9 oz + Captan 80 WDG 2.5 lb	difenoconazole (3, DMI) + cyprodinil (9, AP) + captan (M04) thiophanate-methyl (1, MBC) + captan (M04) thiophanate-methyl (1, MBC) + captan (M04) potassium phosphite (P07, phosphonates) + captan (M04) trifloxystrobin (11, QoI) + captan (M04) trifloxystrobin (11, QoI) + captan (M04)		
19	Untreated inoculated control	-	-	-
20	Untreated non-inoculated control	-	-	-

***Note: The treatments were initiated after primary apple scab season is over and will start from first cover or third cover spray and will continue until ninth cover spray if needed, or until disease incidence data is collected.**
****NA – FRAC code not yet known and/or assigned.**

Spray equipment, fungicide programs and spray dates. For full canopy coverage, all spray treatments were sprayed dilute to drip with 400 gal/A using a tractor-carried sprayer with a 'Friend' brass spray gun #16, nozzle #12 (Pak-Blast 4 x 25-gal custom sprayer, 250 PSI, Rear's Manufacturing, Coburg, OR). This allowed 11.7 Gal./min spray solution flow for good coverage. Spray applications in each spray program in Table 1 were applied on the following dates:
5/23/2022 - 3C (third cover)
6/7/2022 – *inoculation*
6/8/2022 - 4C (fourth cover)
6/21/2022 - 5C (fifth cover)
6/25/2022 - 6C (sixth cover, after 2" rain event)
7/8/2022 - 7C (seventh cover)
7/22/2022 - 8C (eighth cover)

We used spray programs of single active ingredient or its different rate as shown in Table 1 so that we can determine how each of these active ingredients alone can protect against bitter rot during the whole season summer infection pressure of this disease. The list of treatments in Table 1 started at the third cover spray onward, on a 14 to 21-day spray interval depending on the weather patterns (rain amount). To re-apply a cover spray, we used the rule of spraying at 2-week intervals or after 2 inches of rain in single or multiple smaller events, whichever comes first (Aćimović et al. 2020). We applied the treatments up to the 8th cover spray as excessive rain weather patterns required it. We stopped the applications at the 8th cover (6 applications in total) as the first bitter rot symptoms started appearing early, i.e. much before the harvest, thus allowing the rating of the disease and fair evaluation of the treatments.

Pesticide maintenance sprays prior to establishing the trial and during the trial.
3/29/2022: Vanguard 5oz/A + Mancozeb 3lb/A + Bio-Cover Oil 2 Gal/100 gal;
4/6/2022: Inspire Super 12 fl oz/A + Manzate Pro-Stick 3 lbs/A + Assail 4 oz/A;
4/21/2022: Inspire Super 12 fl oz/A + Manzate Pro-Stick 3 lbs/A + Sonoma 10 oz/A;
5/4/2022: Inspire Super 12 fl oz/A + Microthiol Disperss 7 lb/A + Manzate 3lb/A+Indar 8 oz/A;
5/28/2022: Altacor 4.5 oz/A;
6/28/2022: Imidan 5 lb/A + Assail 4 oz/A;
7/15/2022: Voliam Flexi 5 oz/A;

Results. Out of 8 treatments with biorational materials, such as Regalia (both rates), EcoSwing, Actigard, Vacciplant, FungOut, Reliant and Prophyt, none provided satisfactory management of apple bitter rot allowing 37-67% disease incidence on Idared and 11-33% disease incidence on Golden Delicious fruit. In July 2022, all synthetic fungicides including ferbam, captan, ziram, fluazinam (Omega 500), benzovindiflupyr (Aprovia), pyraclostrobin (Cabrio) and trifloxystrobin (Flint Extra), but not kresoxim-methyl (Sovran), were effective with zero to 13% disease incidence on Idared fruit and only 0.6 to 3% disease incidence on Golden Delicious fruit (Fig. 2). Untreated inoculated and untreated non-inoculated controls exhibited 63 and 75% disease incidence on Idared, respectively (Fig. 2). Both controls exhibited 28% disease incidence on Golden Delicious fruit (Fig. 2). Second rating in August (Fig. 3) confirmed these results, but showed more disease incidence develop in biorational material spray programs (78-89% on Idared; 21-48% on Golden Delicious) and in synthetic fungicides (12-39% on Idared; 6-11% on Golden Delicious). The higher disease incidences we recorded on 5 Aug 2022 (Fig. 3) in the effective spray programs from 26 July 2022 (Fig 2) could be due to several reasons:

