Auxins and ABA promote vascular function and reduce bitter pit of ‘Honeycrisp’ apples

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Introduction and Background

- **Bitter pit** is generally thought to be associated with localized Ca deficit
- Ca transport *in-planta* is limited to xylem
- Xylem in fruit undergoes significant strain, progressively rupturing beginning ~30 dafb
- Near the end of the season, relatively little to no xylem connections remain intact and relatively little to no additional Ca is supplied to the fruit
- Bitter pit is also associated with excess Mg and K (Garman and Mathis, 1956; Willis, 1976)
- Ratios of K, Mg, and/or N to Ca have explained ~70% of bitter pit incidence (Marini et al. 2020)

Current Strategies to Mitigate Bitter Pit

- Control biennial bearing and crop load to ~6 to 7 fruit per cm^2 TCA… low crop loads tend to exacerbate bitter pit
- Select rootstocks that do not augment the disorder (i.e., rootstocks that are too vigorous or those with greater affinity to acquire K such as G.41, G.11)… Bud 9, G.214 and G.969 appear better suited
- Frequent foliar applications of Ca (~50% less Ca is delivered to Honeycrisp fruit than Gala; Cheng, 2018)… 3 to 5 lbs of elemental Ca per acre per season
- Reduce potassium levels (K and Mg compete with Ca)… after pre-plant soil preparation the target relative abundance of Ca, Mg and K should be 20:4:1 on an equivalent basis (Cheng and Sazo, 2018) or aim for leaf concentrations of 1 to 1.2 % or reduce maintenance K apps by 30%
- Control N fertilizer and shoot extension growth (P-Ca applications?)

The Effect of Auxin on Xylem

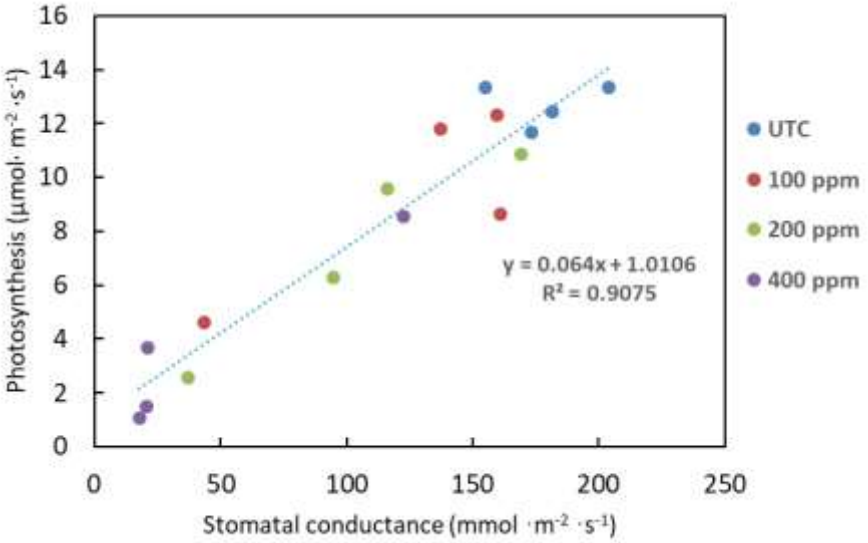
- IAA has been widely known to regulate xylem differentiation; early experiments with Zinnia
- IAA mediates polar Ca transport (Horst, 1980)
- In addition to differentiation, flexibility of xylem tissue is mediated by IAA (Yoshimoto et al. 2016)
- Use of an auxin transport inhibitor, 2,3,5-triiodobenzoic acid, reduced Ca of tomato fruit (Bangerth 1976)

Effect of an Auxin Transport Inhibitor on Xylem Functionality in 'Honeycrisp'

Treatment	Stem Primary	Stem Dorsal	Calyx Primary	Calyx Dorsal
UTC (Unthinned)	3.41	0.56	2.42	0.32
UTC (Thinned)	1.89	0.57	1.56	0.49
TIBA	1.19	0.2	1.1	0.2
P value	*0.035*	*0.354*	*0.137*	*0.373*

The Effect of ABA on Xylem

- ABA reduced transpiration and BER and increased xylem function, water and Ca delivery to tomato fruit (de Freitas et al. 2011)
- ABA regulates a variety of Ca genes and increased Ca allocation to apple fruit (Falchi et al. 2017)
- ABA reduces gas exchange of 'Honeycrisp' leaves (rate dependent), persisting for ~7 d



Wittenbach and Einhorn, unpublished

Hypothesis

Applications of ABA and auxins will increase xylem differentiation and the functional life of vascular tissues in fruit which will, in turn, improve fruit calcium concentration and, ultimately, reduce bitter pit

Materials and Methods

5th leaf 'Honeycrisp'/G.11 trees selected on trunk circ. and bloom

- RCBD: 10 treatments, 5 replicates
- Successive applications: 30, 45, 60 dafb
- Fruit were sampled and dyed throughout the season

