The biological mechanism behind early and late apple bud sports

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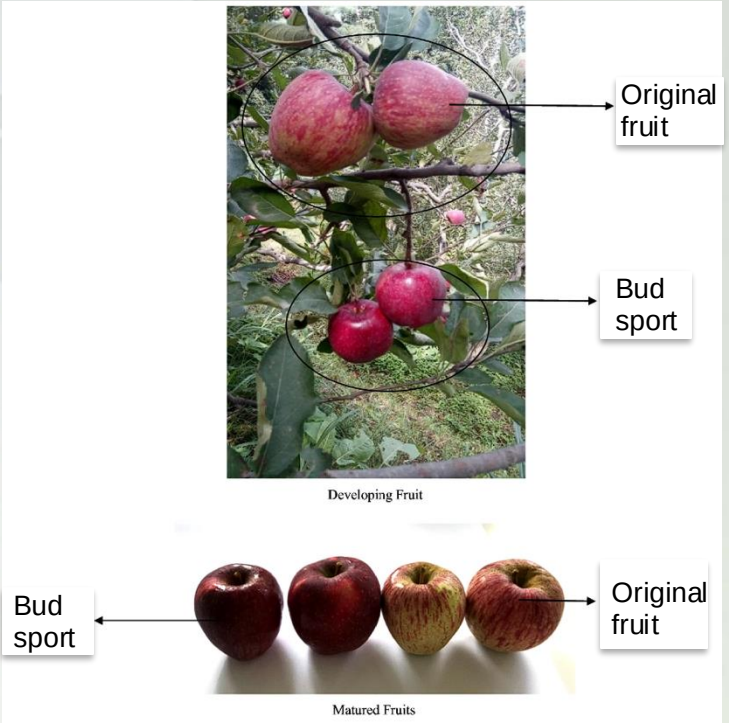
Dr. Ning Jiang
Dr. Berkley Walker

What are apple bud sports?

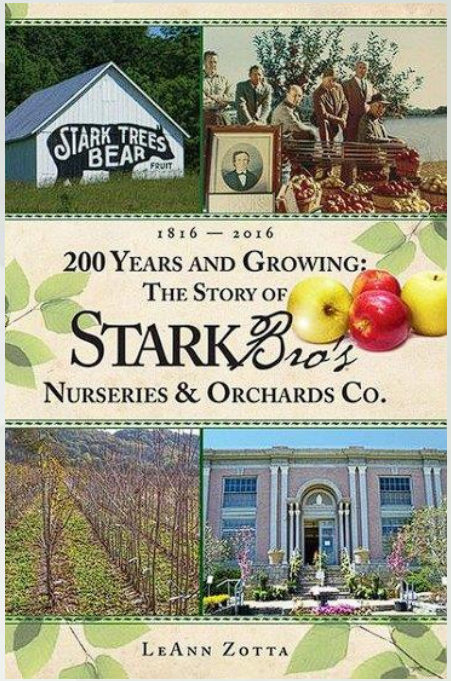
- Stable somatic mutation that leads to valuable phenotypic change in shoot, leaf, flower, or fruit
- Sport phenotypes in fruit include enhanced color sports, size, shape, firmness (storability), and altered harvest time (maturity sports)



https://link-springer-com.proxy1.cl.msu.edu/article/10.1007/s10722-023-01659-9

Background

- June 1923 – The $6000 tree limb – Starking Delicious
- Plant Patent Act enacted in 1930
- By 1936, 1664 fruit tree bud sport patents were issued (Shamel and Pomeroy, 1936)

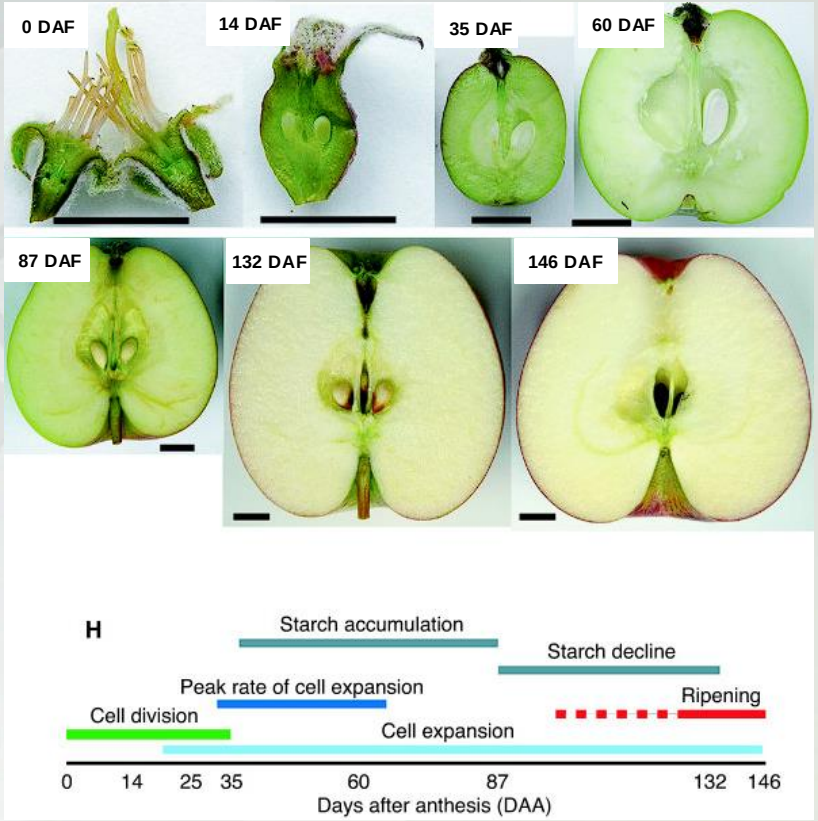


Biological and economic relevance of apple sports

- Growers aren't growing 'Kidd's D-8' (Original 'Gala' cultivar, J.H. Kidd, NZ)
- Sport industry (not football) in U.S. worth billions
- Apple and grape sport mechanisms have been investigated, mostly regarding color
- Despite their value, mechanisms for many popular apple sports' origin are not known, especially maturity sports