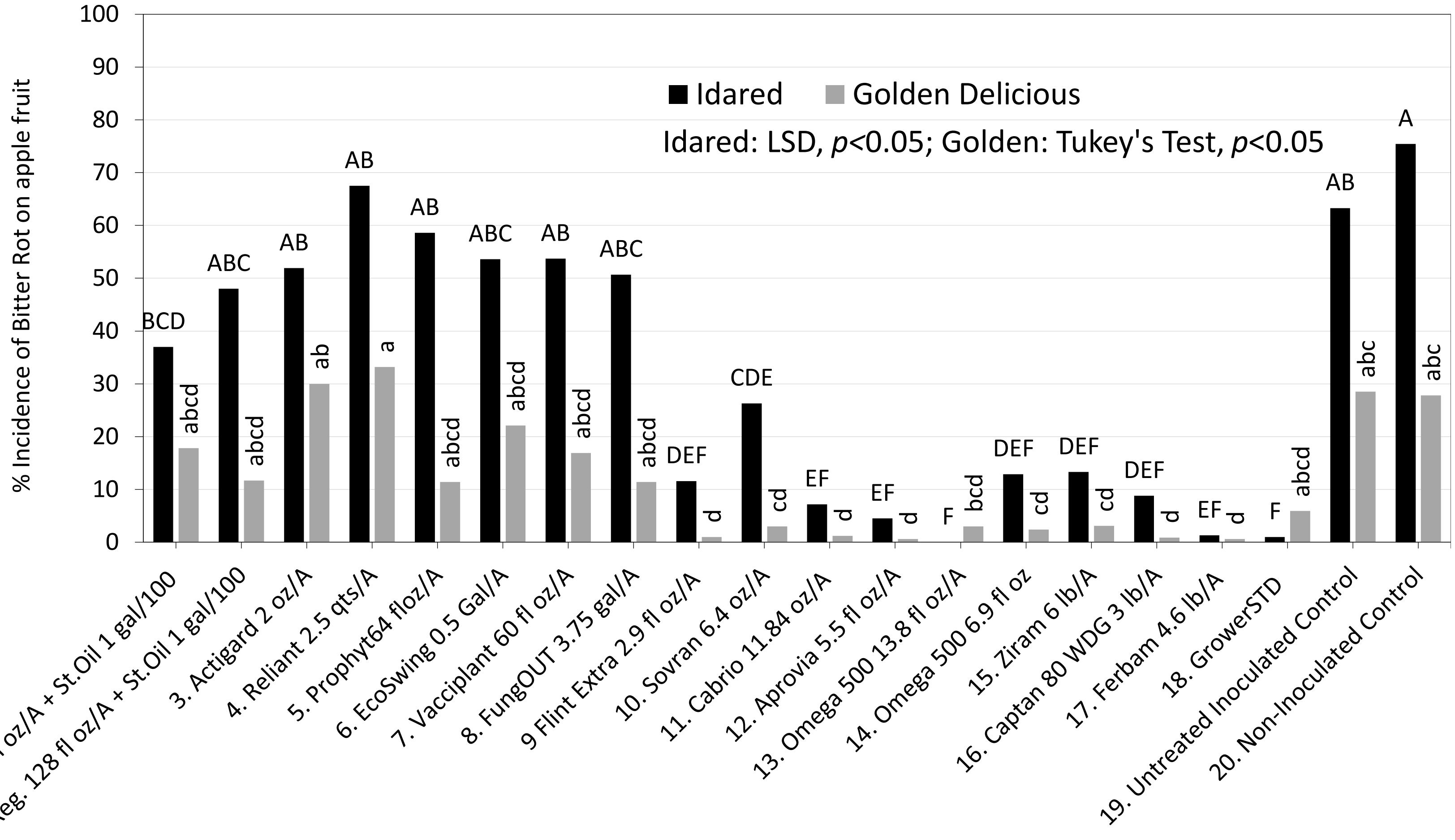

Figure 2. Apple fruit bitter rot control on 26 July 2022 on 'Idared' and 'Golden Delicious', after 6 consecutive summer applications of individual fungicides or their different rates listed in each numbered treatment, except in treatment 18 which consisted of many different fungicides alternated during the summer. Means within each cultivar i.e. bar color followed by different letters are significantly different (α=0.05). Orchard inoculation was performed with *Colletotrichum fructicola* on 7 Jun 2022 for both cultivars. Each mean consists of four replicate trees.

