J. Amer. Soc. Hort. Sci. 148(3):117–125. 2023. https://doi.org/10.21273/JASHS05302-23

Xylem Functionality Is Not a Direct Indicator of Apple Preharvest Fruit Drop

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Keywords. crop load management, *Malus ×domestica*, plant growth regulators

Abstract. Apple (*Malus ×domestica*) growers can incur significant economic losses when fruit drop before they can be harvested [preharvest fruit drop (PFD)]. In some years and cultivars, more than 30% of potential yield can be lost. Growers frequently apply plant bioregulators to reduce PFD, either via delay in maturity [aminoethoxyvinylglycine (AVG), 1-methylcycolpropene] or via inhibition in production of cell hydrolysis enzymes in the fruit pedicel [naphthalene acetic acid (NAA)]. Finding a physiological indicator of PFD would allow growers to assess the susceptibility of fruit to PFD. Due to its lignification, xylem is believed to be the last tissue to break down in the fruit pedicel, leading to PFD. To determine whether loss in xylem functionality can be used as an indicator of PFD potential, studies were conducted in 2020 and 2021 with 'Red Delicious' treated with AVG (132 $\mu L \cdot L^{-1}$), NAA (10 $\mu L \cdot L^{-1}$), and an ethylene-producing compound [ethephon (150 $\mu L \cdot L^{-1}$ in 2020, 200 $\mu L \cdot L^{-1}$ in 2021)] to generate a range of PFD potentials. Xylem functionality was assessed in the fruit cortex. Internal ethylene content (IEC), fruit maturity indices, and PFD rates were quantified weekly throughout the harvest period. Expression of genes encoding for cell hydrolysis enzymes (*MdEG1* and *MdPG2*) was quantified to relate xylem functionality to fruit abscission mechanisms. In 2020 and 2021, AVG reduced PFD compared with the untreated control by decreasing IEC. Although ethephon did not result in higher PFD than untreated fruit, NAA reduced PFD in 2020 but not 2021. For all treatments in both years, there was a linear decrease in xylem functionality throughout the measurement period. Cumulative PFD exponentially decreased as xylem functionality neared zero and the climacteric rise in ethylene began. Concurrent with the rise in IEC and PFD was an increase in the expression of *MdEG1* and *MdPG2* in the fruit pedicel of the control compared with AVG-treated fruit. AVG-treated fruit lost xylem functionality at a similar rate to the untreated control but had lower expression of *MdEG1* and *MdPG2*. These results indicate that xylem functionality is not a sole direct indicator of PFD. The concurrent increase in PFD and expression of *MdEG1*/*MdPG2* supports previous research indicating that these two genes may serve as potential markers for PFD.

Harvesting apple (*Malus ×domestica*) fruit at optimal horticultural maturity is critical to meeting consumer preferences and to maintain quality throughout storage (McCluskey et al. 2007; Palmer et al. 2010). Management during harvest is complicated by uneven ripening, overlapping maturity of cultivars, and lack of labor availability. Exacerbating these complications is the tendency for apples to abscise prematurely before harvest [preharvest fruit drop (PFD)]. PFD can occur up to 4 weeks before harvest and cause yield losses of up to 30% (Arseneault and Cline 2016). Preharvest is the second period in the growing season when fruit abscise from the tree, the first being immature fruitlet abscission 5 to 6 weeks after bloom (Dal Cin et al. 2009), termed "June drop" in the Northern Hemisphere.

Apple fruit abscission occurs along a narrow plane that is six to eight cells in width [abscission zone (AZ)]. The AZ differentiates in the constriction zone (20 to 30 cells in width) at the junction of the pedicel and peduncle. The sequence of cell breakdon in the constriction zone is as follows: cell wall swelling, pectin in the middle lamella dissolving, primary and secondary walls collapsing, and finally, abscission due to weight of the fruit causing vessel and fiber rupture (McCown 1943).

Li et al. (2010) supported the observations of McCown (1943), finding that increased expression of genes encoding cell hydrolysis enzymes—polygalacturonase (PG) and cellulase (EG)—precedes PFD. *MdPG2* and *MdEG1* expression was higher in the AZ of Golden Delicious, a PFD-susceptible cultivar, compared with that in Fuji, which is not prone to PFD (Li et al. 2010). Concurrent with softening in the AZ was an increase in internal ethylene content and expression of ethylene biosynthesis/receptor genes (*MdACS5A*, *MdACO1*, *MdETR2*, *MdERS2*) in 'Golden Delicious' but not 'Fuji' (Li et al. 2010).

Ethylene biosynthesis, characterized by Yang and Hoffman (1984), involves the conversion of S-adenosylmethionine (SAM), a product of methionine metabolism, to 1-aminocyclopropane-1-carboxylate (ACC) and subsequently to ethylene. SAM is converted to ACC by ACC synthase enzymes (ACS), whereas ACC is converted to ethylene by ACC oxidase (ACO). Apples are a climacteric fruit that experience a rise in respiration and ethylene during ripening. The rise in ethylene during climacteric ripening

Received for publication 14 Feb 2023. Accepted for publication 6 Apr 2023. Published online 12 May 2023.
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is due to ethylene biosynthesis transitioning from an autoinhibitory to an autocatalytic program, wherein ethylene increases production of ACS (Barry et al. 2000).

In an analysis of 144 wild and domestic *Malus* cultivars, Sun et al. (2009) found wide ranges of internal ethylene content among cultivars with high and low PFD rates. Sun et al. (2009) concluded that ethylene alone may not serve as the only signal that leads to PFD. In other examples of organ abscission, interaction of ethylene with phytohormones such as auxin regulates progression of AZ activation (Taylor and Whitelaw 2001). For example, molecular analysis of immature apple fruit abscission suggests that the AZ is responsive to ethylene only when auxin transport through the pedicel is inhibited (Botton et al. 2011). To the extent of our knowledge, the role of auxin transport through the pedicel has not been investigated in relation to PFD.

Plant bioregulators (PBRs) can effectively control PFD either through suppression of ethylene biosynthesis [aminoethoxyvinylglycine (AVG)], blocking ethylene perception/action [1-methylcyclopropene (1-MCP)], or indirectly by inhibiting PG and EG enzymes [naphthaleneacetic acid (NAA) (Arseneault and Cline 2016; Greene 2010)]. In molecular analysis of the effects of AVG, 1-MCP, and NAA, Li and Yuan (2008) found that AVG and 1-MCP downregulated the expression of ethylene biosynthesis and receptor genes—*MdACS1*, *MdACO1*, and *MdERS1*—and in turn cell wall disassembly genes, *MdPG2* and *MdEG1*, in the AZ. Meanwhile, NAA increased expression of ethylene biosynthesis genes while still limiting PFD due to downregulation of *MdPG2* and *MdEG1* expression (Li and Yuan 2008). These results are similar to the proposed role of auxin and ethylene in immature fruit abscission. PFD can be limited by either maintaining auxin transport through the pedicel to limit the pedicel's sensitivity to ethylene (e.g., NAA) or by limiting production of ethylene (e.g., AVG and 1-MCP). PG and EG enzymes would then be produced when the pedicel is sensitive to ethylene. Supporting this connection, Ward et al. (1999) detected increases in cellulase in the AZ when fruit were removed from the pedicel. There, removing fruit eliminated the source of auxin traveling through the pedicel resulting in an upregulation of cellulase (Ward et al. 1999). Although these PBRs reliably control PFD, they are applied only in relation to the anticipated harvest date. Identification of an indicator for PFD would allow for assessment of an orchard block's susceptibility to PFD and more efficient harvest management.

