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VIRGINIA APPLE RESEARCH COMMITTEE GRANT REPORTS

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2023 FINAL REPORT TO VIRGINIA APPLE RESEARCH PROGRAM

Testing New Fungicide Options for Bitter Rot Control (second year)

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

Background and Justification. Apple bitter rot disease in Virginia is caused by the fungal pathogens *C. fioriniae*, *C. nymphaeae*, *C. fructicola*, *C. chrysophilum*, *C. siamense* and *C. theobromicola* (Khodadadi et al. 2023). The most frequently found species in Virginia were *C. fructicola, C. chrysophilum* and *C. fioriniae*. These species differ in the optimal temperature for growth, life cycle, virulence and fungicide sensitivity. Knowing the identity of causal *Colletotrichum* species is critical for successful management of bitter rot and the leaf form of this disease called Glomerella leaf spot. Global warming has caused warmer and wetter summers which favor bitter rot outbreaks (Frumhoff et al. 2007; Hayhoe et al. 2007; Aćimović and Meredith 2019). For two years, we visited more than 40 apple orchards in Virginia and determined that in poorly protected commercial orchards the damage from bitter rot ranged from 24 to 83% with most affected cultivars like Enterprise, Goldrush Granny Smith, Fuji, Idared and Honeycrisp (Aćimović and Khodadadi 2021, *unpublished*). One cider apple orchard had damage of 98% (Aćimović and Khodadadi 2021, *personal records*). Infected apple fruit are not accepted for fresh or juice market. Bitter rot can also occur post-harvest in storages leading to 2 – 14% of unmarketable fruit (Biggs & Miller 2001; Sutton et al. 2014; Rosenberger 2016; Peter et al. *unpublished*). In the U.S., bitter rot causes economic losses of up to $282 million per year expressed in today's dollar value (Schrenk and Spaulding 1903; Burrill 1907; CPI Inflation Calculator). A 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).

In the second project year we continued testing the scope in the efficacy of new and classic fungicides to control bitter rot because there is a particular concern for resistance of *Colletotrichum* spp. to the Quinone Outside Inhibitor fungicides (QoI-s), often referred to as strobilurins or FRAC group 11 fungicides. This resistance risk is high for two reasons: (1) commercial farms use strobilurins up to and in some cases over 6 times per season, (2) commercial farms are dependent on the high efficacy of strobilurins for management of bitter rot as the most important summer disease of apples, and (3) there is limited number of fungicides of with other FRAC modes of action to offset the pressure in use of strobilurins.

Plant pathogens are prone to developing resistance to strobilurin (QoI) fungicides, 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 must limit commercial farms to only four applications per season of any fungicide in the QoI or FRAC 11 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 have been used in 5 to 8 applications per year, in some cases due to need to control powdery mildew early and/or late in spring.

The goal of this project was to find more fungicides with different modes of action that can be included in commercial spray programs and alternated with the 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 proactively limit the progression of fungicide resistance among the *Colletotrichum* populations that exists in our region on grapes, for example, causing ripe rot. Based on our previous research on fungicides 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 required more testing in Virginia conditions, where complex of *Colletotrichum* species causing bitter rot is different.

The key questions we wanted to address were can biorational material options like Regalia plus JMS Stylet Oil, 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 selection pressure for resistance occurrence in *Colletotrichum* species. This project tested these soft, biorational 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, M03, M04) during the growing season and thus prevent the development of fungicide resistance in populations of six *Colletotrichum* species to currently overused FRAC 11 group fungicides. 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. 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 and 2022 were.

Project objective. To expand spray material options for bitter rot control during summer with different modes of action by evaluating efficacy of single-fungicide summer spray programs in Table 1 in control of bitter rot for two growing seasons. Our project has potential to yield alternative and organically acceptable 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 24-year-old apple trees, which included 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. Spray programs were replicated on four trees of each cultivar using a completely randomized design (CRD). Each replicate plot consisted of both cultivars stated above.

Orchard fruit inoculation. We prepared *Colletotrichum fructicola* inoculum for this trial by inoculating immature apple fruit of 'Golden Delicious' in the laboratory with *C. fructicola* strain VA-3-73 with mycelial plugs and incubating the fruit at 77°F in the dark for 15 days i.e until bitter rot lesions yielded fungal spores on the fruit surface. Once sporulation was detected, the inoculated fruit were placed in meshed (onion) bags and suspended as inoculum on 30 May 2023 at the top of the canopy of each 'Idared' and 'Golden Delicious' tree in spray programs 1-18 in Table 1 (growth stage: fruit 20-25 mm).

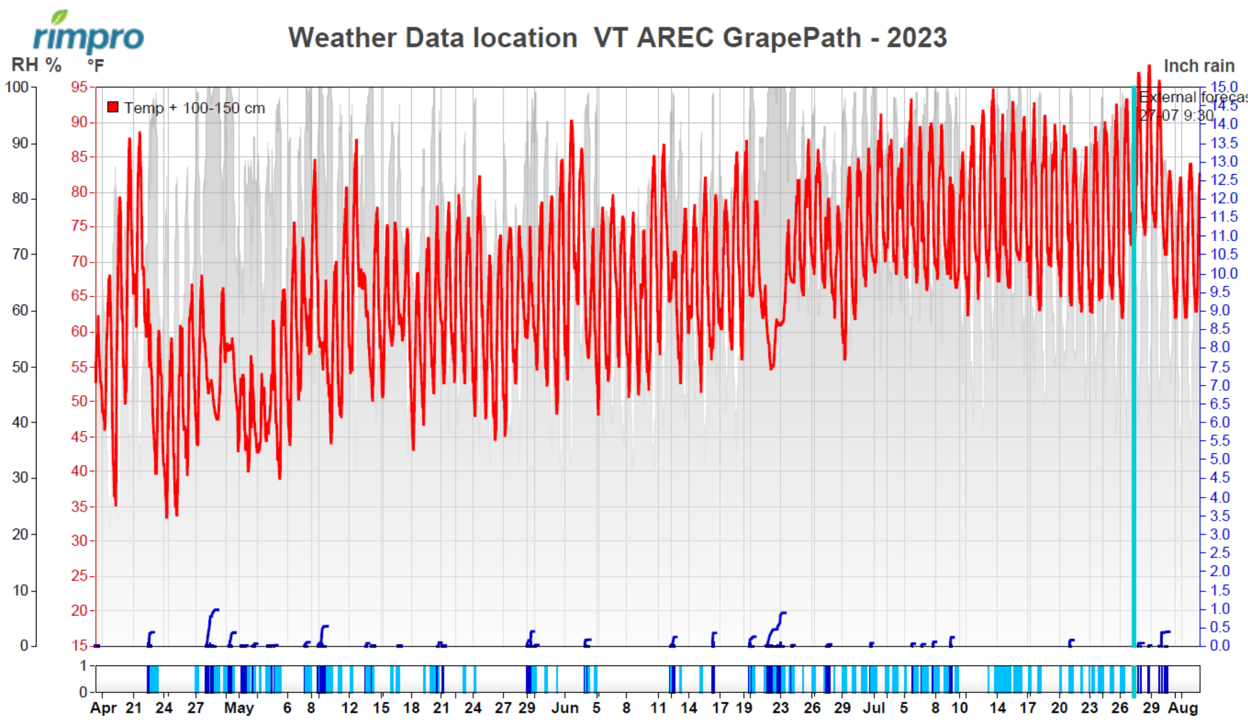

Figure 1. Weather conditions in 2023 during apple bitter rot trial at Winchester, VA with low frequency rain events that allowed enough disease infection periods for apple bitter rot. Source: RIMpro B.V., France, subscription-based service.