Apple phenology

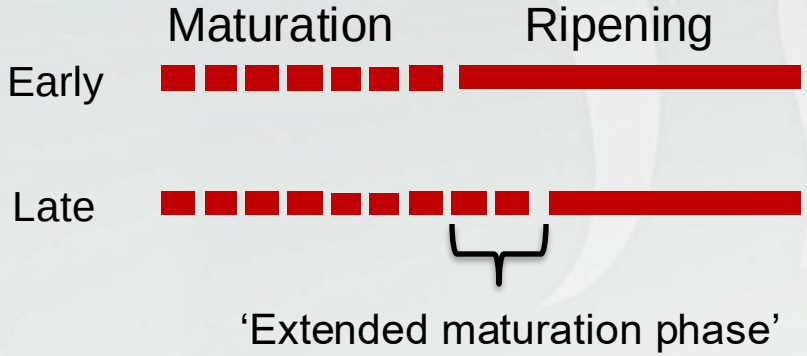
- Developmental stages
  - Flowering
  - Fertilization
  - Cell division
  - Cell Expansion
  - Maturity
  - Ripening

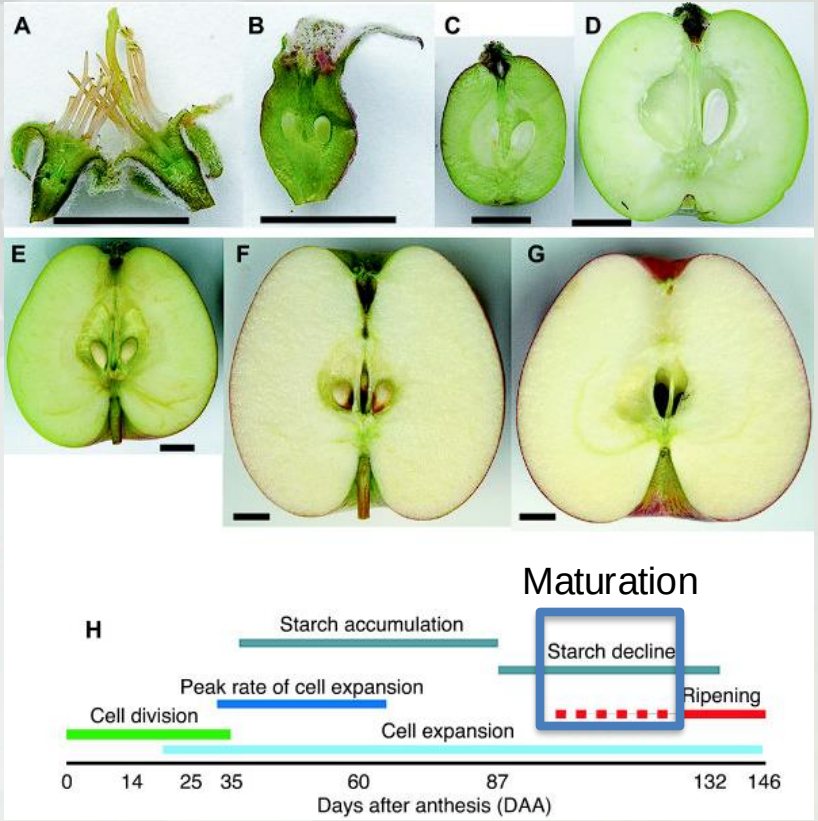


Janssen, Bart & Thodey, Kate & Schaffer, Robert & Alba, Rob & Balakrishnan, Lena & Bishop, Rebecca & Bowen, Judith & Crowhurst, Ross & Gleave, Andrew & Ledger, Susan & McArtney, Steve & Pichler, Franz & Snowden, Kimberley & Ward, Shayna. (2008). Global gene expression analysis of apple fruit development from the floral bud to ripe fruit. BMC plant biology. 8. 16. 10.1186/1471-2229-8-16.

Maturation

- Maturation begins before climacteric ripening.
- It is thought by many that difference in maturity likely a result of ‘extension’ in maturation before climacteric ripening.





Questions

1. Do later harvesting sports exhibit an extended maturation phase before ripening, or do they display longer development?
2. Is leaf photosynthetic rate tied to developmental rate and/or harvest date determination?
3. Which genetic mutation caused early or delayed maturation in our selected bud sports?

Design

- Fruit development
  - Fruit growth, growth rate, and acceleration of growth
  - Ripening: Starch degradation, internal ethylene, color, chlorophyll absorbance, firmness, sugars
- Photosynthesis
  - Leaf photosynthesis diurnal measurements
- Genomics and Transcriptomics
  - Map all cultivar's genomic sequences to 'Gala' haploid genome to search for variants causal of early or delayed maturation phenotype
  - Gene expression analysis to buttress genomic findings

- Gala
  - Original germplasm from parent tree (Kidd's D-8) and bud sport limb (Autumn Gala)
- Fuji
  - September Wonder Fuji-early sport of 'Yataka'
  - Aztec Fuji-chance seedling out of New Zealand
- Pink Lady®
  - 'Maslin' is a bud sport of 'Cripps Pink'

Kidd's D-8

Autumn Gala

September Wonder Fuji

Aztec Fuji

Maslin

Cripps Pink

Cultivar	Autumn Gala	Sep. Wonder Fuji	Maslin
Phenotype	4-5 weeks **later**	5-6 weeks **earlier**	2 weeks **earlier**