Loss of fruit xylem functionality has been hypothesized to be one physiological factor that may indicate susceptibility to PFD. In a comparison of two cultivars McIntosh (prone to PFD) and Gala (not prone to PFD), Arseneault (2016) assessed xylem functionality with an acid fuchsin dye infiltration method. Most of xylem functionality was lost over the duration of the growing season in 'McIntosh' but was retained in 'Gala' as fruit matured (Arseneault 2016). Xylem functionality has been found to decrease throughout the growing season in several apple cultivars. Dražeta et al. (2004) observed loss of xylem functionality throughout fruit development of 'Granny Smith' and 'Braeburn', with 'Braeburn' losing functionality earlier in the season than 'Granny Smith'. Using a pressure bomb to quantify xylem exudation, Lang and Ryan (1994) found that xylem flow peaked 40 to 70 d after bloom and decreased thereafter for 'Cox's Orange Pippin' and 'Royal Gala'. Dysfunctional xylem vessels were more pronounced toward the calyx end of the fruit than in the stem end (Dražeta et al. 2004). Loss in xylem function has been hypothesized to be caused by physical rupture as fruit grow (Dražeta et al. 2004).

It is hypothesized here that loss of xylem functionality precedes the onset of PFD. The objective of the current study was to determine if loss of xylem functionality proximal to harvest could be used as a physiological indicator of PFD potential. Two PBRs commonly used to limit PFD, AVG, and NAA, as well as an ethylene-releasing compound ethephon, were applied to 'Red Delicious' to generate a range of PFD rates. Further, expression of genes related to cell wall breakdown were measured to relate xylem functionality to fruit abscission mechanisms.

Materials and Methods

Plant material. This study was conducted in 2020 and 2021 in mature 'Red Delicious' orchards. In 2020, 'Red Delicious Oregon Spur II'/'M.111' (M.111) trees (planted 2002) at a spacing of 2.7 m between trees and 6.1 m between rows at North Carolina State's Mountain Horticultural Crops Research Station in Mills River, NC, USA (lat. 35.428079°N, long. 82.563295°W, elevation 649 m) were used. A commercial orchard of 'Morgan Spur'/'M.111' (planted 2008) spaced 1.8 m by 4.9 m was used in 2021 (Hendersonville, NC, USA).

Treatments. This study used a completely randomized design with four treatments, five replications, and three tree plots (n = 20). Treatments were made on 26 Aug 2020 and 25 Aug 2021, two weeks before anticipated harvest. Anticipated harvest was based on historical records, and harvest dates of earlier ripening cultivars in the growing season. The following treatments were evaluated: 1) untreated control, 2) 132 $\mu L \cdot L^{-1}$ AVG (ReTain®; Valent BioSciences, Libertyville, IL, USA), 3) 10 $\mu L \cdot L^{-1}$ NAA (PoMaxa®, Valent BioSciences), and 4) 150 $\mu L \cdot L^{-1}$ ethephon in 2020 or 200 $\mu L \cdot L^{-1}$ ethephon in 2021 (Ethephon 2SL; ADAMA, Raleigh, NC, USA). Ethephon and NAA were applied with 0.125% (v/v) nonionic surfactant (Regulaid®; Kalo, Inc., Overland Park, KS, USA). 0.1% (v/v) organosilicone surfactant (Silwett L-77; Helena Agri-Enterprises, LLC, Collierville, TN, USA) was used for AVG applications. These surfactants were added in accordance with local recommendations. The pH of ethephon tank mix was 3.1 in both years. In 2020, treatments were applied with a CO_2-powered hand sprayer to both sides of all trees. In 2021, a backpack-powered air blast sprayer was used. In both years, applications were made with a water volume of 935 $L \cdot ha^{-1}$. Supplemental Table S1 summarizes dates of treatment application and data collection throughout the study.

Fruit drop rates. Fruit drop rates were quantified by counting the number of fruit that had dropped from the center tree of each three-tree plot weekly. After counting of dropped fruit, the area beneath the center tree (nondestructive tree) was raked clean to ensure that the only fruit that were counted had abscised during the previous week. At the conclusion of the study in each year, all remaining fruit from each center tree were harvested to determine the number of fruit that were on the tree at the beginning of the study (initial crop load) and calculate percent drop for each subsequent week (Eq. [1]).

$$\text{Weekly cumulative fruit drop} = (\text{total cumulative dropped fruit} \div \text{initial crop load}) \times 100\% \quad [1]$$

Fruit maturity indices and internal ethylene quantification. From the outside two trees (destructively sampled trees) of each

Fig. 1. Mature 'Red Delicious' apple (*Malus ×domestica*) cortex with functional xylem vessels-stained pink using an acid fuchsin dye. Primary (ventral; blue circles) xylem vessels form in a ring of 10 around the dorsal (green circles) xylem vessels located next to seed locules, maximum of 5.

plot, fruit were collected to track internal ethylene content and maturity throughout the study. Twelve fruit were randomly collected from each plot (n = 240 fruit) weekly and followed the same series of nondestructive and destructive tests: internal ethylene content (IEC), fruit firmness, soluble solids content (SSC), and starch staining pattern. Ten of the 12 fruit per plot were used for internal ethylene content quantification using gas chromatography. A 1-mL gas sample was collected from the core cavity and injected into a gas chromatograph (GC-8A; Shimadzu; Kyoto, Japan) with a 3.175-mm stainless steel column packed with alumina (Supelco, Bellefonte, PA, USA). Fruit firmness measurements were taken on opposite sides of each fruit with a fruit texture analyzer (Güss GS-20; GÜSS Manufacturing Ltd.; Strand, Cape Town, South Africa) equipped with a 11-mm-wide flat probe, 7.9 mm into the fruit (Kupferman and Dasgupta 2001). A juice sample was collected, and SSC was measured with a digital refractometer (3810; Atago, Tokyo, Japan) from pooled sample of the fruit in a plot. Starch staining pattern was rated on a scale of 1 to 8 by cutting the fruit in half transversely and then dipping into an iodine solution (Blanpied and Silsby 1992; Smith et al. 1979).

Xylem functionality. Functional xylem bundles were assessed with an acid fuchsin dye infiltration method (Arseneault 2016; Dražeta et al. 2004). Six fruit per plot (n = 120 fruit) were collected from the orchard before dawn by pruning the spur at the branch junction. Fruit were then brought into the laboratory and the spur was recut in deionized water before being placed into a 0.5% acid fuchsin dye (Sigma-Aldrich, St. Louis, MO, USA). The spur was trimmed with a razor blade so that the length of the spur + pedicel was ∼3 cm. The interval for uptake of dye was 2 h. A fan was placed in front of the fruit to encourage transpiration through the fruit. After infiltration, each fruit was cut in half equatorially, and total number of stained dorsal and primary bundles were counted (Fig. 1).

RNA extraction and gene expression. Fruit cortex and pedicel samples were collected weekly for gene expression analyses. Tissue was collected from three fruit per plot of the destructively sampled trees. Cortex samples were collected by taking a 1-cm slice from the middle of the fruit, cutting that slice in half, removing the peel/pith, dicing the cortical tissue, and placing into a 50-mL canonical tube. Fruit pedicels were diced and placed into a 5-mL centrifuge tube. Immediately upon collection, samples were frozen in liquid nitrogen until they could be transported to a –80 °C freezer for long-term storage.

Total RNA was extracted from frozen tissue following the CTAB method presented by Vashisth et al. (2011). Following tissue grinding in liquid nitrogen, ∼0.8 g of cortex and ∼0.4 g of pedicel tissue was used for RNA extraction. After extraction, RNA concentration and 260:280-nm absorbance was quantified with a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific, Inc., Waltham, MA, USA). One microgram of DNase (Promega Corp, Madison, WI, USA) treated RNA was used for cDNA synthesis in a 20-µL reaction volume (Vashisth et al. 2011). cDNA was diluted 6-fold for quantitative real-time polymerase chain reaction (RT-qPCR).

