ABSTRACT

Title of Thesis:	MAKING APPLES BLUSH: UNDERSTANDING HOW THE COMBINED USE OF REFLECTIVE GROUNDCOVERS AND PLANT GROWTH REGULATORS IMPACT RED SKIN COLORATION AND QUALITY OF ‘HONEYCRISP’ APPLES IN THE MID-ATLANTIC US
	Md Shipon Miah, Master of Science, 2024
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Apples are among the most valuable fruits globally, with 'Honeycrisp' ranking as the top sales-producing cultivar in the US. However, challenges such as insufficient red skin coloration and increased preharvest fruit drop significantly diminish their market value. Reflective groundcovers have been reported to enhance apple skin coloration, while the application of the plant growth regulator AVG reduces fruit drop but may negatively impact skin coloration. Research on the impacts of these practices in mid-Atlantic US-grown apples remains limited. In this two years study, our aims were 1) to evaluate the effect of reflective groundcovers on solar radiation (PPFD, UV) distribution; 2) to assess the combined effect of reflective groundcovers and ethylene inhibitor (AVG) on preharvest fruit drop, ethylene production, red blush percentage, and overall fruit quality; 3) to investigate the combined effect of reflective groundcovers and ethylene inhibitor (AVG) on expression level of key anthocyanin and ethylene biosynthesis related genes; 4) to determine the combined effect of reflective groundcovers and ethylene inhibitors (AVG) in the accumulation of total anthocyanin. Apples underwent four treatment combinations of reflective groundcover (Extenday) and AVG (130 mg L^{-1}). Our findings revealed that Extenday significantly enhanced skin coloration (>75% blush) through increased reflectance of PPFD and UV radiation, along with increased IEC, while also accelerating fruit maturity, i.e., overripening. In fact, Extenday-only treated fruit exhibited the highest upregulation of ethylene and anthocyanin biosynthetic-related genes, as well as total anthocyanins. Conversely, AVG notably reduced fruit drop and decreased IEC, delaying fruit maturity while significantly diminishing red coloration (30–48% blush). AVG treated fruit significantly suppressed the expression of key ethylene and anthocyanin biosynthetic structural and regulatory genes, as well as total anthocyanins. The combined application of Extenday and AVG synergistically decreased fruit drop while enhancing skin coloration (>50% blush), but without inducing overripening. This combination fine-tuned the transcript accumulation of ethylene and anthocyanin biosynthetic-related genes, as well as total anthocyanins, enabling 'Honeycrisp' fruit to exceed 50% blush, while moderately increasing IEC (compared to Extenday-only and control fruit), thus enhancing fruit economic value. Therefore, combining Extenday and AVG can boost the market value for 'Honeycrisp' apples in the mid-Atlantic US.

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by

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Dedication

To my life partner: Thank you for being my rock, even when you were miles away.

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Introduction

The USA is the second largest producer of apples (*Malus* spp.), with a production value of $3,033 million USD (USApple, 2022; USDA, 2022). In commercial year 2021-2022, the total production of apples was 4.92 million metric tons, where the top cultivars were *Malus domestica* 'Gala', 'Red Delicious', 'Honeycrisp', 'Fuji', and 'Granny Smith' with a production share of 18.6%, 13.4%, 11.7%, 11% and 10.3%, respectively (USApple, 2022; USDA, 2022). Eight of the top ten cultivars in the USA are red cultivars, and of these, the production of 'Honeycrisp' has increased the most (136%) in the last five years (USApple, 2022). Regarding red skin color percentage, the USDA standard for red cultivars, such as 'Honeycrisp', requires having at least 50% blush for marketing purposes from 'U.S. Extra Fancy' grade fruit (USDA, 2019). However, the production trend and ripening pattern of 'Honeycrisp' apples are being hampered in the mid-Atlantic region due to insufficient red skin color and preharvest fruit drop. The main factors for insufficient red skin coloration are mainly environmental factors, specifically, hot, and humid weather with uneven light distribution alongside a lack of differential temperature between day and night (Biggs & Peck, 2015; Miller & Greene, 2003; Mogil & Seaman, 2009; Musacchi & Serra, 2018).

Fruit ripening is a complex physiological and biochemical process, which contributes to changes in the fruit's appearance, firmness, and flavor, ultimately making the fruit attractive and palatable for consumers (Bouzayen et al., 2010; Farcuh et al., 2020; Farcuh & Hopfer, 2023; Osorio & Fernie, 2013). 'Honeycrisp' differentiates price range by ~$0.92 USD per pound, which is more than any other apple cultivars (USApple, 2022), making it one of the most profitable cultivars in the USA. Nevertheless, these profitability margins are only possible if the required skin blush percentage is achieved. Therefore, to produce ‘Honeycrisp’ apples that classify for the ‘U.S. Extra Fancy’ grade, favorable temperature, and good distribution of light or solar radiation to the surface of the fruit are required (Iglesias & Alegre, 2009; USDA, 2019). Furthermore, tree architecture alters light distribution, as fruit in the upper canopy receive a greater amount of light than fruits in the lower canopy, which can alter rates of fruit red skin coloration (W. Yang et al., 2021). In response, apple growers in the mid-Atlantic region delay harvesting fruits to expand radiation duration to the surface of the fruit and thus increase red skin blush percentage (Watkins et al., 2005). As a result of prolonged time of fruit hanging on the tree, the fruit becomes overripe and loses firmness, negatively impacting overall fruit quality and storability, as over-mature apple fruits soften faster, abscise from the tree before harvest, and have a shorter shelf-life. These fruits are not suitable for long-term storage and do not categorize as ‘U.S. Extra Fancy’ grade (Drake & Eisele, 1994; USDA, 2019). Previous research showed that delaying harvest also increases fruit drop and, depending on the types of cultivars the drop percentage can vary from less than 20% for late cultivars to more than 50% for early cultivars (Arseneault & Cline, 2016; Harb et al., 2012). Therefore, there is a critical need to develop new practices to improve red skin coloration without preharvest fruit drop of ‘Honeycrisp’ fruits grown in the mid-Atlantic, which will increase the production efficiency, profitability, and sustainability of the industry.

Development of apple red skin coloration

In apples, the appearance of the fruit is primarily determined by the skin color, which is a result of a combination of the underlying background color and the surface color (Bhatt & Pant, 2015; Gao et al., 2021; Toivonen et al., 2019). The skin color is controlled by a complex interplay of photosynthetic and non-photosynthetic pigments, including anthocyanins (Gao et al., 2021; Toivonen et al., 2019). Anthocyanins, which are a group of phenolic compounds that can be biosynthesized through the phenylpropanoid pathway (Figure 1.1), play a key role in apple surface coloration. Anthocyanins biosynthesis is developmentally regulated and occurs at fruitlet state and during fruit ripening. In red cultivars, anthocyanins biosynthesis plays the most important role during the ripening stage (Ubi et al., 2006). A suite of structural and regulatory enzymes and genes are involved in anthocyanin production, including phenylalanine ammonia-lyase (*PAL*), chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*F3H*), dihydroflavonol 4-reductase (*DFR*), leucoanthocyanidin dioxygenase (*LDOX*), and glycosyltransferase (*UFGT*) (Feng et al., 2013; Gao et al., 2021; Mao et al., 2021). The phenylpropanoid pathway followed by flavonoid pathway produces anthocyanins as a final product and starts with phenylalanine. *PAL* produces cinnamic acid then it follows by a series of enzymatic reactions to produce chalcones and starts the flavonoid pathway (Figure 1.1). Then *CHI* converts chalcones to flavanones which are then converted to dihydroflavonols by *F3H*. Dihydroflavonols are then converted to leucoanthocyanidins by *DFR*. Leucoanthocyanidins are then converted to anthocyanidins by *LDOX*. Finally, anthocyanins are being produced from anthocyanidins by *UFGT*. For cultivars like 'Honeycrisp', anthocyanins, specifically cyanidin-3-galactoside, play a key role in determining the red skin coloration of the fruit (Feng et al., 2013; Gao et al., 2021; Ubi et al., 2006). The expression of these genes is also regulated by a transcription factor (TFs) complex i.e., basic helix-loop-helix *(bHLH3)*, myeloblastosis *(MYB10)* with WD40 repeat protein and known as MYB-bHLH-WDR or MBW complex (Farcuh et al., 2022; Zhao et al., 2023). Previous studies related with the anthocyanin pathway revealed that the interaction among TFs of the MBW can be induced by environmental and hormonal factors, and thus the activation of downstream genes (i.e., *DFR, UFGT)* can be maintained to upregulate the biosynthesis of anthocyanin (J.-P. An et al., 2020; X. H. An et al., 2012; Gao et al., 2021; Hu et al., 2019; Zimmermann et al., 2004). Therefore, this research will focus on the anthocyanin (phenylpropanoid) pathway and how genes related to this pathway are affected by preharvest management practices aiming to increasing 'Honeycrisp' red skin coloration.

Environmental factors affecting apple red skin coloration

Anthocyanins accumulation is affected by environmental factors such as temperature, visible light, and ultraviolet radiation (Hengari et al., 2014; Iglesias & Alegre, 2009; W. Liu et al., 2019; Shü et al., 2001). The ideal temperature ranges for red color development in apples are below 15 °C at night and below 25°C during the day, especially four weeks before harvest (Iglesias & Alegre, 2009). Whereas solar radiation is another crucial driving force for red skin color development in apple, and it is composed of radiation with different wavelengths, where plants can use the photosynthetic photon flux density (PPFD) lies in between 400 – 700 nm (Nangle et al., 2016) and ultraviolet (UV) radiation lies in between 280 – 400 nm (Toivonen et al., 2019).

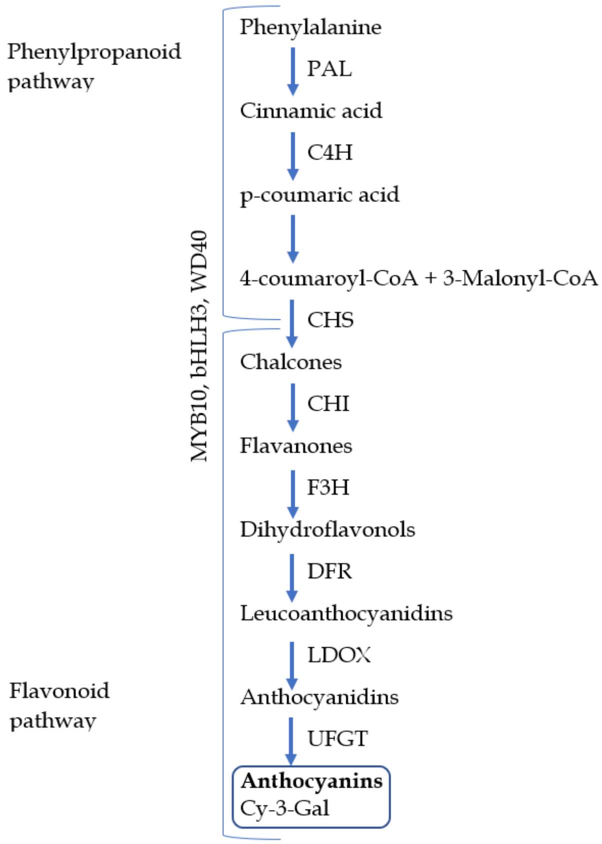

Figure 1.1. Schematic representation of phenylpropanoid and flavonoid biosynthesis pathways adapted from Farcuh et al. (2022). Phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX), UDP glucose-flavonoid 3-O-glucosyltransferase (UFGT). Transcription factors (MYB10, bHLH3, WD40).

When solar radiation reaches the tree canopy some parts of it are intercepted and absorbed by the leaf of the trees while some other parts of it are reflected and diffused in different ways. Both the intensity and wavelength of these radiations and their interception and reflectance significantly affect red skin coloration of apple fruits by promoting the biosynthesis of anthocyanins, specifically cyanidin-3-galactoside (Bassett & Glenn, 2014; Feng et al., 2013; Jaakola, 2013; Musacchi &

Serra, 2018). Moreover, previous studies have shown that several genes in anthocyanin pathway are influenced by the UV radiation (Guo et al., 2010; Park et al., 2007; Toivonen et al., 2019). Therefore, this research project will focus on the distribution and quality concerning the interception and reflection of PPFD and UV radiation on the canopy of the tree, to better understand how PPFD and UV radiation affect anthocyanin biosynthesis.

Hormonal factors affecting apple red skin coloration and fruit drop

In apples, the biosynthesis of anthocyanins is also regulated by hormones such as ethylene, jasmonates, abscisic acid and auxins (Gao et al., 2021; Honda et al., 2014; Whale & Singh, 2007; Zhang et al., 2018; Zhao et al., 2023). Particularly, ethylene which is a plant hormone that plays key roles in regulating different physiological processes in plants including fruit maturation and ripening (Farcuh et al., 2019; Grierson, 2013; Hyun & Kieber, 2005; Qi et al., 2020) has been reported to induce the expression of several genes of the anthocyanin pathway during ripening, thus enhance red skin coloration and fruit drop in several fruits, including apple (Cheng et al., 2016; Farcuh et al., 2022; Zhang et al., 2018). Biosynthesis of ethylene (Figure 1.2) starts with the conversion of methionine to Ado-Met (S-adenosyl-L-methionine) then 1-aminocyclopropane-1-carboxylate (ACC) and finally ethylene (Figure 1.2). Where, ACC synthase *(ACS)* enzyme converts Ado-Met and ACC oxidase *(ACO)* converts ACC to ethylene (Farcuh et al., 2019; Sun et al., 2017). Then ethylene undergoes perception and the final signaling phase. During perception, synthesized ethylene is perceived by the plant cells through specific receptors (Dias et al., 2021; Schaller & Binder, 2017). These receptors are typically membrane-bound proteins that can bind to ethylene molecules, triggering a signaling cascade within the cell. Once ethylene is perceived by the receptors, it initiates a signaling cascade within the cell, leading to various physiological responses such as fruit ripening, flower senescence, or stress responses. This signaling pathway often involves the activation of gene expression and the regulation of various cellular processes. Previous research findings support the idea that both genes (*ACS* and *ACO*) are the most crucial elements in the ethylene biosynthesis pathway (Farcuh et al., 2019; Hyun & Kieber, 2005; X. Yang et al., 2013). Considering the reported link between ethylene and anthocyanin biosynthesis, this research will focus on understanding how the expression of ethylene biosynthesis related genes is being affected by different preharvest management technologies.

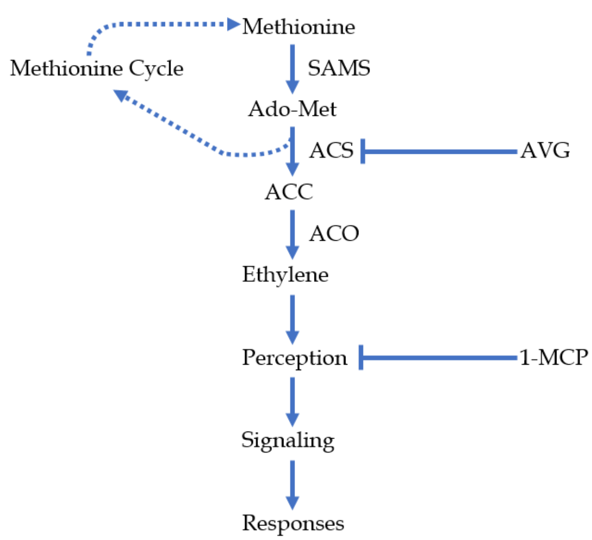

Figure 1.2. Schematic representation of ethylene pathway adapted from Hyun & Kaeber (2005), Schaller & Binder (2017) and Farcuh et al. (2019). S-adenosyl-L-methionine synthase (SMAS), 1-aminocyclopropane-1-carboxylate (ACC) synthase (*ACS*), ACC oxidase (*ACO*).

Available preharvest management technologies for manipulating apple red skin coloration and preharvest fruit drop

Reflective groundcover and plant growth regulators such as ethylene inhibitors are two available technologies for apple production that can influence apple red skin color development and fruit drop percentages, respectively.

Reflective groundcovers are technologies that are designed to cover the ground with the aim of reflecting solar radiation and heat away from the ground, rather than absorbing it. It is typically made from materials with a high reflectivity, such as plastic or metallic sheets, and can be used to help regulate soil temperature and moisture levels. Apart from stress resilience it can also be an important technology to regulate and amplify solar radiation to the fruit's surface, especially UV radiation to the lower canopy (Bassett & Glenn, 2014; Glenn & Puterka, 2007; Jakopič et al., 2010; Miller & Greene, 2003; Mupambi et al., 2021; Toivonen et al., 2019; Ubi et al., 2006). There are several types of reflective groundcovers available to growers, which are manufactured from aluminum or white fabric (Glenn & Puterka, 2007; Mupambi et al., 2021; Overbeck et al., 2013; J.-P. Privé et al., 2011; Weber et al., 2019). The white fabric or woven polythene cover (Extenday™) type can be used for several years (5-7 years), and it can be installed anytime of the year (Hanrahan et al., 2011). Extenday™ is made of woven polyethylene (Extenday™, Aucland, New Zealand), which has a high air and water porosity capacity and can be deployed as a 2 m wide sheet on both sides (left and right) of the orchard's plot (J. P. Privé et al., 2008; Toivonen et al., 2019; Vangdal et al., 2007). It has been reported that this technology has the capability to induce apple red skin coloration significantly by deploying it around 4 weeks before the anticipated commercial harvest (Mupambi et al., 2021; Yildiz et al., 2012). However, research is lacking in understanding how reflective groundcovers affect apple fruit grown in the mid-Atlantic region of the USA.

Additionally, preharvest strategies aimed at manipulating fruit ripening to control fruit drop also exist (Thewes et al., 2021; X. Yang et al., 2013). These strategies involve the use of preharvest plant growth regulators (PGRs) like ethylene inhibitors such as, aminoethoxy-vinyl-glycine or AVG (commercially available as Retain® by Valent Bioscience, IL, USA) and 1-methylcyclopropene or 1-MCP. Both PGRs reduce ethylene biosynthesis, where AVG work in the production level by inhibiting the activity of ACS enzyme in the ethylene pathway, while 1-MCP works in the perception level by interacting with the ethylene receptor, and consequently reducing ethylene biosynthesis (Figure 1.2) (Bisson et al., 2016; Dias et al., 2021; do Amarante et al., 2022; Schaller & Binder, 2017; Sun et al., 2017). For this study, AVG was being used as an ethylene biosynthesis inhibitor PGRs. The application of AVG is recommended to be carried out 4 weeks before the expected commercial harvest (Yildiz et al., 2012). As AVG can delay fruit maturity, apples may remain on the tree for a longer period, extending their exposure to solar radiation, and thus induce red skin coloration (Sun et al., 2017; Yildiz et al., 2012). Although this plant growth regulators are widely used in the mid-Atlantic, to date, no research has examined how the combination of reflective groundcovers (Extenday^{TM}) and application of an ethylene inhibitor (AVG) will affect red skin coloration in 'Honeycrisp' fruit grown under mid-Atlantic conditions. Therefore, this research has been designed to understand the combination of reflective groundcovers (Extenday^{TM}) and ethylene inhibitor (AVG) on 'Honeycrisp' apple fruits grown in the mid-Atlantic US.

We hypothesize that, if the reflective groundcovers reflect the solar radiation back to the canopy of the tree and the application of ethylene inhibitors (AVG) extend the time fruit can hang on the tree by blocking ethylene and delaying ripening, then distribution of solar radiation (PPFD, UV) will be intensified at the lower third canopy level, fruit drop percentage will be decreased, internal ethylene concentration will be minimized, anthocyanin biosynthesis will be increased in the fruit skin, expression of the genes related to anthocyanin and ethylene pathway will be increased and decreased, respectively, and overall fruit quality will be maintained throughout the 'Honeycrisp' ripening period.

Consequently, the combination of these two horticultural management strategies will increase red skin blush percentage without compromising other quality required to produce 'U.S. Extra Fancy' grade 'Honeycrisp' apple. Based on this understanding this study is conducted targeting the following specific objectives.

Specific Objectives

Objective 1. Evaluate the effect of reflective groundcovers on solar radiation (PPFD, UV) distribution.

- **Hypothesis 1.** *If reflective groundcovers can reflect and distribute solar radiation back to the tree canopy, then solar radiation (PPFD, UV) will be* *amplified to the lower third canopy level of the tree compared to trees without reflective groundcovers.*

Objective 2. Evaluate the combined effect of reflective groundcovers and ethylene inhibitor (AVG) on preharvest fruit drop, ethylene production, red blush percentage, and overall fruit quality.

- **Hypothesis 2.** *If the combined effects of reflective groundcovers and the application of an ethylene inhibitor (AVG) will stimulate the solar radiation at the lower third canopy level and extend the time fruit can hang on the tree by blocking ethylene and delaying ripening, then preharvest fruit drop will be minimized, fruit internal ethylene concentration will be lowered, red skin blush percentage will be enhanced without hampering overall fruit quality throughout the ripening period.*

Objective 3. Evaluate the combined effect of reflective groundcovers and ethylene inhibitor (AVG) on expression level of key anthocyanin and ethylene biosynthesis related genes.

- **Hypothesis 3.** *If the combination of reflective groundcovers and application of ethylene inhibitor will enhance the red skin blush percentage and decrease fruit's internal ethylene concentration throughout the ripening period, then the expression of anthocyanin pathway-related genes and ethylene pathway-related genes will be up regulated and down regulated, respectively.*

Objective 4. Determine the combined effect of reflective groundcovers and ethylene inhibitors (AVG) in the accumulation of total anthocyanin.