Active Ingredient	Formula or Product (% a.i.)	Active Ingredient (ppm) (2021)	Active Ingredient (ppm) (2022)
Control	-	-	-
IAA	Pure solid	5	10
		10	20
		20	40
NAA	Fruitone®L	5	2.5
		10	5
		20	10
ABA	ProTone® (20%)	75	62.5
		150	125
		-	250

Materials and Methods

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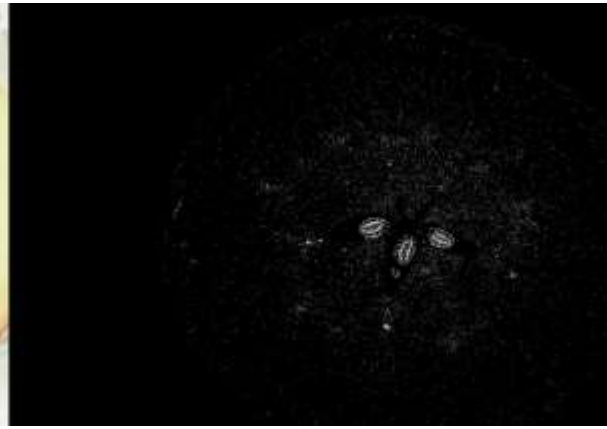
Fruit at 86 DAFB

Primary bundles are the largest type of bundle and provide nutrients to the flesh (10 total)

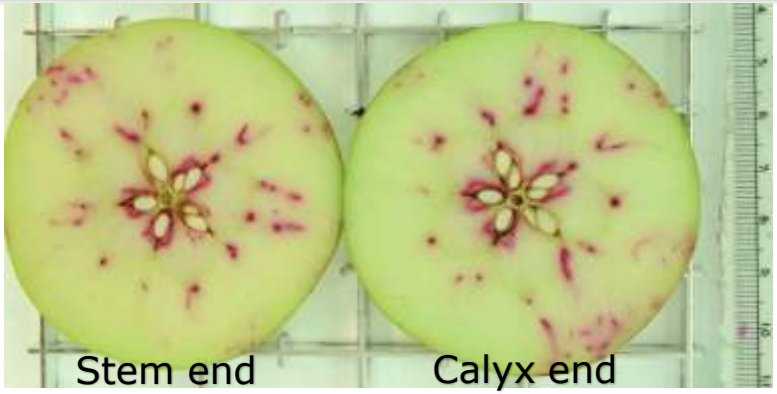
Dorsal bundles are the primary suppliers of nutrients to growing seeds (5 total)