RT-qPCR reactions were carried out in a 12-µL volume with 2 µL of diluted cDNA, 4 µL of 0.2-µM primer mix, and 6 µL of 2X PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Thermo Fisher Scientific, Inc.). Primers used for gene

Table 1. List of gene-specific primers for gene expression analysis of 'Red Delicious' apple (*Malus ×domestica*) cortex and pedicel samples that were treated with aminoethoxyvinylglycine and untreated control.

Gene	Accession no.	Primer sequence (5′ to 3′)[i]
MdGAPDH[ii]	EB146750	TGAGGGCAAGCTGAAGGGTATCTT
		TCAAGTCAACCACACGGGTACTGT
MdEXPA10;1[ii]	MDP0000681724	GGGTGCGGATCTTGCTACG
		GGAGGGCGTTGTTTGGTGGA
MdPG2[iii]	AB210897	CGGTTCAGCCGACAAGTTG
		TACGAGTGAGGAGGAGTAGATGGA
MdEG1[iii]	AY350734	ACCAGAACGATGGATTTCCAGAT
		GTACGTTGCAGGCTCCGAAT
MdPIP1;3[iv]	KY952167	GGATCCATGGAGGGCAAGGAAGAAG
		TCTAGATTAGCTCCTGGACTTGAAAGG

[i] Presented primer sequences are forward (top) and reverse (bottom).
[ii] Primer sequences presented in Dash et al. (2013).
[iii] Primer sequences presented in Li et al. (2010).
[iv] Primer sequence presented in Wang et al. (2017).

Table 2. Summary statistics of cumulative fruit drop, internal ethylene content, and functional xylem bundles for study conducted in 'Red Delicious' (*Malus ×domestica* L. Borkh) each week after treatment (WAT) in Mills River NC, USA (2020) and Hendersonville NC, USA (2021). Whole trees were treated with aminoethoxyvinylglycine (AVG), ethephon, naphthalene acetic acid (NAA), or untreated (CTRL). Treatments were imposed on 26 Aug 2020 and 25 Aug 2021.

		Cumulative fruit drop (%)		Internal ethylene content ($\mu L \cdot L^{-1}$)[i]		Stained primary bundles (no.)		Stained dorsal bundles (no.)	
		Mean ± *SD*		Median ± *SD*		Median ± *SD*		Median ± *SD*	
WAT	Treatment	2020	2021	2020	2021	2020	2021	2020	2021
0	CTRL	0.49 ± 0.36 a[ii]	—	0.02 ± 0.08 b[iii]	—	5.95 ± 2.8 a[iii]	—	3.86 ± 1.24 a[iii]	—
	AVG	0.48 ± 0.65 a	—	0.01 ± 0.04 b	—	5.81 ± 2.89 a	—	3.71 ± 1.42 a	—
	Ethephon	0.33 ± 0.32 a	—	0.18 ± 0.24 a	—	5.54 ± 3.36 a	—	3.46 ± 1.44 a	—
	NAA	0.44 ± 0.54 a	—	0.01 ± 0.18 b	—	3.59 ± 3.18 a	—	2.47 ± 1.7 a	—
1	CTRL	1.22 ± 0.43 a[ii]	2.24 ± 1.28 a[ii]	0.05 ± 0.41 b[iii]	0.05 ± 0.28 b[iii]	4.04 ± 3.41 a[iii]	5.62 ± 3.53 a[iii]	2.65 ± 1.74 a[iii]	2.41 ± 1.55 a[iii]
	AVG	0.78 ± 0.93 a	0.88 ± 1.12 a	0 ± 0.05 b	0.03 ± 0.05 c	4.62 ± 3.86 a	5.41 ± 2.8 a	3.17 ± 1.77 a	2.79 ± 1.72 a
	Ethephon	0.8 ± 0.8 a	1.06 ± 1.16 a	0.15 ± 1.11 a	0.22 ± 1.89 a	4.14 ± 2.79 a	5.48 ± 3.46 a	3.59 ± 1.52 a	2.55 ± 1.7 a
	NAA	1.09 ± 1.01 a	1.2 ± 1.5 a	0.03 ± 0.29 b	0.04 ± 2.23 b	4.7 ± 3.39 a	5.3 ± 2.31 a	3 ± 1.41 a	2.53 ± 1.76 a
2	CTRL	2.19 ± 1.05 a[ii]	4.69 ± 0.79 a[ii]	0.08 ± 0.17 a[iii]	0.24 ± 1.94 a[iii]	4.34 ± 3.52 a[iii]	4.87 ± 3.77 a[iii]	2.97 ± 1.78 a[iii]	2.13 ± 1.74 a[iii]
	AVG	1.27 ± 1.2 a	3.15 ± 2.21 a	0.03 ± 0.09 b	0.04 ± 0.06 b	3.45 ± 3.05 a	4.24 ± 3.45 a	3.21 ± 1.4 a	2.45 ± 2.1 a
	Ethephon	1.91 ± 0.68 a	6.44 ± 3.13 a	0.17 ± 4.84 a	0.3 ± 2.55 a	2.97 ± 2.77 a	4.96 ± 2.93 a	3.24 ± 1.75 a	2.63 ± 1.82 a
	NAA	1.36 ± 0.81 a	3.57 ± 3.6 a	0.09 ± 0.58 a	0.22 ± 1.38 a	2.86 ± 2.35 a	3.81 ± 2.97 a	2.43 ± 1.55 a	2.19 ± 1.66 a
3	CTRL	3.26 ± 1.01 a[ii]	7.72 ± 3.1 a[ii]	0.16 ± 5.73 a[iii]	0.35 ± 2.47 ab[iii]	2.85 ± 2.3 a[iii]	3.47 ± 3.14 a[iii]	2.74 ± 1.65 a[iii]	2.5 ± 2 a[iii]
	AVG	1.79 ± 1.31 a	4.57 ± 2.19 a	0.02 ± 0.06 b	0.04 ± 0.09 c	3.67 ± 3.01 a	4.9 ± 3.09 a	2.93 ± 1.66 a	2.3 ± 1.68 a
	Ethephon	3.4 ± 1.23 a	8.9 ± 2.53 a	0.14 ± 4.04 a	0.2 ± 1.52 b	3.25 ± 3.18 a	4.03 ± 3.58 a	2.54 ± 1.73 a	2.03 ± 1.84 a
	NAA	2.54 ± 1.08 a	5.59 ± 4.14 a	0.15 ± 2.44 a	0.4 ± 7.22 a	2.07 ± 3.07 a	2.97 ± 2.34 a	1.97 ± 1.72 a	1.43 ± 1.41 a
4	CTRL	5.51 ± 0.73 a[ii]	14.47 ± 8.66 a[ii]	0.42 ± 9.76 a[iii]	0.43 ± 16.37 a[iii]	1.13 ± 1.91 b[iii]	1.69 ± 2.74 a[iii]	2 ± 1.84 ab[iii]	1.45 ± 1.62 a[iii]
	AVG	2.23 ± 1.48 a	6.51 ± 2.69 a	0 ± 0.08 b	0.09 ± 0.47 b	3.07 ± 3.13 a	2.63 ± 3.03 a	2.77 ± 1.81 a	1.73 ± 1.55 a
	Ethephon	6.8 ± 3.78 a	12.07 ± 4.62 a	0.27 ± 10.29 a	0.38 ± 3.99 a	0.26 ± 0.53 c	2.34 ± 3.07 a	1.48 ± 1.53 b	1.34 ± 1.34 a
	NAA	3.47 ± 0.85 a	9.41 ± 7.86 a	0.14 ± 8.59 a	1.55 ± 6.96 a	1.03 ± 1.5 bc	1.56 ± 2.81 a	1.53 ± 1.63 b	1.41 ± 1.28 a
5	CTRL	15.58 ± 5.08 a[ii]	28.57 ± 14.53 a[ii]	15.57 ± 28.19 a[iii]	1.35 ± 20.41 b[iii]	0.32 ± 1.02 b[iii]	2.37 ± 3.38 ab[iii]	0.82 ± 1.25 a[iii]	1.48 ± 1.83 ab[iii]
	AVG	4.07 ± 1.38 b	7.2 ± 2.29 b	0.06 ± 0.36 b	0.05 ± 0.2 c	1.43 ± 1.99 a	3.03 ± 3.01 a	1.43 ± 1.45 a	1.79 ± 1.66 a
	Ethephon	15.78 ± 6.18 a	27.27 ± 15 ab	6.41 ± 43.93 a	0.88 ± 13.65 b	0 ± 0 c	1.66 ± 2.45 ab	0.66 ± 0.9 a	1.14 ± 1.77 ab
	NAA	6.66 ± 1.69 b	29.25 ± 10.16 a	20.45 ± 58.29 a	10.22 ± 16.21 a	1.59 ± 2.23 a	0.74 ± 2.09 b	1.21 ± 1.59 a	0.37 ± 0.88 b
6	CTRL	25.97 ± 8.92 a[ii]	53.26 ± 18.51 a[ii]	—	—	—	—	—	—
	AVG	5.03 ± 1.69 b	12.51 ± 2.55 b	—	—	—	—	—	—
	Ethephon	26.21 ± 7.75 a	54.25 ± 12.3 a	—	—	—	—	—	—
	NAA	11.85 ± 2.86 b	60.2 ± 10.32 a	—	—	—	—	—	—
7	CTRL	43.13 ± 6.49 a[ii]	66.11 ± 17 a[ii]	30.52 ± 43.98 a[iii]	—	0.17 ± 0.49 a[iii]	—	0.7 ± 1.18 b[iii]	—
	AVG	8.04 ± 1.13 b	24.99 ± 3.74 b	0.12 ± 9.14 b	—	0.78 ± 1.48 a	—	1.85 ± 1.73 a	—
	Ethephon	44.55 ± 10.65 a	72.79 ± 3.57 a	41.88 ± 51 a	—	0.12 ± 0.33 a	—	0.52 ± 0.92 b	—
	NAA	19.17 ± 5.27 b	76.14 ± 7.01 a	38.3 ± 56.93 a	—	0.1 ± 0.41 a	—	0.52 ± 1.15 b	—
8	CTRL	54.97 ± 6.27 a[ii]	70.08 ± 16.25 a[ii]	—	—	—	—	—	—
	AVG	12.81 ± 3.02 b	28.88 ± 4.6 b	—	—	—	—	—	—
	Ethephon	52.86 ± 8.76 a	76.14 ± 3.82 a	—	—	—	—	—	—
	NAA	23.71 ± 5.53 b	78.2 ± 6.91 a	—	—	—	—	—	—