- **Hypothesis 4.** *If the interaction of reflective groundcovers and ethylene inhibitor application will enhance apple's red skin blush percentage, then total anthocyanin content will be higher in the skin tissue of the fruit throughout the ripening period of 'Honeycrisp' apple.*

Chapter 1: Combining the Use of Reflective Groundcovers and Aminoethoxyvinylglycine to Assess Effects on Skin Color, Preharvest Drop, and Quality of ‘Honeycrisp’ Apples in the Mid-Atlantic US

Abstract

Apples are one of the most valuable fruits worldwide. ‘Honeycrisp’ is the top sales-producing cultivar in the US. Lack of red skin coloration and increased preharvest fruit drop significantly reduce the market value for cultivars such as ‘Honeycrisp’. The use of reflective groundcovers has been shown to enhance apple skin coloration. While the use of plant growth regulator AVG reduces fruit drop, it negatively affects skin coloration. Studies on the impacts of these practices in mid-Atlantic US-grown apples are limited. In this work, for two years, we compared differences in the light environment, fruit drop, internal ethylene concentration (IEC), physicochemical parameters, and skin coloration of ‘Honeycrisp’ apples in the lower third of the canopy. Apples were submitted to four treatment combinations of reflective groundcover (Extenday) and AVG (130 mg L^{-1}). Assessments occurred throughout three ripening stages. Our results demonstrated that Extenday significantly promoted skin coloration (>75% blush) via the increased reflectance of photosynthetic photon flux density and UV radiation, and increased IEC, while also advancing fruit maturity, i.e., overripening. Conversely, AVG significantly minimized fruit drop and decreased IEC, delaying fruit maturity but drastically reducing red coloration (30–48% blush). The combined use of Extenday and AVG had a synergistic effect by decreasing fruit drop while enhancing fruit with >50%

blush, without promoting overripening. Combining Extenday and AVG can boost the market value for ‘Honeycrisp’ apples in the mid-Atlantic US.

1. Introduction

Apple (*Malus domestica* Borkh.) red skin coloration is one of the primary determinants of consumer preference and market value (Funke & Blanke, 2021; Musacchi & Serra, 2018). Therefore, poor red skin coloration is a key factor that can result in downgrading apple fruit as it is associated with poor visual appearance and thus low consumer acceptance (Layne et al., 2002). Furthermore, in recent years, standards for minimum acceptable red skin coloration have increased from 25% to 50% blush across several commercially important cultivars, such as ‘Honeycrisp’ (Kon & Clavet, 2023; USDA Agricultural Marketing Service, n.d.).

Red skin coloration in apples is mainly determined by the content of anthocyanins (Castañeda-Ovando et al., 2009). Anthocyanin accumulation is strongly affected by environmental factors such as temperature and light (Honda & Moriya, 2018; Lancaster, 1992; Ubi et al., 2006). The ideal conditions for red color development in apples correspond to bright, clear days with temperatures of 25 °C and cool nights (15 °C) during 3–4 weeks preharvest (Chen et al., 2021; Iglesias & Alegre, 2009). Nevertheless, due to the hot and humid environmental conditions of the mid-Atlantic region of the US, high-value cultivars such as ‘Honeycrisp’ often produce marginal red skin coloration, not meeting the market standards. Regarding light intensity and wavelength, ultraviolet radiation, intrinsic to sunlight, has shown effects on inducing fruit anthocyanin biosynthesis, thus improving apple red skin coloration (Honda & Moriya, 2018; Toivonen et al., 2019). Light penetration levels can vary widely within the canopy, with less light generally reaching the inner and lower third parts of the canopy while more sunlight tends to be intercepted by the upper and outer parts of the canopy (Layne et al., 2002). Furthermore, considerable light is lost to red fruit color development as it is absorbed by the ground between the orchard rows (Wünsche et al., 1996).

Horticultural practices, such as the use of reflective groundcovers, are used to enhance the tree canopy light environment (Layne et al., 2002; J.-P. Privé et al., 2011). The deployment of reflective groundcovers improves the capacity of apple trees to harness sunlight by reflecting the light that would otherwise be absorbed at the ground surface, back into the canopy (Funke & Blanke, 2021; Kon & Clavet, 2023; Mupambi et al., 2021). This amplifies the cumulative light reaching the apple fruit surface, particularly fruit located in the lower third and inner part of the canopy (Toye, 1995). The deployment of groundcovers around 4 weeks before the anticipated harvest has been reported to increase red skin coloration in apples (Iglesias & Alegre, 2009; Kon & Clavet, 2023; Layne et al., 2002; Miller & Greene, 2003; Mupambi et al., 2021; J. P. Privé et al., 2008; Robinson & Gonzalez, 2023; Toivonen et al., 2019). Despite its proven ability to enhance apple coloration, research on reflective groundcovers has not been widely conducted under the environmental conditions of the US mid-Atlantic region.

In addition to the lack of red skin coloration, ‘Honeycrisp’ apples are prone to preharvest fruit drop, which can begin 4 weeks before the anticipated harvest and prior to the fruit reaching horticultural maturity (Arseneault & Cline, 2018; Irish-Brown et al., 2011; J. Liu et al., 2022). The practice of harvesting fruit early to avoid preharvest fruit drop is not feasible, as immature fruit will not achieve acceptable quality and particularly red skin coloration requirements, leading to poor storability and reduced marketability (Baugher & Schupp, 2010). Therefore, reducing preharvest fruit drop is of critical importance for extending the harvest window and decreasing economic losses in the commercially important cultivar ‘Honeycrisp’ in the mid-Atlantic.

Ethylene has been widely reported as a key ripening-related hormone affecting fruit quality (Burg & Burg, 1965), as well as a primary driver of preharvest fruit drop in apples (Klee, 2004). Fruit quality is determined by multiple irreversible physiological and biochemical modifications that take place as fruit matures (Farcuh et al., 2022). These include modifications in fruit skin color (background and red surface color), texture (flesh softening), and flavor (increase in sugar contents, decrease in organic acids, and changes in aroma volatiles) (Farcuh et al., 2020; Miah et al., 2023; Osorio & Fernie, 2013). In addition, regarding preharvest fruit drop, ethylene can promote the degradation of the cell wall and intercellular tissues in the abscission zone of the pedicel, resulting in fruit drop (Bonghi et al., 2000; Roberts et al., 2002). Furthermore, apple fruit is classified as climacteric, characterized by an upsurge in respiration rates and internal ethylene concentration (IEC) as it ripens (Costa et al., 2005; Farcuh et al., 2018). Nevertheless, ethylene production in apple fruit can vary amongst cultivars, with cultivars producing a higher IEC, presenting a higher susceptibility to preharvest fruit drop (Chu, 1988; Gussman et al., 1993), such as ‘Honeycrisp’ (Arseneault & Cline, 2018; Irish-Brown et al., 2011).

Horticultural practices to reduce preharvest fruit drop currently rely mainly on plant growth regulators such as aminoethoxyvinylglycine (AVG), which inhibit the enzyme that catalyzes the rate-limiting step in ethylene biosynthesis, 1-aminocyclopropane-1-carboxylic acid (ACC) (Arseneault & Cline, 2016; J. Liu et al., 2022). AVG applications 4 weeks before the anticipated harvest have significantly reduced preharvest fruit drop in different cultivars, such as 'Honeycrisp', 'Gala', and 'McIntosh', grown under different environmental conditions (Arseneault & Cline, 2018; Layne et al., 2002; J. Liu et al., 2022; Schupp & Greene, 2004). In addition to reducing fruit drop, AVG has been reported to delay fruit maturity, impacting several fruit-quality-related attributes, such as reducing fruit softening, starch breakdown, and soluble solids contents; maintaining high acidity, and significantly reducing red skin coloration development in different apple cultivars (Arseneault & Cline, 2016, 2018; Boyacı, 2022; Byers, 1997; Greene & Schupp, 2004; Layne et al., 2002; J. Liu et al., 2022; Yuan & Li, 2008). Particularly regarding red skin coloration, endogenous ethylene has been reported to also play a critical role in regulating anthocyanin accumulation (Blankenship & Unrath, 1988; Farcuh et al., 2022; Wang & Dilley, 2001; Whale & Singh, 2007), in addition to environmental factors.

Although earlier research has documented the effects of the use of reflective groundcovers and AVG on apples, investigations of the responses to these strategies under the environmental conditions of the US mid-Atlantic region are lacking. Furthermore, to our knowledge, studies on the impacts of the combination of both horticultural practices on 'Honeycrisp' apples have not yet been conducted under our environmental conditions. Based on the above, the aim of the present work was threefold: first, to evaluate the effect of the reflective groundcover Extenday in light interception and reflectance in a commercial ‘Honeycrisp’ orchard grown under US mid-Atlantic environmental conditions; secondly, to characterize and compare differences in fruit drop, internal ethylene concentration, fruit-quality-related physicochemical parameters, and skin coloration of ‘Honeycrisp’ apples submitted to reflective groundcover Extenday and AVG treatment combinations throughout ripening on the tree; and thirdly, to use multivariate data analysis to identify significant correlations amongst all assessed features.

2. Materials and Methods

2.1. Plant Material and Preharvest Orchard Treatments

A 12-year-old commercial ‘Honeycrisp’/‘M9′ apple orchard located in Aspers, PA (39.96° N, 77.28° W), was used for this study. Tree spacing was 1.5 × 4 m and trees were trained to a central leader system. Four treatments composed of different combinations of the reflective groundcover Extenday (Extenday New Zealand, Auckland, New Zealand) and the plant growth regulator AVG (ReTain, Valent Biosciences Corporation, Libertyville, IL, USA) (Table 1.1) were established during two consecutive production seasons (2021 and 2022). For Extenday (T1, T2), a 3.5 m wide white woven polyethylene reflective groundcover was deployed adjacent to 50 tree plots on each side of the row 4 weeks before the anticipated commercial harvest date and secured according to manufacturer recommendations. Extenday (T1, T2)- and non-Extenday (T3, T4)-treated plots were separated down the tree row by at least 30 trees and separated by 3 rows of trees on either side to mitigate potential cofounding due to altered light reflection in trees adjacent to those applied with the Extenday treatment. The AVG treatment was applied to 20 tree subplots on Extenday (T1) and non-Extenday treatments (T3) and comprised a full-rate (130 mg L^{-1}) application, 4 weeks before the anticipated commercial harvest date. All sprays were mixed with 1.0 mL L^{-1} Silwet-77 organosilicone surfactant before application, which were made using a pressurized orchard sprayer. Additional trees in each plot were used as buffers to manage the potential drift of AVG treatment. A randomized complete block design with four replications was used.

‘Honeycrisp’ fruit maturity indices were monitored throughout the season each year to harvest fruit in the optimal commercial maturity stage using control fruit (T4) as the reference. Fruits were harvested in three different ripening stages on the tree: at optimal commercial harvest (CH) (corresponding to 3 September 2021 and 4 September 2022), 1 week after CH (CH + 1) (corresponding to 10 September 2021 and 11 September 2022), and 2 weeks after CH (CH + 2) (corresponding to 17 September 2021 and 18 September 2022). On each harvest date, for each of the four replications per treatment, a total of twenty-five fruit were harvested from the lower third of the canopy (1.5 m above the ground). Fruits with uniform size and an absence of visual blemishes, bruises, and/or diseases were chosen. After harvest, fruits were quickly transported to the laboratory. Per replication, five fruits were used for the analysis of internal ethylene concentration, while the rest of the fruits were used to assess quality-related physicochemical properties (described below).

Table 1.1. Combination of reflective groundcover and plant growth regulator treatments established in this study.

Treatments	Reflective Groundcover Treatment	Plant Growth Regulator Treatment
Treatment 1 (T1)	Extenday deployed	AVG applied
Treatment 2 (T2)	Extenday deployed	No AVG applied
Treatment 3 (T3)	No Extenday deployed	AVG applied
Treatment 4 (T4)	No Extenday deployed	No AVG applied

2.2. Light Interception and Reflectance Measurements

Light interception and reflectance by the reflective groundcover Extenday (T2) and by the ground or control (T4) were quantified in the middle of the drive row (mid-row) and within the tree canopy (in-canopy), proximal to solar noon on a sunny, cloud-free day and on a cloudy day in each year of this study. Light data were collected on two mid-row positions and two trees at the center of each 20-tree-plot replication. Measurements were collected 1.5 m above the ground. Intercepted light was determined with sensors oriented towards the sun (sky), while light reflectance was quantified by inverting the sensors (facing the ground or the reflective groundcover). Photosynthetic photon flux density (PPFD; 400–700 nm waveband; µmol m^{-2} s^{-1}) was evaluated using an LI-COR LI-191R Line Quantum Sensor attached to an LI-250A Light Meter (LI-COR Environmental, Lincoln, NE, USA). Two measurements of interception and reflectance were taken, with the sensor positioned perpendicular to the row, once each on the north and south side of the trunk. In-canopy measurements were carried out with the distal end of the sensor next to the trunk. Ultraviolet light (UV; 250–400 nm; µmol m^{-2} s^{-1}) was measured with a portable UV meter (FieldScout model 3414F, Spectrum Technologies Inc., Aurora, IL, USA). Four measurements of interception and reflectance were collected for each tree, once at each of the four points around the trunk (north, east, south, and west). In-canopy measurements were performed with the UV meter positioned 15 cm from the trunk.

2.3. Preharvest Fruit Drop Measurements

For each treatment and replication, two weeks before CH, a total of 5 limbs (from different trees) with a total of 20 fruits each were selected and tagged from either side of the trees. Preharvest fruit drop was evaluated by counting the number of tagged fruits weekly starting from one week before CH (1WBCH) to 2 weeks after CH (CH + 2). The percentage of fruit drop was then calculated relative to the initial fruit count per limb.

2.4. Fruit Internal Ethylene Concentration

The internal ethylene concentration (IEC) of each fruit was measured on 1 mL samples of internal gas from the core cavity using a gas chromatograph (GC-2014C, Shimadzu Co., Kyoto, Japan) equipped with an activated alumina column attached to a flame ionization detector as previously described (Farcuh & Hopfer, 2023; Kim et al., 2015; Miah et al., 2023). Nitrogen (N_2) was used as the carrier gas at a flow rate of 30 mL min^{-1}, while O_2 and H_2 were used to create the flame of the detector at a flow rate of 300 and 30 mL min^{-1}, respectively. Injector, detector, and oven temperatures were set at 140, 150, and 80 °C, respectively.

2.5. Fruit-Quality-Related Measurements

Fruit weight, skin color, index of absorbance difference (IAD), skin red blush percentage, flesh firmness, starch pattern index (SPI), soluble solids contents (SSCs), and titratable acidity (TA) were measured. Fruit weight was quantified using an electronic balance (Sartorius, AG Gottingen, Germany). Skin color was assayed on the two opposite sides of each fruit along the equatorial axes, and the red-green (a*) and yellow-blue (b*) values were measured using a colorimeter (Konica Minolta CR400 Chroma Meter, Konica Minolta Sensing, Inc., Osaka, Japan). Hue angle (hue°), representing changes in primary colors, was calculated as h = arctan(a*/b*) (Infante et al., 2008). The index of absorbance difference ($I_{AD} = A_{670} - A_{720}$; DA-Meter, TR Turoni, Forli, Italy) was measured on fruit skin by averaging the values recorded on three spots on each apple fruit (Ziosi et al., 2008). Flesh firmness was measured on the two opposite peeled sides of each fruit using a TA.XT Plus Connect texture analyzer (Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 50 kg loadcell and analyzed with the Exponent TE32 (v6.0, Texture Technologies Corp., Scarsdale, NY, USA) software fitted with an 11.1 mm diameter probe. The SPI of each fruit cut at the equator was assessed using the Cornell generic chart where 1 = 100%-iodine-stained starch and 8 = 0%-stained starch (Blanpied & Silsby, 1992). To determine SSC and TA, a wedge from each fruit was removed and pooled to create a composite sample of each replication. Juice was extracted from these composite samples with a hand press and filtered through cheesecloth. SSC was determined by using a digital hand-held refractometer (Atago, Tokyo, Japan) and expressed as %, whereas TA was computed by automatic titration (855 Robotic Titrosampler; Metrohm, Riverview, FL, USA) with a 0.1 N sodium hydroxide solution to an end point to pH 8.2, expressed as % malic acid (Farcuh et al., 2018, 2020).

2.6. Statistical Analysis

Response variables were modeled using generalized linear mixed models including treatments and evaluation periods as fixed factors, as well as blocks as a random factor to determine the statistical significance of the interactions and main effects (analysis of variance, ANOVA). When the analysis was statistically significant, the separation of means was carried out using Tukey's HSD test at a significance level of 5%.

Pearson's correlation coefficients, using mean-centered data, were calculated for each pairwise-combination of evaluated parameters. PCA, which was applied to reduce the dimensionality of the data, was visualized through a 'biplot' graph, thus representing the relationships among the variables (preharvest fruit drop, IEC, physicochemical measurements, and fruit skin color) and the assessed treatments and evaluation periods. The Scree test was used to select the number of principal components that captured most of the variation. The software package JMP (ver 15.2, SAS Institute) was used for all the statistical analyses.

3. Results

3.1. Effect of the Reflective Groundcover Extenday on Light Interception and Reflectance

Strong and consistent differences on mid-row and in-canopy PPFD and UV radiation were observed between the reflective groundcover Extenday-only treatment (T2) and by the ground or control treatment (T4) on both cloudy and sunny days, throughout the two evaluated years (Tables 1.2 and 1.3). Mid-row and in-canopy-intercepted PPFD and UV radiation results showed that, for both years, values were significantly higher in sunny than in cloudy days, but there were no differences between treatments. Conversely, in the case of mid-row and in-canopy-reflected PPFD and UV radiation, differences were observed between treatments as well as between sunny and cloudy days. The Extenday-only (T2) treatment assessed on the sunny days displayed the significantly highest reflected PPFD and UV radiation values, followed by this same treatment measured on the cloudy days, and subsequently by the ground or control treatment (T4) evaluated on the sunny days. The significantly lowest values for reflected PPFD and UV radiation were for the ground or control treatment (T4) assessed on the cloudy days (Tables 1.2 and 1.3). Mid-row measurements of PPFD and UV radiation reflected from Extenday were 5–20 times greater than those of the ground treatment, while in-canopy measurements of PPFD and UV radiation reflected from Extenday were 5–12 times greater than those of the ground treatment (Tables 1.2 and 1.3) considering cloudy and sunny days, in both evaluated years.

Table 1.2. Effect of the reflective groundcover Extenday on intercepted and reflected PPFD and UV radiation in the middle of the drive row (mid-row) and within the canopy (in-canopy) of 'Honeycrisp' trees on a sunny and on a cloudy day in Aspers, PA, in 2021.

PPFD (400–700 nm; µmol m^{-2} s^{-1})								
	Mid-Row				In-Canopy [z]			
	Intercepted [y]		Reflected [x]		Intercepted		Reflected	
Treatment [w]	Cloudy Day	Sunny Day	Cloudy day	Sunny Day	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day
T2	332 ± 34.8 b	1305 ± 30.9 a	130 ± 15.3 b	468 ± 13.2 a	130 ± 10.3 b	470 ± 20.1 a	61 ± 7.1 b	154 ± 12.1 a
T4	328 ± 24.6 b	1288 ± 24.1 a	11 ± 1.1 d	62 ± 8.8 c	126 ± 8.6 b	458 ± 15.2 a	5 ± 0.5 d	30 ± 2.7 c
UV (250–400 nm; µmol m^{-2} s^{-1})								
	Mid-Row				In-Canopy [v]			
	Intercepted		Reflected		Intercepted		Reflected	
Treatment	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day
T2	50 ± 4.1 b	152 ± 8.5 a	21 ± 1.7 b	42 ± 1.4 a	11 ± 1.5 b	34 ± 9.6 a	6 ± 1.0 b	13 ± 2.4 a
T4	46 ± 2.8 b	153 ± 9.1 a	2 ± 0.1 d	7 ± 0.3 c	9 ± 1.1 b	27 ± 5.2 a	0.5 ± 0.1 d	2 ± 0.3 c

PPFD, photosynthetic photon flux density; UV, ultraviolet radiation. [z] In-canopy PPFD measurements were carried out with the distal end of the sensor next to the trunk; [y] intercepted light was determined with sensors oriented towards the sun (sky) approximately 1.5 m above the ground; [x] reflected light was quantified by inverting the sensors (facing the ground or the reflective groundcover); [w] codes for treatments are described in Table 1.1; [v] in-canopy UV measurements were performed with the UV meter positioned 15 cm from the trunk. Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

Table 1.3. Effect of the reflective groundcover Extenday on intercepted and reflected PPFD and UV radiation in the middle of the drive row (mid-row) and within the canopy (in-canopy) of 'Honeycrisp' trees on a sunny and on a cloudy day in Aspers, PA, in 2022.