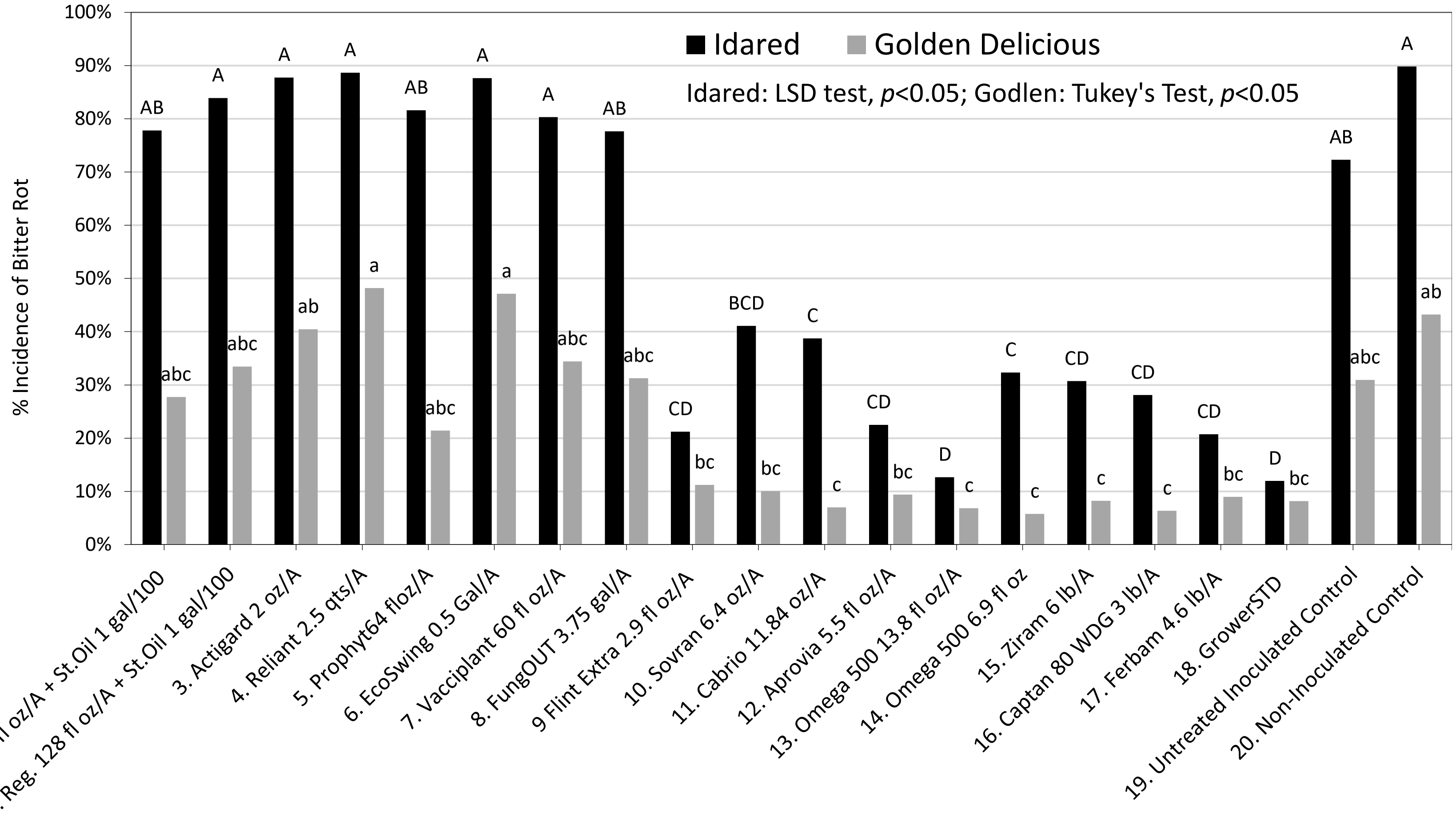

Figure 3. Apple fruit bitter rot control on 5 August 2022 on ‘Idared’ and ‘Golden Delicious’, after 6 consecutive summer applications of individual fungicides or their different rates listed in each numbered treatment, except in treatment 18 which consisted of many different fungicides alternated during the summer. Means within each cultivar i.e. bar color followed by different letters are significantly different (α=0.05). Orchard inoculation was performed with *Colletotrichum fructicola* on 7 Jun 2022 for both cultivars. Each mean consists of four replicate trees.

(1) Infections of bitter rot that have occurred after the last experiment cover applied on 22 July 2022, which was 14 days during which 1.87 to 2.26 inches of rain occurred, as per two on-site NEWA weather stations. However, this might not true as Grower Standard (treatment #18 was fairly effective on 5 Aug 2022 disease rating).
(2) Infections of bitter rot that have established during the 3.29-inch rain event on 22-23 June 2022. However, this might not true as Grower standard (treatment #18 was effective on 5 Aug 2022 too).
(3) Ferbam, captan, ziram are contact fungicides so they are prone to wash-off by rain and the excessive rain amounts in 2022 shown in Fig. 1 likely led to their lower efficacy on 5 Aug 2022 due to rain-driven wash-off reducing the residue amounts.
(4) Low rate of Omega 500 (6.9 fl oz/A) and poor formulation of Cabrio deeming them less effective.
(5) Emergence of low-level resistance in *Colletotrichum* species to pyraclostrobin (Cabrio) and to kresoxim-methyl (Sovran).
(6) Higher rainfastness of the tested fungicides i.e. their formulations, when applied alone and during the excessively rainy season, might have led to their lower efficacy.

The same spray programs in Table 1 will be repeated in 2023 in continuation of this project so that the consistency of the presented results from 2022 is tested in one more growing season before we can report biorational fungicides as ineffective (treatments #1-8). Therefore, the most effective fungicides against bitter rot in Virginia are ferbam, captan, ziram, fluazinam (Omega 500), benzovindiflupyr (Aprovia), pyraclostrobin (Merivon, Pristine), and trifloxystrobin (Flint Extra).

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