[i] Data transformed for statistical analysis by taking the natural log of internal ethylene content.
[ii] Treatments within each WAT and year separated using Tukey's honest significant difference at $\alpha = 0.05$.
[iii] Treatments within each WAT and year separated using Kruskal–Wallis test at $\alpha = 0.05$.

expression analysis are listed in Table 1. RT-qPCR reactions were carried out in a real-time PCR machine (Stratagene Mx3005P; Agilent Technologies, Inc., Santa Clara, CA, USA). The thermal profile for RT-qPCR reactions were as follows: 50 °C for 2 min; 95 °C for 5 min; 40 cycles of 95 °C for 30 s then 60 °C for 1 min. A denaturation program was performed at the end of the PCR to determine primer specificity.

Relative quantity of expression (RQ) of each gene was calculated by first determining primer efficiency (Eff) in LinRegPCR (Ramakers et al. 2003; Ruijter et al. 2009). Then cycle threshold values (Ct) were determined for each sample (MxPro, Agilent Technologies, Inc.). RQ values were calculated using the following equation: $RQ = 1/Eff^{Ct}$. RQ values of genes of interest were then normalized to the expression of the reference gene *MdGAPDH*, generating normalized relative quantity of expression (NRQ) values. *MdGAPDH* and *MdACTIN* were both tested as housekeeping genes, but expression of *MdACTIN* was sporadic across samples. NRQ values were then transformed using $\log_2$ for statistical analysis. Expression of target genes in the fruit pedicel are presented relative to the expression of the gene for the AVG treatment on 28 Sep 2021.

Data analysis. All data analysis was done in R (R Foundation for Statistical Computing, Vienna, Austria). Differences among treatments for initial crop load were tested with analysis of variance. Mean separation was conducted between treatments on each measurement date for cumulative fruit abscission, internal ethylene content, maturity indices, and stained xylem vessels. Tukey's honest significant difference was performed for parametric data. For nonparametric data, Kruskal–Wallis rank sum test was used to detect treatment differences. Treatment separation for internal ethylene content was analyzed on a natural log scale, but untransformed values are presented. Second-order polynomial regression models were built using lm function in the Stats R package (R Foundation for Statistical Computing) between cumulative fruit drop and total xylem functionality, as well as cumulative fruit drop and internal ethylene content. Regression models were built for each treatment and year combination. $\log_2$ transformed NRQ values were compared within each tissue type and sampling date for untreated control and AVG-treated samples using paired *t* tests.

Results

Fruit drop. Initial crop load was not different between treatments in 2020 ($P = 0.4$) and 2021 ($P = 0.221$). In both years, there were low levels of PFD (10% to 15%) until ~3 weeks after treatment [WAT (Table 2)]. AVG suppressed PFD compared with the control and ethephon treatments from 4 WAT onward. At the same stage, NAA-treated fruit had lower PFD compared with the control and ethephon in 2020, but not 2021. Ethephon did not increase PFD compared with the control at any measurement date in both years.

Cumulative PFD reached ~50% for the untreated control and ethephon-treated fruit by the end of each study period, 21 Oct 2020