PPFD (400–700 nm; µmol m^{-2} s^{-1})								
	Mid-Row				In-Canopy [z]			
	Intercepted [y]		Reflected [x]		Intercepted		Reflected	
Treatments [w]	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day
T2	642 ± 21.6 b	1620 ± 44.1 a	435 ± 25.6 b	779 ± 20.3 a	252 ± 18.5 b	584 ± 19.3 a	145 ± 11.4 b	268 ± 16.7 a
T4	635 ± 28.4 b	1599 ± 39.9 a	20 ± 3.3 d	97 ± 16.2 c	248 ± 15.1 b	576 ± 12.9 a	12 ± 1.1 d	51 ± 7.2 c
UV (250–400 nm; µmol m^{-2} s^{-1})								
	Mid-Row				In-Canopy [v]			
	Intercepted		Reflected		Intercepted		Reflected	
Treatments	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day	Cloudy Day	Sunny Day
T2	68 ± 3.4 b	173 ± 14.4 a	43 ± 2.9 b	68 ± 7.3 a	16 ± 2.5 b	40 ± 11.6 a	13 ± 2.3 b	24 ± 3.0 a
T4	61 ± 4.2 b	172 ± 19.8 a	4 ± 1.3 d	15 ± 2.1 c	13 ± 1.7 b	34 ± 9.7 a	1.5 ± 0.3 d	5 ± 0.4 c

PPFD, photosynthetic photon flux density, UV, ultraviolet radiation. [z] In-canopy PPFD measurements were carried out with the distal end of the sensor next to the trunk; [y] intercepted light was determined with sensors oriented towards the sun (sky) approximately 1.5 m above the ground; [x] reflected light was quantified by inverting the sensors (facing the ground or the reflective groundcover); [w] codes for treatments are described in Table 1.1; [v] in-canopy UV measurements were performed with the UV meter positioned 15 cm from the trunk. Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

3.2. Effects of Extenday and AVG Treatment Combinations on 'Honeycrisp' Preharvest Fruit Drop

In 2021, there was no difference in fruit drop percentage amongst Extenday and AVG treatment combinations at 1WBCH (Figure 1.3A), but the fruit drop percentage started to increase significantly for all treatments from this period onwards. The Extenday-only (T2) treatment and the control (T4) displayed comparable levels of fruit drop that were around two times higher than treatments where AVG was applied (T1 and T3), from CH onwards. In the last assessment period (CH + 2), the treatments that did not receive AVG, T2 and T4, displayed fruit drop values ~30%, while T1 and T3, which did receive AVG, presented fruit drop values ~15%.

For 2022, differences in preharvest fruit drop amongst the four assayed treatment combinations were significant only for the evaluation periods of CH + 1 and CH + 2 (Figure 1.3B). Consistent with the results from 2021, fruit drop percentages started to increase significantly for all treatments from 1WBCH onwards. Furthermore, treatments lacking AVG application, T2 and T4, displayed fruit drop percentages that were ~1.5 times higher than treatments which received AVG applications, T1 and T3, from CH + 1 forward. During the last assessment period (CH +2), T2 and T4 displayed fruit drop values ~25%, while T1 and T3, which did receive AVG, presented significantly lower fruit drop values ~15%.

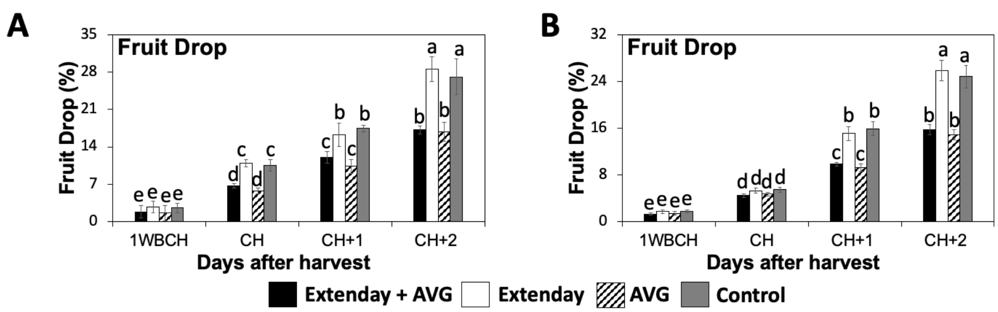

Figure 1.3. Effect of Extenday and AVG treatment combinations on preharvest fruit drop of 'Honeycrisp' apples grown in Aspers, PA. Fruit drop percentages were assessed in (A) 2021 and (B) 2022. Apples were evaluated 1 week before optimal commercial harvest (1WBCH), at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's HSD test.

3.3. Internal Ethylene Concentration and Physicochemical Properties of 'Honeycrisp' Fruit Submitted to Extenday and AVG Treatment Combinations

In general, Extenday and AVG treatment combinations had a consistently significant effect on IEC and most physicochemical properties of 'Honeycrisp' fruit across the two assessed years (Tables 1.4 and 1.5). Regarding IEC, all treatment combinations displayed significant increases as the fruit ripened on the tree (Tables

1.4 and 1.5), and therefore, fruit assessed at CH + 2 presented the highest IEC for each treatment, with respect to the other two evaluation periods. Within each evaluation period, the highest ethylene concentrations occurred with fruits submitted to Extenday-only (T2), followed by the control (T4), Extenday + AVG (T1), and finally AVG-only (T3), which presented the lowest ethylene concentration values. The exception was for 2022, where, at CH, fruits submitted to T2 displayed no significant differences with respect to T4 (Table 1.5).

Table 1.4. Effects of Extenday and AVG combinations on internal ethylene concentration and physicochemical properties of 'Honeycrisp' fruit harvested in three different ripening stages on the tree in Aspers, PA, in 2021.

Treatment [w]	Ethylene (μL L^{-1})			Firmness (N)			SPI (1 to 8)			SSC (%)			TA (% Malic Acid)		
	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2
T1	1.4 ± 0.3 g	2.7 ± 0.4 f	10.4 ± 1.3 c	78.4 ± 2.1 ab	68.5 ± 1.6 c	63.5 ± 2.4 d	3.9 ± 0.4 h	6.0 ± 0.4 e	7.1 ± 0.4 b	13.1 ± 0.3 de	13.4 ± 0.3 cd	13.5 ± 0.4 cd	0.50 ± 0.01 b	0.45 ± 0.01 c	0.39 ± 0.02 de
T2	4.1 ± 0.9 e	6.5 ± 0.9 d	17.3 ± 1.1 a	63 ± 2.6 d	58 ± 2.7 e	57.1 ± 2.7 e	6.0 ± 0.3 e	7.0 ± 0.3 b	7.8 ± 0.7 a	13.7 ± 0.2 b	14.4 ± 0.3 ab	14.6 ± 0.9 a	0.37 ± 0.02 e	0.33 ± 0.03 f	0.30 ± 0.02 fg
T3	0.5 ± 0.2 h	1.4 ± 0.2 g	6.4 ± 0.6 d	80.1 ± 2.7 a	74 ± 2.2 b	62.6 ± 2.3 d	3.2 ± 0.4 i	5.3 ± 0.4 f	6.5 ± 0.5 cd	12.5 ± 0.1 f	13.1 ± 0.2 de	13.2 ± 0.7 d	0.55 ± 0.02 a	0.50 ± 0.03 b	0.42 ± 0.02 cd
T4	2.8 ± 0.6 f	4.3 ± 0.8 e	14.4 ± 0.9 b	68.7 ± 2.8 c	62.5 ± 2.6 d	62.0 ± 2.5 d	4.6 ± 0.4 g	6.5 ± 0.4 cd	7.8 ± 0.6 a	13.4 ± 0.2 cd	13.8 ± 0.2 b	14.2 ± 0.4 ab	0.44 ± 0.02 c	0.37 ± 0.01 e	0.33 ± 0.01 f

[w] Codes for treatments are described in Table 1.1. Apples were harvested at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). N, Newton; SPI, starch pattern index (1–8 scale); SSC, soluble solids content; TA, titratable acidity. Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

Table 1.5. Effects of Extenday and AVG combinations on internal ethylene concentration and physicochemical properties of 'Honeycrisp' fruit harvested in three different ripening stages on the tree in Aspers, PA, in 2022.

Treatment [w]	Ethylene (μL L^{-1})			Firmness (N)			SPI (1 to 8)			SSC (%)			TA (% malic acid)		
	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2
T1	2.9 ± 0.7 g	3.8 ± 0.8 f	11.3 ± 0.8 c	76.2 ± 3.2 a	66.4 ± 2.2 b	61.6 ± 1.8 c	4.4 ± 0.6 d	5.6 ± 0.3 c	7.3 ± 0.7 ab	13.7 ± 0.2 bc	14.0 ± 0.3 b	14.2 ± 0.7 ab	0.47 ± 0.02 b	0.42 ± 0.02 bc	0.37 ± 0.02 cd
T2	5.5 ± 0.7 e	8.1 ± 0.6 d	20.7 ± 1.5 a	61.1 ± 2.4 c	55.9 ± 3.1 d	55.0 ± 1.8 d	6.7 ± 0.8 b	7.6 ± 0.5 a	7.9 ± 0.5 a	14.1 ± 0.6 b	14.3 ± 0.4 ab	14.7 ± 1.2 a	0.34 ± 0.01 d	0.30 ± 0.02 de	0.28 ± 0.01 e
T3	1.0 ± 0.1 h	2.5 ± 0.4 g	8.1 ± 0.8 e	76.1 ± 1.6 a	69.8 ± 1.8 ab	61.0 ± 2.5 c	3.4 ± 0.2 e	4.7 ± 0.6 d	6.7 ± 0.4 b	13.4 ± 0.4 c	13.9 ± 0.5 b	14 ± 0.5 b	0.52 ± 0.01 a	0.47 ± 0.01 b	0.40 ± 0.01 c
T4	4.5 ± 0.3 ef	5.9 ± 0.9 e	15.8 ± 0.6 b	66.9 ± 2.1 b	61.2 ± 3.1 c	56.1 ± 3.1 d	5.3 ± 0.5 c	6.8 ± 0.4 b	7.8 ± 0.8 a	14 ± 0.6 b	14.3 ± 0.5 ab	14.5 ± 0.7 a	0.4 ± 0.01 c	0.35 ± 0.02 d	0.31 ± 0.01 de

[w] Codes for treatments are described in Table 1.1. Apples were harvested at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). N, Newton; SPI, starch pattern index (1–8 scale); SSC, soluble solids content; TA, titratable acidity. Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

Throughout all the evaluation periods and treatments, fruit weight was not significantly altered, and thus no significant differences were observed (data not shown).

Fruit flesh firmness decreased as fruits ripened on the tree for all treatment combinations in both years (Tables 1.4 and 1.5). In general, flesh firmness values were highest at CH for Extenday + AVG (T1) and AVG-only (T3) (76–80 N), followed by the control (T4) and finally by Extenday-only (T2), which exhibited the significantly lowest values (61–63 N) for 2021 and 2022 assessments. These differences in treatment combinations were also observed for CH + 1, although in 2021, there was a significant difference between AVG-only (T3) and Extenday + AVG (T1) that was not observed in any other evaluation period or year (Table 1.4). Extenday-only (T2) assessed in CH + 1 and at CH + 2 evaluation periods presented the lowest flesh firmness values (<60 N) amongst all treatments and evaluation periods. Particularly in 2022, the control (T4) assessed at CH + 2 did not significantly differ from Extenday-only (T2) (<60 N) but did differ from Extenday + AVG (T1) and AVG-only (T3) (>60 N) (Table 1.5).

Extenday and AVG treatment combinations also affected the starch pattern index (Tables 1.4 and 1.5). As the fruit ripened on the tree, the SPI values significantly increased (indicative of a lower starch content) for all treatment combinations in both years. At CH and CH + 1, SPI values were lowest for AVG-only (T3), followed by Extenday + AVG (T1) and the control (T4), and the highest

SPI values were obtained for Extenday-only (T2), which displayed 1.3–2 times higher SPI values than AVG-only (T3) when considering both years. Fruits evaluated at CH + 2 presented the same trends as described for the other evaluation periods. The exception was for 2021, where Extenday-only (T2) and the control (T4) did not differ significantly (SPI ~7.8) (Table 1.4), and for 2022, where Extenday + AVG (T1; SPI ~7.3) exhibited no differences with any of the other assayed treatment combinations, although AVG-only (T3; SPI ~6.7) did differ from Extenday-only (T2) and the control (T4; SPI ~7.8) (Table 1.5).

In both years, SSC significantly increased throughout the three ripening evaluation periods for all treatment combinations, except for Extenday + AVG (T1), which still presented an increasing trend, but was not significant (Tables 1.4 and 1.5). In 2021, within the evaluation periods, in most cases, Extenday-only (T2) and the control (T4) displayed significantly higher values than Extenday + AVG (T1) and AVG-only (T3), which, for CH + 2, corresponded to 13.2–13.5% and 14.2–14.6%, respectively (Table 1.4). In 2022, values for AVG-only (T3) were statistically lower than for Extenday-only (T2) and the control (T4) during CH and CH + 2, with values ranging from 13.4 to 14% for the former and 14 to 14.7% for the latter (Table 1.5).

Titratable acidity (TA) values significantly decreased throughout the different assayed evaluation periods of ripening on the tree for all treatment combinations (Tables 1.4 and 1.5). During both years, at CH, AVG-only (T3) presented the significantly highest acidity values (~0.55), followed by Extenday + AVG (T1; ~0.47–0.5), the control (T4; ~0.4), and lastly Extenday-only (T2; < 0.4). In 2021, these differences amongst treatments combinations were maintained through CH + 1, although at CH + 2, no significant differences were displayed between Extenday + AVG (T1) and AVG-only (T3) (~0.4) as well as between Extenday-only (T2) and the control (T4) (~0.3) (Table 1.4). In 2022, in the CH+ 1 and CH +2 evaluation periods, no significant differences were observed between Extenday + AVG (T1) and AVG-only (T3) nor between Extenday-only (T2) and the control (T4) (Table 1.5).

3.4. Effects of Extenday and AVG Treatment Combinations on 'Honeycrisp' Skin Coloration

Extenday and AVG treatment combinations displayed significant effects on surface red skin color as well as background skin coloration in 'Honeycrisp' apples throughout the two years of evaluation (Tables 1.6 and 1.7 and Figure 1.4).

Table 1.6. Effects of Extenday and AVG combinations on surface and background skin coloration of 'Honeycrisp' fruit harvested in three different ripening stages on the tree in Aspers, PA, in 2021.

Treatment [w]	Surface Skin Color (Hue°)			Red Blush (%)			Background Skin Color (Hue°)			Index of Absorbance Difference (I_{AD})		
	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2
T1	48.7 ± 1.5 c	43.3 ± 1.3 d	38.1 ± 1.3 e	58 ± 2.5 e	65 ± 2.9 cd	69 ± 2.1 c	105.1 ± 2.7 c	98.2 ± 2.1 e	93.4 ± 2.2 f	0.7 ± 0.03 c	0.6 ± 0.04 d	0.4 ± 0.02 f
T2	43.4 ± 1.1 d	38.2 ± 1.2 e	34.1 ± 1.3 f	75 ± 2.3 b	80 ± 3.1 ab	85 ± 3.2 a	101.1 ± 2.1 d	93.9 ± 2.3 f	86.9 ± 1.9 g	0.5 ± 0.04 e	0.4 ± 0.01 f	0.3 ± 0.01 g
T3	66.9 ± 1.9 a	55.1 ± 1.8 b	48.5 ± 2.1 c	30 ± 2.1 i	35 ± 2.5 h	45 ± 2.3 f	114.2 ± 2.6 a	113.5 ± 2.7 a	104.2 ± 2.2 c	1.0 ± 0.04 a	0.8 ± 0.04 b	0.6 ± 0.02 d
T4	55.3 ± 1.8 b	47.3 ± 1.2 c	43.7 ± 1.7 d	40 ± 2.9 g	46 ± 2.8 f	58 ± 2.1 e	108.9 ± 2.1 b	104.6 ± 1.7 c	96.6 ± 2.9 ef	0.8 ± 0.02 b	0.7 ± 0.03 c	0.5 ± 0.01 e

[w] Codes for treatments are described in Table 1.1. Apples were harvested at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

Table 1.7. Effects of Extenday and AVG combinations on surface and background skin coloration of ‘Honeycrisp’ fruit harvested in three different ripening stages on the tree in Aspers, PA, in 2022.

Treatment [w]	Surface Skin Color (Hue°)			Red Blush (%)			Background Skin Color (Hue°)			Index of Absorbance Difference (I_{AD})		
	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2	CH	CH + 1	CH + 2
T1	44.9 ± 1.9 c	39.5 ± 1.1 d	35.6 ± 1.2 e	64 ± 2.8 d	68 ± 3.5 cd	72 ± 2.4 c	104.2± 2.9 c	96.4 ± 2.6 ef	92.6 ± 2.5 fg	0.6 ± 0.04 c	0.5 ± 0.03 d	0.3 ± 0.03 f
T2	37.8 ± 1.5 de	34.9 ± 0.9 e	31.6 ± 1.1 f	80 ± 2.5 b	85 ± 3.4 ab	90 ± 4.9 a	98.2± 2.3 e	91.5 ± 2.1 g	86.1 ± 2.3 h	0.5 ± 0.03 d	0.4 ± 0.02 e	0.2 ± 0.03 g
T3	64.5 ± 2.2 a	50.6 ± 2.4 b	44.1 ± 1.8 c	32 ± 2.9 h	40 ± 2.2 g	48 ± 2.7 e	112.8± 2.4 a	110.1 ±3 .7 ab	100.4 ± 2.2 de	0.9 ± 0.03 a	0.7 ± 0.02 b	0.5 ± 0.03 d
T4	52.6 ± 2.6 b	43.9 ± 1.6 c	40.3 ± 1.4 d	44 ± 1.7 f	49 ± 2.6 e	63 ± 2.5 d	107.1± 2.4 b	102.2 ± 2.4 cd	94.8 ± 2.7 f	0.7 ± 0.02 b	0.6 ± 0.04 c	0.4 ± 0.03 e

[w] Codes for treatments are described in Table 1.1. Apples were harvested at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey’s test.

Figure 1.4. Effects of Extenday and AVG treatment combinations on ‘Honeycrisp’ fruit coloration at commercial harvest in Aspers, PA, in 2022.

As the fruits ripened on the tree, the surface skin hue angle values significantly decreased (indicative of a higher red coloration) in all treatment combinations in 2021 and 2022 (Tables 1.6 and 1.7). For all evaluation periods and in both years, surface skin hue angle values were highest for AVG-only (T3), followed by the control (T4) and Extenday + AVG (T1), and finally, the statistically lowest values were for Extenday-only (T2). In agreement with the results for surface skin hue angle, the assessment of skin blush percentage showed that from CH to CH + 2, there was a significant increase in this parameter for all treatment combinations in both assayed years (Tables 1.6 and 1.7). Furthermore, the significantly highest skin blush percentage was consistently obtained for Extenday-only (T2; 75–90%), followed by Extenday + AVG (T1; 58–72%) and the control (T4; 40–63%), and finally, the lowest values were displayed by AVG-only (T3; 30–48%). It is important to note that the control (T4) only reached ~50% skin blush in 'Honeycrisp' in fruit harvested at CH + 1, while AVG-only (T3) only achieved it in fruit harvested at CH + 2 (Tables 1.6 and 1.7).

Background skin color hue values as well as the index of absorbance difference (I_{AD}) significantly decreased (indicative of a change in color from green to yellow, and to an increase in chlorophyll degradation, respectively), in all treatment combinations and both evaluated years, as fruit ripened on the tree (Tables 1.6 and 1.7). Consistently for 2021 and 2022, as well as for all the assessed evaluation periods, AVG-only (T3) displayed the significantly highest values, followed by the control (T4) and Extenday + AVG (T1), which also differed amongst them. The significantly lowest values for background skin color hue and I_{AD} were for 'Honeycrisp' fruit from Extenday-only (T2) in all cases, evidencing the highest rate of chlorophyll disappearance.

3.5. Relationships among Fruit Drop, Ethylene Concentration, Physicochemical Properties, and Fruit Color of 'Honeycrisp' Apple Fruit Submitted to Extenday and AVG Treatment Combinations

Correlation coefficients were calculated (Table 1.8) and a Principal Component Analysis (PCA) was performed (Figure 1.5) to visualize the relationships among all the parameters assessed in 'Honeycrisp' fruit and described above during the two assayed production seasons.

Table 1.8. Pearson correlation coefficients among all assessed features in 'Honeycrisp' fruit including fruit drop, internal ethylene concentration (IEC), physicochemical parameters, and skin coloration.

Feature	Fruit Drop	IEC	Firmness	SPI	SSC	TA	Surface Hue	Skin Blush	Background Hue	I_{AD}
Fruit drop	1.00	0.94 *	−0.89 *	0.88 *	0.78 *	−0.89 *	−0.69 *	0.63 *	−0.82 *	−0.77 *
IEC		1.00	−0.80 *	0.79 *	0.77 *	−0.85 *	−0.70 *	0.63 *	−0.85 *	−0.79 *
Firmness			1.00	−0.96 *	−0.83 *	0.95 *	0.81 *	−0.67 *	0.82 *	0.84 *
SPI				1.00	0.83 *	−0.91 *	−0.89 *	0.70 *	−0.86 *	−0.89 *
SSC					1.00	−0.89 *	−0.75 *	0.69 *	−0.73 *	−0.74 *
TA						1.00	0.83 *	−0.75 *	0.85 *	0.86 *
Surface Hue							1.00	−0.90 *	0.92 *	0.97 *
Skin blush								1.00	−0.86 *	−0.89 *
Background Hue									1.00	0.97 *
I_{AD}										1.00

Internal ethylene concentration (IEC), starch pattern index (SPI), soluble solids content (SSC), titratable acidity (TA), index of absorbance difference (I_{AD}). All correlations shown are significant (*; $p \leq 0.05$).