Xylem networks throughout the cortex

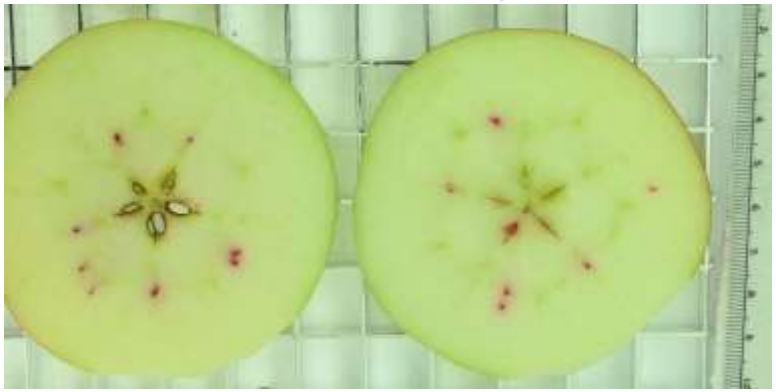


Results: Xylem Function

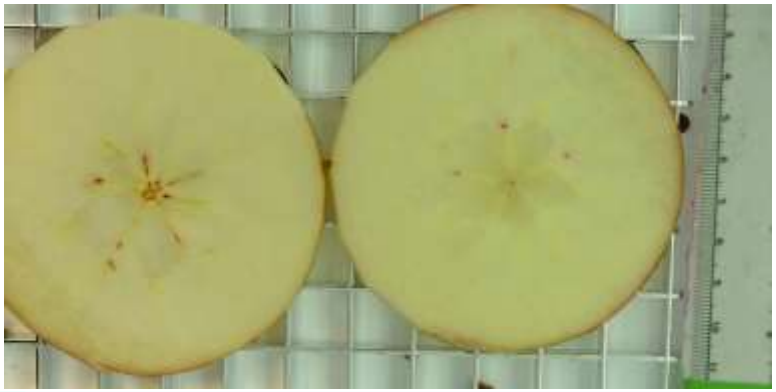
86 DAFB

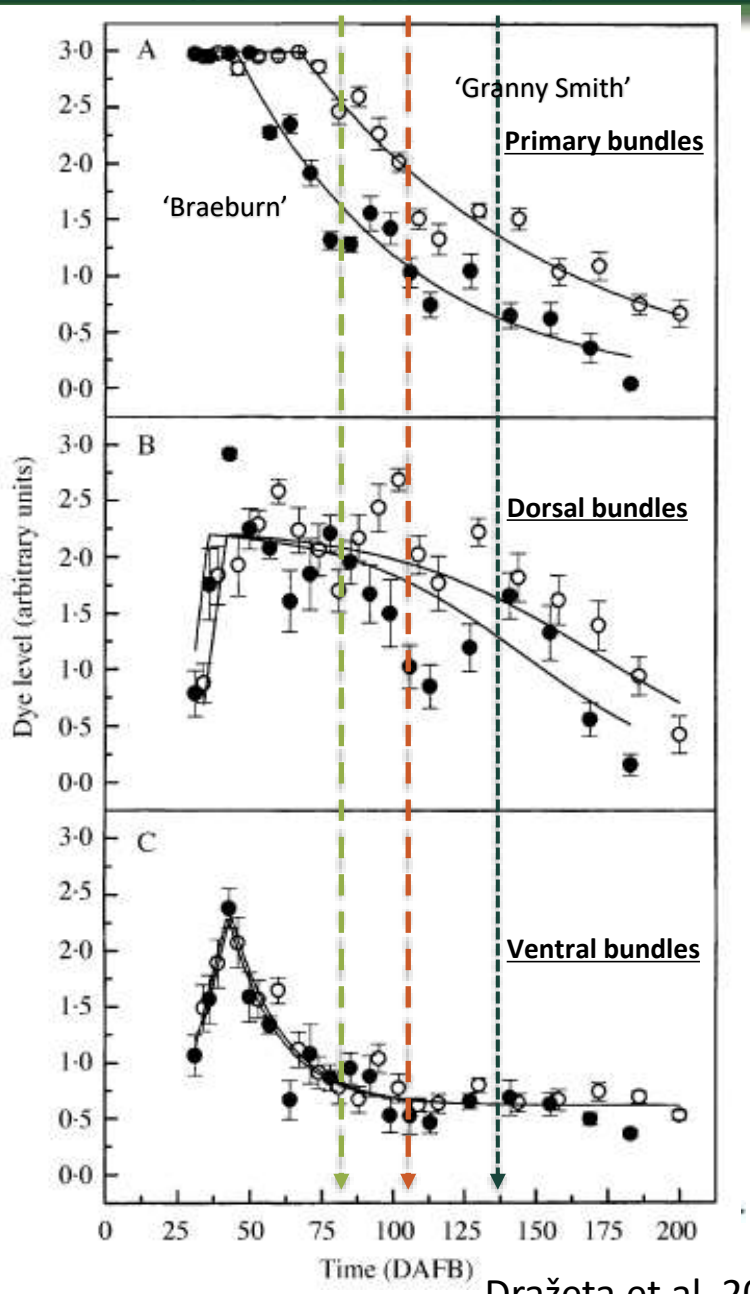


107 DAFB



136 DAFB





Dražeta et al. 2004

Results: Rates of Xylem Dysfunction