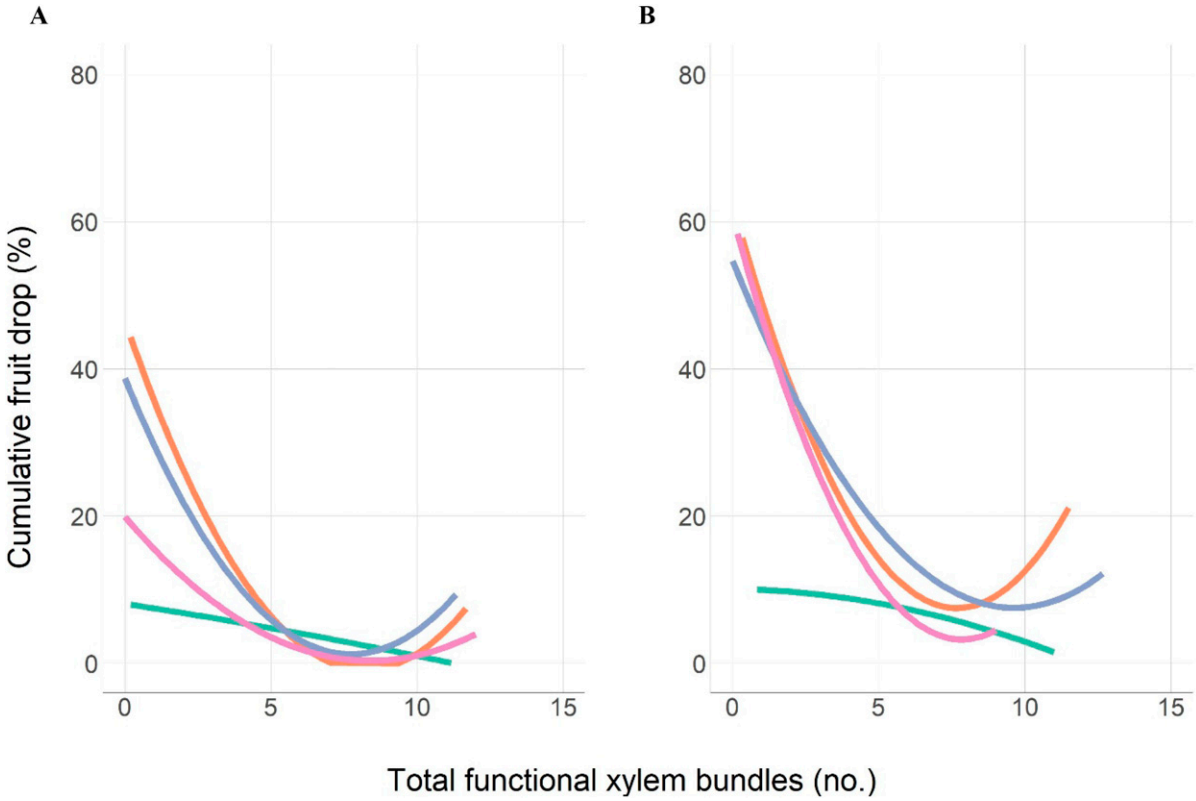

Fig. 2. Second-order polynomial regression curves between total number of stained xylem vessels and cumulative percent fruit drop of the subsequent week following xylem functionality test in 2020 (**A**) and 2021 (**B**). Four treatments: aminoethoxyvinylglycine [AVG (teal)], ethephon (blue), and naphthalene acetic acid [NAA (pink)] and an untreated control (orange) were imposed on 26 Aug 2020 and 25 Aug 2021, 2 weeks before anticipated harvest, to a mature block of 'Red Delicious' apple (*Malus* ×*domestica*). Regression calculated from the mean for each replication (five replications, complete randomized design) on each measurement date (2020, n = 140; 2021, n = 100). In 2020, untreated control: $r^2 = 0.676$, $P < 0.001$; AVG: $r^2 = 0.289$, $P < 0.01$; ethephon: $r^2 = 0.571$, $P < 0.001$; NAA: $r^2 = 0.601$, $P < 0.001$. In 2021, untreated control: $r^2 = 0.525$, $P < 0.001$; AVG: $r^2 = 0.299$, $P < 0.05$; ethephon: $r^2 = 0.494$, $P < 0.001$; NAA: $r^2 = 0.702$, $P < 0.001$.

and 11 Oct 2021 (Table 2). Approximately 15% of AVG-treated fruit had abscised by the end of the study in both years (Table 2). Nearly 25% of NAA-treated fruit abscised in 2020 (Fig. 2A), whereas 60% abscised in 2021 (Table 2).

Fruit maturity/internal ethylene content. IEC increased exponentially throughout the study period in 2020 and 2021 for the untreated control, ethephon, and NAA-treated fruit. In both years, ethephon-treated fruit did have a higher IEC than all other treatments in the first week following application (Table 2). There were no significant differences in IEC between the untreated control and ethephon from 2 WAT onward in both years. Compared with the untreated control and ethephon, NAA had no difference in IEC from this point onward in 2020 (Table 2) but had higher IEC 4 WAT (Table 2). AVG-treated fruit had lower IEC compared with all other treatments from 2 weeks after treatment application onward in both years (Table 2).

Starch staining rating increased throughout the measurement period from ~2 to 8 in both years (Table 3). In 2020, ethephon-treated fruit had greater starch degradation than NAA and AVG-treated fruit shortly after treatment application until the week of harvest. Ethephon treatments resulted in greater starch degradation than in the untreated control at 2 WAT (Table 3). In 2020, AVG-treated fruit had decreased starch degradation than all other treatments from 4 WAT onward (Table 3). In 2021, starch staining rating was lower for AVG compared with only ethephon from 1 WAT onward (Table 3). From 2 WAT, in 2020, and 1 WAT, in 2021, there was no significant difference between NAA- and ethephon-treated fruit (Table 3). In 2021, NAA had significantly higher starch degradation than the untreated control the week of harvest and 4 WAT (Table 3). SSC had a similar pattern to starch degradation increasing for all treatments throughout the measurement period in both years (Table 3). In 2020, ethephon-treated fruit had higher SSC at 0 WAT than all other treatments (Table 3). The only significant difference between treatments in 2021 was that ethephon-treated fruit had higher SSC than AVG-treated and the untreated control at 1 WAT (Table 3).

Throughout the measurement periods in both years, fruit firmness decreased for all treatments (Table 3). In 2020, NAA-treated fruit had the lowest firmness by the end of the measurement period. This decrease in firmness was evident from the 2 WAT, except at 3 WAT when there was no significant difference between NAA- and ethephon-treated fruit (Table 3). In 2020, AVG-treated fruit had higher firmness than NAA-treated fruit at 3 WAT, NAA- and ethephon-treated fruit at 4 WAT, and all three other treatments at 6 WAT (Table 3). There were fewer significant differences

Table 3. Summary statistics for fruit maturity metrics soluble solids content, starch staining index, and fruit firmness for study conducted in 'Red Delicious' (*Malus ×domestica* L. Borkh) each week after treatment (WAT) in Mills River NC, USA (2020) and Hendersonville NC, USA (2021). Whole trees were treated with aminoethoxyvinylglycine (AVG), ethephon, naphthalene acetic acid (NAA), or untreated (CTRL). Treatments were imposed on 26 Aug 2020 and 25 Aug 2021.