Methods – Crop Load Management

Every other cluster removed

Remaining spurs thinned to king fruit

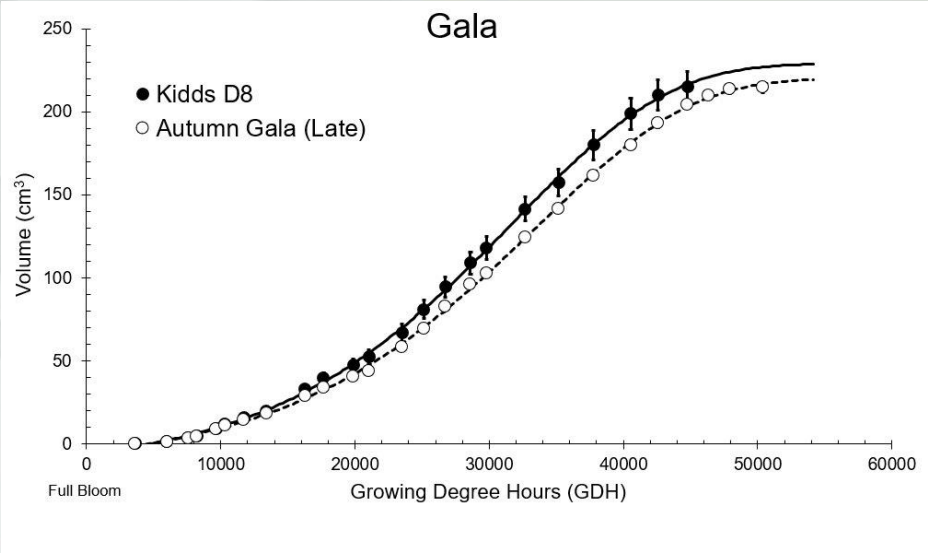
Fruit then thinned per limb if necessary

- Fruit Growth
  - 5 trees
  - 5 fruit per tree
  - Fruit measurements taken twice weekly over the whole growing season

Larson, James & Perkins, Penelope & Ma, Guoying & Kon, Thomas. (2023). Quantification and Prediction with Near Infrared Spectroscopy of Carbohydrates throughout Apple Fruit Development. Horticulturae. 9. 279. 10.3390/horticulturae9020279.

Methods – Curve Fits

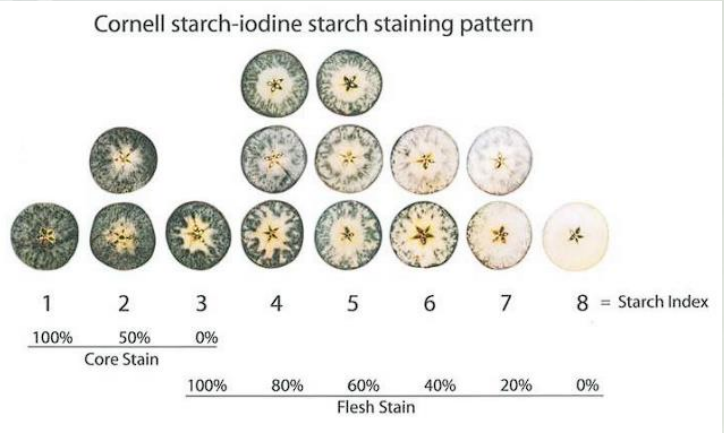
- 'TableCurve 2.0, Jandel Scientific, San Rafael, CA'
  - Manual curve fits for all 6 cultivar's growth curves (150 curves).
  - Accurate – Let us transform to growth rate and acceleration
  - Example: All generated growth curves fit the data at an R^2 value = 0.999 and above
  - Weibull equation 'y=a+b(1-exp(-((x+d*ln(2)^(1/e)/d)^e))' where x=GDH