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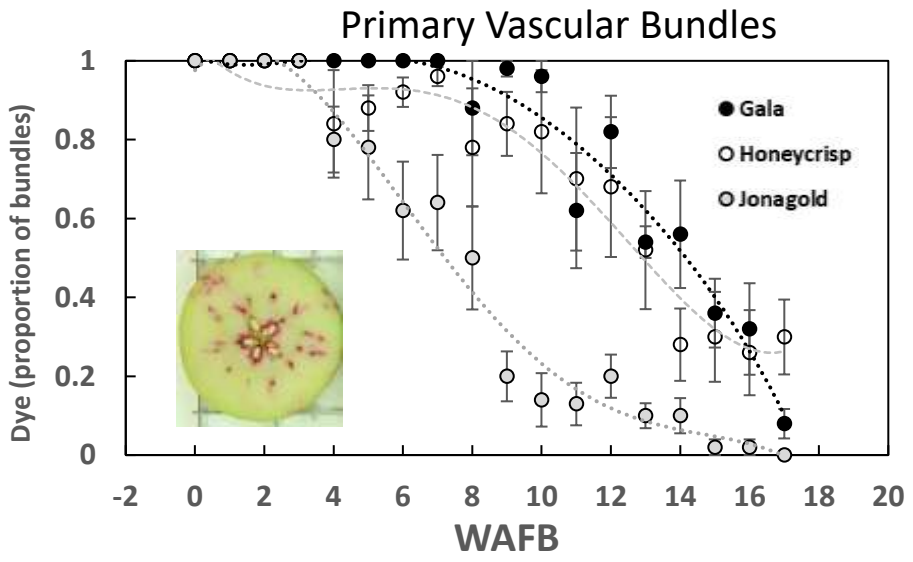
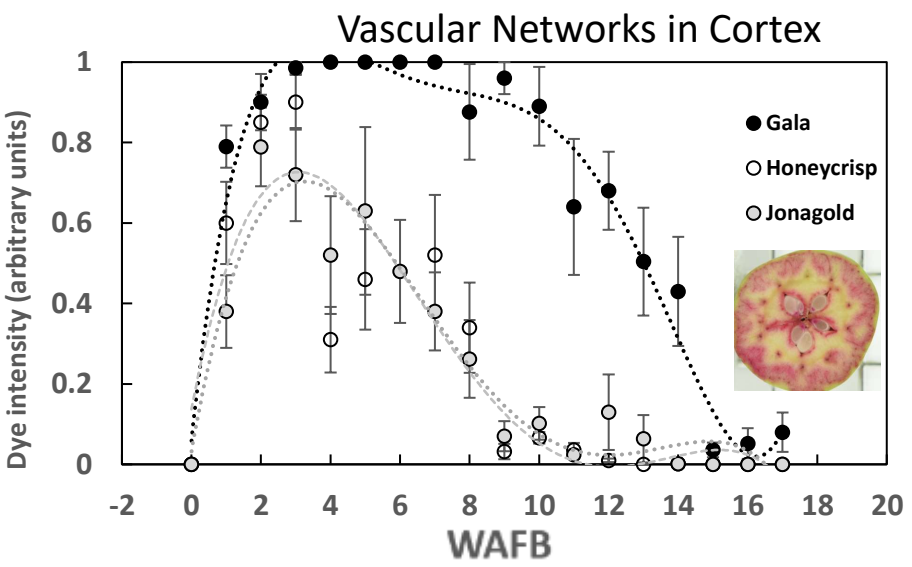
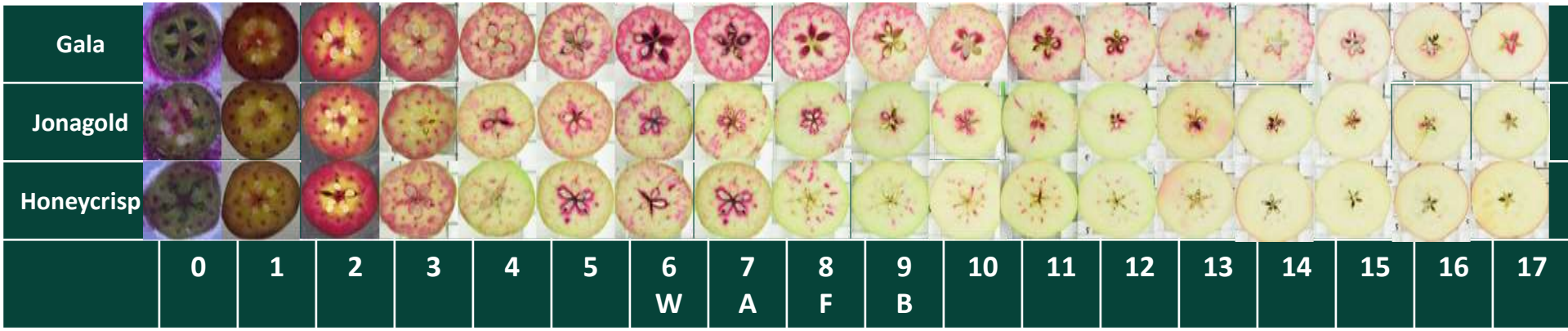


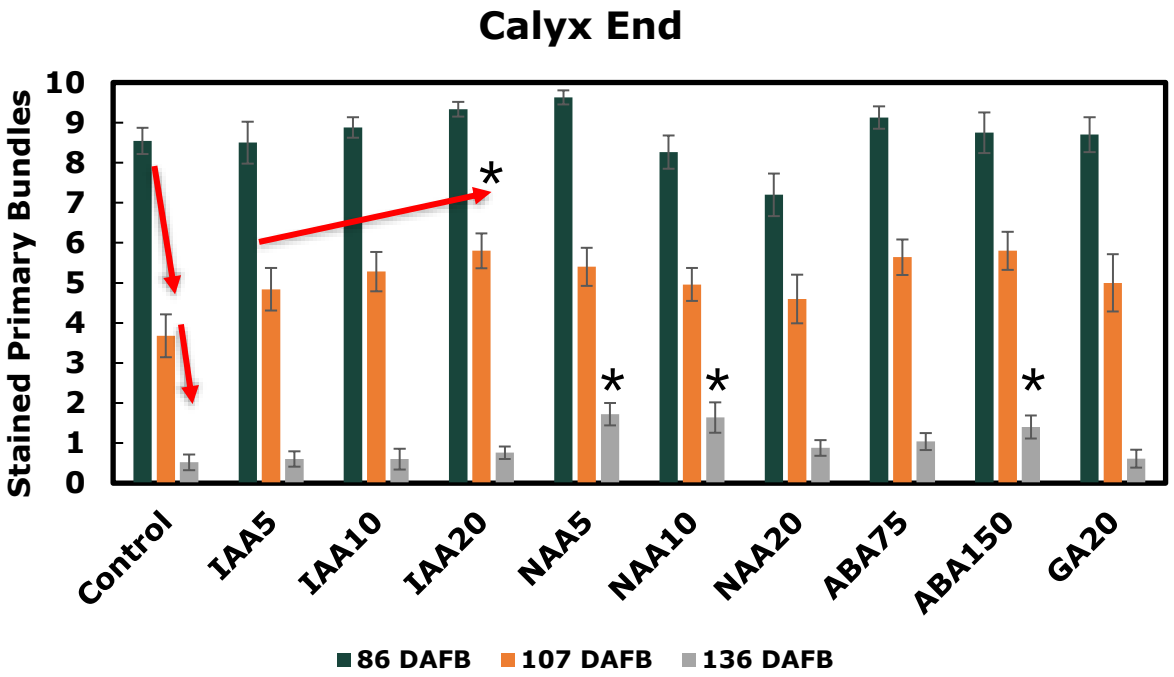




- Transport limits of Honeycrisp apple appear to be associated with the backroads and not the freeways