		Soluble solids content (%)		Starch staining index		Fruit firmness (N)	
		Mean ± *SD*		Mean ± *SD*		Median ± *SD*	
WAT	Treatment	2020	2021	2020	2021	2020	2021
0	CTRL	9.32 ± 0.19 a[i]	8.5 ± 0.42 a[i]	2.54 ± 0.77 a[ii]	2.65 ± 1.13 a[ii]	74.7 ± 5.2 a[ii]	73.6 ± 4.8 a[i]
	AVG	9.18 ± 0.68 a	8.8 ± 0.27 a	2.62 ± 0.92 a	2.31 ± 0.7 a	72.7 ± 5.6 a	74.5 ± 4.6 a
	Ethephon	9.3 ± 0.4 a	8.4 ± 0.21 a	2.48 ± 0.73 a	2.39 ± 0.56 a	74.2 ± 5.6 a	74.1 ± 4.2 a
	NAA	9.22 ± 0.5 a	8.76 ± 0.28 a	2.25 ± 0.91 a	2.32 ± 0.57 a	73.4 ± 4.4 a	73.2 ± 3.6 a
1	CTRL	9.7 ± 0.38 b[i]	8.92 ± 0.64 a[i]	2.73 ± 0.94 ab[ii]	3.19 ± 1.59 a[ii]	72.1 ± 4.2 a[ii]	72.6 ± 4.9 a[ii]
	AVG	9.48 ± 0.47 b	9.04 ± 0.41 a	2.58 ± 0.91 b	3.4 ± 1.35 a	71.3 ± 4.6 a	71.8 ± 3.8 ab
	Ethephon	10.48 ± 0.45 a	9.3 ± 0.45 a	3.02 ± 0.89 a	3.49 ± 1.32 a	70.9 ± 4.5 a	70.9 ± 4 ab
	NAA	9.56 ± 0.34 b	9.24 ± 0.41 a	2.53 ± 0.94 b	3.6 ± 1.48 a	70.6 ± 4 a	71.1 ± 4.5 b
2	CTRL	10.42 ± 0.34 b[i]	9.52 ± 0.81 b[i]	3.35 ± 1.1 b[ii]	5.03 ± 1.61 b[ii]	70.8 ± 3.7 a[ii]	71 ± 4.8 a[ii]
	AVG	10.2 ± 0.65 b	9.3 ± 0.32 b	3.14 ± 1.12 b	4.95 ± 1.43 b	69.7 ± 6 a	70.1 ± 4.8 a
	Ethephon	11.42 ± 0.28 a	10.42 ± 0.29 a	4.35 ± 1.52 a	5.75 ± 1.72 a	68.7 ± 4.5 a	69.6 ± 4.2 a
	NAA	10.42 ± 0.51 b	9.66 ± 0.35 ab	3.23 ± 1.28 b	5.56 ± 1.56 ab	69.9 ± 3.9 a	70.8 ± 4.4 a
3	CTRL	10.96 ± 0.51 a[i]	10.4 ± 0.95 a[i]	2.73 ± 0.94 ab[ii]	5.66 ± 1.83 b[ii]	69.8 ± 4.8 a[ii]	69.6 ± 5.2 a[i]
	AVG	10.36 ± 0.9 a	10.66 ± 0.83 a	2.58 ± 0.91 b	5.48 ± 1.68 b	68.6 ± 4.8 a	69.7 ± 4.3 a
	Ethephon	11.44 ± 0.7 a	10.76 ± 0.59 a	3.02 ± 0.89 a	6.75 ± 1.61 a	68.4 ± 4.4 a	69.2 ± 4.3 a
	NAA	10.86 ± 0.8 a	10.54 ± 0.48 a	2.53 ± 0.94 b	6.47 ± 1.62 a	66.5 ± 3.9 b	68.1 ± 5.2 a
4	CTRL	11.78 ± 0.54 ab[i]	10.1 ± 1.09 a[i]	5.14 ± 1.68 a[ii]	6.47 ± 1.68 ab[ii]	66.9 ± 4.2 a[ii]	68.7 ± 4.3 a[i]
	AVG	11.06 ± 0.53 b	9.9 ± 0.41 a	5.08 ± 1.51 a	6.4 ± 1.34 b	66.8 ± 6.1 a	65.7 ± 3.9 a
	Ethephon	12.06 ± 0.17 a	10.62 ± 0.75 a	5.77 ± 1.67 a	7 ± 1.34 ab	65.8 ± 6.8 ab	66.7 ± 4 a
	NAA	11.46 ± 0.7 ab	10.76 ± 0.51 a	5.29 ± 1.86 a	6.73 ± 1.48 a	64.5 ± 5.1 b	66.9 ± 5.2 a
5	CTRL	12.74 ± 0.56 a[i]	11.32 ± 1.44 a[ii]	6.69 ± 1.19 a[ii]	7.12 ± 1.24 bc[ii]	65 ± 5.2 ab[ii]	66.2 ± 5.8 ab[ii]
	AVG	11.5 ± 0.46 a	10.64 ± 0.26 a	5.89 ± 1.55 b	6.93 ± 0.96 c	66.1 ± 4.1 a	67.5 ± 4.8 a
	Ethephon	12.84 ± 0.88 a	10.94 ± 0.8 a	6.88 ± 0.82 a	7.33 ± 1.1 b	63.7 ± 3.7 b	66.6 ± 5.4 ab
	NAA	12.82 ± 1.1 a	11.68 ± 0.59 a	6.79 ± 1.07 a	7.81 ± 0.43 a	60.1 ± 5.1 c	63.9 ± 5.3 b
7	CTRL	13.82 ± 0.72 a[i]	—	7.58 ± 0.92 a[ii]	—	56.6 ± 8.7 b[ii]	—
	AVG[i]	12.58 ± 1.11 a	—	6.62 ± 1.26 b	—	62.4 ± 6.3 a	—
	Ethephon	13.82 ± 0.93 a	—	7.64 ± 0.48 a	—	57.3 ± 9 b	—
	NAA	13.76 ± 1.15 a	—	7.74 ± 0.52 a	—	51.6 ± 9.8 c	—

[i] Treatments within each WAT and year separated using Tukey's honest significant difference at $\alpha = 0.05$.
[ii] Treatments within each WAT and year separated using Kruskal–Wallis test at $\alpha = 0.05$.

between treatments in 2021, only with AVG having higher firmness than NAA-treated fruit at 0 WAT and 4 WAT (Table 3).

Xylem functionality. Overall, the number of functional xylem vessels in the fruit assessed by acid fuchsin dye decreased throughout the measurement period for all treatments in both years (Table 2). Nearly six of 10 primary bundles were functional at the beginning of the study in both years. At 5 WAT, there was on average less than one and approximately two functional primary bundles in 2020 and 2021, respectively (Table 2). A similar rate of decrease in xylem functionality occurred in dorsal bundles, with nearly four and three of five bundles functional in 2020 and 2021, respectively (Table 2). In 2020 there was <1 functional dorsal bundle at the end of the study and ~1.5 in 2021 (Table 2).

In 2020, AVG-treated fruit had more functional primary bundles than all other treatments and more functional dorsal bundles than NAA- and ethephon-treated fruit at 5 WAT (Table 2). There were a higher number of functional primary bundles in AVG- and NAA-treated fruit at 4 WAT and dorsal bundles in AVG-treated fruit at 6 WAT in 2020 (Table 2). In 2021, the only significant differences between treatments were that AVG-treated fruit had a higher number of functional primary and dorsal bundles than NAA-treated fruit 4 WAT (Table 2).

Relationships among fruit drop, xylem functionality, and internal ethylene content. There was an exponential increase in cumulative PFD in both years as total functional xylem bundles decreased (Fig. 2) and IEC increased (Fig. 3). In 2020, the R^2 values between cumulative PFD and xylem functionality was 0.676 [untreated control ($P < 0.001$)], 0.289 [AVG-treated ($P < 0.01$)], 0.571 [ethephon-treated ($P < 0.001$)], and 0.601 [NAA-treated ($P < 0.001$)]. In 2021, the R^2 for each treatment was 0.525 for the untreated control ($P < 0.001$), 0.299 for AVG-treated ($P < 0.05$), 0.494 for ethephon-treated ($P < 0.001$), and 0.702 for NAA-treated ($P < 0.001$). The R^2 between IEC and cumulative PFD was 0.855 [untreated control ($P < 0.001$)], 0.588 [AVG-treated ($P < 0.001$)], 0.773 [ethephon-treated ($P < 0.001$)], and 0.655 [NAA-treated ($P < 0.001$)] in 2020. In 2021, the R^2 for each treatment was 0.532 for the untreated control ($P < 0.001$), 0.286 for AVG-treated ($P < 0.05$), 0.526 for ethephon-treated ($P < 0.001$), and 0.719 for NAA-treated ($P < 0.001$).

Gene expression. Expression of *MdEG1* and *MdPG2* in fruit pedicels significantly differed between untreated control and AVG treatments at 5 WAT (Fig. 4). On this measurement date, expression of *MdEG1* was nearly 7-fold higher in the untreated control compared with AVG (Fig. 4A). Similarly, there was over 40-fold higher expression of *MdPG2* for the untreated control compared with the AVG treatment at 5 WAT (Fig. 4B). Expression of *MdEG1* and *MdPG2* were not detected in fruit cortex samples that were collected on 23 and 28 Sep 2021 (data not shown). *MdEXPA10;1* was inconsistently expressed in both pedicel and cortex tissues on all collection dates (data not shown). There was no detectable expression of *MdPIP1;3* in most of the samples analyzed (data not shown).