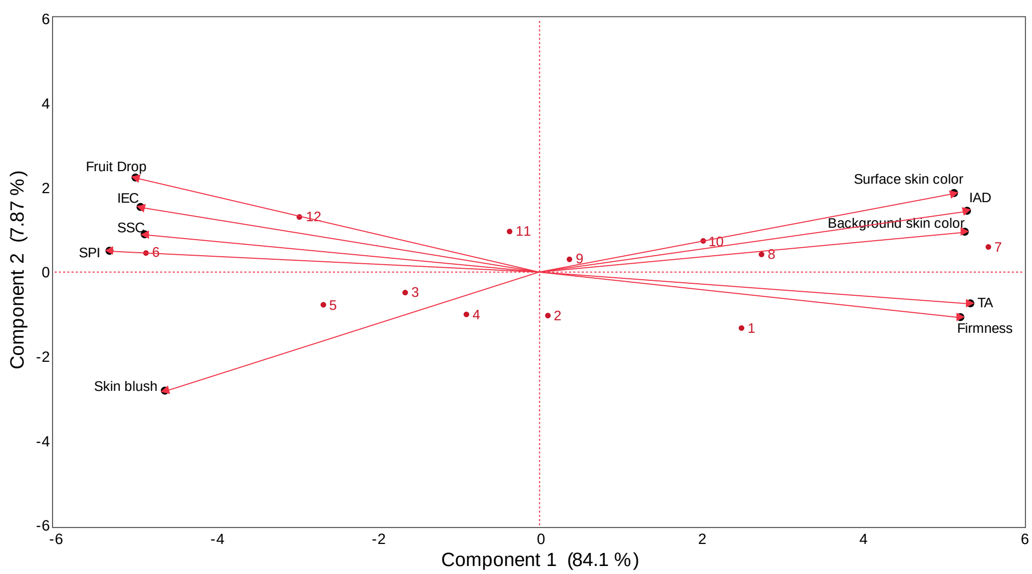

Figure 1.5. Biplot from Principal Component Analysis of data obtained from fruit drop, internal ethylene concentration, physicochemical parameters, and skin coloration of 'Honeycrisp' apples submitted to different Extenday and AVG treatments combinations throughout ripening on the tree. Internal ethylene concentration (IEC), starch pattern index (SPI), soluble solids content (SSC), titratable acidity (TA), index of absorbance difference (IAD). Numbers correspond to the different treatments and ripening stages on the tree that were assayed (1 (T1_CH),

2 (T1_CH + 1), 3 (T1_CH + 2), 4 (T2_CH), 5 (T2_CH + 1), 6 (T2_CH + 2), 7 (T3_CH), 8 (T3_CH + 1), 9 (T3_CH + 2), 10 (T4_CH), 11 (T4_CH + 1), 12 (T4_CH + 2)). Codes for treatments are described in Table 1.1.

Fruit drop significantly and positively correlated with IEC (r = 0.94), SPI (r = 0.88), SSC (r = 0.78), and skin blush (r = 0.63) but was negatively associated with fruit flesh firmness (r = −0.89), titratable acidity (r = −0.89), color-related features of the surface and background skin hue angles (r = −0.69 and r = −0.82, respectively), and I_{AD} (r = −0.77).

IEC was significantly and positively correlated with SPI (r = 0.79), SSC (r = 0.77), and skin blush (r = 0.63) (Table 1.8), while it negatively correlated with flesh firmness (r = −0.80), titratable acidity (r = −0.85), surface and background skin hue angles (r = −0.70 and r = −0.85, respectively), and I_{AD} (r = −0.79).

The parameter of flesh firmness was positively associated with titratable acidity (r = 0.95), the color parameters of surface and background skin hue angles (r = 0.81 and r = 0.82, respectively), as well as I_{AD} (r = 0.84). Furthermore, there was a significant negative correlation between flesh firmness and assessed fruit physicochemical properties such as SPI (r = −0.96) and SSC (r = −0.83), as well as with skin blush (r = −0.67).

The starch pattern index presented positive correlations with SSC (r = 0.83) and skin blush (r = 0.70) but was negatively associated with titratable acidity (r = −0.91), skin and flesh color hue (r = −0.89 and r = −0.86, respectively), and I_{AD} (r = −0.89) (Table 1.8). SSC correlated positively with skin blush (r = 0.69) and negatively with titratable acidity (−0.89), surface and background skin hue angles (r = −0.75 and r = −0.73, respectively), and the index of absorbance difference (r =

−0.74). Titratable acidity, on the other hand, was positively associated with the color features of surface and background skin hue angles (r = −0.83 and r = −0.85, respectively) and the index of absorbance difference (r = −0.86), but negatively associated with fruit skin blush (r = −0.75).

Amongst the color-related parameters, surface skin hue angle was positively correlated with background skin hue angle (r = 0.92) and I_{AD} (r = 0.97), and negatively correlated with skin blush (r = −0.90). On the other hand, skin blush displayed significantly negative correlations with background skin hue angles (r = −0.86) and I_{AD} (r = −0.89).

The PCA showed that the first and second principal components explained 84.1% (Component 1) and 7.87% (Component 2) of the observed variation (91.9% total), respectively (Figure 1.5). Along the first principal component, the separation of the Extenday and AVG treatments combinations was driven by preharvest fruit drop, IEC, SSC, SPI, and skin blush on the negative side of the axis (associated with treatments Extenday + AVG (T1; at CH + 2), Extenday-only (T2; all evaluation periods), and the control (T4; at CH + 1 and CH + 2)) and by surface and background skin hue values, IAD, titratable acidity, and firmness on the positive side of the axis (associated with treatments Extenday + AVG (T1; at CH and CH + 1), AVG-only (T3; all evaluation periods), and the control (T4; at CH) (Figure 1.5)).

4. Discussion

There are numerous factors influencing red skin coloration in apples, including environmental factors such as temperature and light, as well as plant growth regulators such as AVG, among others (Blankenship & Unrath, 1988; Chen et al., 2021; Honda & Moriya, 2018; Lancaster, 1992; J. Liu et al., 2022; Musacchi & Serra, 2018; Toivonen et al., 2019; Whale & Singh, 2007). Regarding light, although there are various reports indicating that the reflective groundcover Extenday has improved red skin coloration in apples (Iglesias & Alegre, 2009; Kon & Clavet, 2023; Mupambi et al., 2021; J. P. Privé et al., 2008; Robinson & Gonzalez, 2023; Toivonen et al., 2019), studies evaluating its effects on fruit maturity and quality are inconsistent, and have been lacking under the environmental conditions of the mid-Atlantic of the US in 'Honeycrisp' apples. Regarding AVG, it has been reported that it negatively affects red skin coloration development but can effectively delay maturity and reduce preharvest fruit drop (Arseneault & Cline, 2016; Boyacı, 2022; Greene, 2005; Layne et al., 2002; J. Liu et al., 2022; Schupp & Greene, 2004; Wang & Dilley, 2001). However, there is limited information about the effects of Extenday and AVG treatment combinations on fruit drop, fruit maturity and quality, and particularly the skin coloration of 'Honeycrisp' apples grown under the hot and humid weather of the mid-Atlantic. In the present study, there was a general trend for decreased preharvest fruit drop and increased red skin coloration (>50% blush) without the promotion of overripening in fruit harvested from the lower third of the canopy, with the combined use of the reflective groundcover Extenday and the plant growth regulator AVG, consistent throughout two consecutive years.

4.1. Extenday reflects photosynthetic photon flux density (PPFD) and ultraviolet (UV) radiation back to the canopy

Ultraviolet radiation is known to improve red skin coloration in fruits, including apples (Charles & Arul, 2007; Dong et al., 1995; Honda & Moriya, 2018; Toivonen et al., 2019). In agreement with our results, the use of Extenday in other regions has also shown an increase in reflected UV radiation from the orchard ground back onto the trees as compared to control trees (Kon & Clavet, 2023; Toivonen et al., 2019). These results support the significantly higher red skin coloration attained by Extenday-only-treated fruit in our work, which displayed >75% skin blush in the first assayed ripening stage (CH). Red color development on apple fruits results from the accumulation of anthocyanin pigments, which have been demonstrated to be highly influenced by UV radiation, as most enzymes involved in the anthocyanin biosynthesis pathway are light-inducible (Chen et al., 2021; Vimolmangkang et al., 2014). Consistent with our results, increased reflected PPFD by the reflective groundcover Extenday was also observed in previous studies (Iglesias & Alegre, 2009; Kon & Clavet, 2023; J. P. Privé et al., 2008; Robinson & Gonzalez, 2023; Toivonen et al., 2019). Nevertheless, it is important to mention that excessive PPFD and UV radiation in other apple-producing regions, such as the Pacific Northwest of the US (hot and dry climate), can have negative impacts on 'Honeycrisp' fruit, such as the development of sunburn (Mupambi et al., 2021). In the current study, under our environmental conditions, we did not observe sunburn incidence with the use of the reflective groundcover Extenday in any of the treatments.

4.2. Aminoethoxyvinylglycine minimizes preharvest fruit drop by reduction of ethylene biosynthesis, independent of reflective groundcover

Preharvest fruit drop is a major concern in apple production in many regions, including the mid-Atlantic (Arseneault & Cline, 2018; Irish-Brown et al., 2011; J. Liu et al., 2022). Plant growth regulators, such as AVG, are widely used to prevent fruit drop due to their capacity of inhibiting ethylene biosynthesis (Arseneault & Cline, 2016; Yuan & Li, 2008). This is supported by the positive correlation obtained between fruit drop and IEC in this work. Consistent with our results, the application of AVG at full-rate three-to-four weeks before harvest was significantly effective at minimizing preharvest fruit drop in ‘Honeycrisp’ (Arseneault & Cline, 2018), ‘Gala’ (Layne et al., 2002; J. Liu et al., 2022), and ‘McIntosh’ (Schupp & Greene, 2004). In ‘McIntosh’ apples, it has been reported that once applied, the onset of action time for AVG to manifest and significantly reduce fruit drop is between 10 and 14 days (Greene, 2005). In this study, the application of AVG consistently decreased preharvest fruit drop in ‘Honeycrisp’ apples, independent of the use of the reflective groundcover Extenday, suggesting that the use of the latter does not significantly impact apple fruit drop.

4.3. The combined Extenday and Aminoethoxyvinylglycine treatment maintains fruit physicochemical properties by promoting fruit maturation without inducing overripening

As apple fruit display a climacteric fruit ripening behavior, and thus IEC has been reported to play a key role in controlling fruit maturity (Costa et al., 2005; Farcuh et al., 2018; Miah et al., 2023), attempts to control fruit drop by reducing ethylene biosynthesis via AVG are expected to delay fruit maturity. In this work, AVG-treated ‘Honeycrisp’ apples displayed the significantly lowest IEC, which affected several quality-related physicochemical attributes.

In agreement with previous studies (Arseneault & Cline, 2018; Boyacı, 2022; Byers, 1997; Greene, 2005; Layne et al., 2002; J. Liu et al., 2022), AVG delayed fruit flesh softening and starch breakdown, reduced soluble solids contents, and maintained the highest acidity values throughout ripening. Consistent with our results, AVG has been reported to have no effect on apple fruit weight (Greene, 2002; J. Liu et al., 2022; Schupp & Greene, 2004), suggesting that this is an ethylene-independent trait. Likewise, regarding the use of the reflective groundcover Extenday, no significant effects on apple fruit weight have been reported (Iglesias & Alegre, 2009; Kon & Clavet, 2023), supporting our results. Nevertheless, the use of Extenday has shown inconsistent results in terms of its effect on fruit maturity and quality. In this study, Extenday deployment hastened fruit maturity by increasing ethylene production in 'Honeycrisp' apples, decreasing flesh firmness and acidity, but increasing starch degradation as well as soluble solid contents, in accordance with previous findings in peaches (Layne et al., 2001) and apples (Overbeck et al., 2013). However, other studies have reported no effects on fruit-maturity-related attributes due to Extenday deployment (Funke & Blanke, 2021; Iglesias & Alegre, 2009; Kon & Clavet, 2023; Layne et al., 2002; Mupambi et al., 2021; J.-P. Privé et al., 2011). This variability in findings amongst studies regarding the impact of Extenday on apple fruit maturity and physicochemical characteristics can most likely be attributed to the differences in growing conditions, apple cultivars, management practices, and the time interval in which the reflective groundcover Extenday is in place. Moreover, in this study, when combining Extenday + AVG under US mid-Atlantic environmental conditions, 'Honeycrisp' fruit displayed an intermediate fruit maturity, i.e., significantly advanced with respect to AVG-only-treated apples but, at the same time, significantly delayed as compared to control and Extenday-only-treated fruit. The latter is indicative of an interaction between both Extenday and AVG that is impacting fruit quality properties by advancing maturity, but not overly stimulating the ripening process, and thus avoiding overripening as the fruit is left hanging on the tree. This is of key importance as it suggests that the combined Extenday + AVG treatment could be improving subsequent fruit storability and shelf-life capacity, which has not been accounted for in this study, but is currently under investigation.

4.4. The combined Extenday and Aminoethoxyvinylglycine treatment increases fruit skin coloration at commercial harvest without enhancing fruit overripening

Fruit red skin coloration is of major importance for fruit quality, as it is directly tied to consumer preference and market value (Musacchi & Serra, 2018). As discussed above, apple skin red coloration is a result of the accumulation of anthocyanins, which, in addition to environmental factors, such as UV radiation, is also partially regulated by endogenous ethylene (Blankenship & Unrath, 1988; Farcuh et al., 2022; Wang & Dilley, 2001; Whale & Singh, 2007). This is supported by the positive correlations between IEC and skin blush, as well as the negative correlations between IEC and hue angle, obtained in this study. AVG application at full-rate three-to-four weeks before harvest in ‘Gala’, ‘Red Delicious’, ‘Jonagold’, ‘Honeycrisp’, and ‘Red Chief’ apples has been reported to significantly reduce red skin coloration (Arseneault & Cline, 2018; Boyacı, 2022; Layne et al., 2002; J. Liu et al., 2022), consistent with our results, where the required minimum 50% skin blush for fruit to be marketable was only attained in ‘Honeycrisp’ fruit in the last assayed ripening stage (CH + 2). On the other hand, in this work, the deployment of the reflective groundcover Extenday significantly boosted ‘Honeycrisp’ red skin coloration to >75% skin blush in the first assayed ripening stage (CH), in agreement with other studies conducted in different regions and cultivars (Iglesias & Alegre, 2009; Kon & Clavet, 2023; Mupambi et al., 2021; Overbeck et al., 2013; J.-P. Privé et al., 2011; J. P. Privé et al., 2008; Robinson & Gonzalez, 2023; Toivonen et al., 2019). Furthermore, the combined treatment of Extenday + AVG significantly enhanced the red skin coloration of ‘Honeycrisp’ apples, which displayed >50% red blush in CH under mid-Atlantic environmental conditions, suggesting an interaction between both horticultural practices. This is of crucial importance as fruit from the upper sun-exposed third of the canopy are typically redder than fruit located in the lower third of the canopy (Layne et al., 2002). However, the combined treatment of Extenday + AVG could ensure that fruit from the lower canopy actually pack out in premium grades, while not increasing fruit drop or fruit overripening, therefore increasing total crop value and profitability, and justifying the economic investment in the reflective groundcover Extenday.

Additionally, changes from green to yellow in the background color of apples, through a decrease in the values of hue angle as well as a result of chlorophyll disappearance (I_{AD}), have been shown to be associated with increased fruit maturity (Miah et al., 2023; Ziosi et al., 2008). These results support the significantly negative correlations between I_{AD} and IEC obtained in this work, and are consistent with other studies in apples (Mahdavi et al., 2022; Miah et al., 2023; Wang & Dilley, 2001) and peaches (Farcuh & Hopfer, 2023; Ziosi et al., 2008). The significantly highest and lowest hue angle and I_{AD} values for AVG-only- and Extenday-only-treated ‘Honeycrisp’ apples, respectively, are indicative of the delay and advancement of background color changes, respectively. Nonetheless, the combined Extenday + AVG treatment in ‘Honeycrisp’ apples presented an intermediate background color change, similar to what was observed for fruit maturity and quality-related physicochemical parameters, supporting an advancement in fruit maturity that does not translate into fruit overripening.

4.5. Extenday and Aminoethoxyvinylglycine treatment combinations on 'Honeycrisp' fruit skin color, preharvest drop, and quality under Mid-Atlantic conditions

In this study, the distribution of each Extenday and AVG treatment combination/evaluation period along component 1 of the PCA is supported by the AVG-only-treated ‘Honeycrisp’ fruit displaying the significantly lowest IEC, most delayed fruit maturity, and reduced fruit drop but a drastically inhibited red skin coloration in all evaluation periods; followed by the Extenday + AVG treatment in ‘Honeycrisp’ fruit exhibiting an intermediate positioning in terms of IEC, fruit maturity (not leading to overripe fruit), and fruit drop, while significantly enhancing red skin coloration above the required marketable minimum (>50% blush) in all evaluation periods; by the control ‘Honeycrisp’ fruit showing a significantly increased IEC, fruit maturity (leading to overripe fruit towards the later assessed evaluation periods), and fruit drop, with a considerably hindered red skin coloration in the first evaluation periods; and finally by the Extenday-only-treated ‘Honeycrisp’ fruit, presenting the significantly highest IEC, most advanced fruit maturity (leading to overripe fruit), increased preharvest fruit drop, yet a promoted red skin coloration in all evaluation periods (>75% blush). However, the results of this study may only be applicable for fruit grown under US mid-Atlantic conditions. Thus, this work needs to be replicated in major production regions of ‘Honeycrisp’ with different environmental conditions, such as the Pacific Northwest, to assess the transferability of these outcomes between regions. Furthermore, these results are specific for

‘Honeycrisp’ fruit and future work is required to include a wide range of cultivars to assess the robustness of these results.

5. Conclusions

‘Honeycrisp’ fruit located in the lower third of the canopy and submitted to different combinations of Extenday and AVG treatments under US mid-Atlantic environmental conditions over two consecutive years revealed that Extenday deployment can significantly promote apple red skin coloration (>75% blush) via an increased reflected PPFD and UV radiation (>10 times as compared to the control) as well as via an increased IEC, while also advancing fruit maturity, i.e., overripening. The application of AVG, conversely, negatively impacted apple red skin coloration (30–48% blush), minimized fruit drop in half as compared to the control, and effectively decreased IEC, thus delaying fruit maturity in terms of fruit firmness, starch degradation, SSC, and acidity. We demonstrated that the combined use of Extenday and AVG treatments had a synergistic effect and decreased preharvest fruit drop to the same levels as AVG-only while reducing overripening, without sacrificing red skin coloration development (which reached >58% blush). Moreover, the combined use of these treatments would decrease fruit drop while increasing the uniformity of fruit maturity and the proportion of fruit with >50% blush, consequently decreasing harvest labor input and boosting total crop value and profitability. Further research is warranted for assessing the consistency of these results across contrasting environments, different cultivars, as well as after postharvest storage.

Chapter 2: Expression of key ethylene and anthocyanin biosynthetic genes of ‘Honeycrisp’ apples subjected to the combined use of reflective groundcovers and Aminoethoxyvinylglycine in the Mid-Atlantic US

Abstract

Decreased profitability of important apple cultivars such as ‘Honeycrisp’ results from poor red skin coloration and high fruit drop, in the mid-Atlantic US. Apple red skin coloration is determined by anthocyanin concentration. Reflective groundcovers promote red skin coloration, whereas aminoethoxyvinylglycine (AVG) decreases ethylene production and fruit drop, reducing coloration. Although our previous study showed that combinations of these practices impact fruit drop, quality and color, research is lacking on understanding their effects at the gene and metabolite levels. In this work, for two years, we compared differences in internal ethylene concentration (IEC), red skin coloration, transcript accumulation of key ethylene and anthocyanin biosynthetic pathway-related genes, and total anthocyanin concentration of ‘Honeycrisp’. Fruit were treated with combinations of reflective groundcover (Extenday) and AVG (130 mg L^{-1}) and assessed throughout ripening. Extenday-only treated fruit displayed the highest upregulation of ethylene and anthocyanin biosynthetic-related genes, and total anthocyanins, exceeding the required minimum 50% blush, while boosting IEC. In contrast, AVG-only significantly decreased the expression of key ethylene and anthocyanin biosynthetic structural and regulatory genes and total anthocyanins, preventing apples from reaching 50% blush, while decreasing IEC. Combination of Extenday x AVG fine-tuned transcript accumulation of ethylene and anthocyanin biosynthetic-related genes

as well as total anthocyanins, allowing ‘Honeycrisp’ fruit to exceed 50% blush, while increasing IEC moderately (as compared to Extenday-only and control fruit), enhancing fruit economic value.

1. Introduction

Red skin coloration in apple (Malus domestica Borkh) fruit is generally associated with a better marketability, consumer acceptability and a higher profitability (Ban et al., 2007; Musacchi & Serra, 2018). Additionally, at least 50% blush is required for commercialization of economically significant apple cultivars, including the top sales-producing cultivar ‘Honeycrisp’ (Kon & Clavet, 2023; Miah & Farcuh, 2024; USDA Agricultural Marketing Service, n.d.).

Total anthocyanin concentration is a key component of apple red skin coloration (Castañeda-Ovando et al., 2009). Anthocyanins are classified as phenolic compounds resultant from the phenylpropanoid pathway (Feng et al., 2013). Besides to their critical role in apple red skin coloration, anthocyanins also contribute to shielding fruit from photooxidative damage, while can also aid in the removal of free radicals, consequently contributing to reduce cancer and coronary diseases, among others (Boyer & Liu, 2004; Hyson, 2011; Li & Cheng, 2008; Rupasinghe, 2020). The presence of anthocyanins is usually higher in apple skin when compared to other tissues such as fruit flesh (Kunradi Vieira et al., 2009; Łata et al., 2009).

The anthocyanin biosynthesis pathway consists of numerous steps, starting with the precursor phenylalanine. Important enzymes that participate in this pathway comprise phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX), and UDP glucose-flavonoid 3-O-glucosyltransferase (UFGT) (Xie et al., 2011). Furthermore, regulatory genes, such as the transcription factor *MdMYB10*, have been reported to play key roles in red skin coloration by controlling the transcript accumulation of the structural genes involved in the anthocyanin biosynthetic pathway (Ban et al., 2007; Espley et al., 2007, 2009; Ryu et al., 2022; Telias et al., 2011). The expression of *MdMYB10* has been reported to be increased during the accumulation of anthocyanins in apple fruit (Espley et al., 2007).