Disease rating. The fruit bitter rot incidence was visually rated twice on 24 and 31 July 2023, thus much before the usual harvest dates for both cultivars because the favorable weather conditions allowed multiple *Colletotrichum* infection periods very early and throughout the summer (Fig. 1). The first rating of 24 July 2023 was performed at the time when apple bitter rot symptoms were first uniformly visible across the northern Virginia region and in the untreated controls in the experimental orchard in Winchester. We rated fruit the second time on 31 July 2023 to capture whether the disease incidence has changed (increased) or not in comparison to the first rating. The mean percent bitter rot incidence on apple fruit was calculated from the number of apple fruit with bitter rot lesions versus the number of apple fruit without lesions in a per fruit cluster basis, totaling to 200 fruit for each cultivar and spray program (50 fruit per each tree replicate, from approximately 20 fruit clusters). Disease incidences on fruit for each spray program were subjected to LSD or Tukey's HSD test ($\alpha = 0.05$) for a completely randomized design in SAS Studio (SAS Institute Inc., Cary, NC).

Table 1. Spray programs for control of apple bitter rot evaluated in 2023 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 spray applications were initiated after primary apple scab season was over and were started from first or third cover spray and were continued until ninth cover spray if needed, or until disease incidence data was collected.**
****NA – FRAC code not yet known and/or assigned.**

Spray equipment, fungicide programs and spray dates. For full canopy coverage, all applications were sprayed dilute to drip with 400 gal/A using a tractor-carried sprayer with a

brass 'Friend' spray gun #16, nozzle #10 connected to a Pak-Blast hybrid 4 x 25-gal sprayer (250 PSI, Rear's Manufacturing, Coburg, OR). This allowed 6.9 gal/min spray solution flow securing satisfactory tree canopy penetration and coverage. Spray applications in each spray program in Table 1 were applied on the following dates:
5/27/2023 - 3C
5/30/2023 – **inoculation with *C. fructicola***
6/9/2023 - 4C
6/21/2023 - 5C
7/5/2023 - 6C
7/19/2023 - 7C

We used spray programs of single active ingredient or their different rates throughout the summer of 2023 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 summer infection pressure of this disease. The list of spray programs 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 came first (Aćimović et al. 2020). The primary reason for using this rule is that fully developed leaf and fruit canopy can hold much more fungicide residues, thus being more durable i.e. longer lasting through rain. We stopped the applications at the 7th cover (5 applications in total) as the first bitter rot symptoms in untreated inoculated and untreated uninoculated control trees appeared on 5 July 2023, i.e., much before the usual calendar harvest dates, thus allowing us to rate the disease at the end of July for fair evaluation of spray program efficacy.

Pesticide maintenance sprays prior to establishing the trial and during the trial.
3/29/2023: Avaunt 6oz/A + Vanguard 5 oz/A + Manzate 3lb/A;
4/6/2023: FireWall 32 oz/A; 4/26/2023: Assail 8oz/A + Manzate 3lb/A + Sonoma 20EW 4oz/A;
5/10/2023: Manzate 3 lb/A + Altacor 4 oz/A; 5/24/2023: Movento 9 fl oz/A + Altacor 4 oz/A + Regulaid 1 pint/100 gals;
5/26/2023: Trionic 4SC + Movento 9 fl oz/A + Altacor 4 oz/A;
6/2/2023: Refine 3.5 at 4 fl oz/100 + Regulaid 1 pt/100 gals;
6/8/2023: Refine 4 fl oz/A;
6/13/2023: Refine 4 fl oz/A + Beseige 12 fl oz/A; 6/29/2023: Beseige 12 fl oz/A;
7/12/2023: Beseige 12 fl oz/A.

Results. Untreated inoculated and untreated non-inoculated controls exhibited 62.2 and 63.4% disease incidence, respectively, in first rating on Idared (Fig. 2), and 57.3 and 59.4% in the second rating, respectively, (Fig. 3). Both controls exhibited 29.6 and 21.3% in the first rating and 20.9 and 20.2% disease incidence in second rating on Golden Delicious fruit, respectively (Figs 2 and 3). The lower incidences in the second rating in these controls could be attributed to the losses of fruit from fruit drop due to bitter rot.

Out of 8 spray programs with biorational materials, such as Regalia (two rates), EcoSwing, Actigard, Vacciplant, FungOut, Reliant and Prophyt, only the low rate of Regalia (64 fl oz/A) with Stylet oil provided statistically significant control of apple bitter rot of 57.6 to 56.5% (ratings on 24 and 31 July, respectively). This success could be attributed to a drier 2023 summer with lower frequency of rain events in comparison to 2022 summer. It is debatable whether this level of control would be acceptable for commercial growers, but it opens a question whether a single or few applications of Regalia at 64 fl oz/A could be included in the summer spray programs on commercial farms in alternation with synthetic fungicides to reduce the overall seasonal number of synthetic fungicide spray applications. Due to Regalia's different mode of action to synthetic fungicides, its applications in commercial spray programs alternated with synthetic fungicides could also contribute to the reduction of resistance selection pressure in *Colletotrichum* species by the excessively used QoI fungicides. It remains to be determined why the higher rate of Regalia (128 fl oz/A, spray program #2) did not provide an expected higher efficacy in comparison to Regalia 64 fl oz/A spray program #1, but it has been recorded before that some biorational materials due to their plant immunity mode of action often act the opposite from synthetic fungicides: the lower rates show better efficacy than higher rates (e.g. Vacciplant, UPL, Ltd). All other biorational spray programs we tested (#2 to #8) allowed unacceptable 31-54% disease incidence levels on Idared and 12-19% disease incidence levels on 'Golden Delicious' fruit.

In July 2023, all synthetic fungicides including ferbam, captan, ziram, fluazinam (Omega 500), benzovindiflupyr (Aprovia), pyraclostrobin (Cabrio), trifloxystrobin (Flint Extra), and kresoxim-methyl (Sovran), were effective with 2.3-2.7 to 12.9-13.1% disease incidence on Idared fruit (Figs 2 and 3) and only 0.0 to 2.3-2.9% disease incidence on Golden Delicious fruit (Figs 2 and 3). On 'Golden Delicious', there were only numerically higher disease incidences in the untreated controls (#19, #20) in comparison to all biorational spray treatments (#1 to #8) but no statistically significant differences. This confirmed on another cultivar ('Golden Delicious') no efficacy of biorational materials in bitter rot control. In contrast, all (Fig. 2) and almost all (Fig. 3) synthetic fungicide spray programs reduced significantly disease incidence on 'Golden Delicious' when compared to the untreated controls (#19, #20). Lower disease incidences in the untreated controls (#19, #20) on 'Golden Delicious' versus Idared' are likely because of combination of several factors: lower susceptibility of skin to *Colletotrichum* infection of 'Golden Delicious' versus Idared' and different fruit ripening times.

In the second rating of 31 July (Fig. 3) the efficacy results from first rating of 24 July were more solidly confirmed than in the case of the 2022 results, but in biorational spray programs #2, 3, 4 6 and 8 we saw development of slightly more disease incidence. In contrast, in spray programs #1, 5 and 7 had a slightly lower disease incidence in the second rating of 31 July in comparison to the rating one on 24 July (possibly because of fruit drop). For synthetic materials in spray programs #9 to 18 the disease incidences were largely similar between the two ratings of 24 and 31 July. This is the opposite to the prevailing trend we saw in the 2022 evaluation results of the same spray programs which clearly showed the higher disease incidences recorded in the second disease rating on 5 Aug 2022 for the effective spray programs in comparison to the first rating of 26 July 2022. By observing results from two ratings in 2023, this can lead us to a solid conclusion that the weakening of efficacy for synthetic fungicides we saw in 2022 was because the higher amounts of rainwater and frequency of rain events in 2022 in comparison to 2023, led to faster depletion of fungicide residues in the tree canopy. Thus, we can conclude that in wet years the effective fungicides must be applied at intervals shorter than 14 days between the cover sprays to prevent more rapid wash-off of residues by rain and thus loss of efficacy.