Discussion

Red Delicious has been noted to be a cultivar that is prone to PFD (Irish-Brown et al. 2011). In the current study, fruit drop of

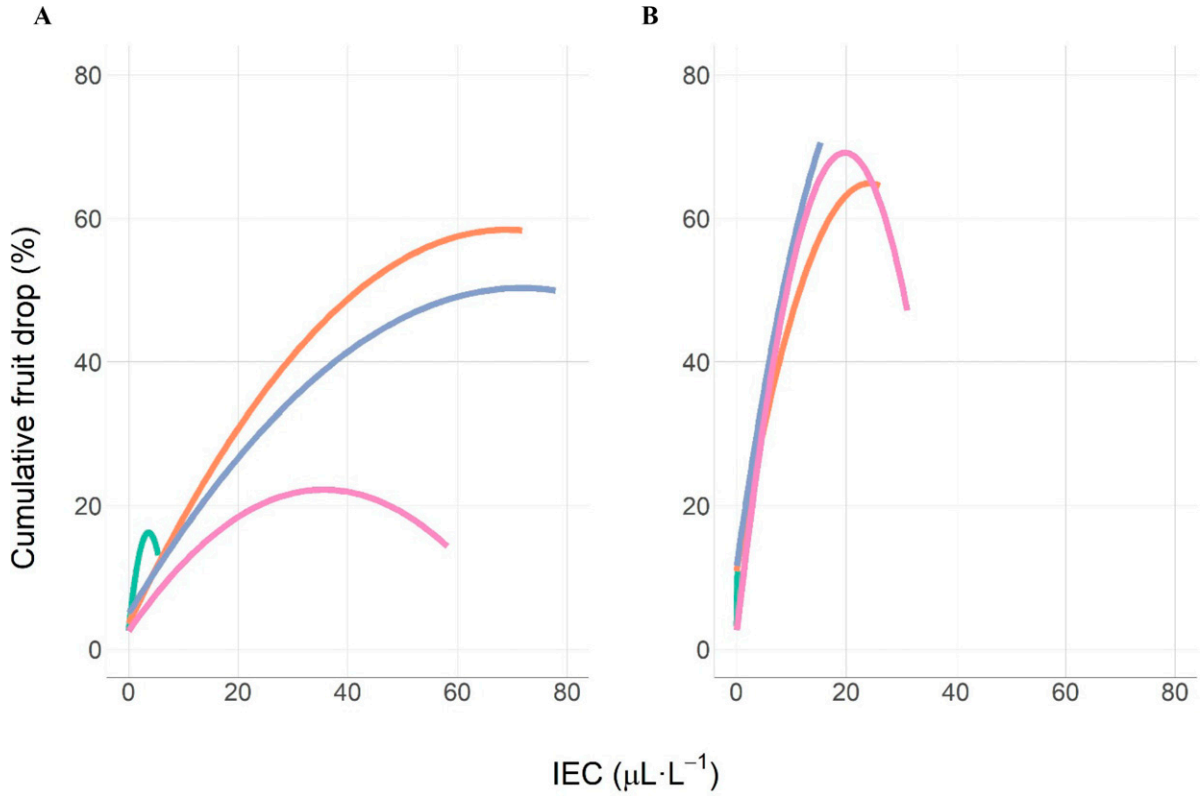

Fig. 3. Second-order polynomial regression curves between internal ethylene content (IEC) and cumulative percent fruit drop of the subsequent week following ethylene quantification 2020 (**A**) and 2021 (**B**). Four treatments: aminoethoxyvinylglycine [AVG (teal)], ethephon (blue), and naphthalene acetic acid [NAA (pink)] and an untreated control (orange) were imposed on 26 Aug 2020 and 25 Aug 2021, 2 weeks before anticipated harvest, to a mature block of 'Red Delicious' apple (*Malus ×domestica*). Each point is the mean for each replication (five replications, complete randomized design) on each measurement date (2020, n = 140; 2021, n = 100). In 2020, untreated control: $r^2 = 0.855$, $P < 0.001$; AVG: $r^2 = 0.588$, $P < 0.001$; ethephon: $r^2 = 0.733$, $P < 0.001$; NAA: $r^2 = 0.655$, $P < 0.001$. In 2021, untreated control: $r^2 = 0.532$, $P < 0.001$; AVG: $r^2 = 0.286$, $P < 0.05$; ethephon: $r^2 = 0.526$, $P < 0.001$; NAA: $r^2 = 0.719$, $P < 0.001$.

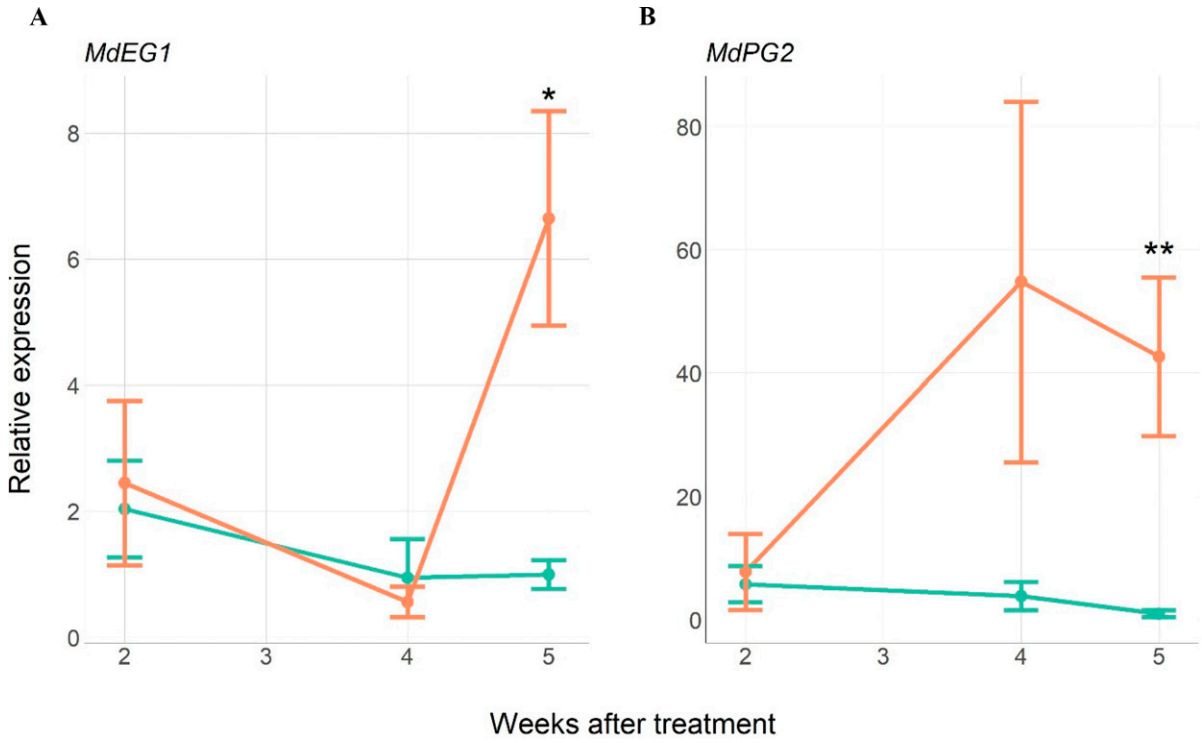

Fig. 4. Relative expression of *MdEG1* (**A**) and *MdPG2* (**B**) in fruit pedicel of 'Red Delicious' apple (*Malus* ×*domestica*) treated with aminoethoxyvinylglycine [AVG (teal)] and untreated control (orange) throughout the measurement period in 2021. The pedicels of three fruit per five replications were collected each sampling date. Treatments were applied 25 Aug 2021. Fruit pedicels collected from a mature orchard in Hendersonville, NC, USA. Quantification of target genes were normalized to quantification of housekeeping gene *MdGADPH*. Expression of the target genes relative to the expression of the AVG treatment on 28 Sep 2021. Paired *t*-0 used to compare expression of target gene at each sampling date between untreated control and AVG treatments. $*P < 0.05$; $**P < 0.005$.