The biosynthesis of anthocyanins primarily takes place during fruit ripening (Honda et al., 2002). Other factors, such as light (Honda & Moriya, 2018; Lancaster, 1992; Ubi et al., 2006), as well as ethylene production (Blankenship & Unrath, 1988; Farcuh et al., 2022; Honda & Moriya, 2018; Lancaster, 1992; Ubi et al., 2006; Wang & Dilley, 2001; Whale et al., 2008; Whale & Singh, 2007) have been shown to drastically impact anthocyanin concentration. Particularly regarding light, accumulation of anthocyanins and consequently increased red skin coloration has been shown to be affected by wavelength (stimulated via fruit exposure to ultraviolet radiation) and light intensity (Charles & Arul, 2007; Chen et al., 2021; Dong et al., 1995; Honda & Moriya, 2018; Toivonen et al., 2019). Light has been reported to induce the expression of MYB transcription factors as well as of the structural anthocyanin biosynthetic genes (Allan et al., 2008; Ban et al., 2007; Ju et al., 1999; Kondo et al., 2002; Takos et al., 2006; Vimolmangkang et al., 2014; Xu et al., 2012).

Reflective groundcovers have been shown to increase the light that reaches the apple fruit surface by enhancing light reflection (including ultraviolet radiation) from the orchard floor towards the tree canopy (Funke & Blanke, 2021; Kon & Clavet, 2023; Layne et al., 2002; Miah & Farcuh, 2024; Mupambi et al., 2021; J.-P. Privé et al., 2011; Toivonen et al., 2019; Toye, 1995). Consequently, the use of reflective groundcovers has been reported to promote red skin coloration in multiple apple cultivars grown under different environmental conditions (Iglesias & Alegre, 2009; Kon & Clavet, 2023; Layne et al., 2002; Miah & Farcuh, 2024; Miller & Greene, 2003; Mupambi et al., 2021; J. P. Privé et al., 2008; Robinson & Gonzalez, 2023; Toivonen et al., 2019). Although earlier studies have documented the effectiveness of reflective groundcovers on enhancing apple red skin coloration, research to evaluate the effect of this practice on the expression of anthocyanin biosynthetic pathway- related genes and total anthocyanin concentration is scarce and has not been conducted under the environmental conditions of the US mid-Atlantic region.

The production of the hormone ethylene regulates anthocyanin biosynthesis (Blankenship & Unrath, 1988; Chen et al., 2021; Farcuh et al., 2022; Shafiq et al., 2014; Wang & Dilley, 2001; Whale & Singh, 2007). Previous studies have shown that the initiation of anthocyanin biosynthesis and ethylene production concur (Faragher & Brohier, 1984). However, ethylene production can also enhance fruit ripening, directly impacting fruit quality (Burg & Burg, 1965; Farcuh et al., 2017, 2019, 2020; Miah et al., 2023; Miah & Farcuh, 2024), and at the same time can promote apple preharvest fruit drop (Arseneault & Cline, 2016). The biosynthesis of ethylene has been significantly studied in fruit that present a climacteric behavior, such as apple, which display a rise in their rate of respiration and internal ethylene concentration (IEC) as ripening progresses (Brumos, 2021; Costa et al., 2005; Miah et al., 2023). Ethylene biosynthesis is driven by 1-aminocyclopropane-1-carboxylate synthase (ACS) which converts S-adenosyl-L-methionine (SAM) to 1-aminocyclopropane-1-carboxylate (ACC) and subsequently by the action of ACC oxidase (ACO) which drives the oxidation of ACC to ethylene (Cherian et al., 2014; S. F. Yang & Hoffman, 1984). Moreover, IEC has been shown to vary largely within apple cultivars, with high ethylene producing cultivars such as 'Honeycrisp' reported to be subject to higher ripening and preharvest fruit drop rates when compared to other lower ethylene producing cultivars (Arseneault & Cline, 2018; Chu, 1988; Gussman et al., 1993; Irish-Brown et al., 2011).

Aminoethoxyvinylglycine (AVG) is widely used in the apple industry as it been shown to delay apple ripening rates and to reduce preharvest fruit drop by significantly decreasing ethylene production via hampering ACS activity (Arseneault & Cline, 2016; Layne et al., 2002; J. Liu et al., 2022; Whale et al., 2008; S. F. Yang & Hoffman, 1984). Earlier studies have reported that the use of AVG has significantly delayed ripening and decreased preharvest fruit drop, but at the same time has negatively impacted red skin coloration in 'McIntosh', 'Honeycrisp' and 'Gala' cultivars, grown under different environmental conditions (Arseneault & Cline, 2018; Boyacı, 2022; Byers, 1997; Greene, 2005; Greene & Schupp, 2004; Layne et al., 2002; J. Liu et al., 2022; Miah & Farcuh, 2024; Yuan & Li, 2008). Nevertheless, studies assessing the effect of AVG on ethylene and anthocyanin biosynthetic pathway-related genes and total anthocyanin concentration throughout ripening are lacking for mid-Atlantic grown 'Honeycrisp' apples.

Research targeting the effects of the combined use of reflective groundcovers and AVG is limited. In our previous work, we demonstrated that the combination of both horticultural practices could impact fruit drop, quality as well as skin color of 'Honeycrisp' apples grown in the Mid-Atlantic US (Miah & Farcuh, 2024). However, to our knowledge, studies on the impacts of these treatment combinations on 'Honeycrisp' key ethylene and anthocyanin biosynthesis-related transcript accumulation as well as total anthocyanin concentration under our environmental conditions, have not yet been conducted. Based on the above, the aim of the present work was two-fold: first, to characterize and compare differences in IEC, red skin coloration, transcript accumulation of key ethylene and anthocyanin biosynthetic pathway-related genes and transcription factors, as well as total anthocyanin concentration of 'Honeycrisp' apples subjected to treatment combinations of reflective groundcover Extenday and AVG during ripening on the tree in the mid-Atlantic US; and secondly, to use multivariate data analysis to detect important correlations amongst all assessed variables.

2. Materials and Methods

2.1. Plant material and preharvest orchard treatments

This study is a continuation of our previous chapter (Miah & Farcuh, 2024) and in the present work we used the same plant material and preharvest orchard treatments (Table 1.1) as described in chapter 1.

To collect fruit at the optimal commercial maturity stage, fruit maturity indices were examined each year of the study for 'Honeycrisp' fruit during the season (Miah & Farcuh, 2024). Evaluation periods or harvest dates consisted on three different ripening stages on the tree: optimal commercial harvest (CH), 1 week after CH (CH + 1) and 2 weeks after CH (CH + 2). The same number of replications per treatment (four) and number of fruit per replication (twenty-five) as described in our previous work (Miah & Farcuh, 2024) were used in the present study. For each replication, internal ethylene concentration was assessed in five fruit, which were additionally washed, peeled, and for which skin tissue was pooled together, frozen and homogenized in liquid nitrogen, and stored at —80 °C for further analysis; the remaining fruit were used to assess apple skin red coloration.

2.2. Fruit internal ethylene concentration and color measurements

Fruit internal ethylene concentration (IEC) was measured on 1 mL samples of internal gas from the core cavity of each fruit using a gas chromatograph (GC-2014C, Shimadzu Co., Kyoto, Japan) as described before (Farcuh & Hopfer, 2023; Miah et al., 2023; Miah & Farcuh, 2024). Apple fruit red skin coloration was assessed as previously described (Infante et al., 2008; Miah et al., 2023; Miah & Farcuh, 2024).

2.3. Real-time quantitative RT-PCR Analysis

RNA was isolated from apple fruit skin from each of the four replicates for each treatment and at each harvest date using the cetyltrimethylammonium bromide (CTAB)/NaCl method (Chang et al., 1993), with some modifications (Farcuh et al., 2018; Kim et al., 2015). First-strand complementary DNA synthesis, primer design, and quantitative PCR were performed as described before (Kim et al., 2015). The sets of primers used for the amplification of the different target genes are listed in Table S1. Analysis of the relative gene expression was performed according to the Comparative

Cycle Threshold Method (Livak & Schmittgen, 2001). Actin (*MdACT*) was used as a reference gene.

2.4. Total Anthocyanin Quantification

Total anthocyanins in apple fruit skin from each of the four replicates for each treatment and at each harvest date were quantified following the method previously described (Whale & Singh, 2007). Absorbance was recorded at 530 nm using a Cary 60 UV–Vis (Agilent Technologies, Palo Alto, CA, USA) spectrophotometer. Concentration of total anthocyanin in the skin samples were determined by using molar extinction coefficient (i.e., 3.43 x 104) for idaein chloride (Siegelman & Hendricks, 1958) and were expressed as μg g^{-1} fresh weight (i.e., μg g^{-1} FW).

2.5. Statistical analysis

Response variables were modeled using generalized linear mixed models including treatments and ripening stages as fixed factors, and block as a random factor to determine the statistical significance of the interactions and main effects (analysis of variance, ANOVA). When the analysis was statistically significant, separation of means was carried out using Tukey's HSD test at a significance level of 5%.

Pearson's correlation coefficients, using mean-centered data, were calculated for each pairwise-combination of evaluated parameters. PCA, which was applied to reduce the dimensionality of the data, was visualized through a 'biplot' graph, thus representing the relationships among the variables (IEC, skin color measurements, gene expression values, anthocyanin contents) and the assessed treatments and evaluation periods. The Scree test was used to select the number of principal components that captured most of the variation. The software package JMP (ver 15.2, SAS Institute) was used for all the statistical analyses.

3. Results

3.1. Effects of Extenday and AVG treatment combinations on 'Honeycrisp' internal ethylene concentration and red skin coloration

In this study, strong and consistent differences were observed in internal ethylene concentration (IEC) and red skin coloration amongst the different Extenday and AVG treatment combinations for the two evaluated production seasons (Figure 2.1).

For all assayed treatments, IEC values significantly augmented throughout the three assayed ripening stages (Figure 2.1A, D). In general, for each assessed ripening stage, Extenday-only (T2) treated fruit always displayed the highest IEC, followed by control (T4) fruit, next by the combined Extenday + AVG (T1) treatment, while AVG-only (T3) treated fruit presented the statistically lowest IEC. This was consistent in both years, excluding CH in 2022, where T2 and T4 presented no differences (Figure 2.1D).

Red skin coloration significantly increased throughout the three assayed ripening stages for all assessed treatments, in both years, considering surface skin hue angle and skin blush percentage measurements (Figure 2.1B, C, E, F). In general, for each assessed ripening stage, the AVG-only (T3) treated fruit presented the statistically highest values for surface skin hue angle, followed by control (T4) fruit, next by Extenday + AVG (T1), while Extenday-only (T2) presented the lowest values (Figure 2.1B, E). Results for skin blush percentage were consistent with skin hue angle evaluations. For each assessed ripening stage, the Extenday-only (T2) treated fruit always displayed the highest skin blush percentage (> 75%), followed by the combined

Extenday + AVG (T1) treatment (> 50%), next by the control (T4) treated fruit (40-60%), while AVG-only (T3) treated fruit presented the statistically lowest skin blush percentage, only reaching the minimum required 50% blush at the last assessed ripening stage (CH + 2) (Figure 2.1C, F).

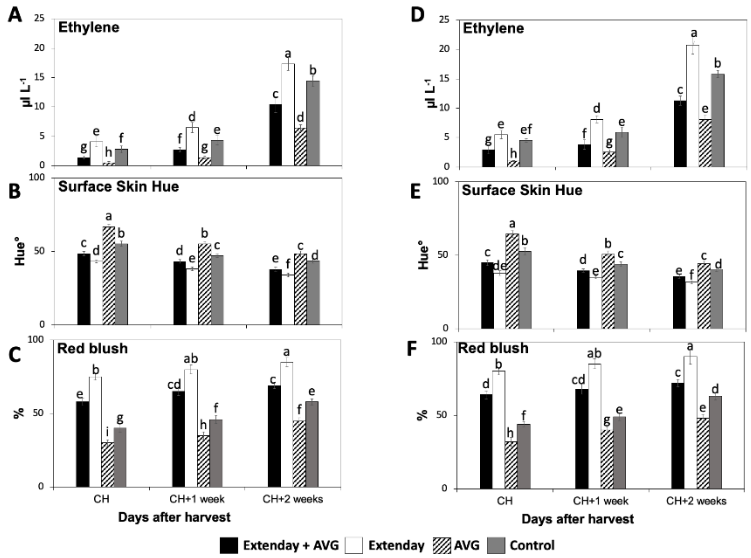

Figure 2.1. Effects of Extenday and AVG combinations on internal ethylene concentration (IEC) and red skin coloration of 'Honeycrisp' fruit grown in Aspers, PA in (A- C) 2021 and (D- F) 2022. Apples were evaluated at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's HSD test.

3.2. Effects of Extenday and AVG treatment combinations on key ethylene and anthocyanin biosynthetic pathway-related genes and transcription factors associated with their regulation

3.2.1. Ethylene biosynthetic genes

The expression of the two key genes involved in the biosynthesis of ethylene, *MdACS1* and *MdACO1* were assessed in 'Honeycrisp' fruit subjected to different Extenday and AVG treatment combinations, for two years.

The expression of *MdACS1* and *MdACO1* significantly increased throughout the three assayed ripening stages for all assessed treatments consistently in both years (Figure 2.2), following a similar pattern as observed for IEC (Figure 2.1A, D). In general, for each assessed ripening stage, Extenday-only (T2) treated fruit always displayed the statistically highest transcript accumulation for *MdACS1* and *MdACO1*, followed by control (T4) fruit, next by the combined Extenday + AVG (T1) treatment, while AVG-only (T3) treated fruit displayed the lowest transcript accumulation for the above-mentioned genes.

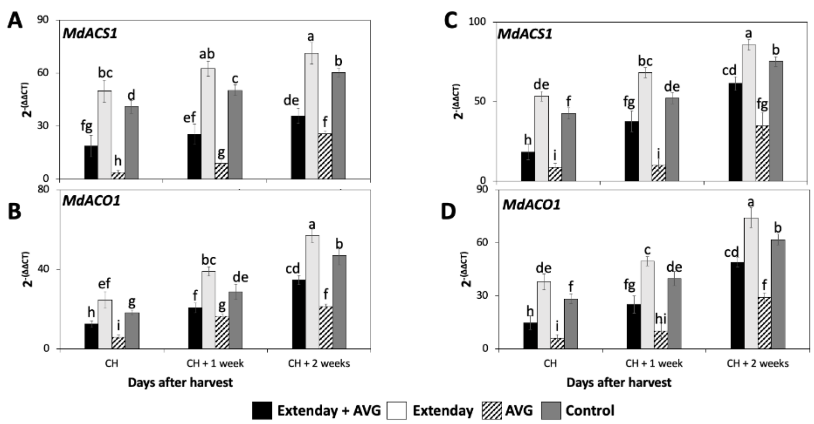

Figure 2.2. Effects of Extenday and AVG combinations on relative gene expression levels of ethylene biosynthetic genes of 'Honeycrisp' fruit grown in Aspers, PA in (A-B) 2021 and (C-D) 2022. Apples were evaluated at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's HSD test. 1-aminocyclopropane- carboxylase synthase (ACS), 1-aminocyclopropane- carboxylase oxidase (ACO).

3.2.2. Anthocyanin biosynthetic genes and transcription factors associated with its regulation

The expression profiles of seven key structural genes involved in anthocyanin biosynthesis, including *MdPAL, MdCHS, MdCHI, MdF3H, MdDFR, MdLDOX* and

MdUFGT, and one key transcription factor, *MdMYB10*, were assessed in 'Honeycrisp' fruit submitted to different Extenday and AVG treatment combinations, for two years.

All key structural genes and the transcription factor that were evaluated in this study for all treatment combinations displayed a significant increase in expression profile throughout the three assayed ripening stages, i.e., from CH to CH + 2 (Figure 2.3). In general, regarding the seven key structural genes, for each assessed ripening stage, the Extenday-only (T2) treated fruit always displayed the statistically higher transcript accumulation, followed by the combined Extenday + AVG (T1) treatment, next by the control (T4) treated fruit, while AVG-only (T3) treated fruit presented the significantly lowest gene expression values. This was consistent in both years, excluding 2021, where, at CH + 2, fruit submitted to T1 displayed no significant differences with respect to T4 for *MdCHI* and *MdDFR* transcript accumulation (Figure 2.3C, E). For 2022, at CH and CH + 1, fruit submitted to T1 presented no differences as compared to T4 for *MdF3H* expression profiles (Figure 2.3L), while at CH + 2, transcript accumulation of *MdDFR* was the same for T1 and T4 (Figure 2.3M).

The transcription factor *MdMYB10*, within each evaluation period, and consistently for both years, followed the same expression pattern as the seven assayed structural anthocyanin biosynthesis genes, i.e., the Extenday-only (T2) treated fruit always displayed the statistically higher transcript accumulation, followed by the combined Extenday + AVG (T1) treatment, next by the control (T4) treated fruit, while AVG-only (T3) treated fruit presented the significantly lowest *MdMYB10* expression values (Figure 2.3H, P). This was consistent in both years, excluding 2022, where at

CH + 1, fruit submitted to T1 and T4 displayed no significant differences between them (Figure 2.3P).

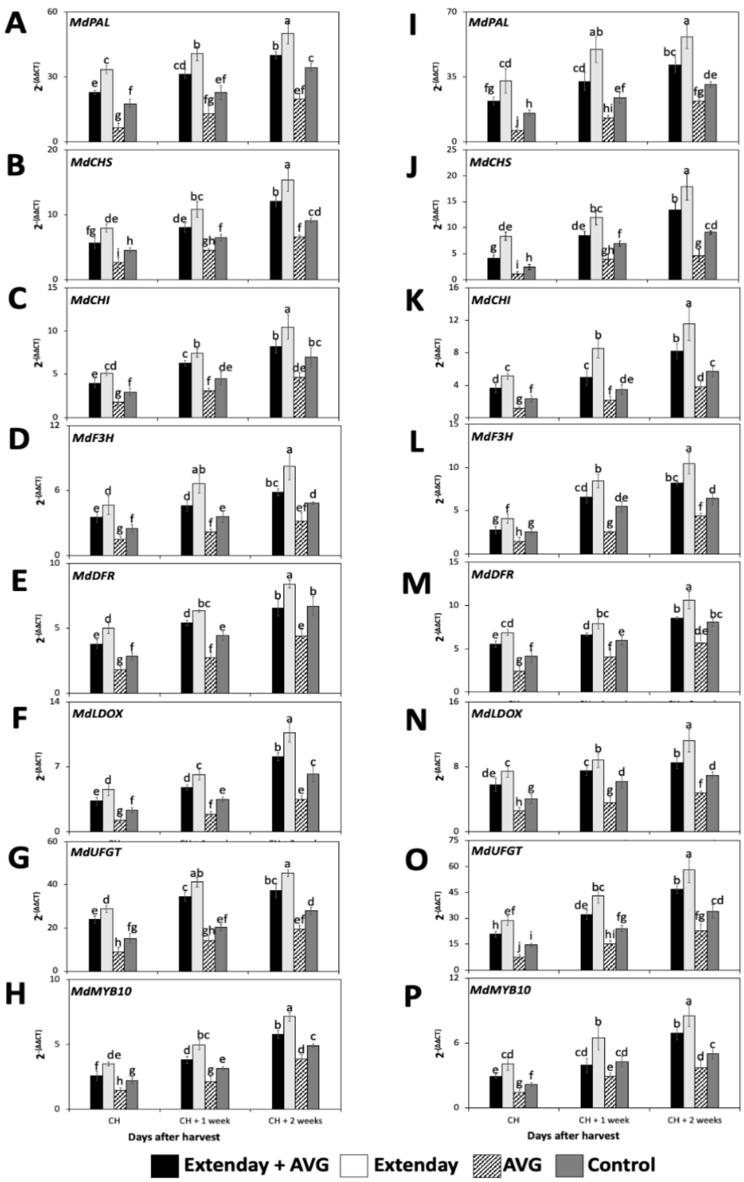

Figure 2.3. Effects of Extenday and AVG combinations on relative gene expression levels of anthocyanin structural biosynthetic genes and *MdMYB10* of 'Honeycrisp' fruit grown in Aspers, PA in (A-H) 2021 and (I-P) 2022. Apples were evaluated at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's HSD test. Phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-

hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX), UDP glucose-flavonoid 3-O-glucosyltransferase (UFGT).

3.3. Effects of Extenday and AVG treatment combinations on 'Honeycrisp' total anthocyanin concentration

Total anthocyanin concentration significantly increased throughout the three assayed ripening stages in this study, resulting in a 1.4 to 2.5-fold increase from CH to CH + 2 considering all treatment combinations in both years (Figure 2.4). At all evaluation periods, the Extenday-only (T2) treated fruit always displayed the significantly highest concentration of total anthocyanins (ranging between 170 and 270 μg g^{-1}), followed by the combined Extenday + AVG (T1) treatment (ranging between 100 and 200 μg g-1), next by the control (T4) treated fruit (ranging between 70 and 130 μg g^{-1}), while AVG-only (T3) treated fruit presented the significantly lowest values (ranging between 40 and 100 μg g^{-1}), consistently in both assayed years (Figure 2.44).

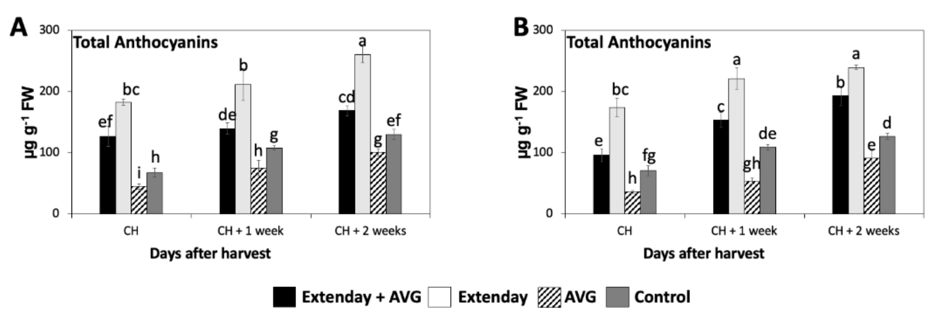

Figure 2.4. Effects of Extenday and AVG combinations on total anthocyanin concentration of 'Honeycrisp' fruit grown in Aspers, PA in (A) 2021 and (B) 2022. Apples were evaluated at optimal commercial harvest (CH), 1 week after CH (CH + 1), and 2 weeks after CH (CH + 2). Values are means ± standard error. Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's HSD test.