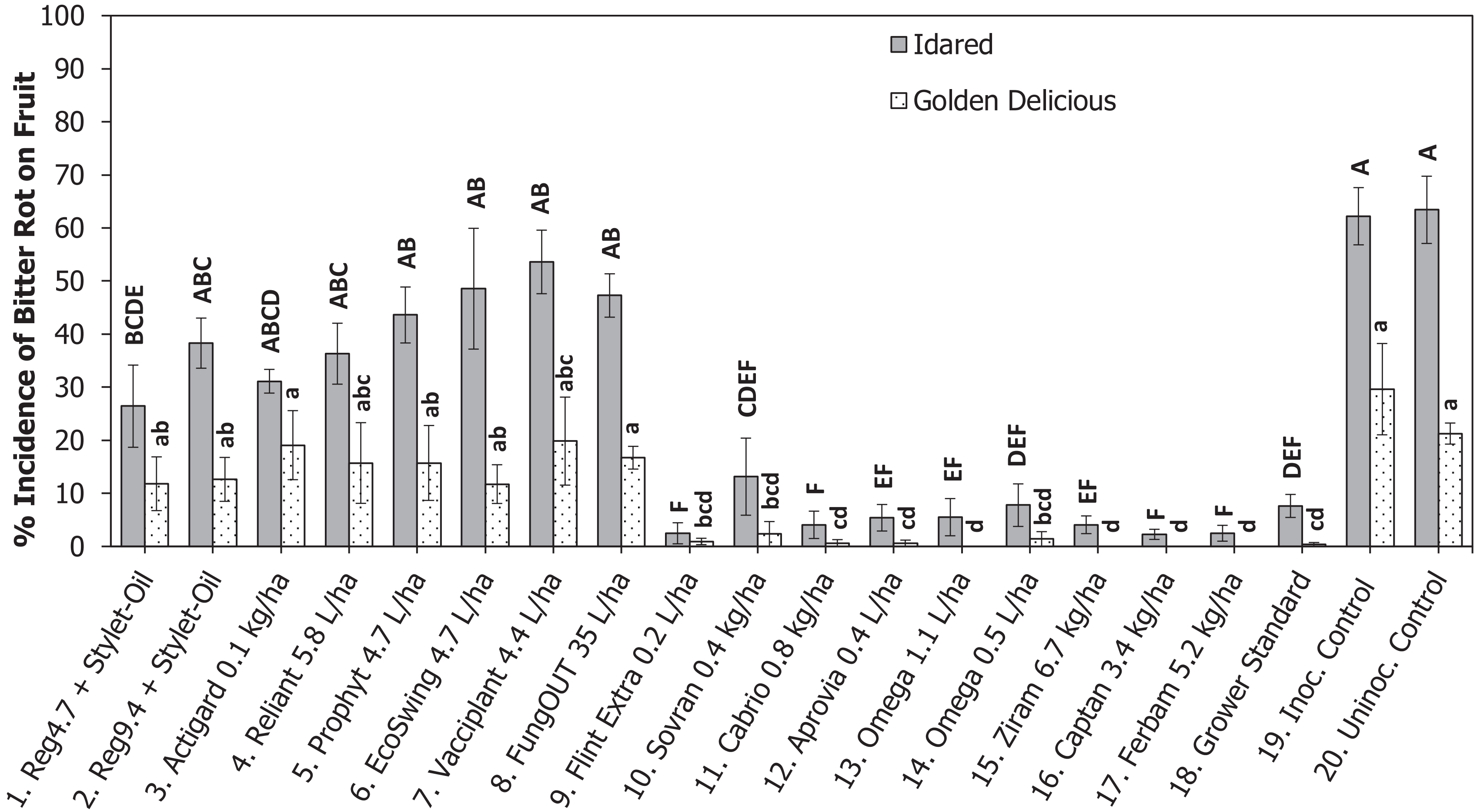

Figure 2. Apple fruit bitter rot control on 24 July 2023 on 'Idared' and 'Golden Delicious', after 5 consecutive summer applications of individual fungicides or their different rates listed in each numbered spray program, except in spray program 18 which consisted of many different fungicides. Means within each cultivar i.e., bar color followed by different letters are significantly different (Tukey test, α=0.05). Orchard inoculation was performed with *Colletotrichum fructicola* on 30 May 2023 for both cultivars. Each mean consists of four replicate trees. Error bars are standard error of the mean. Note: the fungicide amounts used are the same as in Tabe 1 and Figure 2 below but are shown here in Liters per hectare.

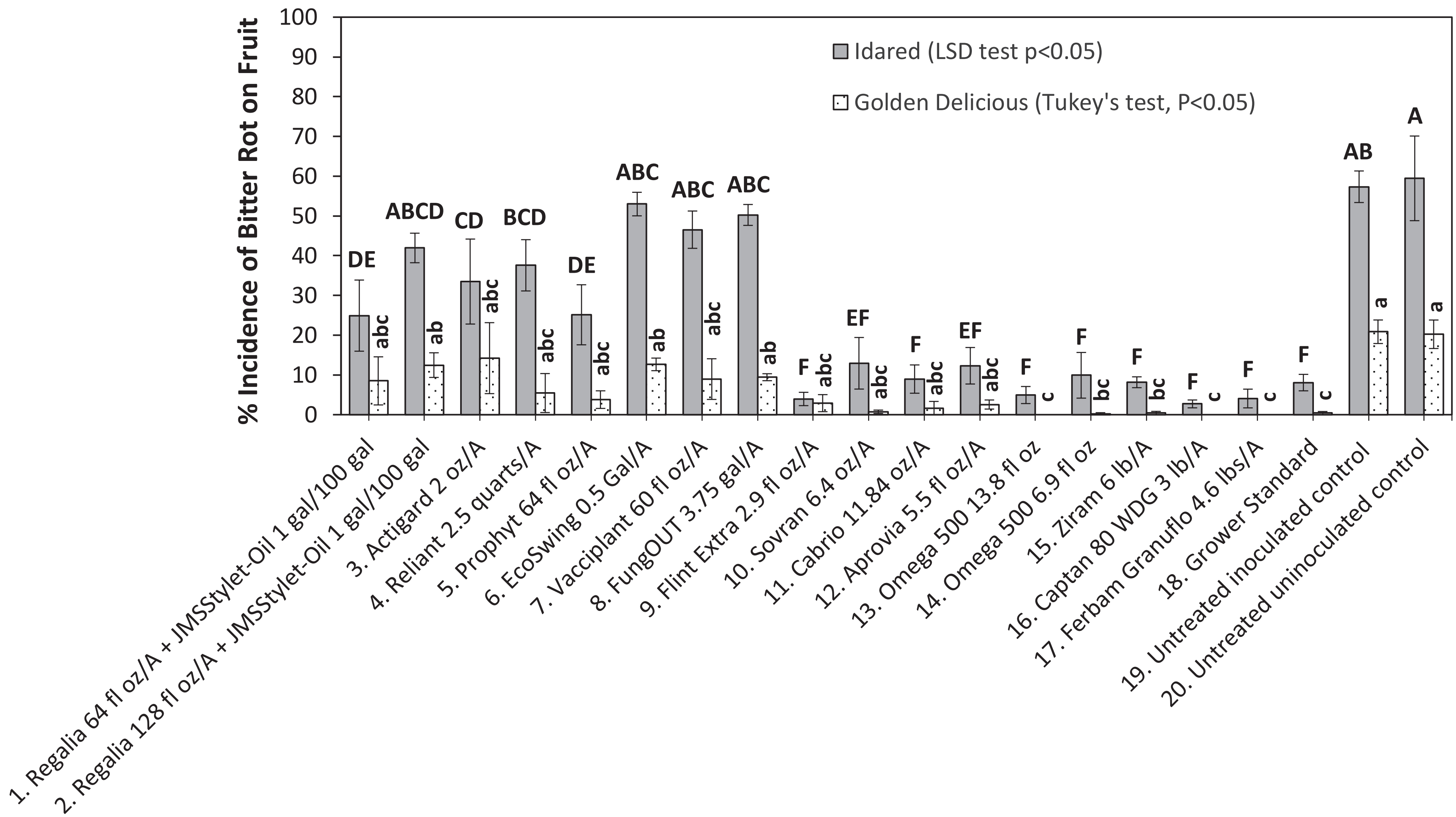

Figure 3. Apple fruit bitter rot control on 31 July 2023 on 'Idared' and 'Golden Delicious', after 5 consecutive summer applications of individual fungicides or their different rates listed in each numbered spray program, except in spray program 18 which consisted of many different fungicides. Means 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 30 May 2023 for both cultivars. Each mean consists of four replicate trees. Error bars represent standard error of the mean.