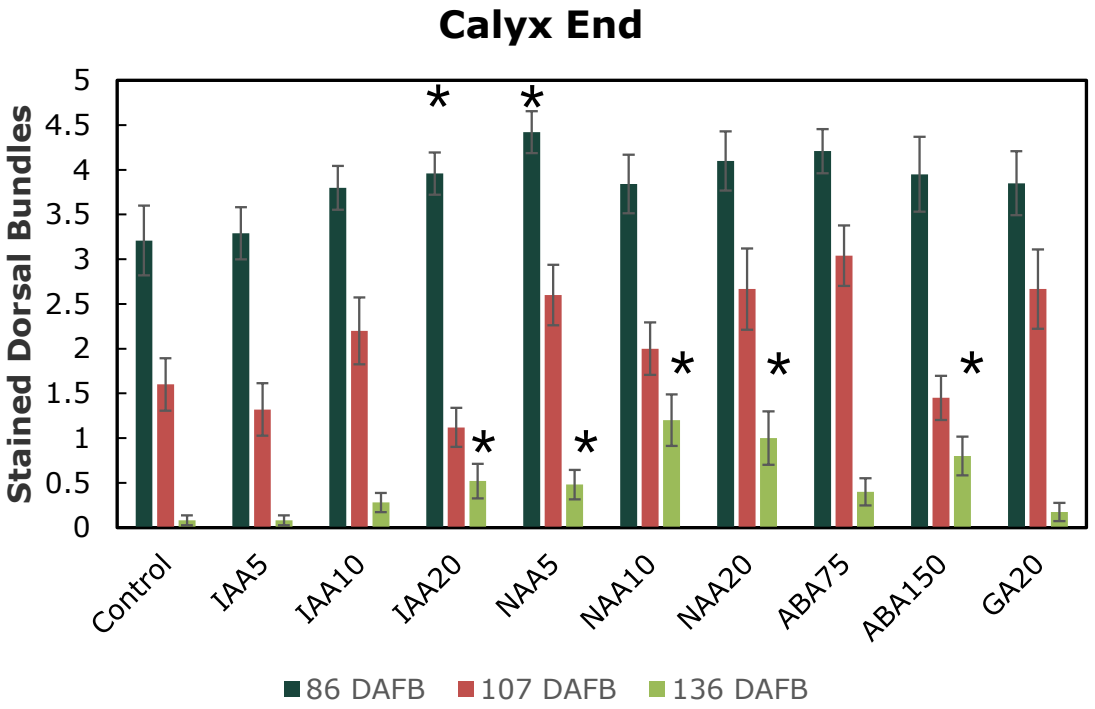
Griffith and Einhorn, *Sci Hort, 2022*

Results: Primary Bundles



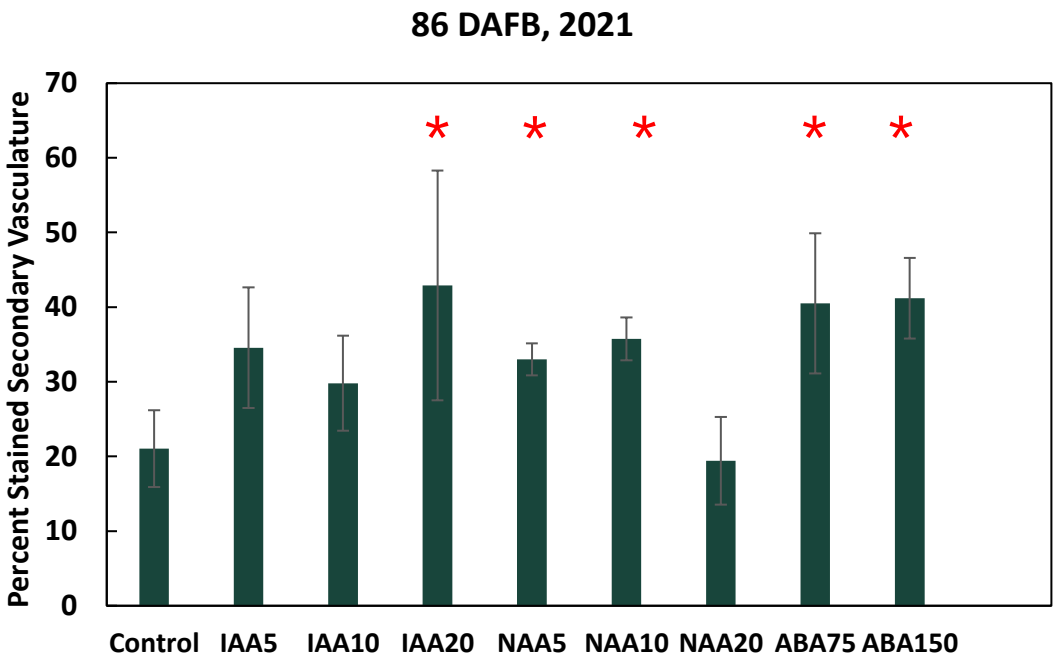
- Primary bundle functionality decreased with time (dafb) and distance from the stem
- IAA 20 ppm increased the number of functional bundles in a dose dependent manner at 86 and 107 dafb but not at 136 dafb (harvest)
- ABA and NAA 5 and 10 ppm increased the number of functional bundles at mid and late timings; increasing NAA rate had a negative effect on bundles