3.4. Relationships among ethylene concentration, red skin coloration, key ethylene and anthocyanin biosynthetic- related genes, and anthocyanin

concentration of ‘Honeycrisp’ apple fruit submitted to Extenday and AVG treatment combinations

Calculation of correlation coefficients was performed (Table 2.1) and a Principal Component Analysis (PCA) (Figure 2.5) was conducted considering all the evaluated parameters in this study for ‘Honeycrisp’ apple fruit during 2021 and 2022.

IEC displayed a significant and positive correlation with skin blush ($r = 0.63$), ethylene biosynthetic-related genes (*MdACS1, MdACO1*; $r \geq 0.92$), anthocyanin biosynthetic-related genes (*MdPAL, MdCHS, MdCHI, MdF3H, MdDFR, MdLDOX, MdUFGT*; $r \geq 0.80$) and transcription factors (*MdMYB10*; $r = 0.91$), as well as with total anthocyanin concentration ($r = 0.71$), while IEC was negatively correlated with surface skin hue angle ($r = -0.69$) (Table 2.1).

Amongst the color-related parameters, surface skin hue angle was negatively correlated with skin blush ($r = -0.90$), ethylene biosynthetic-related genes ($r \leq -0.80$), anthocyanin biosynthetic-related genes ($r \leq -0.90$) and *MdMYB10* ($r = -0.90$), as well as with total anthocyanin concentration ($r = -0.90$). On the other hand, skin blush displayed significantly positive correlations with ethylene biosynthetic-related genes ($r \geq 0.72$), anthocyanin biosynthetic-related genes ($r \geq 0.83$) and *MdMYB10* ($r = 0.81$), as well as with total anthocyanin concentration ($r = 0.96$).

Ethylene biosynthetic-related genes presented positive correlations between them ($r = 0.98$), as well as with anthocyanin biosynthetic-related genes ($r \geq 0.84$) and *MdMYB10* ($r \geq 0.90$), and with total anthocyanin concentration ($r \geq 0.81$).

All assayed anthocyanin biosynthetic-related genes positively associated amongst them ($r \geq 0.96$), and with the anthocyanin-related transcription factor *MdMYB10* ($r \geq 0.95$), as well as with total anthocyanin concentration ($r \geq 0.92$).

Additionally, the anthocyanin-related transcription factor *MdMYB10* positively correlated with total anthocyanin concentration (r = 0.90) (Table 2.1).

Table 2.1. Pearson correlation coefficients among all assessed features in ‘Honeycrisp’ fruit including internal ethylene concentration (IEC), red skin coloration, key ethylene and anthocyanin biosynthetic- related genes, and total anthocyanin concentration.

Feature	**IEC**	**Surface Hue**	**Skin Blush**	***ACS1***	***ACO1***	***PAL***	***CHS***	***CHI***	***F3H***	***DFR***	***LDOX***	***UFGT***	***MYB10***	**Total Antho-cyanins**
IEC	1.00	−0.69 *	0.63 *	0.92 *	0.96 *	0.80 *	0.86 *	0.86 *	0.83 *	0.89 *	0.86 *	0.80*	0.91 *	0.71 *
Surface Hue		1.00	−0.90 *	−0.80 *	−0.81 *	−0.90 *	−0.90 *	−0.90 *	−0.90 *	−0.90 *	−0.90 *	−0.90 *	−0.90 *	−0.90 *
Skin Blush			1.00	0.75 *	0.72 *	0.94 *	0.83 *	0.86 *	0.86 *	0.87 *	0.89 *	0.91 *	0.81 *	0.96 *
ACS1				1.00	0.98 *	0.87 *	0.87 *	0.87 *	0.89 *	0.90 *	0.88 *	0.84 *	0.90 *	0.83 *
ACO1					1.00	0.87 *	0.89 *	0.89 *	0.90 *	0.92 *	0.89 *	0.85 *	0.93 *	0.81 *
PAL						1.00	0.96 *	0.97 *	0.98 *	0.97 *	0.98 *	0.99 *	0.95 *	0.98 *
CHS							1.00	0.99 *	0.98 *	0.97 *	0.99 *	0.98 *	0.99 *	0.92 *
CHI								1.00	0.98 *	0.97 *	0.99 *	0.99 *	0.99 *	0.93 *
F3H									1.00	0.96 *	0.97 *	0.98 *	0.97 *	0.94 *
DFR										1.00	0.98 *	0.97 *	0.98 *	0.92 *
LDOX											1.00	0.99 *	0.97 *	0.94 *
UFGT												1.00	0.96 *	0.96 *
MYB10													1.00	0.90 *
Total Anthocyanins														1.00

All correlations shown are significant (*; P ≤0.05).

The first and second principal components of the PCA explained 91.2% (Component 1) and 5.14% (Component 2) of the total observed variation (96.3%) (Figure 2.5). The separation of the different treatment combinations assayed in this study along the first principal component of the PCA was determined by surface skin hue values on the negative side of the axis (associated with treatments Extenday + AVG (T1; at CH), AVG-only (T3; all evaluation periods), and the control (T4; at CH and CH + 1)) and by IEC, skin blush, all assessed ethylene and anthocyanin biosynthetic-related genes, and the *MdMYB10* transcription factor, and total anthocyanin concentration on the positive side of the axis (associated with treatments Extenday + AVG (T1; at CH + 1 and CH + 2), Extenday-only (T2; all evaluation periods), and the control (T4; at CH + 2) (Figure 2.5)).

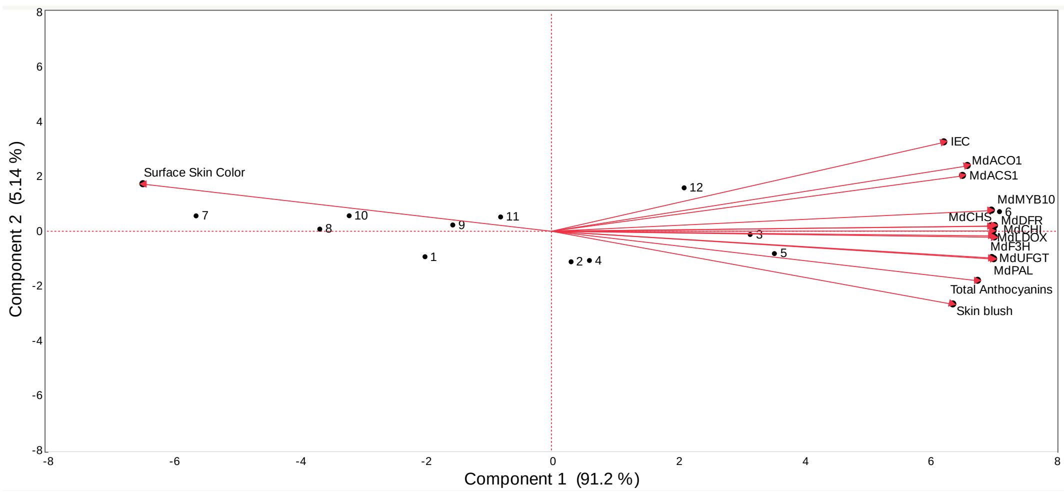

Figure 2.5. Biplot from Principal Component Analysis of data obtained from internal ethylene concentration (IEC), red skin coloration, key ethylene and anthocyanin biosynthetic-related genes, and total anthocyanin concentration of 'Honeycrisp' apples submitted to different Extenday and AVG treatments combinations throughout three ripening stages. Numbers correspond to the different treatments and ripening stages that were assayed (1 (T1_CH), 2 (T1_CH + 1), 3 (T1_CH + 2), 4 (T2_CH), 5 (T2_CH + 1), 6 (T2_CH + 2), 7 (T3_CH), 8 (T3_CH + 1), 9 (T3_CH + 2), 10 (T4_CH), 11 (T4_CH + 1), 12 (T4_CH + 2)). Codes for genes are described in Figures 2.2 and 2.3.

4. Discussion

Anthocyanin accumulation is of major significance throughout apple fruit ripening due to its direct influence on red skin coloration, which is associated with a higher apple acceptability and profitability (Miah & Farcuh, 2024; Musacchi & Serra, 2018). Anthocyanin concentration has been shown to be significantly impacted by light (Honda & Moriya, 2018; Lancaster, 1992; Ubi et al., 2006) as well as by ethylene production (Blankenship & Unrath, 1988; Farcuh et al., 2022; Wang & Dilley, 2001; Whale et al., 2008; Whale & Singh, 2007), among others. In terms of light, the reflective groundcover Extenday has been shown to increase the light that reaches the apple fruit surface (Layne et al., 2002; Miah & Farcuh, 2024; J.-P. Privé et al., 2011) and thus enhance red skin coloration in apples (Iglesias & Alegre, 2009; Kon & Clavet, 2023; Layne et al., 2002; Miah & Farcuh, 2024; Miller & Greene, 2003; Mupambi et al., 2021; J. P. Privé et al., 2008; Robinson & Gonzalez, 2023; Toivonen et al., 2019), but to also increase ethylene concentration, hastening fruit maturity (Crisosto et al., 1999; Layne et al., 2001; Overbeck et al., 2013). In terms of ethylene, the use of AVG, which has been shown to delay apple ripening rates and to reduce preharvest fruit drop by significantly decreasing ethylene production, has also been reported to negatively impact apple red skin coloration (Arseneault & Cline, 2016; Boyacı, 2022; Greene, 2005; Greene & Schupp, 2004; Layne et al., 2002; J. Liu et al., 2022; Miah & Farcuh, 2024; Wang & Dilley, 2001). In our previous chapter (Miah & Farcuh, 2024) we demonstrated that the combined use of Extenday and AVG treatments could impact fruit drop, quality, as well as skin color of 'Honeycrisp' apples grown under the hot and humid weather of the mid-Atlantic. However, it is still unknown how these treatment combinations impact expression of key ethylene and anthocyanin biosynthetic genes as well as total anthocyanin concentration of 'Honeycrisp' apples in the mid-Atlantic US. In this work, the combined use of the reflective groundcover Extenday and the plant growth regulator AVG enhanced total anthocyanin concentration as well as expression of key anthocyanin biosynthetic genes and the transcription factor *MdMYB10*, translating into an increased red skin coloration (> 50% blush at CH); moreover, it didn't significantly promote transcript accumulation of key ethylene biosynthetic-related genes nor ethylene concentration to the levels observed in fruit subjected to

Extenday-only or control treatments. The later can explain the lack of fruit overripening we observed in our previous study (Miah & Farcuh, 2024) in 'Honeycrisp' fruit harvested from the lower third of the canopy and subjected to the combined Extenday and AVG treatment (T1). These results were consistent throughout two consecutive years of study.

4.1 The use of Extenday upregulates the expression of anthocyanin regulatory and structural genes during fruit ripening

The biosynthesis of anthocyanins in red apple cultivars such as 'Honeycrisp' has been shown to be regulated at the developmental level and to primarily take place during fruit ripening (Honda et al., 2002). It has been reported that there is a progressive increase in total anthocyanin accumulation (Ubi et al., 2006) as well as in the expression profiles of the key structural genes (*MdCHS, MdF3H, MdDFR, MdLDOX* and *MdUFGT*) and transcription factors (*MdMYB10*) involved in anthocyanin biosynthesis (Chen et al., 2021; Kondo et al., 2002) during fruit ripening. This is consistent with the results for all assayed treatments in this study. Nonetheless, Extenday-only treated fruit displayed the highest expression levels for anthocyanin biosynthetic-related genes, followed by Extenday + AVG treatment, while the lowest expression was for AVG-only. These results suggest that the degree of upregulation of all assayed structural genes for anthocyanin synthesis and for *MdMYB10*, within each examined ripening stage, varied significantly with the use of different treatment combinations of these horticultural practices.

The anthocyanin production in apple skin requires the presence of light reaching the surface of the fruit (Feng et al., 2013; Lancaster, 1992; Saure, 1990). In apples, as well as in other fruit, ultraviolet radiation is known to stimulate fruit anthocyanin biosynthesis (Charles & Arul, 2007; Dong et al., 1995; Honda & Moriya, 2018; Toivonen et al., 2019). Previous research has shown that the use of the reflective groundcover Extenday significantly increases the reflected ultraviolet radiation from the orchard floor towards the tree canopy (Kon & Clavet, 2023; Miah & Farcuh, 2024; Toivonen et al., 2019). The increased presence of light in the tree canopy can induce anthocyanin biosynthesis and accumulation in apples indirectly, by boosting photosynthesis and increasing the supply of assimilates diverted to sink tissues such as fruit, which consequently provide the substrate for anthocyanin biosynthesis; or directly, via Extenday promoting anthocyanin biosynthesis, as it is known that the majority of the anthocyanin biosynthetic genes and enzymes are significantly upregulated by light (Chen et al., 2021; Ju et al., 1999; Vimolmangkang et al., 2014). The latter is in agreement with our results, as the expression levels of the seven assayed structural genes (*MdPAL, MdCHS, MdCHI, MdF3H, MdDFR, MdLDOX* and *MdUFGT*) and the key transcription factor *MdMYB10* involved in anthocyanin biosynthesis were markedly and coordinately upregulated in Extenday-only treated fruit, followed by the combined Extenday + AVG treatment, as compared to the non-Extenday treatments, which displayed the lowest transcript accumulation. Similar results were also observed in 'Ambrosia' apples subjected to Extenday treatment during ripening (Toivonen et al., 2019). Consistent with our results, it has been reported that when comparing apples subjected to higher and lower light intensity treatments, there was a significantly increased expression of anthocyanin biosynthetic-related genes in the former than in the latter conditions (J.-P. An et al., 2020).

4.2 Extenday enhances expression of transcription factor *MdMYB10* which controls the expression of structural anthocyanin biosynthesis genes

Regulatory genes, such as the transcription factor *MdMYB10*, have been reported to be critical in apple red skin coloration as they control the expression levels of the structural genes involved in the anthocyanin biosynthetic pathway (Ban et al., 2007; Espley et al., 2007, 2009; Ryu et al., 2022; Telias et al., 2011). This is supported by the positive correlations obtained amongst *MdMYB10* and all assayed structural anthocyanin biosynthetic-related genes in this work. Furthermore, and consistent with previous studies in apples (Honda et al., 2002; Lister et al., 1996; Toivonen et al., 2019) there was a positive correlation between total anthocyanin concentration and the transcript accumulation of the assayed structural genes involved in anthocyanin biosynthesis and *MdMYB10*. Moreover, the expression levels of these key anthocyanin biosynthesis-related genes displayed corresponding increases in total anthocyanin concentration and in red skin coloration (supported by their positive correlations with skin blush and negative correlations with surface skin hue angle). This is consistent with reports in 'Ambrosia' apple subjected to Extenday deployment (Toivonen et al., 2019) and with 'Fortune' apple fruit in response to increased sunlight exposure (Feng et al., 2013). Likewise, in 'Gala' apples anthocyanin accumulation has been shown to be enhanced with the use of Extenday (Overbeck et al., 2013).

4.3. The combined Extenday and Aminoethoxyvinylglycine treatment fine-tunes transcript accumulation of ethylene and anthocyanin biosynthesis- related genes

Ethylene production is also known to play a role in regulating the accumulation of anthocyanins (Blankenship & Unrath, 1988; Chen et al., 2021; Faragher & Brohier, 1984; Farcuh et al., 2022; Shafiq et al., 2014; Wang & Dilley, 2001; Whale & Singh, 2007). This is supported by the positive correlations obtained in this study amongst IEC with ethylene and anthocyanin biosynthetic-related gene expression, *MdMYB10* transcript profiles, total anthocyanin concentration as well as with skin blush, and by the negative correlation between IEC and surface skin hue angle. The use of reflective groundcovers has been previously shown to increase IEC, boosting anthocyanin accumulation and thus red skin coloration, but also hastening fruit maturity and therefore fruit overripening (Ju et al., 1999; Layne et al., 2001; Miah & Farcuh, 2024; Overbeck et al., 2013). Inversely, the use of AVG, which hinders the activity of ACS activity (Arseneault & Cline, 2016; J. Liu et al., 2022), can reduce IEC, delaying fruit maturity as well as red skin coloration, as has been shown in several apple cultivars such as 'Gala', 'Jonagold' and 'Cripps Pink' (Layne et al., 2002; J. Liu et al., 2022; Phan-Thien et al., 2004; Wang & Dilley, 2001), 'McIntosh' (Stover et al., 2003), 'Red Delicious' and 'Red Chief' (Boyacı, 2022) as well as 'Honeycrisp' (Arseneault & Cline, 2018; Miah & Farcuh, 2024). Consistent with our results, the AVG-only and the Extenday-only treated apples presented the significantly lowest and highest transcript accumulation for *MdACS1* and *MdACO1*, as well as for all assayed anthocyanin biosynthetic-related genes and total anthocyanin concentration at all stages, respectively. These results support the differences obtained in IEC between these treatments, as well as explain the delay of the fruit subjected to the AVG-only treatment to reach the required minimum 50% skin blush (which was only reached at CH + 2). The combined Extenday + AVG treatment displayed gene expression profile values that were positioned in-between the above-mentioned treatments, which for *MdACS1* and *MdACO1* were only higher than AVG-only treated fruit, and for anthocyanin biosynthetic-related genes and total anthocyanin concentration were higher than control and AVG-only treated fruit, but lower than Extenday-only treated apples. Nevertheless, the combined Extenday + AVG treated fruit still reached the required 50% blush at CH, therefore, emphasizing a potential interactive effect of both horticultural practices. In fact, a synergistic interaction between *MdMYB10* and the ethylene biosynthesis genes, *MdACS1* and *MdACO1*, has been reported, which can then activate the downstream structural genes associated to the anthocyanin biosynthetic pathway leading to anthocyanin accumulation (J.-P. An et al., 2018; Awad & De Jager, 2002; Wang & Dilley, 2001; Yu et al., 2022). Our results suggest that the degree of fine-tunning in transcript accumulation of ethylene and anthocyanin biosynthesis-related genes in the combined Extenday + AVG treatment allows 'Honeycrisp' to comply with the required 50% blush at CH and at the same time to advance their maturity throughout ripening, while avoiding overripening or increased fruit drop, as shown to occur with fruit subjected to Extenday-only in our previous study (Miah & Farcuh, 2024).

4.4 Extenday and Aminoethoxyvinylglycine treatment combinations on 'Honeycrisp' fruit ethylene concentration, total anthocyanin concentration, and transcript accumulation of ethylene and anthocyanin biosynthesis pathway-related genes

Concerning the PCA, the positioning of the different treatment combinations/ ripening stages along component 1 is supported by the AVG-only treated 'Honeycrisp' fruit exhibiting the significantly lowest *MdACS1* and *MdACO1* expression levels, IEC, red skin coloration, transcript accumulation of anthocyanin biosynthetic-related genes, and total anthocyanin concentration in all assessed ripening stages; by the fruit subjected to the combined Extenday + AVG treatment presenting a transitional positioning in terms of ethylene biosynthesis-related gene expression and IEC, but still reaching the required minimum 50% blush at CH, explained by the increased transcript accumulation of anthocyanin biosynthesis-related genes and total anthocyanin concentration; by the control fruit displaying a significant upregulation of *MdACS1* and *MdACO1* and increased IEC as compared to the AVG-only and Extenday + AVG treatments, but with a fruit blush that does not reach the minimum 50% requirement at CH explained by a decreased expression level of anthocyanin biosynthetic-related genes, *MdMYB10* and total anthocyanin contents as compared to the Extenday treatments; and by the fruit subjected to the Extenday-only treatment showing the significantly highest *MdACS1* and *MdACO1* expression levels, IEC, red skin coloration, transcript accumulation of anthocyanin biosynthetic-related genes, and total anthocyanin concentration in all assayed ripening stages. However, and as reported in our previous work (Miah & Farcuh, 2024), Extenday-only treated fruit also displayed the most advanced fruit maturity, i.e. overripening, followed by control 'Honeycrisp' fruit, with both treatments exhibiting the highest preharvest fruit drop under US mid-Atlantic conditions. The effect of these treatment combinations on other economically important apple cultivars besides 'Honeycrisp' grown under environmental conditions different than the mid-Atlantic US is currently under investigation.

5. Conclusions

Overall, treatment combinations of the reflective groundcover Extenday and the plant growth regulator AVG, assayed for two production seasons, significantly altered key ethylene and anthocyanin biosynthesis-related gene expression, transcript accumulation of the transcription factor *MdMYB10* as well as total anthocyanin concentration, throughout ripening of ‘Honeycrisp’ apples grown in the mid-Atlantic US. Correspondingly, these treatments impacted red skin coloration and IEC. Extenday-only treated fruit displayed the highest upregulation of ethylene and anthocyanin biosynthetic-related genes, *MdMYB10* as well as of total anthocyanin concentration, allowing apples to exceed the required minimum 50% blush at all assayed stages, while also promoting IEC, to a level that has been shown to induce overripening. On the other hand, AVG-only treated fruit exhibited the significantly lowest expression of key ethylene and anthocyanin biosynthetic structural and regulatory genes together with total anthocyanin concentration, preventing apples to reach the required minimum 50% blush until the last assayed ripening stage (CH + 2), while also decreasing IEC, which has been shown to significantly delay ripening. Fruit treated with the combined use of Extenday x AVG fine-tuned the transcript accumulation of ethylene and anthocyanin biosynthetic-related genes as well as of total anthocyanin concentration. ‘Honeycrisp’ fruit under Extenday x AVG treatment reached the required 50% blush at CH, but moderately increased IEC (as compared to only-Extenday and control treatments) advancing their maturity throughout ripening, while avoiding overripening, as previously shown. Hence, increasing ‘Honeycrisp’ market value and acceptability.