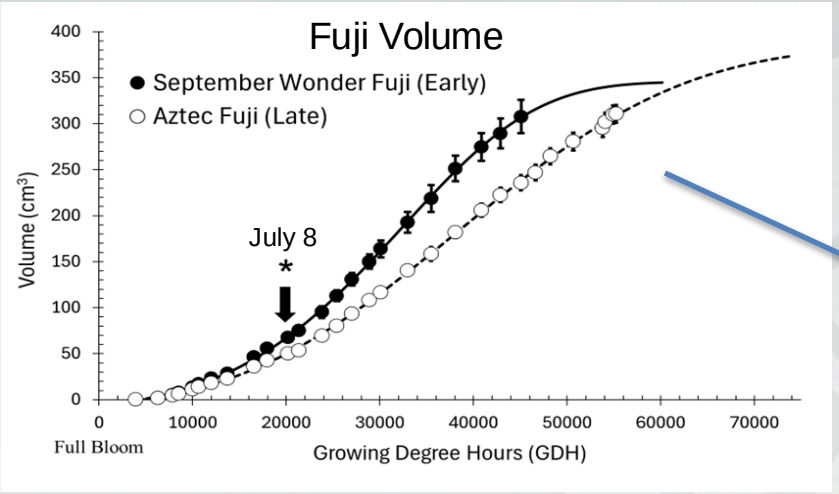


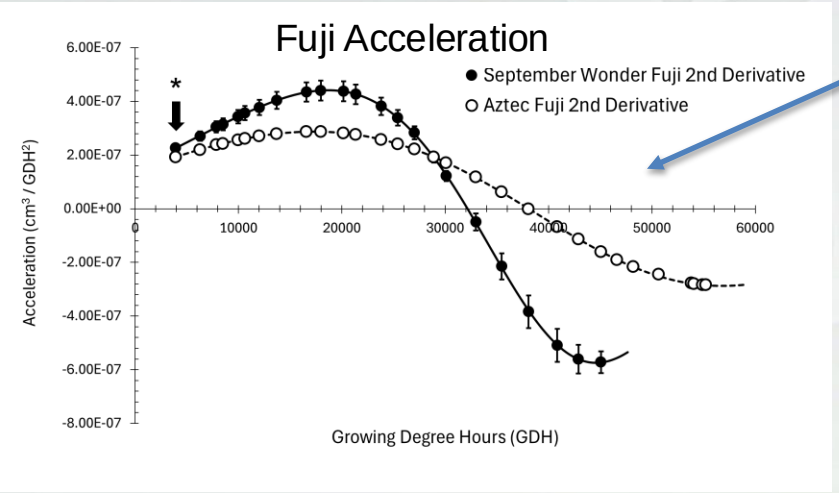
Variables	a	b	c	d	e	R^2
Kidd's D-8	-14.1656	243.15592	28605.070	167182.70	15.402599	0.99949319
Autumn Gala	-14.1674	234.23149	29912.333	366756.62	33.084848	0.99959610
September Wonder Fuji	-14.5170	360.12959	30418.000	73339.687	6.0632131	0.99969120
Aztec Fuji	-5.2252	391.10507	39198.310	53966.1351	2.9420340	0.99964431
Maslin	-3.4059	323.740647	41548.613	52884.155	2.3926701	0.99978296
Cripps Pink	-25.5178	257.20485	37093.037	222948.29	11.699273	0.99970823

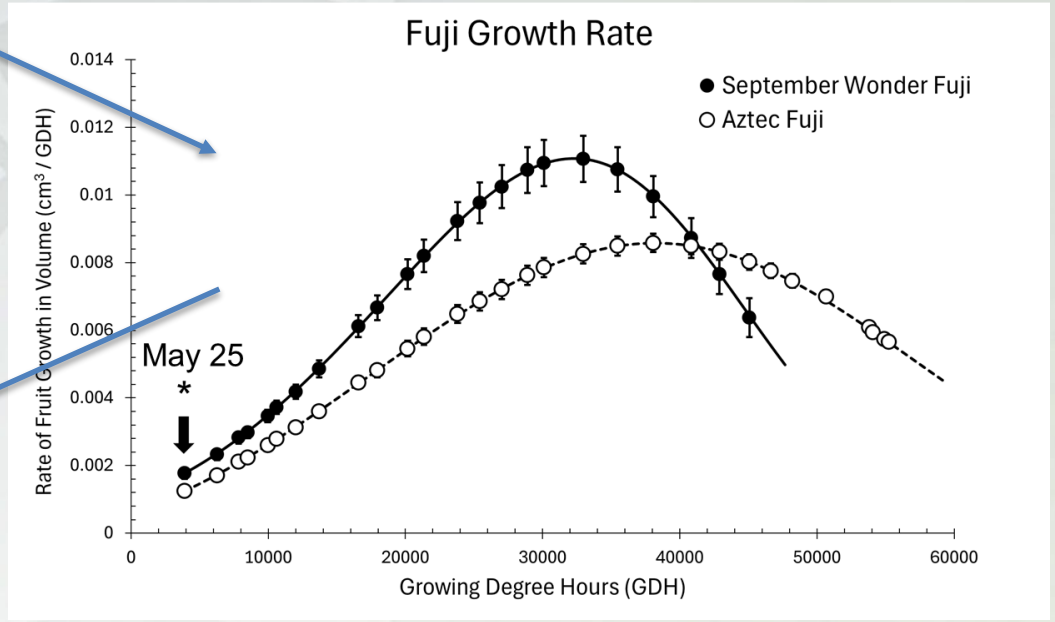
Methods – Ripening

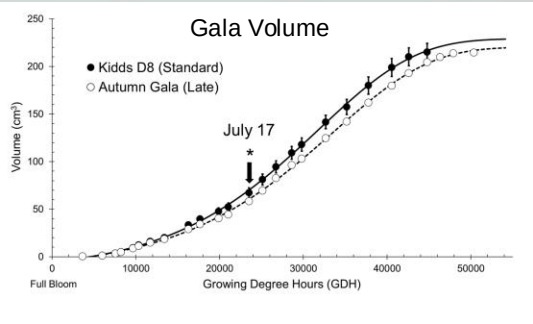
- Starch degradation analysis
  - Cornell Starch Index (1-8)
    - (Blanpied & Silsby, 1992).
  - Average index of ~4 is typical commercial harvest
  - Harvest dates established for each cultivar by an index of 4

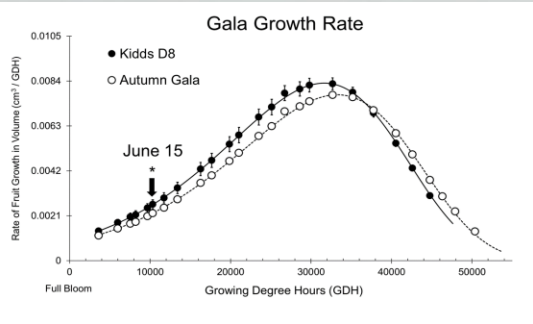


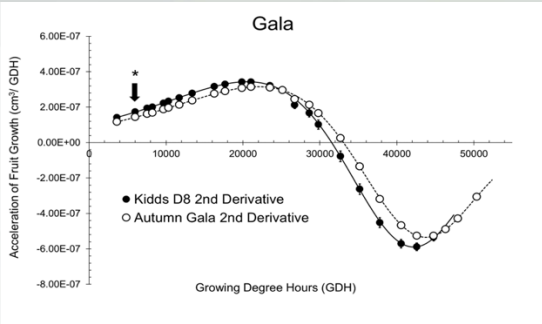

Fuji Volume
September Wonder Fuji (Early)
Aztec Fuji (Late)
July 8
*
Volume (cm³)
Full Bloom
Growing Degree Hours (GDH)