By comparing the consistency of the spray programs in Table 1 from both experiment years (2022, 2023) we can conclude that biorational fungicides we tested are ineffective for apple bitter rot control and thus are not recommended for commercial farm use (spray programs #1-8). Therefore, the most effective fungicides against bitter rot in Virginia are multi-site fungicides captan, ziram, ferbam, and single-site fungicides fluazinam (Omega 500), benzovindiflupyr (Aprovia), pyraclostrobin (Merivon, Pristine), and trifloxystrobin (Flint Extra). Based on our results, in drier years like 2023, even kresoxim-methyl (Sovran) can perform equally well as other synthetic 11 fungicide but cannot be relied upon in wet years like 2022

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Maintaining and Evaluating Apple Seedlings from Honeycrisp X Cameo Crossings: Tree Growth and Fruit Quality Parameters

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Duration of project: One year, May 1, 2023 – April 30, 2024

A. Background and justification:

The major apple breeding programs in the United States currently focus on developing either dwarfing and disease-resistant rootstocks (such as Geneva® rootstocks) or elite scion varieties with superior fruit quality traits. Rootstock breeding is managed by Cornell University, while scion breeding programs are primarily managed by Washington State University and the University of Minnesota. Additionally, several high-quality apple varieties are also developed and controlled by grower clubs and profit organizations. While Geneva® rootstocks have become available to other apple-growing states, the newly released scion varieties (including SweetTango®, Cosmic Crisp®, JazzTM, and EnvyTM) from both public and private sectors have become restricted to certain states and/or stakeholders. This exclusivity given to certain states and stakeholders to exclusively produce high-quality apple varieties is expected to shift market dynamics towards higher demand for these varieties in the long term, leaving publicly accessible cultivars (such as Fuji, Gala, and Red Delicious) with limited marketable advantage and consumer preference. Recent advances in genomics and bioinformatics have produced a plethora of valuable knowledge about the genetic control of major fruit quality traits (such as fruit color, firmness, and crispness), which has opened the door for integrating DNA-informed technologies (such as marker-assisted selection (MAS)) into modern breeding programs. These new technologies are expected to shorten the breeding cycle and significantly reduce the cost of land and labor needed for planting, maintenance, and evaluation of progenies (seedlings) from various crosses. In light of these advancements, a Virginia-based apple breeding program was launched at the AHS Jr. AREC in Winchester in 2018. The main goal of this program is to produce apple varieties with outstanding eating quality, good appearance, and enhanced storability through integrating traditional and DNA-assisted breeding strategies, thereby enhancing the marketable competitive advantage of Virginia's fresh market apple industry.

B. Objectives:

1: Maintenance of apple seedlings resulting from the 2018 and 2019 'Honeycrisp' X 'Cameo' crosses.

2: Evaluation of seedlings from the 2018 'Honeycrisp' X 'Cameo' crossings for tree growth and fruit quality parameters.

C. Approach

The Virginia apple breeding program was initiated in 2018 through the crossing of two high-quality apple varieties, 'Honeycrisp' and 'Cameo,' known for their exceptional eating-quality characteristics. The crossing was performed by hand-pollinating 400 'Cameo' flowers with 'Honeycrisp' pollen, harvesting 117 fruits, and obtaining 321 seeds. The stratification of seeds for three months in a cold environment was followed by their planting in a potting soil mix and maintenance in a greenhouse for five months (January to May) until they reached a height of 3-4 ft. Subsequently, the seedlings (215) were moved to a high-tunnel shade for three months (May to August) before being bud-grafted to B.9 rootstocks (2-3 buds per rootstock). The rootstocks were generously donated by Mr. Bill Mackintosh of Mackintosh Fruit Farm, and the skilled grafting was carried out by Mr. Raul Godinez and his team at Countryside Farm and Nurseries, Inc. In November 2019, sleeping-eye trees were transplanted into the permeant orchard, spaced at 2'x12', and supported by a 1-inch metal conduit. Beginning in the summer of 2023, all seedlings underwent thorough evaluations focusing on growth and yield parameters. Additionally, a detailed assessment of fruit quality traits was conducted, which included measurements of fruit weight, diameter, firmness, color, total soluble solids (TSS), pH, and starch content.

To increase the number of seedlings and allow for marker-assisted selection/breeding (MAS/MAB), a reciprocal crossing of 'Cameo' and 'Honeycrisp' was conducted in 2019. Specifically, 400 'Honeycrisp' flowers were emasculated and pollinated manually with 'Cameo' pollen, resulting in the harvest of 185 fruits and the extraction of 1320 seeds. These seeds were cold stratified and kept in the greenhouse, as outlined previously. The 1300 resulting seedlings were subjected to PCR screening to identify seedlings with genetic markers associated with low-medium ethylene content and exclude those with markers linked with high-ethylene. Those seedlings with desirable markers/traits were bud-grafted to B.9 in August 2021 and maintained in the permanent orchard at the AHS Jr. AREC as outlined above. Evaluation of the seedlings for tree growth and fruit quality parameters will commence in 2025-2027.

D. Results

For the 2023 growing season, in the assessment of a breeding progeny from the 2018 crossbreeding, fruit production was observed in 99 trees, accounting for approximately 64% of the total. Detailed evaluations were conducted on a sample set, with results averaged from five apples per tree to ensure statistical relevance. The data encompassed a range of morphological and quality parameters, summarized as follows:

- **Fruit color at harvest assessed by the DA meter**: The DA at harvest varied from 0.2 to 0.92, with an average of 0.61.
- **Fruit Count**: The number of fruits per tree ranged widely from 1 to 101, with an average production of 28 fruits per tree.
- **Harvest Period:** The harvesting window extended from August 15 to October 12, with the bulk of the fruits typically being ready by August.
- **Physical Characteristics:**
    - **Weight:** Fruit weights spanned from 54.6g to 337.67g, with an average weight of 158.36g.
    - **Diameter:** The diameters of the apples ranged from 50mm to 90.5mm, averaging at 71mm.
    - **Firmness:** The firmness of the apples was recorded between 13.01 lbs and 24.89 lbs, with an average firmness of 18.37 lbs.
- **Chemical Characteristics:**
    - **Brix** (Total Soluble Solids): The Brix values ranged from 11.04% to 18.00%, with a mean of 14.77%, indicative of the sweetness level.
    - **Starch Index**: The starch index, which indicates the ripeness, varied from 2.2 to 8, with an average of 6.
- **Sensory Attributes:**

- **Taste**: The taste profile varied from very tart to very sweet, with the majority of the sampled fruits classified as sweet.
- **Color**: The range of colors observed in the progeny varied from Granny Smith-green to Golden Delicious-yellow, accompanied by typical red HC streaking. Additionally, light HC streaking was noted, interspersed with blotches of deep red, yellow, and green, showcasing a diverse color palette within the progeny (Figure 1).