Overall conclusion and future directions

The research indicates that utilizing Extenday and AVG treatments can significantly influence the quality of apple fruit, including its red skin coloration, maturity, and susceptibility to preharvest fruit drop. While Extenday treatment alone enhances red skin color and accelerates fruit maturation, it also poses the risk of overripening. Conversely, AVG treatment delays maturation and reduces fruit drop. However, combining Extenday and AVG treatments synergistically reduces fruit drop, adjusts (fine-tune) the expression of crucial genes involved in ethylene and anthocyanin production, and enhances red skin coloration without compromising fruit quality (Figure 2.6, 2.7). These findings suggest promising aspects of optimizing fruit production techniques to enhance crop yield and profitability. Nonetheless, further research is necessary to validate these findings across various environmental conditions, apple cultivars, and postharvest storage scenarios. Additionally, exploring the long-term implications and economic viability of these treatments would be beneficial for their practical implementation in apple orchards.

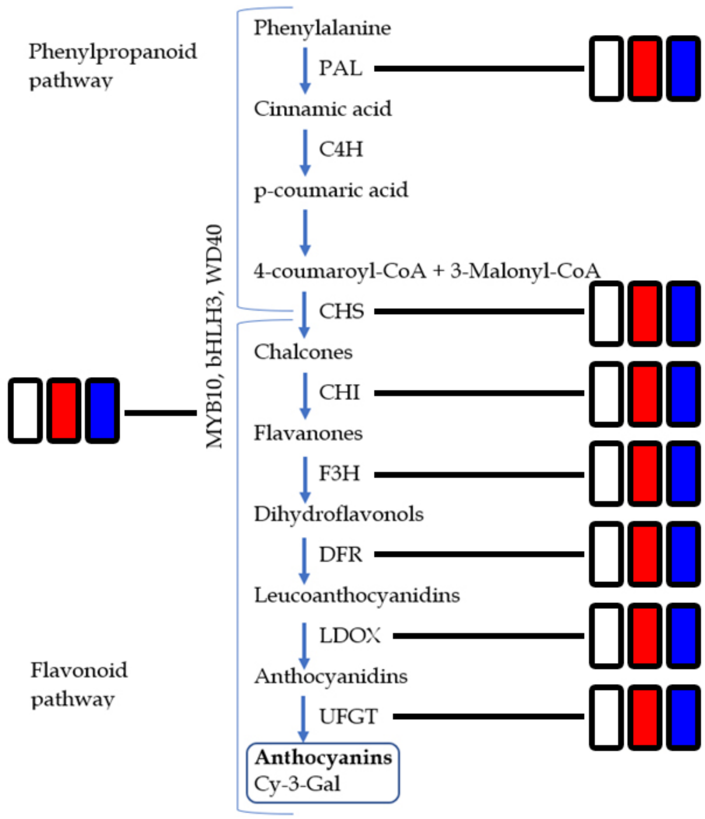

Figure 2.6. Schematic summary of anthocyanin biosynthesis related pathway genes expression submitted to T1 (Extenday+AVG), T2 (Extenday-only), and T3 (AVG-only). Thick triple bars indicate T1, T2 and T3, respectively from left to right. While white, red or blue color of the thick bars indicate gene expression levels that are fine-tuned, induced or reduced, respectively.

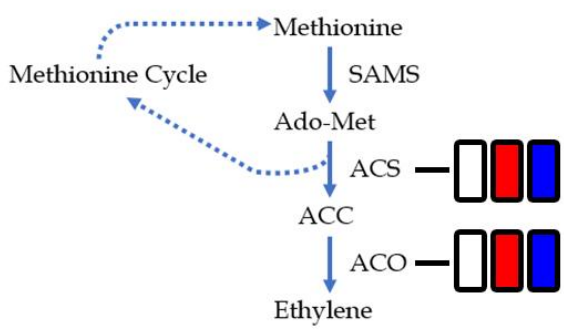

Figure 2.7. Schematic summary of ethylene biosynthesis related pathway genes expression submitted to T1 (Extenday+AVG), T2 (Extenday-only), and T3 (AVG-only). Thick triple bars indicate T1, T2 and T3, respectively from left to right. While white, red or blue color of the thick bars indicate gene expression levels that are fine-tuned, induced or reduced, respectively.

Appendices

Supplementary Table S1. Primers used in qRT-PCR

Gene name	Description	Primer orientation	Primer sequence (5' to 3')
MdPAL	Phenylalanine ammonia-lyase	Forward	GTGCTGTGGAGTCCCCGCTT
		Reverse	GGTGA GGCTCTCTCCGCCAAGT
MdCHS	Chalcone synthase	Forward	GGAGACAACTGGAGAAGGACTGGAA
		Reverse	CGACATTGATACTGGTGTCTTCA
MdCHI	Chalcone isomerase	Forward	GGGATAACCTCGCGGCCAAA
		Reverse	GCATCCATGCCGGAAGCTACAA
MdF3H	Flavanone 3-hydroxylase	Forward	TGGAAGCTTGTGAGGACTGGGGT
		Reverse	CTCCTCCGATGGCAAATCAAAGA
MdDFR	Dihydroflavonol 4-reductase	Forward	GATAGGGTTTGAGTTCAAGTA
		Reverse	TCTCCTCAGCAGCCTCAGTTTTCT
MdLDOX	Leucoanthocyanidin dioxygenase	Forward	CCAAGTGAAGCGGGTTGTGCT
		Reverse	CAAAGCAGGCGGACAGGAGTAGC
MdUFGT	UDP glucose-flavonoid 3- o -glucosyl transferase	Forward	CCACCGCCCTTCCAAACACTCT
		Reverse	CACCCTTATGTTACGCGGCATGT
MdMYB10	Transcription factor	Forward	TGCCTGGACTCGAGAGGAAGACA
		Reverse	CCTGTTTCCCAAAAGCCTGTGAA
MdACS1	1-aminocyclopropane-carboxylase (ACC) synthase	Forward	CTCCTCCTTTCCTTCGTTGA
		Reverse	ACCATGTCGTCGTTGGAGTAG
MdACO1	ACC oxidase	Forward	ATCAATGATGCTTGTGAGAACTG
		Reverse	GGTCTTCTTGTAGTGATCCTTGG
MdACT	Actin	Forward	TGACCGAATGAGCAAGGAAATTACT
		Reverse	TACTCAGCTTTGGCAATCCACATC

Bibliography

Allan, A. C., Hellens, R. P., & Laing, W. A. (2008). MYB transcription factors that colour our fruit. *Trends in Plant Science*, *13*(3), 99–102.

An, J.-P., Liu, Y.-J., Zhang, X.-W., Bi, S.-Q., Wang, X.-F., You, C.-X., & Hao, Y.-J. (2020). Dynamic regulation of anthocyanin biosynthesis at different light intensities by the BT2-TCP46-MYB1 module in apple. *Journal of Experimental Botany*, *71*(10), 3094–3109.

An, J.-P., Wang, X.-F., Li, Y.-Y., Song, L.-Q., Zhao, L.-L., You, C.-X., & Hao, Y.-J. (2018). EIN3-LIKE1, MYB1, and ETHYLENE RESPONSE FACTOR3 act in a regulatory loop that synergistically modulates ethylene biosynthesis and anthocyanin accumulation. *Plant Physiology*, *178*(2), 808–823.

An, X. H., Tian, Y., Chen, K. Q., Wang, X. F., & Hao, Y. J. (2012). The apple WD40 protein MdTTG1 interacts with bHLH but not MYB proteins to regulate anthocyanin accumulation. *Journal of Plant Physiology*, *169*(7), 710–717. https://doi.org/10.1016/J.JPLPH.2012.01.015

Arseneault, M. H., & Cline, J. A. (2016). A review of apple preharvest fruit drop and practices for horticultural management. *Scientia Horticulturae*, *211*, 40–52.

Arseneault, M. H., & Cline, J. A. (2018). AVG, NAA, boron, and magnesium influence preharvest fruit drop and fruit quality of ‘Honeycrisp’apples. *Canadian Journal of Plant Science*, *98*(3), 741–752.

Awad, M. A., & De Jager, A. (2002). Formation of flavonoids, especially anthocyanin and chlorogenic acid in ‘Jonagold’ apple skin: influences of growth regulators and fruit maturity. *Scientia Horticulturae*, *93*(3–4), 257–266. https://doi.org/10.1016/S0304-4238(01)00333-8

Ban, Y., Honda, C., Hatsuyama, Y., Igarashi, M., Bessho, H., & Moriguchi, T. (2007). Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin. *Plant and Cell Physiology*, *48*(7), 958–970.

Bassett, C. L., & Glenn, D. M. (2014). Reflective Films and Expression of Light-regulated Genes in Field-grown Apple. In *J. AMER. SOC. HORT. SCI* (Vol. 139, Issue 4).

Baugher, T. A., & Schupp, J. R. (2010). Relationship Between’Honeycrisp’Crop Load and Sensory Panel Evaluations of the Fruit. *Journal of the American Pomological Society*, *64*(4), 226.

Bhatt, A. K., & Pant, D. (2015). Automatic apple grading model development based on back propagation neural network and machine vision, and its performance evaluation. *AI and Society*, *30*(1), 45–56. https://doi.org/10.1007/s00146-013-0516-5

Biggs, A. R., & Peck, G. M. (2015). Managing Bitter Pit in ‘Honeycrisp’ Apples Grown in the Mid-Atlantic United States with Foliar-applied Calcium Chloride and Some Alternatives. *HortTechnology Hortte*, *25*(3), 385–391. https://doi.org/10.21273/HORTTECH.25.3.385

Bisson, M., Kessenbrock, M., Müller, L., Hofmann, A., Schmitz, F., Cristescu, S. M., & Groth, G. (2016). Peptides interfering with protein-protein interactions in the ethylene signaling pathway delay tomato fruit ripening. *Scientific Reports*, *6*(1),

1–11.
Blankenship, S. M., & Unrath, C. R. (1988). PAL and ethylene content during maturation of red and golden delicious apples. *Phytochemistry*, *27*(4), 1001–1003.
Blanpied, G. D., & Silsby, J. (1992). Predicting Harvest Date Windows for Apples. In *A cornell cooperative extension* (pp. 7–9). Cornell Cooperative Extension.
Bonghi, C., Tonutti, P., & Ramina, A. (2000). Biochemical and molecular aspects of fruitlet abscission. *Plant Growth Regulation*, *31*, 35–42.
Bouzayen, M., Latché, A., Nath, P., & Pech, J. C. (2010). Mechanism of Fruit Ripening. In E. C. Pua & M. R. Davey (Eds.), *Plant Developmental Biology - Biotechnological Perspectives: Volume 1* (pp. 319–339). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-02301-9_16
Boyacı, S. (2022). Effect of Aminoethoxyvinylglycine (AVG) Applications on Pre-Harvest Drop and Fruit Quality of 'Red Delicious, Red Chief'Apple Cultivar. *Erwerbs-Obstbau*, *64*(3), 395–400.
Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal*, *3*(1), 1–15.
Brumos, J. (2021). Gene regulation in climacteric fruit ripening. *Current Opinion in Plant Biology*, *63*, 102042.
Burg, S. P., & Burg, E. A. (1965). Ethylene action and the ripening of fruits: Ethylene influences the growth and development of plants and is the hormone which initiates fruit ripening. *Science*, *148*(3674), 1190–1196. https://doi.org/10.1126/science.148.3674.1190
Byers, R. E. (1997). Effects of aminoethoxyvinylglycine (AVG) on preharvest fruit drop, maturity, and cracking of several apple cultivars. *Journal of Tree Fruit Production*, *2*(1), 77–97.
Castañeda-Ovando, A., Pacheco-Hernández, M. de L., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. In *Food Chemistry* (Vol. 113, Issue 4, pp. 859–871). Elsevier. https://doi.org/10.1016/j.foodchem.2008.09.001
Chang, S., Puryear, J., & Cairney, J. (1993). A simple and efficient method for isolating RNA from pine trees. *Plant Molecular Biology Reporter*, *11*(2), 113–116. https://doi.org/10.1007/BF02670468
Charles, M. T., & Arul, J. (2007). UV treatment of fresh fruits and vegetables for improved quality: a status report. *Stewart Postharvest Review*, *3*(3), 1–8.
Chen, Z., Yu, L., Liu, W., Zhang, J., Wang, N., & Chen, X. (2021). Research progress of fruit color development in apple (Malus domestica Borkh.). *Plant Physiology and Biochemistry*, *162*, 267–279.
Cheng, Y., Liu, L., Yuan, C., & Guan, J. (2016). Molecular Characterization of Ethylene-Regulated Anthocyanin Biosynthesis in Plums During Fruit Ripening. *Plant Molecular Biology Reporter*, *34*(4), 777–785. https://doi.org/10.1007/s11105-015-0963-x
Cherian, S., Figueroa, C. R., & Nair, H. (2014). 'Movers and shakers' in the regulation of fruit ripening: a cross-dissection of climacteric versus non-climacteric fruit. *Journal of Experimental Botany*, *65*(17), 4705–4722.
Chu, C. L. (1988). Internal ethylene concentration of 'McIntosh','Northern

Spy’,‘Empire’,‘Mutsu’, and ‘Idared’apples during the harvest season. *Journal of the American Society for Horticultural Science*, *113*(2), 226–229.
Costa, F., Stella, S., Van de Weg, W. E., Guerra, W., Cecchinel, M., Dallavia, J., Koller, B., & Sansavini, S. (2005). Role of the genes Md-ACO1 and Md-ACS1 in ethylene production and shelf life of apple (Malus domestica Borkh). *Euphytica*, *141*, 181–190.
Crisosto, C. H., Mitchell, F. G., & Ju, Z. (1999). Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. *HortScience*, *34*(6), 1116–1118. https://doi.org/10.21273/HORTSCI.34.6.1116
Dias, C., Ribeiro, T., Rodrigues, A. C., Ferrante, A., Vasconcelos, M. W., & Pintado, M. (2021). Improving the ripening process after 1-MCP application: Implications and strategies. *Trends in Food Science & Technology*, *113*, 382–396. https://doi.org/https://doi.org/10.1016/j.tifs.2021.05.012
do Amarante, C. V. T., Argenta, L. C., de Freitas, S. T., & Steffens, C. A. (2022). Efficiency of pre-harvest application of 1-MCP (Harvista^{TM} 1.3 SC) to delay maturation of ‘Cripps Pink’apple fruit. *Scientia Horticulturae*, *293*, 110715.
Dong, Y., Mitra, D., Kootstra, A., Lister, C., & Lancaster, J. (1995). Postharvest stimulation of skin color in Royal Gala apple. *Journal of the American Society for Horticultural Science*, *120*(1), 95–100.
Drake, S. R., & Eisele, T. A. (1994). *Influence of Harvest Date and Controlled Atmosphere Storage Delay on the Color and Quality of “Delicious” Apples Stored in a Purge-type Controlled-atmosphere Environment* (Vol. 4, Issue 3).
Espley, R. V, Brendolise, C., Chagne, D., Kutty-Amma, S., Green, S., Volz, R., Putterill, J., Schouten, H. J., Gardiner, S. E., & Hellens, R. P. (2009). Multiple repeats of a promoter segment causes transcription factor autoregulation in red apples. *The Plant Cell*, *21*(1), 168–183.
Espley, R. V, Hellens, R. P., Putterill, J., Stevenson, D. E., Kutty-Amma, S., & Allan, A. C. (2007). Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. *The Plant Journal*, *49*(3), 414–427.
Faragher, J. D., & Brohier, R. L. (1984). Anthocyanin accumulation in apple skin during ripening: Regulation by ethylene and phenylalanine ammonia-lyase. *Scientia Horticulturae*, *22*(1–2), 89–96. https://doi.org/10.1016/0304-4238(84)90087-6
Farcuh, M., Copes, B., Le-Navenec, G., Marroquin, J., Cantu, D., Bradford, K. J., Guinard, J.-X., & Van Deynze, A. (2020). Sensory, physicochemical and volatile compound analysis of short and long shelf-life melon (Cucumis melo L.) genotypes at harvest and after postharvest storage. *Food Chemistry: X*, *8*. https://doi.org/10.1016/j.fochx.2020.100107
Farcuh, M., & Hopfer, H. (2023). Aroma volatiles as predictors of chilling injury development during peach (Prunus persica (L) Batsch) cold storage and subsequent shelf-life. *Postharvest Biology and Technology*, *195*, 112137.
Farcuh, M., Li, B., Rivero, R. M., Shlizerman, L., Sadka, A., & Blumwald, E. (2017). Sugar metabolism reprogramming in a non-climacteric bud mutant of a climacteric plum fruit during development on the tree. *Journal of Experimental Botany*, *68*(21–22). https://doi.org/10.1093/jxb/erx391
Farcuh, M., Rivero, R. M., Sadka, A., & Blumwald, E. (2018). Ethylene regulation of

sugar metabolism in climacteric and non-climacteric plums. *Postharvest Biology and Technology*, *139*. https://doi.org/10.1016/j.postharvbio.2018.01.012
Farcuh, M., Tajima, H., Lerno, L. A., & Blumwald, E. (2022). Changes in ethylene and sugar metabolism regulate flavonoid composition in climacteric and non-climacteric plums during postharvest storage. *Food Chemistry: Molecular Sciences*, 100075.
Farcuh, M., Toubiana, D., Sade, N., Rivero, R. M., Doron-Faigenboim, A., Nambara, E., Sadka, A., & Blumwald, E. (2019). Hormone balance in a climacteric plum fruit and its non-climacteric bud mutant during ripening. *Plant Science*, *280*. https://doi.org/10.1016/j.plantsci.2018.11.001
Feng, F., Li, M., Ma, F., & Cheng, L. (2013). Phenylpropanoid metabolites and expression of key genes involved in anthocyanin biosynthesis in the shaded peel of apple fruit in response to sun exposure. *Plant Physiology and Biochemistry*, *69*, 54–61.
Funke, K., & Blanke, M. (2021). Spatial and temporal enhancement of colour development in apples subjected to reflective material in the Southern Hemisphere. *Horticulturae*, *7*(1), 2.
Gao, H. N., Jiang, H., Cui, J. Y., You, C. X., & Li, Y. Y. (2021). Review: The effects of hormones and environmental factors on anthocyanin biosynthesis in apple. *Plant Science*, *312*, 111024. https://doi.org/10.1016/J.PLANTSCI.2021.111024
Glenn, D. M., & Puterka, G. J. (2007). The Use of Plastic Films and Sprayable Reflective Particle Films to Increase Light Penetration in Apple Canopies and Improve Apple Color and Weight. *HortScience Horts*, *42*(1), 91–96. https://doi.org/10.21273/HORTSCI.42.1.91
Greene, D. W. (2002). Preharvest drop control and maturity of 'Delicious' apples as affected by aminoethoxyvinylglycine (AVG). *Journal of Tree Fruit Production*, *3*(1), 1–10.
Greene, D. W. (2005). Time of aminoethoxyvinylglycine application influences preharvest drop and fruit quality of'McIntosh'apples. *HortScience*, *40*(7), 2056–2060.
Greene, D. W., & Schupp, J. R. (2004). Effect of Aminoethoxyvinylglycine (AVG) on Preharvest Drop, Fruit Quality, and Maturation ofMcIntosh'Apples. II. Effect of Timing and Concentration Relationships and Spray Volume. *HortScience*, *39*(5), 1036–1041.
Grierson, D. (2013). Ethylene and the Control of Fruit Ripening. *The Molecular Biology and Biochemistry of Fruit Ripening*, 43–73. https://doi.org/10.1002/9781118593714.CH3
Guo, J., Han, W., & Wang, M.-H. (2010). Ultraviolet and environmental stresses involved in the induction and regulation of anthocyanin biosynthesis: A review. *African Journal of Biotechnology*, *7*(25), 4966–4972. https://doi.org/10.4314/ajb.v7i25.59709
Gussman, C. D., Goffreda, J. C., & Gianfagna, T. J. (1993). Ethylene production and fruit-softening rates in several apple fruit ripening variants. *HortScience*, *28*(2), 135–137.
Hanrahan, I., Schmidt, T. R., Castillo, F., & Mcferson, J. R. (2011). *Reflective Ground Covers Increase Yields of Target Fruit of Apple and Pear*.

Harb, J., Gapper, N. E., Giovannoni, J. J., & Watkins, C. B. (2012). Molecular analysis of softening and ethylene synthesis and signaling pathways in a non-softening apple cultivar, "Honeycrisp" and a rapidly softening cultivar, "McIntosh." *Postharvest Biology and Technology*, *64*(1), 94–103. https://doi.org/10.1016/j.postharvbio.2011.10.001

Hengari, S., Theron, K. I., Midgley, S. J. E., & Steyn, W. J. (2014). Response of apple (Malus domestica Borkh.) fruit peel photosystems to heat stress coupled with moderate photosynthetic active radiation at different fruit developmental stages. *Scientia Horticulturae*, *178*(1), 154–162. https://doi.org/10.1016/j.scienta.2014.08.019

Honda, C., Bessho, H., Murai, M., Iwanami, H., Moriya, S., Abe, K., Wada, M., Moriya-Tanaka, Y., Hayama, H., & Tatsuki, M. (2014). Effect of Temperature on Anthocyanin Synthesis and Ethylene Production in the Fruit of Early-and Medium-maturing Apple Cultivars during Ripening Stages. In *HORTSCIENCE* (Vol. 49, Issue 12).