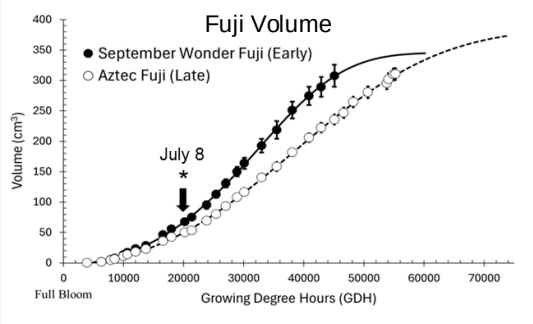

Fuji Acceleration
September Wonder Fuji 2nd Derivative
Aztec Fuji 2nd Derivative
*
Acceleration (cm³ / GDH²)
Growing Degree Hours (GDH)

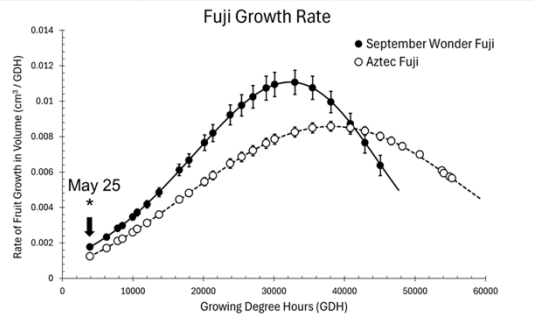

Fuji Growth Rate
September Wonder Fuji
Aztec Fuji
May 25
*
Rate of Fruit Growth in Volume (cm³ / GDH)
Growing Degree Hours (GDH)

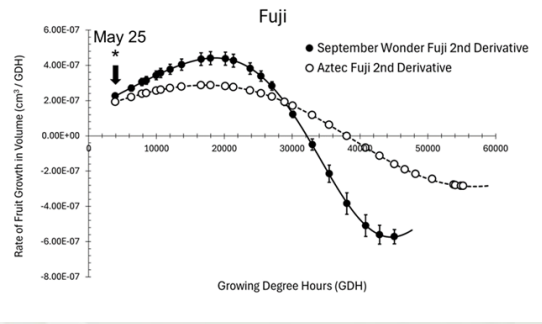

Gala Volume
Kidds D8 (Standard)
Autumn Gala (Late)
July 17
Volume (cm³)
Full Bloom
Growing Degree Hours (GDH)

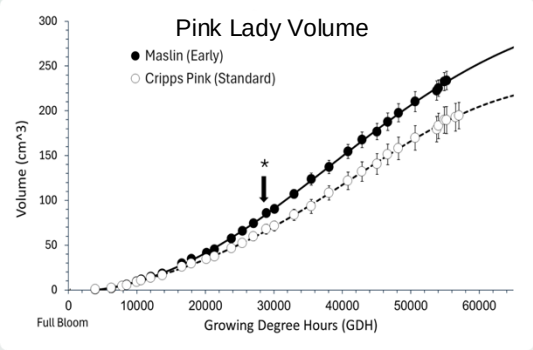

Gala Growth Rate
Kidds D8
Autumn Gala
June 15
Rate of Fruit Growth in Volume (cm³ / GDH)
Full Bloom
Growing Degree Hours (GDH)

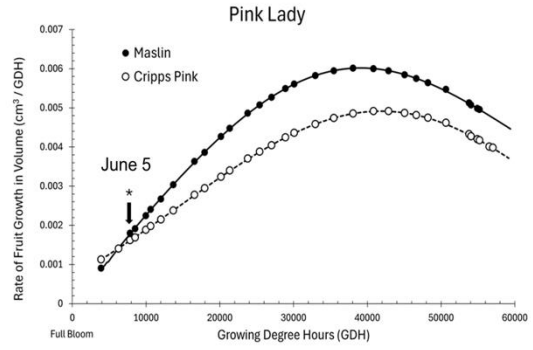

Gala
Kidds D8 2nd Derivative
Autumn Gala 2nd Derivative
Acceleration of Fruit Growth (cm³/ GDH)
Growing Degree Hours (GDH)

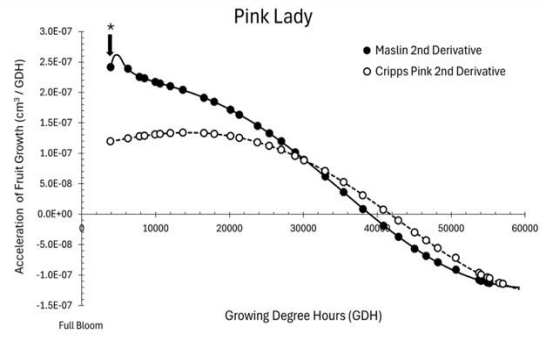

Fuji Volume
September Wonder Fuji (Early)
Aztec Fuji (Late)
July 8
Volume (cm³)
Full Bloom
Growing Degree Hours (GDH)


Fuji Growth Rate
September Wonder Fuji
Aztec Fuji
May 25
Rate of Fruit Growth in Volume (cm³ / GDH)
Growing Degree Hours (GDH)


Fuji
May 25
September Wonder Fuji 2nd Derivative
Aztec Fuji 2nd Derivative
Rate of Fruit Growth in Volume (cm³ / GDH)
Growing Degree Hours (GDH)

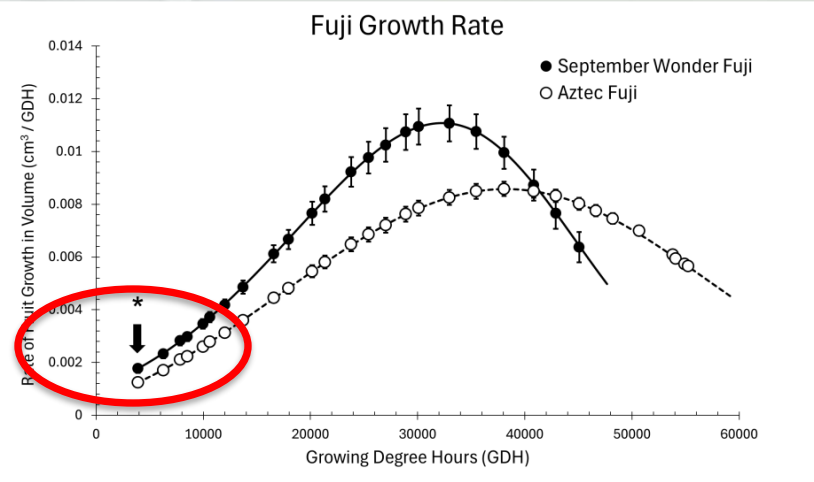

Pink Lady Volume
Maslin (Early)
Cripps Pink (Standard)
Volume (cm^3)
Full Bloom
Growing Degree Hours (GDH)