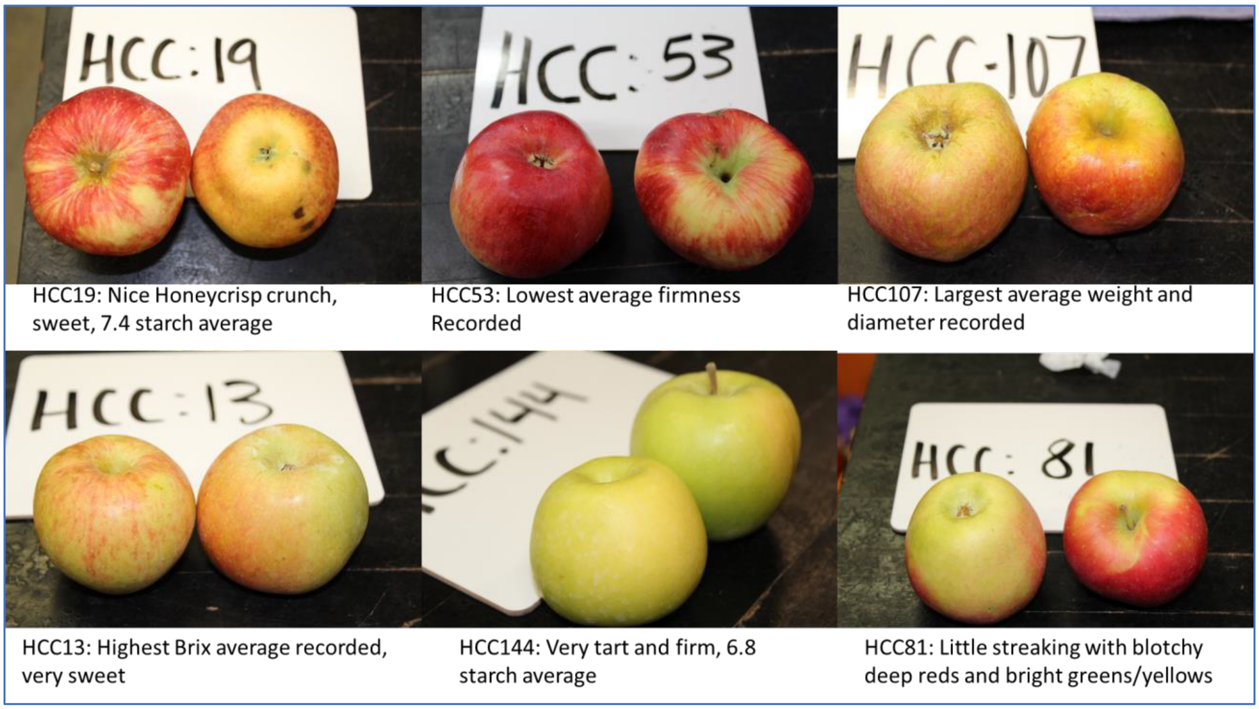

Figure 1: Examples of the diversity in physical and chemical characteristics observed in the seedling progeny resulting from the 2018 Honeycrisp x Cameo crossbreeding.

Pre-Harvest Treatments for Improving Apple Fruit Coloration: Investigating the Potential of PGRs and Phosphorus-Containing Compounds

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Duration of project: One year, May 1, 2023 – April 30, 2024

A. Background and justification:

Red coloration is a crucial quality parameter that contributes significantly to the market value of apples. This coloration is primarily attributed to anthocyanins, which accumulate in the vacuoles of apple skin cells, while flavonols and proanthocyanidins also contribute to color development. The regulation of anthocyanin biosynthesis in apple fruit is a developmentally regulated process that occurs in two peaks (Lancaster and Dougall, 1992). During the fruitlet stage, both red and non-red cultivars display the first peak, while the second peak is exclusive to red cultivars and occurs during the ripening fruit stage. Apple peel consists of nearly five different anthocyanins, with cyanidin 3-galactoside (cy3-gal) being the most abundant pigment, accounting for 80% of the total anthocyanins identified (Lancaster and Dougall, 1992; Gómez-Cordovés et al., 1996). The accumulation of anthocyanins is typically limited to the skin of the fruit and plays a crucial role in cultivar differentiation, while also contributing to the numerous health benefits associated with apple fruit. Various factors such as light, ethylene, temperature, nitrogen fertilization, wounding, bagging, and chemical applications can significantly influence fruit color and anthocyanin biosynthesis.

The enhancement of fruit red color is primarily achieved through the use of ethylene, which is typically applied to fruits in the form of its commercial product, Ethrel. This application promotes the maturity of the fruit, allows for early harvesting, and intensifies the red color of the fruit skin (Ban et al., 2007). However, the use of ethephon may result in reduced storage potential of the fruit, particularly regarding the reduction in flesh firmness (Brackmann et al., 2014; Brackmann et al., 2015). Consequently, recent studies have focused on identifying alternative chemical products that can stimulate the accumulation of anthocyanins in fruit skin without the adverse impacts of maturity acceleration, ethylene production, and reduced storage potential (Bizjak et al., 2013).

A study conducted by Brighenti et al. (2017) demonstrated that the application of prohydrojasmonate (a form of jasmonic acid, JA) and abscisic acid (ABA) as plant growth regulators enhanced the red coloration and chlorophyll degradation in 'Gala Standard' apples without reducing flesh firmness. These regulators were applied at a rate of 400 mg L-1 approximately 20 days before harvest and positively impacted fruit skin color without compromising fruit firmness. The positive effects of JA and ABA were also observed in the United States for Fuji and Buckeye Gala cultivars (Rudell & Mattheis, 2008; Francescatto, 2013), as well as in Turkey and Italy for Gala cultivar and its clones Mondial and Brookfield (Vizzotto et al., 2013; Atay, 2015). Blush and ProTone are trade names under which both prohydrojasmonate and ABA, respectively, are marketed for commercial use in apples.

The use of phosphorus-containing compounds has been documented to increase anthocyanin concentration and enhance fruit color (Gómez-Cordovés et al., 1996; Larrigaudiere et al., 1996; Li et al., 2002; Bizjak et al. 2013). Phostrade Ca (Pho Ca), a liquid fertilizer with a high concentration of phosphorus, along with small amounts of calcium and nitrogen, is recommended for foliar application during fruit formation and maturation stages. Similarly, Seniphos, another phosphorus-containing compound, was found to stimulate anthocyanin accumulation in apples without inducing ethylene production or premature ripening (Larrigaudiere et al., 1996). Furthermore, it does not affect storage life, as observed in the case of ethephon (Li et al., 2002). Li et al. (2002) proposed that the observed enhancement in anthocyanin formation and red coloration of apple skin is due to increased activity of PAL and CHI enzymes. Larrigaudiere et al. (1996) also reported a rapid increase in PAL activity that directly correlated with anthocyanin biosynthesis. Nonetheless, the exact mechanism by which P-containing compounds influence color development is not yet fully understood and warrants further investigation. Phostrade Ca and Seniphos are labeled for use in apples and are usually sprayed within five weeks of commercial harvest to enhance color formation.

B. Objectives:

1. Explore the possibility of improving apple fruit coloration through pre-harvest treatments with phosphorus-containing compounds.
2. Assess the impact of plant growth regulators on enhancing red color development while reducing pre-harvest drop in 'Honeycrisp' and 'Gala' apple varieties.

C. Materials and Methods:

A field trial was conducted at the ASH Jr. AREC in Winchester to assess the effectiveness of eight plant growth regulators (PGRs) alone and in combination with ReTain in enhancing fruit color and reducing pre-harvest fruit drop in 'Honeycrisp' and 'Gala' apple varieties. The trial comprised 18 treatments, including an untreated control where ReTain was sprayed once (3 weeks before harvest date; WBHD), while all other PGRs were sprayed three times (3WBHD, 2WBHD, 1WBHD) (Table 1). Trees were randomly assigned to each treatment, with six replicates for 'Honeycrisp' (three for fruit drop and fruit quality analysis, three for molecular and biochemical analysis) and three replicates for 'Gala' apple varieties. The spray volume for each treatment was adjusted based on 100 gal/acre, and Regulaid at 2 pt/100 gal was mixed with all treatments. It's important to mention that in the case of 'Gala' apples, PGRs were applied individually due to constraints such as limited block size and tree availability, and were not combined with ReTain.

To analyze pre-harvest fruit drop, an initial count of all fruit from the trees/branches was conducted at 4WBHD and continued until 2 weeks after the harvest date (WAHD) for both apple cultivars (Table 2a, b). Peel samples were collected from Honeycrisp apples 24 hours after each spray for molecular (gene expression) and biochemical (anthocyanin and other metabolites) analyses (see Table 2a). At harvest and 2WAHD, fruits were collected from both 'Honeycrisp' and 'Gala' varieties for quality assessment (Table 2a, b). Recorded fruit quality attributes included firmness, weight, diameter, brix, starch content, and changes in chlorophyll (IAD), with data collected from five fruits per replicate.