Results: Dorsal Bundles



- Dorsal bundle functionality decreased with time (dafb) but not with distance from the stem end
- IAA 20 ppm, NAA (all rates) and ABA increased the number of functional dorsal bundles at 136 dafb (harvest)

Results: Xylem Networks in Cortex



- 20 ppm IAA, NAA and ABA increased secondary vasculature functionality to the greatest degree
- A relatively small sample population (*n=5*) per treatment led to wide variation around the average values

Results: IAA Effects Are Transient

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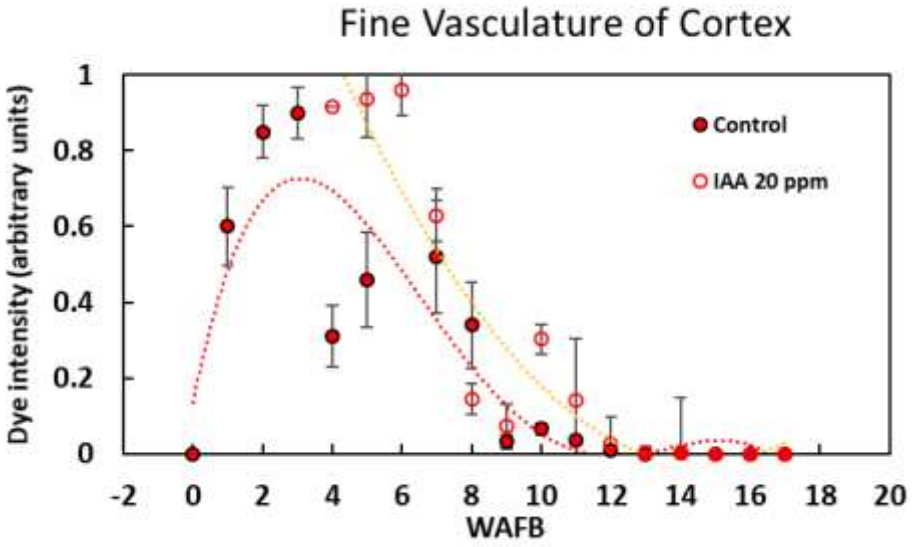
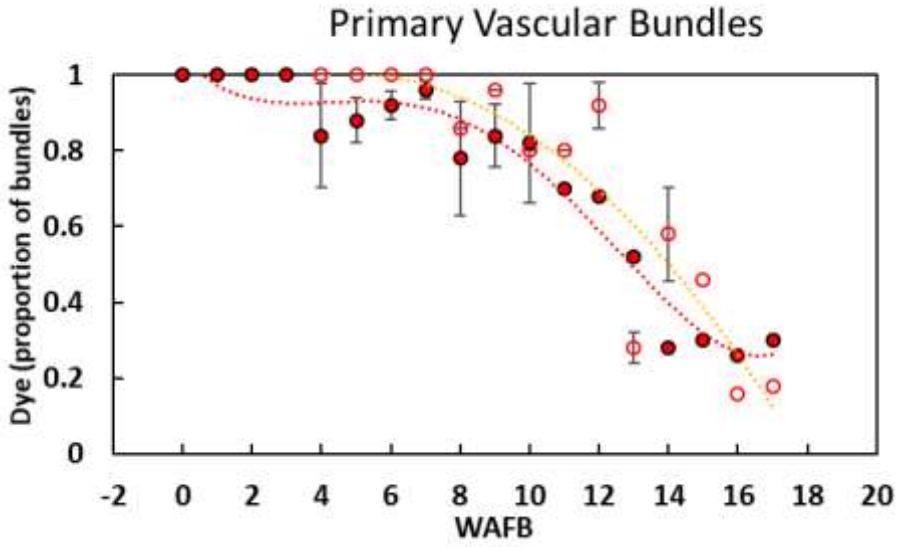
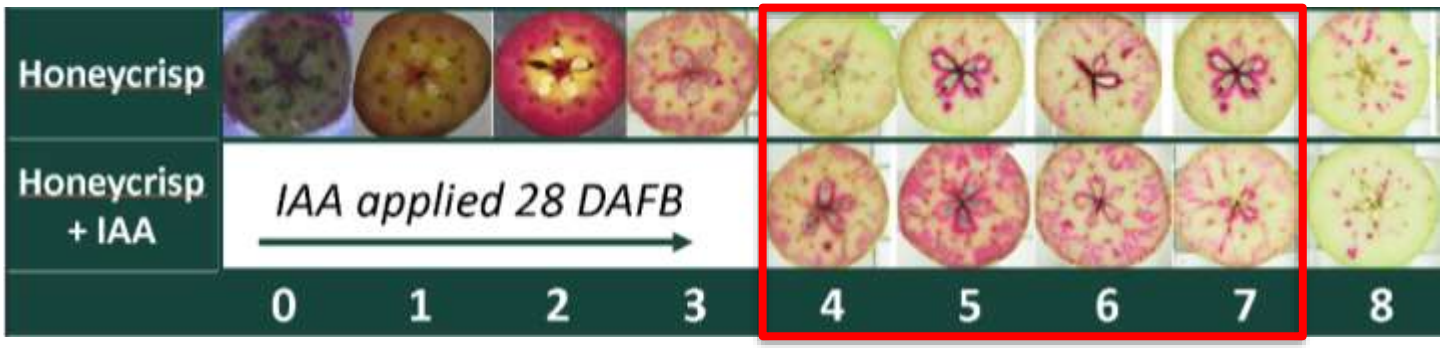
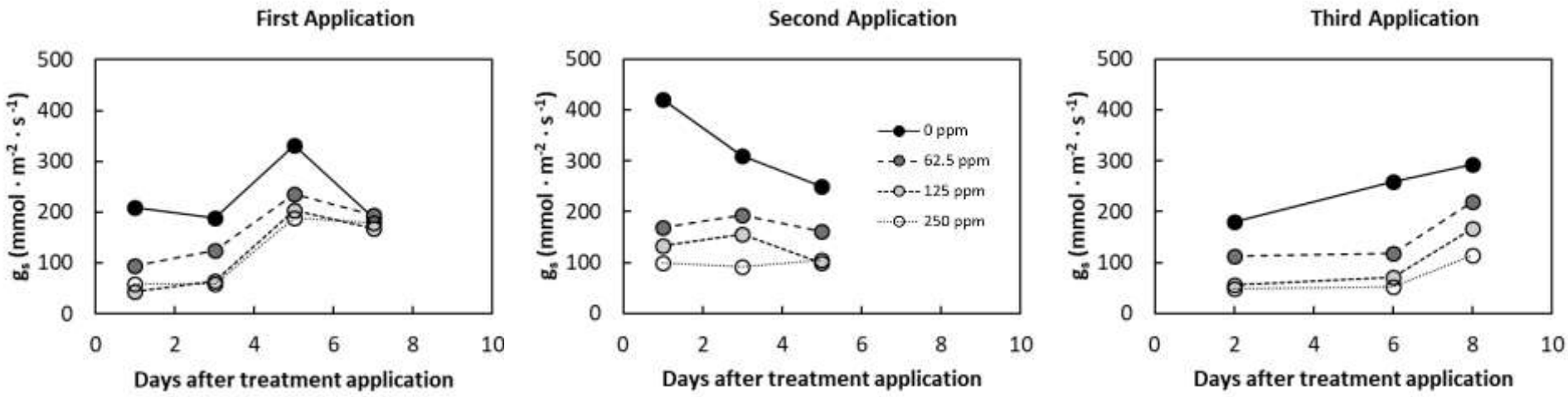