Pink Lady
Maslin
Cripps Pink
June 5
Rate of Fruit Growth in Volume (cm³ / GDH)
Full Bloom
Growing Degree Hours (GDH)


Pink Lady
Maslin 2nd Derivative
Cripps Pink 2nd Derivative
Acceleration of Fruit Growth (cm³ / GDH)
Full Bloom
Growing Degree Hours (GDH)

Fruit Development Conclusions

- Early cultivars display a higher rate of fruit development than late cultivars.
- Significant difference of fruit developmental rate found in exponential phase of fruit growth
- Maturity time appears to be predetermined early in fruit development



Starch degradation results

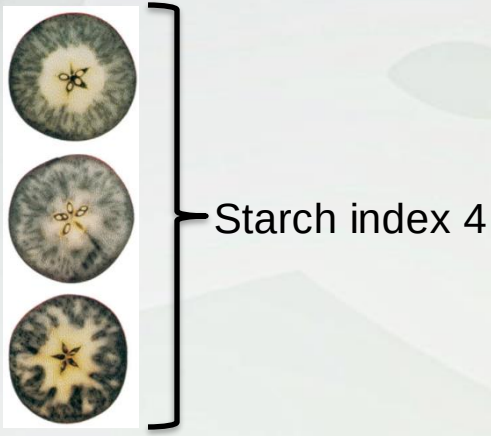
Gala		Fuji	
Standard	Late	Early	Standard
Kidd's D-8	Autumn Gala	Sep. Wonder	Aztec Fuji

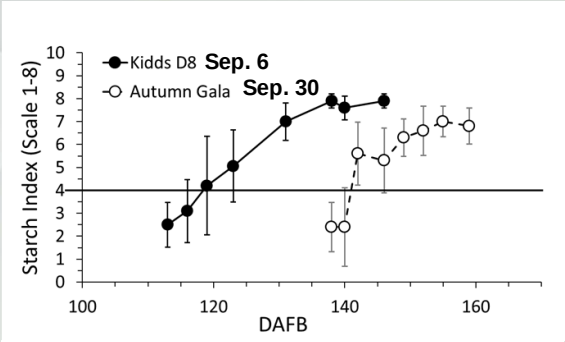
24 days apart

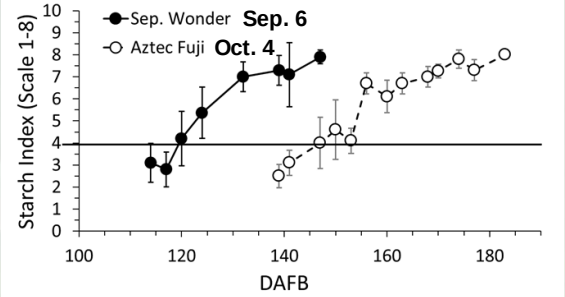
28 days apart

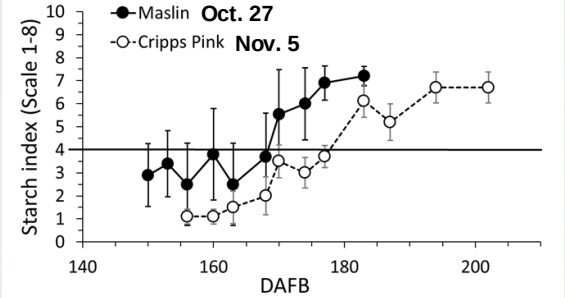
Pink Lady	
Early	Standard
Maslin	Cripps Pink