Table 1. List of Plant Growth Regulators (PGRs) and Their Active Ingredients in the Study

Trt #	Treatments	Active ingredients
1	Accede	1-Aminocyclopropanecarboxylic acid (ACC)
2	ProTone SG	S-Abscisic Acid (ABA)
3	Blush	Prohydrojasmon (PDJ)
4	Motivate	Ethephon (ETH)
5	Seniphos	P2O5-N-Ca
6	Actigard 50WG	Acibenzolar-S-methyl (ASM)
7	Refine	1-Naphthaleneacetic Acid (NAA)
8	OxiDate 2	Hydrogen Peroxide, Peroxyacetic Acid
9	ReTain	Aminoethoxyvinylglycine (AVG)
10	ReTain + Accede	
11	ReTain + ProTone	
12	ReTain + Blush	
13	ReTain + Motivate	
14	ReTain + Seniphos	
15	ReTain + Actigard	
16	ReTain + Refine	
17	ReTain + Oxidate	
18	Control	

Table 2a. Timeline of Data Collection Activities for 'Honeycrisp' Apples

Activities	4WBHD	3WBHD	2WBHD	1WBHD	Harvest	1WAHD	2WAHD
Counting							
Time of PGR Application							
Sampling for Mol/Bio							
Anthocyanin Quantification							
Sampling for Fruit Quality							
Ethylene Quantification							

Table 2b. Timeline of Data Collection Activities for 'Gala' Apples

Activities	4WBHD	3WBHD	2WBHD	1WBHD	Harvest	1WAHD	2WAHD
Counting							
Time of Application							
Sampling for Fruit Quality							

Results:

Impact of PGR Combinations on Enhancing Fruit Color in 'Honeycrisp' and 'Gala' Apples

At harvest and 2 weeks after harvest date (2WAHD), the application of ReTain+Accede significantly increased fruit color in 'Honeycrisp' apples, as evidenced by lower DA meter (IAD) readings (0.33 at harvest and 0.14 at 2WAHD), indicating a higher breakdown of chlorophyll (Table 3a). In contrast, ReTain and control treatments exhibited significantly higher DA meter readings (0.77 and 0.86, respectively) at harvest, indicating less breakdown of chlorophyll, resulting in a more greenish color and less red coloration. However, at 2WAHD, while ReTain continued to show a higher DA meter reading (0.66), the control treatment showed a lower DA meter reading (0.26), similar to the ReTain+Accede combination. Among other treatments, there were no significant differences in color formation, and their DA meter readings were not significant, except for ReTain+Oxidate (at harvest) and ReTain+Seniphos (at 2WAHD), both showing a greener hue compared to the control.

For Gala apples, at harvest, Motivate and Accede significantly increased fruit color, as indicated by lower DA meter readings (0.15 and 0.18, respectively), suggesting a greater breakdown of chlorophyll. At 2WAHD, only Accede (0.01) maintained this effect (Table 3b). Changes in fruit color were also visually observed at both harvest and 2WAHD.

Table 3a. Chlorophyll Breakdown in 'Honeycrisp' Apples: DA Meter Readings (IAD) Across Different Treatment Combinations

Treatment name	DA meter (I_{AD}) at harvest	DA meter (I_{AD}) at 2WAHD
Accede	0.46 d	0.29 efg
Accede+ReTain	0.33 d	0.14 g
Actigard	0.85 abc	0.46 cde
Actigard+ReTain	0.83 abc	0.53 bc
Blush	0.84 abc	0.32 def
Blush+ReTain	0.91 ab	0.58 abc
Motivate	0.44 d	0.23 fg
Motivate+ReTain	0.69 c	0.44 cde
Oxidate	0.87 ab	0.35 def
Oxidate+ReTain	0.95 a	0.57 abc

Protone	0.70 c	0.48 cd
Protone+ReTain	0.78 bc	0.45 cde
Refine	0.86 ab	0.52 bc
Refine+ReTain	0.93 ab	0.56 bc
ReTain	0.77 bc	0.66 ab
Seniphos	0.87 ab	0.48 cd
Seniphos+ReTain	0.88 ab	0.74 a
Control	0.86 ab	0.26 fg

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

Table 3a. Chlorophyll Breakdown in 'Gala' Apples: DA Meter Readings (IAD) Across Different Treatment Combinations

Treatment name	DA meter (I_{AD}) at harvest	DA meter (I_{AD}) at 2WAHD
Accede	0.18 d	0.01 b
Actigard	0.42 abc	0.09 a
Blush	0.47 ab	0.09 a
Motivate	0.15 d	0.04 ab
Oxidate	0.30 cd	0.06 ab
Protone	0.38 bc	0.07 ab
Refine	0.37 bc	0.04 ab
Seniphos	0.47 ab	0.06 ab
Control	0.58 a	0.08 a

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

Analysis of Fruit Drop at Harvest and Two Weeks After Harvest Date

At harvest, the lowest fruit drop (%) was observed in the Refine treatment (16.43%), while the highest fruit drop occurred in Oxidate and Motivate treatments (37.13% and 35.93%, respectively) for 'Honeycrisp' apples (Table 4a). Notably, there was a significant reduction in fruit drop percentage in the Accede+ReTain treatment (35.72%) compared to the control. Two weeks after the harvest date, Refine again showed the lowest fruit drop (26.97%), while Motivate and the control exhibited the highest fruit drop percentages (54.03% and 54.00%, respectively). There was a noticeable reduction in fruit drop percentage in the Accede+ReTain treatment (46.30%) compared to the control, consistent with its effect on enhancing fruit color. None of the other treatments for 'Honeycrisp' apples showed a significant difference in fruit drop compared to the control at both harvest and 2 weeks after harvest.

For 'Gala' apples, both at harvest and 2 weeks after harvest, Refine exhibited significantly lower fruit drop percentages (18.11% and 37.01%, respectively) compared to the control (Table 4b). Refine also showed the highest percentage drop reduction over control, both at harvest and 2 weeks after harvest (38.81% and 27.20%, respectively).

Table 4a. Percentage of Fruit Drop and Reduction in Fruit Drop Compared to Control for 'Honeycrisp' Apples at Harvest and Two Weeks Post-Harvest

Treatment name	At harvest		Two weeks after harvest date	
	%Fruit drop	%Fruit drop reduction over control	%Fruit drop	%Fruit drop reduction over control
Accede	20.80 ab	31.20	41.80 abcd	22.59
Accede+ReTain	19.43 ab	35.72	29.00 cd	46.30
Actigard	23.23 ab	23.15	51.27 ab	5.06
Actigard+ReTain	27.97 ab	7.50	43.07 abcd	20.25
Blush	22.33 ab	26.13	35.47 bcd	34.32
Blush+ReTain	30.03 ab	0.66	43.83 abcd	18.83
Motivate	35.93 a	-18.85	54.03 a	-0.06
Motivate+ReTain	20.63 ab	31.75	42.80 abcd	20.74
Oxidate	37.13 a	-22.82	50.00 ab	7.41
Oxidate+ReTain	26.07 ab	13.78	39.20 abcd	27.41
Protone	22.30 ab	26.24	36.53 abcd	32.35
Protone+ReTain	28.33 ab	6.28	48.77 ab	9.69
Refine	16.43 b	45.64	26.97 d	50.06
Refine+ReTain	33.03 ab	-9.26	45.40 abc	15.93
Retain	19.50 ab	35.50	28.37 cd	47.47
Seniphos	23.73 ab	21.50	41.53 abcd	23.09
Seniphos+ReTain	18.43 ab	39.03	39.47 abcd	26.91
Control	30.23 ab	-	54.00 a	-

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

Table 4b. Percentage of Fruit Drop and Reduction in Fruit Drop Compared to Control for 'Gala' Apples at Harvest and Two Weeks Post-Harvest