- The effect of 20 ppm IAA on increasing fruit xylem was quite marked, but it is highly transient suggesting that newly laid xylem is subject to the same strain as xylem in non-treated fruit, rendering its functional life short

Results: Stomatal Effects of ABA



- ABA reduced stomatal conductance according to rate
- ABA action on stomata may increase the calcium delivery to fruit (via reduced transpiration)
- Plausibly a stomatal effect is additive to the effect of ABA on xylem (previously demonstrated for tomato) though likely less of a role than xylem

Results: Bitter Pit (2021)

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Treatment	Rate	Bitter Pit Rating (0 to 3)	
		Harvest	+14 d PH period
Control		0.72	1.11
IAA	5 ppm	0.27 *	0.4 *
IAA	10 ppm	0.26 *	0.45
IAA	20 ppm	0.26 **	0.32 *
Significant (Linear)		0.036	NS
Adjusted R-squared		0.29	0.18
Control		0.72	1.11
NAA	5 ppm	0.37	0.6
NAA	10 ppm	0.37	0.59
NAA	20 ppm	0.29 ·	0.85
Significant (Linear)		NS	NS
Adjusted R-squared			
Control		0.72	1.11
ABA	75 ppm	0.23 ·	0.31 ·
ABA	150 ppm	0.27 ·	0.56
Significant (Linear)		NS	NS
Adjusted R-squared			

· , *, ** represent significance at $P<0.1$, 0.05, and 0.01, respectively.