'Red Delicious' was not substantial for the untreated control until 3 WAT in either year of the study. This result concurs with previous PFD studies on 'Red Delicious' that found ∼29% cumulative fruit drop at 3 WAT (Greene 2002; Unrath et al. 2009). AVG limiting fruit drop throughout the measurement period in both years also aligns with previous studies (Greene 2002; Li and Yuan 2008; Li et al. 2010). NAA has been found to be ineffective in cultivars that produce high levels of ethylene [Robinson et al. 2010, as cited in Arseneault and Cline 2016]. The difference in ethylene levels between 2020 and 2021 may explain the lack of fruit drop control with NAA in 2021. In the current study at 2 WAT the IEC of NAA treated fruit was 0.09 $\mu L \cdot L^{-1}$ in 2020, whereas in 2021 it was >2-fold higher at 0.22 $\mu L \cdot L^{-1}$. Higher IEC in 2021 suggests that fruit were more susceptible to PFD in 2021. This trend was evident as nearly 30% of untreated fruit had dropped by 5 WAT in 2021, but less than 20% had dropped in 2020 at the same time point. There was likely not any difference in NAA metabolism between years as average daily temperature was 24.5 °C in 2020 and 24.7 °C in 2021 for the week following application.

IEC corresponded with cumulative fruit drop. The climacteric rise in IEC occurred in the untreated control, NAA, and ethephon at 4 WAT in both years. For the untreated control, IEC accounted for from 53% to 85% of the variance in cumulative PFD over the entire measurement period. These data support previous findings that ethylene is involved in apple fruit abscission mechanisms (Li and Yuan 2008; Sun et al. 2009). Li et al. (2010) found a concurrent increase in expression of genes related to ethylene biosynthesis (i.e., *MdACS5A* and *MdACO1*) and genes that encode for cell wall disassembly-related enzymes [i.e., polygalacturonsae (*MdPG2*) and endogluconase (*MdEG1*)]. The results of this study substantiated this finding as the expression of *MdEG1* and *MdPG2* were both greater in the pedicels of untreated control vs. AVG-treated fruit at 5 WAT in 2021. This coincided with the rapid increase in cumulative fruit drop in the untreated control seen in both years. These results indicate that at the stage of maturity that fruit drop occurred in this study, there is a direct relationship between IEC, breakdown of the fruit pedicel, and fruit drop.

There was a linear decrease in xylem functionality within fruit throughout the measurement period in both years. Contrasting with IEC that exponentially increased concurrently with fruit pedicel breakdown and cumulative fruit drop, the constant decrease in functional xylem bundles is independent of cell wall breakdown in the pedicel. Although both treatments lost xylem functionality at a similar rate, there was a marked difference in R^2 values between total xylem functionality and cumulative PFD for untreated control and AVG treatments. For the untreated control total xylem functionality explained 67% (2020) and 52% (2021) of the variance in cumulative PFD. Although only 29% (2020) and 30% (2021) of the variance in cumulative PFD was explained by xylem functionality for the AVG treatment. The initial hypothesis that xylem functionality could be used as a sole predictor of PFD is not supported by these findings.

Expression of genes related to cell wall disassembly in the pedicel was not increased until after the onset of loss of xylem functionality. Expression of *MdEG1* and *MdPG2* was not higher in pedicels of untreated control compared with that in AVG treated fruit until 5 WAT. This marked and rapid increase in cell wall hydrolysis is unlikely to be directly associated with loss of xylem functionality as the latter decreased linearly. Lang and Ryan (1994) reported that loss of xylem functionality throughout the growing season in apple fruit is

due to physical rupture of vessels due to the growing fruit stretching vessels. The current study did not directly measure fruit growth rate.

An alternative explanation for loss of functionality is cavitation of the xylem. As fruit develop, stomata lose functionality of guard cells, remaining open, and develop into lenticels (Blanke and Lenz 1989). Xylem sap flow markedly decreases toward harvest (Lang and Ryan 1994). Together, the lack of stomatal closure and decreases in transpiration suggest that fruit xylem would be susceptible to embolism. Microscopic examination of xylem throughout the pedicel and length of the fruit would attribute loss of xylem functionality to either physical rupture or embolism.

Conclusions and Future Directions

The findings of this study did not support the hypothesis that loss of xylem functionality directly leads to PFD. Treatments that effectively limited PFD (AVG in 2020 and 2021; NAA in 2020) did not consistently maintain higher xylem functionality than treatments with substantial PFD. Loss of xylem functionality was not coincident with cell wall hydrolysis in the fruit pedicel. IEC was a better indicator than xylem functionality at the advanced maturity where fruit drop occurred in this study. Increases in IEC, expression of cell-wall disassembly genes, and cumulative fruit drop were evident. Further analysis is necessary to determine if *MdEG1* and *MdPG2* in the pedicel/AZ can serve as useful markers to predict fruit drop in addition to ethylene biosynthesis-based tools. Together, such tools can help predict susceptibility to PFD.

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Supplemental Table S1. Summary of dates of application and data collection in 2020 and 2021 for study conducted in 'Red Delicious' apple (*Malus ×domestica*).

	Drop count		Fruit maturity parameters		Internal ethylene content		Xylem functionality		Collection for gene expression	
WAT[i]	2020[ii]	2021[iii]	2020	2021	2020	2021	2020	2021	2020	2021
0	25 Aug	—	28 Aug	24 Aug	28 Aug	—	27 Aug	—	27 Aug	23 Aug
1	1 Sep	29 Aug	1 Sep	31 Aug	1 Sep	30 Aug	2 Sep	1 Sep	1 Sep	29 Aug
2	8 Sep	9 Sep	7 Sep	8 Sep	7 Sep	7 Sep	9 Sep	8 Sep	8 Sep	7 Sep
3	15 Sep	15 Sep	14 Sep	14 Sep	14 Sep	13 Sep	16 Sep	15 Sep	15 Sep	13 Sep
4	22 Sep	23 Sep	21 Sep	21 Sep	21 Sep	20 Sep	23 Sep	22 Sep	22 Sep	20 Sep
5	29 Sep	28 Sep	29 Sep	29 Sep	29 Sep	27 Sep	30 Sep	29 Sep	30 Sep	27 Sep
6	7 Oct	11 Oct	—	—	—	—	—	—	7 Oct	—
7	13 Oct	20 Oct	12 Oct	—	12 Oct	—	14 Oct	—	13 Oct	—
8	21 Oct	25 Oct	—	—	—	—	—	—	20 Oct	—

[i] WAT = weeks after treatment. Treatments were an imposed on 26 Aug 2020 and 25 Aug 2021. Whole trees were treated with untreated control, aminoethoxyvinylglycine, naphthalene acetic acid, or ethephon.
[ii] Study conducted at Mountain Horticultural Crops Research and Extension Center (Mills River, NC, USA).
[iii] Study conducted at commercial grower's site in Hendersonville NC, USA.