Treatment name	At harvest		Two weeks after harvest date	
	%Fruit drop	%Fruit drop reduction over control	%Fruit drop	%Fruit drop reduction over control
Accede	30.20 a	-2.06	60.43 a	-18.87
Actigard	28.58 ab	3.41	57.21 ab	-12.54
Blush	22.21 ab	24.95	47.35 d	6.85
Motivate	30.68 a	-3.69	55.43 abc	-9.03
Oxidate	23.76 ab	19.71	47.70 cd	6.16
Protone	25.45 ab	14.00	46.33 d	8.87
Refine	18.11 b	38.81	37.01 e	27.20
Seniphos	25.78 ab	12.87	47.93 cd	5.72
Control	29.59 a	-	50.83 bcd	-

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

Assessment of Fruit Quality for Different Treatments

At harvest, for firmness Protone+ReTain, Oxidate+ReTain and Refine+ReTain had significantly higher values (17.39 lbf, 17.36 lbf and 17.34 lbf, respectively) whereas at 2WAHD Seniphos+ReTain was the highest player (16.91 lbf) (Table 5a,b). The diameter was statistically higher in Oxidate (73.30 mm) and Actigard (73.28 mm) at harvest and in ReTain (76.08 mm) at 2WAHD. The fruit weight was also significantly varied among treatments showing higher weight in Accede (152.37 g) and Oxidate (152.07 g) at harvest and in ReTain (181.20 g) at 2WAHD. Brix values were significantly different among treatments showing highest value for Seniphos+ReTain (12.87) at harvest and for Blush (12.63) at 2WAHD. Strach grading values also showed significant differences among treatment showing higher values for Motivate (6.00), Accede+ReTain (6.00) and Motivate+ReTain (6.00) at harvest and for Accede (6.00), Motivate (6.00) and Accede+ReTain (6.00) at 2WAHD.

For Gala apples, both at harvest and 2WAHD, there was significantly lower firmness in Accede (14.85 lbf and 11.00 lbf, respectively) which showed promising fruit color earlier (Table 5c,d). The diameter was statistically significant at harvest showing the highest in Protone (71.93 mm), however, 2WAHD there was no significant difference in diameter compared to control. The fruit weight was significantly lower in Motivate (125.53 g) at harvest, no significant difference was observed compared to control at 2WAHD. At harvest, Accede showed higher Brix (13.20) which is not statistically significant compared to control (12.69) and similar scenario was observed for Seniphos (13.57) which was statistically identical to control (13.31). At harvest, comparatively higher Starch value was for Motivate (7.87) and at 2WAHD, statistically significant higher value of Starch was observed in Refine (7.93), Accede (7.83), Motivate (7.77), Oxidate (7.77) and Blush (7.73) compared to control (7.33).

Assessment of Fruit Quality for Different Treatments

At harvest, Protone+ReTain, Oxidate+ReTain, and Refine+ReTain exhibited significantly higher firmness values (17.39 lbf, 17.36 lbf, and 17.34 lbf, respectively), while at 2 weeks after harvest date (2WAHD), Seniphos+ReTain showed the highest firmness (16.91 lbf) (Table 5a, b). Diameter measurements were statistically higher in Oxidate (73.30 mm) and Actigard (73.28 mm) at harvest, and in ReTain (76.08 mm) at 2WAHD. Fruit weight also significantly varied among treatments, with higher weights observed in Accede (152.37 g) and Oxidate (152.07 g) at harvest, and in ReTain (181.20 g) at 2WAHD. Brix values differed significantly among treatments, with the highest value observed for Seniphos+ReTain (12.87) at harvest and for Blush (12.63) at 2WAHD. Starch grading values also showed significant differences among treatments, with higher values for Motivate (6.00), Accede+ReTain (6.00), and Motivate+ReTain (6.00) at harvest, and for Accede (6.00), Motivate (6.00), and Accede+ReTain (6.00) at 2WAHD.

For Gala apples, both at harvest and 2WAHD, significantly lower firmness was observed in Accede (14.85 lbf and 11.00 lbf, respectively), which exhibited promising fruit color earlier (Table 5c, d).

Diameter measurements were statistically significant at harvest, with the highest observed in Protone (71.93 mm). However, at 2WAHD, there was no significant difference in diameter compared to the control. Fruit weight was significantly lower in Motivate (125.53 g) at harvest, with no significant difference observed compared to the control at 2WAHD. At harvest, Accede showed higher Brix (13.20), which was not statistically significant compared to the control (12.69), similar to Seniphos (13.57), which was statistically identical to the control (13.31). Comparatively higher starch values were observed for Motivate (7.87) at harvest, and at 2WAHD, statistically significant higher values were observed in Refine (7.93), Accede (7.83), Motivate (7.77), Oxidate (7.77), and Blush (7.73) compared to the control (7.33).

Table 5a. Comparative Analysis of Fruit Quality Parameters in 'Honeycrisp' Apples Under Different Treatments at Harvest

Treatment name	Firmness (lbf)	Diameter (mm)	Weight (g)	Brix	Starch
Accede	17.15 ab	72.90 abc	152.37 a	11.65 cdef	5.80 ab
Accede+ReTain	17.00 ab	69.53 defg	131.33 cdefg	12.78 ab	6.00 a
Actigard	15.96 cd	73.28 a	142.77 abcd	11.68 cdef	5.30 bcde
Actigard+ReTain	16.37 bcd	70.50 bcdef	139.77 abcde	12.30 abcd	4.80 efg
Blush	16.85 abc	71.83 abcd	145.00 abc	11.40 def	5.50 abcd
Blush+ReTain	17.10 ab	68.75 efg	124.43 efg	11.88 bcde	5.23 bcde
Control	16.43 bcd	69.35 defg	134.43 bcdef	10.91 efg	4.43 g
Motivate	17.14 ab	71.13 abcde	140.33 abcde	10.77 fg	6.00 a
Motivate+ReTain	17.21 ab	67.48 g	121.53 fg	11.80 bcde	6.00 a
Oxidate	16.62 abc	73.30 a	152.07 a	11.74 cdef	4.60 fg
Oxidate+ReTain	17.36 a	68.70 efg	124.47 efg	12.25 abcd	4.63 fg
Protone	17.02 ab	69.47 defg	134.07 bcdef	10.28 g	5.37 bcde
Protone+ReTain	17.39 a	67.25 g	117.53 g	11.42 def	5.30 bcde
Refine	16.32 bcd	70.40 cdef	132.10 bcdefg	11.79 bcde	5.60 abc
Refine+ReTain	17.34 a	67.85 fg	121.27 fg	12.41 abc	4.93 defg
ReTain	16.87 ab	69.07 efg	119.30 fg	12.35 abcd	5.50 abcd
Seniphos	15.57 d	73.18 ab	148.07 ab	11.03 efg	5.57 abc
Seniphos+ReTain	17.11 ab	68.65 efg	127.30 defg	12.87 a	5.10 cdef

Table 5b. Comparative Analysis of Fruit Quality Parameters in 'Honeycrisp' Apples Under Different Treatments at Harvest at 2 WAHD

Treatment name	Firmness (lbf)	Diameter (mm)	Weight (g)	Brix	Starch
Accede	14.95 g	74.27 ab	160.83 ab	11.62 bcde	6.00 a
Accede+ReTain	15.05 fg	69.60 defg	132.87 cd	11.53 de	6.00 a
Actigard	15.98 abcdef	68.90 efg	127.03 d	11.60 cde	5.67 abc
Actigard+ReTain	15.65 cdefg	72.92 abcd	152.77 bc	11.70 bcde	5.40 bcd
Blush	16.27 abcd	70.63 cdef	139.53 cd	12.63 a	5.70 abc