9 days apart









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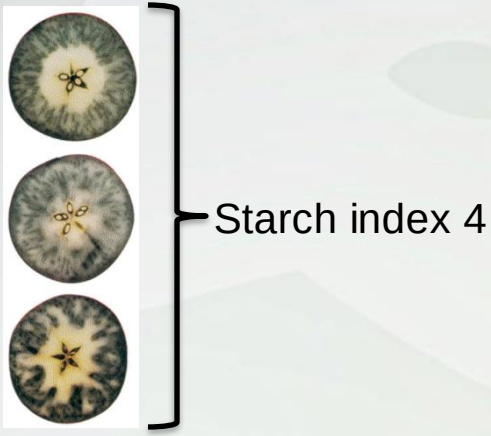
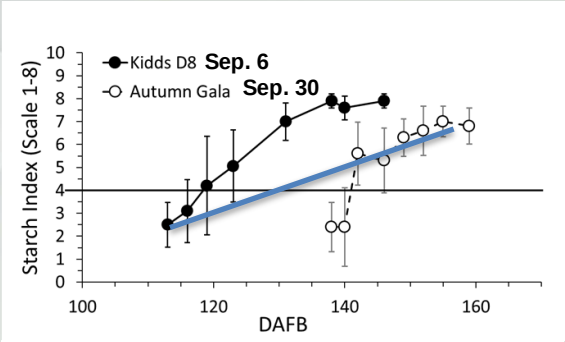
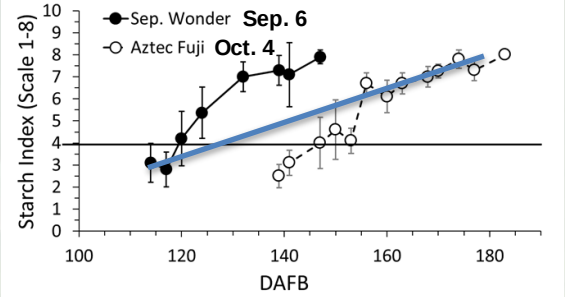
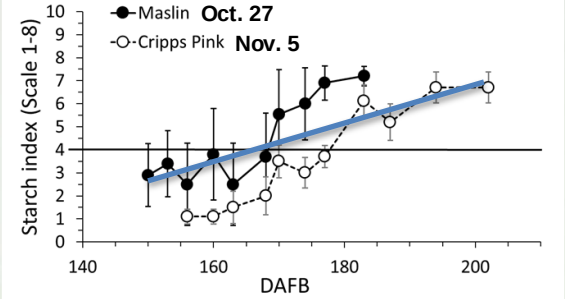
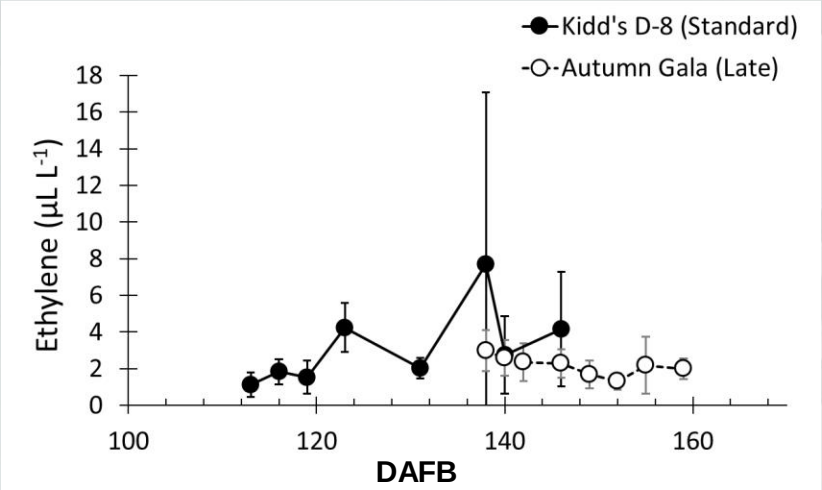
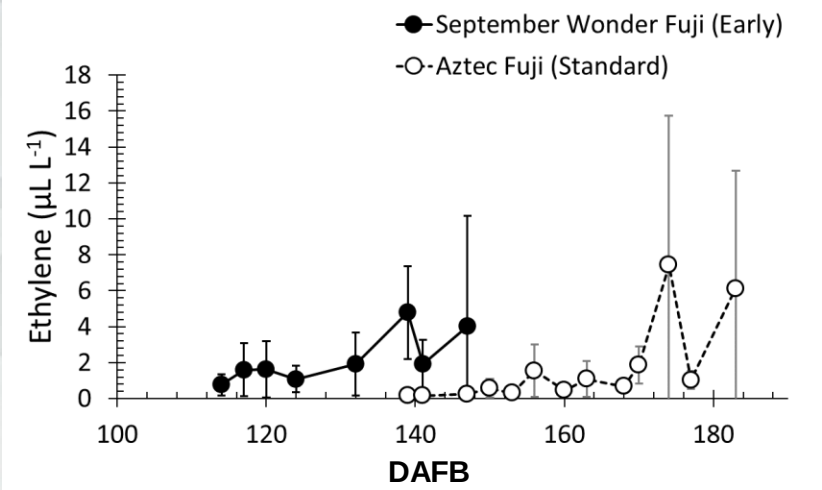