0 1 2 3

Beaudry and Yildiz-Ocal, 2012

- **IAA and ABA reduced bitter pit by ~65% at harvest and after PH**
- Lower rates of NAA reduced bitter pit by ~50% at harvest and PH but had *P* values just over 0.1

Results: Bitter Pit (2021 and 2022)

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Chayce et al. assessed >45,000 fruit for bp

Treatment		2021			2022			
AI	ppm	Fruit weight (g)	Harvest (%)	Storage (%)	Fruit weight (g)	Harvest (%)	Storage (%)	Cropload Adjusted BP
Control	-	322 a	32.9	45.2	242	28.6	34.9	21.6
IAA	5	305 a	15.7*	19.3*	-	-	-	-
	10	304 a	14.5*	25.7*	253	30.1	37.1	22.5
	20	290 ab	7.3*	19.8*	228	18.8*	21.2*	9
	40	-	-	-	245	22.9*	31.5	34.7
NAA	2.5	-	-	-	235	29.5	35.3	25.1
	5	280 ab	18.3*	29.0*	235	28.2	33.4	25.8
	10	258 ab	17.6*	26.3*	223	20.1*	22.3*	10.5
	20	226 b	15.0*	35.1	-	-	-	-
ABA	62.5	-	-	-	239	25.8*	26.1*	17.7
	75	292 ab	11.0*	17.0*	-	-	-	-
	125	-	-	-	241	21.0*	22.7*	11.5
	150	306 a	13.2*	31.3*	-	-	-	-
	250	-	-	-	224	16.4*	24.1*	11.9
TIBA	30		-	-		35.5	50.8	25.1

- **2021: IAA, NAA and ABA reduced bitter pit ~75% to 50% at harvest and 60% to 30% after a PH treatment**
- **2022: IAA, NAA and ABA reduced bitter pit ~45% to 27% at harvest and 35% to 40% after 3 months RA storage**

Results: Bitter Pit (2022)

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	Control	IAA 20	NAA 10	ABA 125	ABA 250
BP	35 %	21 %	22 %	23 %	24 %

Treatment		2021			2022			
AI	ppm	Fruit weight (g)	Harvest (%)	Storage (%)	Fruit weight (g)	Harvest (%)	Storage (%)	Cropload Adjusted BP
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TIBA	30		-	-		35.5	50.8	25.1

Current Strategies to Mitigate Bitter Pit

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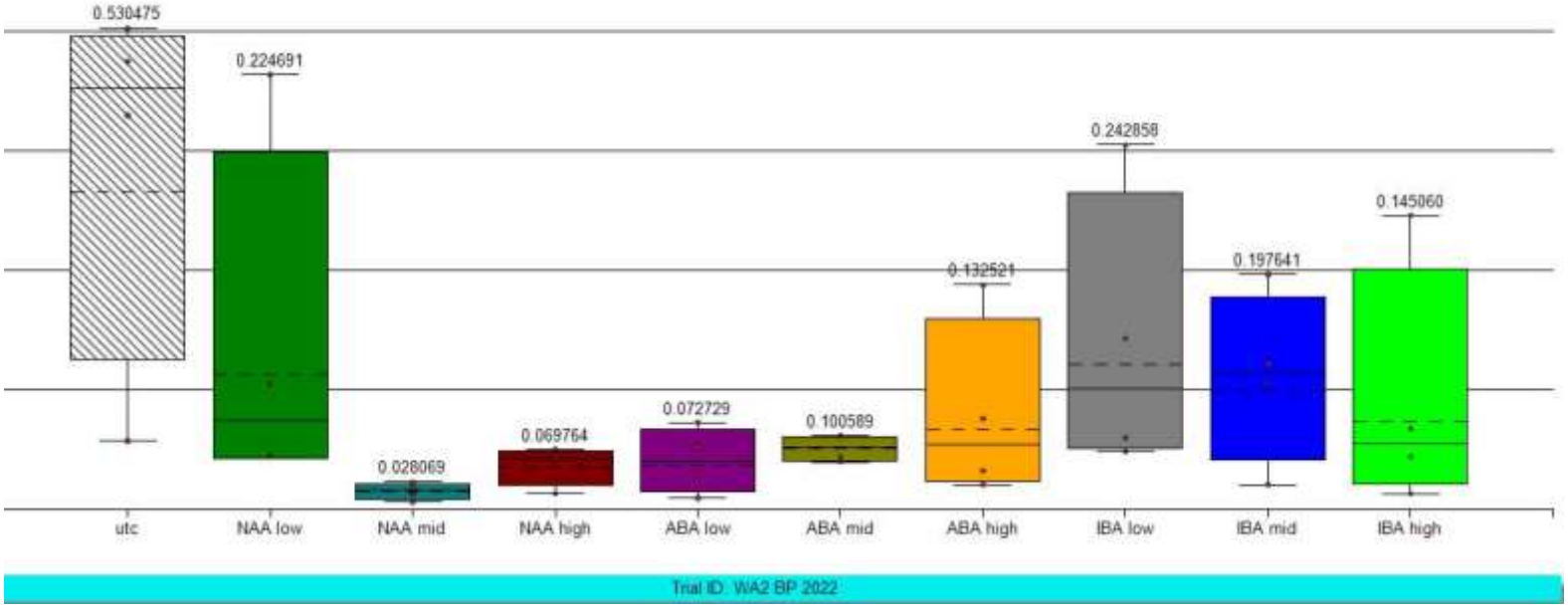
- PGRs (based on the past two years of research)
- Fine Americas has a 2ee temporary label amendment (this spring) for NAA treatment of bitter pit- Michigan will be on the Federal label
- VBC will have a label for ABA (Protone) for 2024 season (it will likely be approved too late for legal use this year)



Data compliments of Drew Hubbard

Thank you for your attention

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