Blush+ReTain	16.77 ab	67.20 g	122.10 d	11.42 de	5.53 bc
Control	16.05 abcde	69.13 efg	132.70 cd	11.45 de	5.83 ab
Motivate	15.47 defg	73.53 abc	162.40 ab	11.96 abcde	6.00 a
Motivate+ReTain	15.94 bcdef	69.08 efg	134.93 cd	11.19 e	5.83 ab
Oxidate	16.46 abc	72.70 bcd	150.67 bc	12.50 abc	5.67 abc
Oxidate+ReTain	16.31 abcd	72.10 bcde	150.67 bc	12.53 ab	5.47 bcd
Protone	15.96 bcdef	70.98 bcdef	142.20 bcd	11.62 bcde	5.67 abc
Protone+ReTain	16.14 abcde	68.20 fg	134.37 cd	11.60 cde	5.67 abc
Refine	16.41 abc	70.20 cdefg	132.37 cd	12.29 abcd	5.63 abc
Refine+ReTain	16.46 abc	70.13 defg	138.47 cd	11.09 e	5.60 abc
ReTain	15.32 efg	76.08 a	181.20 a	12.23 abcd	5.03 d
Seniphos	15.62 cdefg	71.57 bcde	142.00 bcd	11.83 abcde	5.77 abc
Seniphos+ReTain	16.91 a	69.63 defg	136.23 cd	11.89 abcde	5.33 cd

Table 5c. Comparative Analysis of Fruit Quality Parameters in 'Gala' Apples Under Different Treatments at Harvest

Treatment name	**Firmness (lbf)**	**Diameter (mm)**	**Weight (g)**	**Brix**	**Starch**
Accede	14.85 d	69.38 bc	143.50 ab	13.20 a	7.30 ab
Actigard	17.30 ab	68.97 bc	142.53 ab	11.48 c	6.53 de
Blush	16.55 abc	69.08 bc	141.67 ab	12.62 a	6.70 cde
Control	17.96 a	69.28 bc	145.73 ab	12.69 a	6.23 e
Motivate	15.73 cd	66.03 d	125.53 c	12.68 a	7.87 a
Oxidate	16.11 bcd	70.45 ab	149.17 a	12.64 a	7.20 bc
Protone	15.92 bcd	71.93 a	154.83 a	12.53 a	7.03 bcd
Refine	16.24 bcd	67.77 cd	131.37 bc	12.40 ab	7.17 bc
Seniphos	17.79 a	69.48 abc	149.33 a	11.57 bc	6.97 bcd

Table 5d. Comparative Analysis of Fruit Quality Parameters in 'Gala' Apples Under Different Treatments at Harvest at 2 WAHD

Treatment name	**Firmness (lbf)**	**Diameter (mm)**	**Weight (g)**	**Brix**	**Starch**
Accede	11.00 c	71.93 a	165.17 a	12.99 a	7.83 a
Actigard	13.87 a	67.83 b	138.27 b	13.02 a	7.67 ab
Blush	12.86 ab	68.57 ab	139.73 b	12.78 a	7.73 a
Control	13.69 a	68.98 ab	145.60 ab	13.31 a	7.33 b
Motivate	14.37 a	67.17 b	136.60 b	12.70 a	7.77 a
Oxidate	12.62 abc	69.52 ab	143.07 ab	12.89 a	7.77 a
Protone	12.95 ab	68.45 ab	139.40 b	12.89 a	7.70 ab
Refine	11.60 bc	66.50 b	122.80 b	11.69 b	7.93 a
Seniphos	13.88 a	67.43 b	132.90 b	13.57 a	7.60 ab

Effect of Selected Treatments on Levels of Anthocyanin and Ethylene Content at Different Time Points

The skin red coloration for apples is primarily attributed to anthocyanins. There was no significant difference in anthocyanin content at three and two weeks before harvest (WBHD). However, at one week before harvest, anthocyanin content was significantly higher in Accede (296.87 ng/L), Motivate (259.76 ng/L), and Accede+ReTain (231.93 ng/L) compared to the control (41.75 ng/L) (Figure 1). ReTain showed a significantly lower level of anthocyanin, similar to the control at 1WBHD.

At one week after the harvest date (1WAHD), significantly lower levels of ethylene content were found in Accede (96.11 ppm) and Accede+ReTain (92.14 ppm) compared to the control (113.62 ppm) (Figure 2).

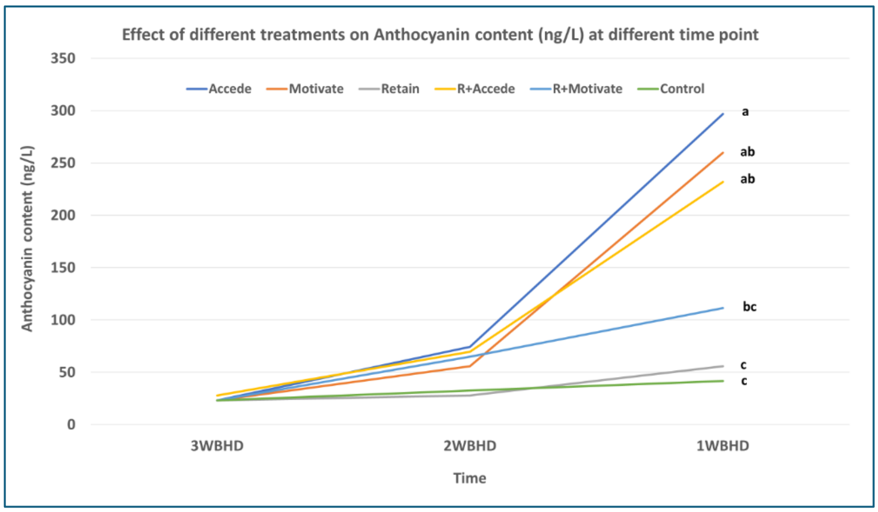

Figure 1. Comparative analysis of Anthocyanin level of six selected treatments at three different time points

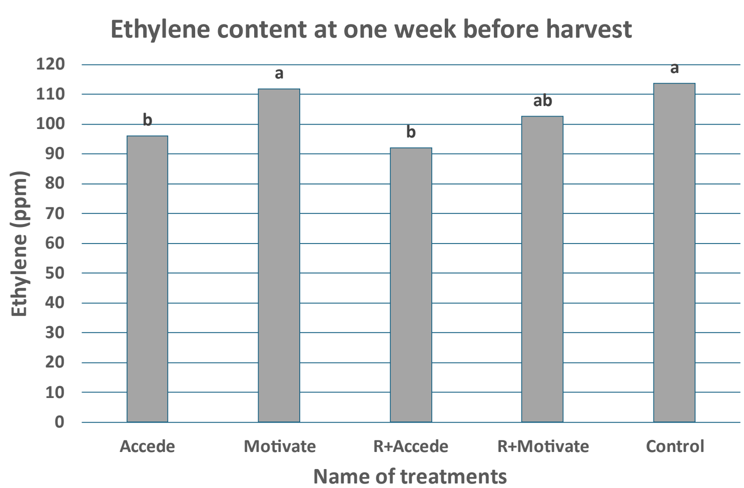

Figure 2. Changes on Ethylene content at one week after harvest for selected treatments

Conclusion

The study comprehensively examined the effects of various plant growth regulator (PGR) combinations on enhancing fruit color, reducing fruit drop, and assessing fruit quality in ‘Honeycrisp’ and ‘Gala’ apples. Key findings include the significant increase in fruit color observed with ReTain+Accede combinations in ‘Honeycrisp’, as well as Motivate and Accede in ‘Gala’ apples. Accede+ReTain notably reduced fruit drop at harvest and two weeks later, indicating improved fruit retention. Additionally, certain treatments demonstrated higher firmness values, while variations in fruit weight, diameter, Brix values, and starch grading were observed. Biochemical analyses revealed higher anthocyanin content with Accede, Motivate, and Accede+ReTain treatments, suggesting enhanced red coloration. Lower ethylene levels in Accede and Accede+ReTain treatments indicated a potential delay in ripening. Overall, the study highlights the potential of specific PGR combinations in enhancing fruit color, reducing fruit drop, and improving fruit quality attributes in both apple varieties. However, further research is needed to optimize application strategies for maximum effectiveness.

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