Honda, C., Kotoda, N., Wada, M., Kondo, S., Kobayashi, S., Soejima, J., Zhang, Z., Tsuda, T., & Moriguchi, T. (2002). Anthocyanin biosynthetic genes are coordinately expressed during red coloration in apple skin. *Plant Physiology and Biochemistry*, *40*(11), 955–962.

Honda, C., & Moriya, S. (2018). Anthocyanin biosynthesis in apple fruit. *The Horticulture Journal*, *87*(3), 305–314.

Hu, D. G., Yu, J. Q., Han, P. L., Xie, X. Bin, Sun, C. H., Zhang, Q. Y., Wang, J. H., & Hao, Y. J. (2019). The regulatory module MdPUB29-MdbHLH3 connects ethylene biosynthesis with fruit quality in apple. *New Phytologist*, *221*(4), 1966–1982. https://doi.org/10.1111/NPH.15511

Hyson, D. A. (2011). A comprehensive review of apples and apple components and their relationship to human health. *Advances in Nutrition*, *2*(5), 408–420.

Hyun, S. C., & Kieber, J. J. (2005). Eto Brute? Role of ACS turnover in regulating ethylene biosynthesis. *Trends in Plant Science*, *10*(6), 291–296. https://doi.org/10.1016/J.TPLANTS.2005.04.006

Iglesias, I., & Alegre, S. (2009). The effects of reflective film on fruit color, quality, canopy light distribution, and profitability of "Mondial Gala" apples. *HortTechnology*, *19*(3), 488–498. https://doi.org/10.21273/hortsci.19.3.488

Infante, R., Farcuh, M., & Meneses, C. (2008). Monitoring the sensorial quality and aroma through an electronic nose in peaches during cold storage. *Journal of the Science of Food and Agriculture*, *88*(12). https://doi.org/10.1002/jsfa.3316

Irish-Brown, A., Schwallier, P., Shane, B., & Tritten, B. (2011). Why does apple fruit drop prematurely? *Michigan State University Extension, East Lansing, MI*.

Jaakola, L. (2013). New insights into the regulation of anthocyanin biosynthesis in fruits. In *Trends in Plant Science* (Vol. 18, Issue 9, pp. 477–483). https://doi.org/10.1016/j.tplants.2013.06.003

Jakopič, J., ˇ tampar, F. S., & Veberič, R. (2010). Influence of Hail Net and Reflective Foil on Cyanidin Glycosides and Quercetin Glycosides in "Fuji" Apple Skin. In *HORTSCIENCE* (Vol. 45, Issue 10).

Ju, Z., Duan, Y., & Ju, Z. (1999). Effects of covering the orchard floor with reflecting films on pigment accumulation and fruit coloration inFuji'apples. *Scientia*

Horticulturae, *82*(1–2), 47–56.
Kim, H., Farcuh, M., Cohen, Y., Crisosto, C., Sadka, A., & Blumwald, E. (2015). Non-climacteric ripening and sorbitol homeostasis in plum fruits. *Plant Science*, *231*, 30–39. https://doi.org/10.1016/j.plantsci.2014.11.002
Klee, H. J. (2004). Ethylene signal transduction. Moving beyond Arabidopsis. *Plant Physiology*, *135*(2), 660–667.
Kon, T. M., & Clavet, C. D. (2023). Enhancing Red Fruit Coloration of Apples in the Southeastern US with Reflective Fabrics. *Horticulturae*, *9*(10), 1125.
Kondo, S., Hiraoka, K., Kobayashi, S., Honda, C., & Terahara, N. (2002). Changes in the expression of anthocyanin biosynthetic genes during apple development. *Journal of the American Society for Horticultural Science*, *127*(6), 971–976.
Kunradi Vieira, F. G., da Silva Campelo Borges, G., Copetti, C., Valdemiro Gonzaga, L., da Costa Nunes, E., & Fett, R. (2009). Activity and contents of polyphenolic antioxidants in the whole fruit, flesh and peel of three apple cultivars. *Archivos Latinoamericanos de Nutrición*, *59*(1), 101–106.
Lancaster, J. E. (1992). Regulation of skin color in apples. *Critical Reviews in Plant Sciences*, *10*(6), 487–502. https://doi.org/10.1080/07352689209382324
Łata, B., Trampczynska, A., & Paczesna, J. (2009). Cultivar variation in apple peel and whole fruit phenolic composition. *Scientia Horticulturae*, *121*(2), 176–181.
Layne, D. R., Jiang, Z., & Rushing, J. W. (2001). Tree fruit reflective film improves red skin coloration and advances maturity in peach. *HortTechnology*, *11*(2), 234–242.
Layne, D. R., Jiang, Z., & Rushing, J. W. (2002). The Influence of Reflective Film and ReTain on Red Skin Coloration and Maturity ofGala'Apples. *HortTechnology*, *12*(4), 640–645.
Li, P., & Cheng, L. (2008). The shaded side of apple fruit becomes more sensitive to photoinhibition with fruit development. *Physiologia Plantarum*, *134*(2), 282–292.
Lister, C. E., Lancaster, J. E., & Walker, J. R. L. (1996). Developmental changes in enzymes of flavonoid biosynthesis in the skins of red and green apple cultivars. *Journal of the Science of Food and Agriculture*, *71*(3), 313–320.
Liu, J., Islam, M. T., & Sherif, S. M. (2022). Effects of Aminoethoxyvinylglycine (AVG) and 1-Methylcyclopropene (1-MCP) on the Pre-Harvest Drop Rate, Fruit Quality, and Stem-End Splitting in 'Gala'Apples. *Horticulturae*, *8*(12), 1100.
Liu, W., Wang, Y., Sun, J., Jiang, H., Xu, H., Wang, N., Jiang, S., Fang, H., Zhang, Z., Wang, Y. L., & Chen, X. (2019). MdMYBDL1 employed by MdHY5 increases anthocyanin accumulation via repression of MdMYB16/308 in apple. *Plant Science*, *283*, 32–40. https://doi.org/10.1016/j.plantsci.2019.01.016
Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, *25*(4), 402–408.
Mahdavi, S., Fallahi, E., & Fazio, G. (2022). The Influence of Rootstock on Fruit Ethylene, Respiration, Index of Absorbance Difference, Fruit Quality, and Production of 'Aztec Fuji'Apple under a Full-crop Condition. *HortScience*, *57*(1), 1–9.
Mao, Z., Jiang, H., Wang, S., Wang, Y., Yu, L., Zou, Q., Liu, W., Jiang, S., Wang,

N., Zhang, Z., & Chen, X. (2021). The MdHY5-MdWRKY41-MdMYB transcription factor cascade regulates the anthocyanin and proanthocyanidin biosynthesis in red-fleshed apple. *Plant Science*, *306*. https://doi.org/10.1016/j.plantsci.2021.110848

Miah, M. S., & Farcuh, M. (2024). Combining the Use of Reflective Groundcovers and Aminoethoxyvinylglycine to Assess Effects on Skin Color, Preharvest Drop, and Quality of 'Honeycrisp'Apples in the Mid-Atlantic US. *Horticulturae*, *10*(2), 179.

Miah, M. S., Hinson, C., & Farcuh, M. (2023). Assessing Fruit Maturity and Quality of 'Buckeye Gala'Grown on a Diverse Panel of Apple (Malus domestica Borkh.) Rootstocks in Western Maryland. *Agronomy*, *13*(10), 2528.

Miller, S. S., & Greene, G. M. (2003). The use of reflective film and ethephon to improve red skin color of apples in the Mid-Atlantic region of the United States. *HortTechnology*, *13*(1), 90–99.

Mogil, H. M., & Seaman, K. L. (2009). The Climate and Weather of Delaware, Maryland, and Washington, D.C. *Weatherwise*, *62*(4), 16–23. https://doi.org/10.3200/WEWI.62.4.16-23

Mupambi, G., Valverdi, N. A., Camargo-Alvarez, H., Reid, M., Kalcsits, L., Schmidt, T., Castillo, F., & Toye, J. (2021). Reflective Groundcover Improves Fruit Skin Color in 'Honeycrisp'Apples Grown under Protective Netting. *Horttechnology*, *31*(5), 607–614.

Musacchi, S., & Serra, S. (2018). Apple fruit quality: Overview on pre-harvest factors. *Scientia Horticulturae*, *234*, 409–430.

Nangle, E. J., Gardner, D. S., Metzger, J. D., Petrella, D. P., Danneberger, T. K., Rodriguez-Saona, L., & Cisar, J. L. (2016). Cool-season Turfgrass Color and Growth Habit Response to Elevated Levels of Ultraviolet-B Radiation. In *HORTSCIENCE* (Vol. 51, Issue 4).

Osorio, S., & Fernie, A. R. (2013). Biochemistry of fruit ripening. *The Molecular Biology and Biochemistry of Fruit Ripening*, 1–19. https://doi.org/10.1002/9781118593714.ch1

Overbeck, V., Schmitz-Eiberger, M. A., & Blanke, M. M. (2013). Reflective mulch enhances ripening and health compounds in apple fruit. *Journal of the Science of Food and Agriculture*, *93*(10), 2575–2579.

Park, J. S., Choung, M. G., Kim, J. B., Hahn, B. S., Kim, J. B., Bae, S. C., Roh, K. H., Kim, Y. H., Cheon, C. I., Sung, M. K., & Cho, K. J. (2007). Genes up-regulated during red coloration in UV-B irradiated lettuce leaves. *Plant Cell Reports*, *26*(4), 507–516. https://doi.org/10.1007/S00299-006-0255-X/FIGURES/4

Phan-Thien, K. Y., Wargo, J. M., Mitchell, L. W., Collett, M. G., & Rath, A. C. (2004). Delay in ripening of Gala and Pink Lady apples in commercial orchards following pre-harvest applications of aminoethoxyvinylglycine. *Australian Journal of Experimental Agriculture*, *44*(8), 807–812.

Privé, J.-P., Russell, L., & LeBlanc, A. (2011). Impact of reflective groundcover on growth, flowering, yield and fruit quality in Gala apples in New Brunswick. *Canadian Journal of Plant Science*, *91*(4), 765–772.

Privé, J. P., Russell, L., & Leblanc, A. (2008). Use of Extenday reflective groundcover in production of ‘Gala’apples (Malus domestica) in New Brunswick, Canada: 1. Impact on canopy microclimate and leaf gas exchange. *New Zealand Journal of Crop and Horticultural Science*, *36*(4), 221–231.

Qi, W., Wang, H., Zhou, Z., Yang, P., Wu, W., Li, Z., & Li, X. (2020). Ethylene Emission as a Potential Indicator of Fuji Apple Flavor Quality Evaluation Under Low Temperature. *Horticultural Plant Journal*, *6*(4), 231–239. https://doi.org/10.1016/J.HPJ.2020.03.007

Roberts, J. A., Elliott, K. A., & Gonzalez-Carranza, Z. H. (2002). Abscission, dehiscence, and other cell separation processes. *Annual Review of Plant Biology*, *53*(1), 131–158.

Robinson, T. L., & Gonzalez, L. (2023). Effect of different reflective ground covers on light reflection and on the coloring of apples at harvest. *Acta Hortic. 1366*, 385–392. https://doi.org/https://doi.org/10.17660/ActaHortic.2023.1366.47

Rupasinghe, H. P. (2020). Special issue “flavonoids and their disease prevention and treatment potential”: recent advances and future perspectives. *Molecules*, *25*(20), 4746.

Ryu, J.-A., Duan, S., Gil, C. S., Jeong, H. Y., Lee, C., Kang, I.-K., & Eom, S. H. (2022). Combined UV-B and methyl jasmonate treatments enhance postharvest pigmentation of “Fuji” apples. *Postharvest Biology and Technology*, *190*, 111938.

Saure, M. C. (1990). External control of anthocyanin formation in apple. In *Scientia Horticulturae* (Vol. 42, Issue 3, pp. 181–218). Elsevier. https://doi.org/10.1016/0304-4238(90)90082-P

Schaller, G. E., & Binder, B. M. (2017). *Inhibitors of Ethylene Biosynthesis and Signaling BT - Ethylene Signaling: Methods and Protocols* (B. M. Binder & G. Eric Schaller (eds.); pp. 223–235). Springer New York. https://doi.org/10.1007/978-1-4939-6854-1_15

Schupp, J. R., & Greene, D. W. (2004). Effect of Aminoethoxyvinylglycine (AVG) on Preharvest Drop, Fruit Quality, and Maturation ofMcIntosh’Apples. I. Concentration and Timing of Dilute Applications of AVG. *HortScience*, *39*(5), 1030–1035.

Shafiq, M., Singh, Z., & Khan, A. S. (2014). Pre-harvest ethephon application and training systems affect colour development, accumulation of flavonoids and fruit quality of’Cripps pink’apple. *Australian Journal of Crop Science*, *8*(12), 1579–1589.

Shü, Z.-H., Chu, C.-C., Hwang, L.-J., & Shieh, C.-S. (2001). Light, Temperature, and Sucrose Affect Color, Diameter, and Soluble Solids of Disks of Wax Apple Fruit Skin. In *HORTSCIENCE* (Vol. 36, Issue 2).

Siegelman, H. W., & Hendricks, S. B. (1958). Photocontrol of anthocyanin synthesis in apple skin. *Plant Physiology*, *33*(3), 185.

Stover, E., Fargione, M. J., Watkins, C. B., & Iungerman, K. A. (2003). Harvest Management of ’Marshall McIntosh’Apples: Effects of AVG, NAA, Ethephon, and Summer Pruning on Preharvest Drop and Fruit Quality. *HortScience*, *38*(6), 1093–1099.

Sun, X., Li, Y., He, W., Ji, C., Xia, P., Wang, Y., Du, S., Li, H., Raikhel, N., Xiao, J., & Guo, H. (2017). Pyrazinamide and derivatives block ethylene biosynthesis by

inhibiting ACC oxidase. *Nature Communications*, *8*(1), 15758. https://doi.org/10.1038/ncomms15758
Takos, A. M., Jaffé, F. W., Jacob, S. R., Bogs, J., Robinson, S. P., & Walker, A. R. (2006). Light-induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples. *Plant Physiology*, *142*(3), 1216–1232.
Telias, A., Lin-Wang, K., Stevenson, D. E., Cooney, J. M., Hellens, R. P., Allan, A. C., Hoover, E. E., & Bradeen, J. M. (2011). Apple skin patterning is associated with differential expression of MYB10. *BMC Plant Biology*, *11*(1), 1–15.
Thewes, F. R., Balkees, B. M., Büchele, F., Wünsche, J. N., Neuwald, D. A., & Brackmann, A. (2021). Ethanol vapor treatment inhibits apple ripening at room temperature even with the presence of ethylene. *Postharvest Biology and Technology*, *173*, 111415. https://doi.org/10.1016/J.POSTHARVBIO.2020.111415
Toivonen, P., Stoochnoff, J., Usher, K., Lu, C., Wiersma, P. A., & Zhou, C. (2019). Biochemical and gene expression involved in red blush color development in 'Ambrosia'apple. *Journal of the American Society for Horticultural Science*, *144*(3), 164–171.
Toye, J. (1995). Reflective mulches—New Zealand leads the way. *The Orchardist*, *68*(8), 58–60.
Ubi, B. E., Honda, C., Bessho, H., Kondo, S., Wada, M., Kobayashi, S., & Moriguchi, T. (2006). Expression analysis of anthocyanin biosynthetic genes in apple skin: Effect of UV-B and temperature. *Plant Science*, *170*(3), 571–578. https://doi.org/10.1016/j.plantsci.2005.10.009
USApple. (2022). *USAppleIndustryOutlook2021*. https://usapple.org/wp-content/uploads/2021/08/USAppleIndustryOutlook2021.pdf
USDA. (2019). *Marketing and Regulatory Programs Agricultural Marketing Service Specialty Crops Program Specialty Crops Inspection Division United States Standards for Grades of Apples*.
USDA. (2022). *NATIONAL APPLE PROCESSING REPORT*.
USDA Agricultural Marketing Service. (n.d.). *Apples Grades and Standards*. Https://Www.Ams.Usda.Gov/Grades-Standards/Apple-Grades-Standards.
Vangdal, E., Meland, M., & Hjeltnes, S. H. (2007). Reflective mulch (Extenday) in fruit orchards-preliminary results. *Acta Horticulturae*.
Vimolmangkang, S., Zheng, D., Han, Y., Khan, M. A., Soria-Guerra, R. E., & Korban, S. S. (2014). Transcriptome analysis of the exocarp of apple fruit identifies light-induced genes involved in red color pigmentation. *Gene*, *534*(1), 78–87.
Wang, Z., & Dilley, D. R. (2001). Aminoethoxyvinylglycine, combined with ethephon, can enhance red color development without over-ripening apples. *HortScience*, *36*(2), 328–331.
Watkins, C. B., Erkan, M., Nock, J. F., Iungerman, K. A., Beaudry, R. M., & Moran, R. E. (2005). Harvest Date Effects on Maturity, Quality, and Storage Disorders of "Honeycrisp" Apples. In *HORTSCIENCE* (Vol. 40, Issue 1).
Weber, S., Damerow, L., Kunz, A., & Blanke, M. (2019). Anthocyanin synthesis and light utilisation can be enhanced by reflective mulch – Visualisation of light penetration into a tree canopy. *Journal of Plant Physiology*, *233*, 52–57.

https://doi.org/10.1016/j.jplph.2018.12.008
Whale, S. K., & Singh, Z. (2007). Endogenous ethylene and color development in the skin of 'Pink Lady'apple. *Journal of the American Society for Horticultural Science*, *132*(1), 20–28.
Whale, S. K., Singh, Z., Behboudian, M. H., Janes, J., & Dhaliwal, S. S. (2008). Fruit quality in 'Cripp's Pink'apple, especially colour, as affected by preharvest sprays of aminoethoxyvinylglycine and ethephon. *Scientia Horticulturae*, *115*(4), 342–351.
Wünsche, J. N., Lakso, A. N., Robinson, T. L., Lenz, F., & Denning, S. S. (1996). The bases of productivity in apple production systems: the role of light interception by different shoot types. *Journal of the American Society for Horticultural Science*, *121*(5), 886–893.
Xie, R., Zheng, L., He, S., Zheng, Y., Yi, S., & Deng, L. (2011). Anthocyanin biosynthesis in fruit tree crops: Genes and their regulation. *African Journal of Biotechnology*, *10*(86), 19890–19897.
Xu, Y., Feng, S., Jiao, Q., Liu, C., Zhang, W., Chen, W., & Chen, X. (2012). Comparison of MdMYB1 sequences and expression of anthocyanin biosynthetic and regulatory genes between Malus domestica Borkh. cultivar 'Ralls' and its blushed sport. *Euphytica*, *185*, 157–170.
Yang, S. F., & Hoffman, N. E. (1984). Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology*, *35*(1), 155–189.
Yang, W., Ma, X., Ma, D., Shi, J., Hussain, S., Han, M., Costes, E., & Zhang, D. (2021). Modeling canopy photosynthesis and light interception partitioning among shoots in bi-axis and single-axis apple trees (Malus domestica Borkh.). *Trees - Structure and Function*, *35*(3), 845–861. https://doi.org/10.1007/s00468-021-02085-z
Yang, X., Song, J., Campbell-Palmer, L., Fillmore, S., & Zhang, Z. (2013). Effect of ethylene and 1-MCP on expression of genes involved in ethylene biosynthesis and perception during ripening of apple fruit. *Postharvest Biology and Technology*, *78*, 55–66. https://doi.org/10.1016/J.POSTHARVBIO.2012.11.012
Yildiz, K., Ozturk, B., & Ozkan, Y. (2012). Effects of aminoethoxyvinylglycine (AVG) on preharvest fruit drop, fruit maturity, and quality of 'Red Chief' apple. *Scientia Horticulturae*, *144*, 121–124. https://doi.org/10.1016/J.SCIENTA.2012.07.005
Yu, J., Qiu, K., Sun, W., Yang, T., Wu, T., Song, T., Zhang, J., Yao, Y., & Tian, J. (2022). A long noncoding RNA functions in high-light-induced anthocyanin accumulation in apple by activating ethylene synthesis. *Plant Physiology*, *189*(1), 66–83.
Yuan, R., & Li, J. (2008). Effect of sprayable 1-MCP, AVG, and NAA on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of 'Delicious' apples. *HortScience*, *43*(5), 1454–1460.
Zhang, J., Xu, H., Wang, N., Jiang, S., Fang, H., Zhang, Z., Yang, G., Wang, Y., Su, M., & Xu, L. (2018). The ethylene response factor MdERF1B regulates anthocyanin and proanthocyanidin biosynthesis in apple. *Plant Molecular Biology*, *98*, 205–218.
Zhao, Y., Sun, J., Cherono, S., An, J.-P., Allan, A. C., & Han, Y. (2023). Colorful

hues: insight into the mechanisms of anthocyanin pigmentation in fruit. *Plant Physiology*. https://doi.org/10.1093/PLPHYS/KIAD160

Zimmermann, I. M., Heim, M. A., Weisshaar, B., & Uhrig, J. F. (2004). Comprehensive identification of Arabidopsis thaliana MYB transcription factors interacting with R/B-like BHLH proteins. *Plant Journal*, *40*(1), 22–34. https://doi.org/10.1111/J.1365-313X.2004.02183.X

Ziosi, V., Noferini, M., Fiori, G., Tadiello, A., Trainotti, L., Casadoro, G., & Costa, G. (2008). A new index based on vis spectroscopy to characterize the progression of ripening in peach fruit. *Postharvest Biology and Technology*, *49*(3), 319–329. https://doi.org/10.1016/j.postharvbio.2008.01.017