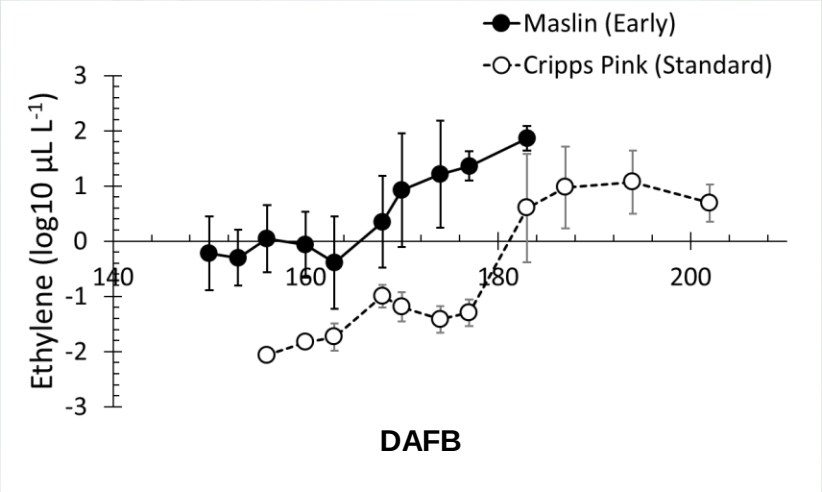






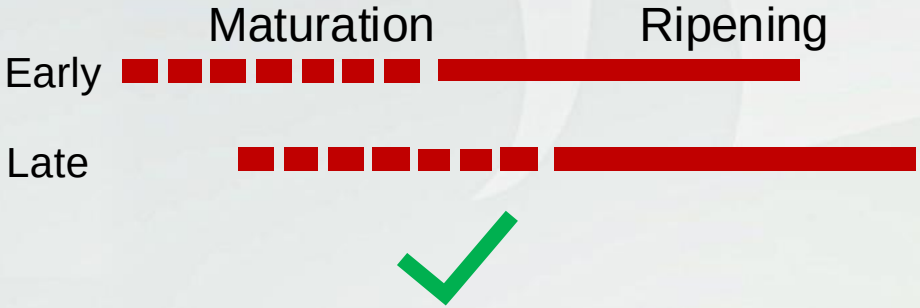

Kidd's D-8 (Standard)
Autumn Gala (Late)
Ethylene (µL L-1)
DAFB

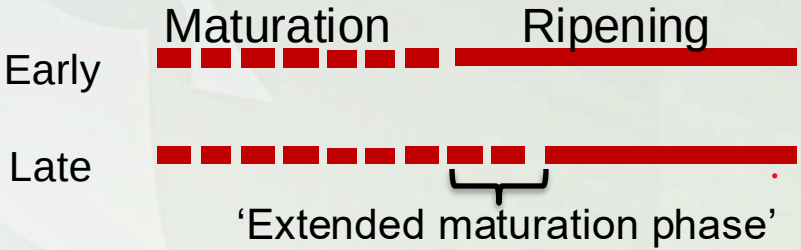

September Wonder Fuji (Early)
Aztec Fuji (Standard)
Ethylene (µL L-1)
DAFB


Maslin (Early)
Cripps Pink (Standard)
Ethylene (log10 µL L-1)
DAFB

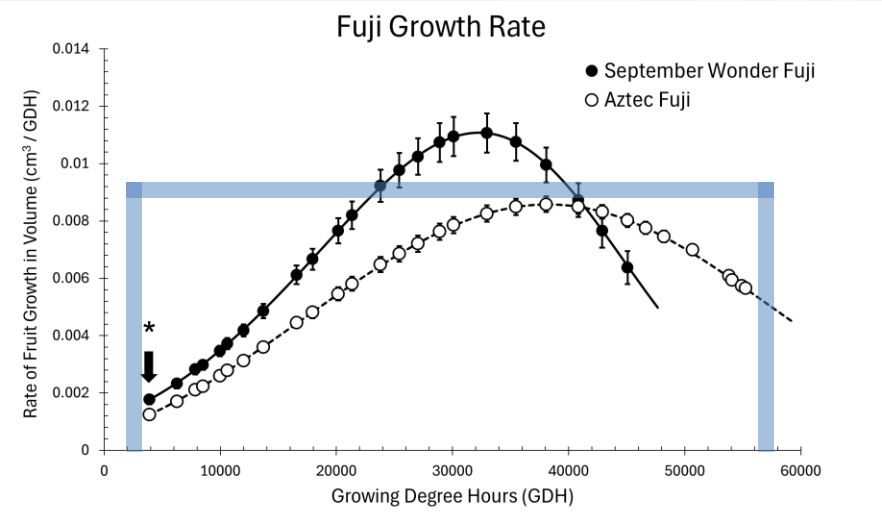
Maturity Analysis Conclusions

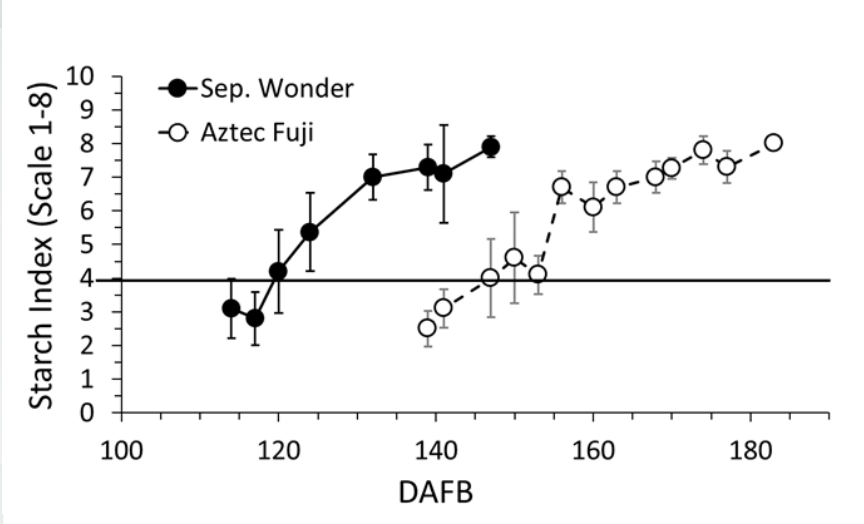
- No evidence of a ‘paused’ or drawn-out maturation.
- No difference in ripening rate was observed in ripening of early and late cultivars; the two cultivars began ripening at different times.





Advancement/delay in maturity is not due strictly to maturation rate, but to a compressed/stretched window of development.





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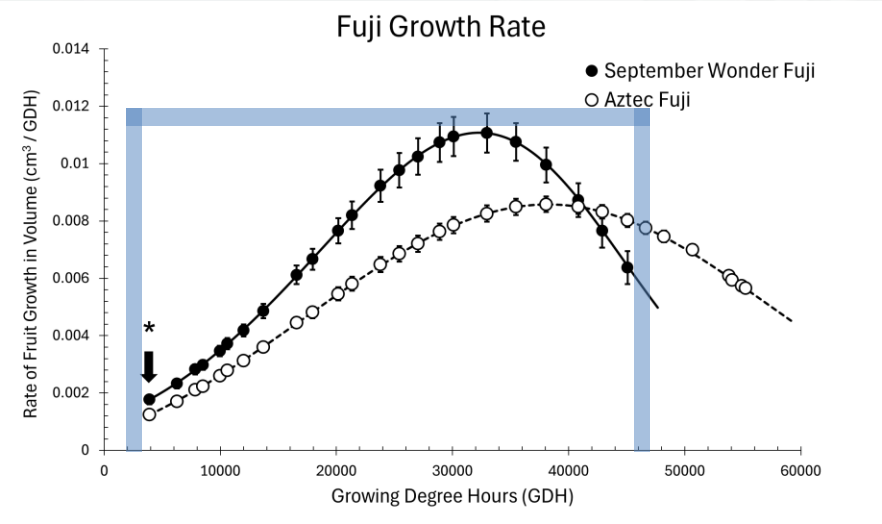
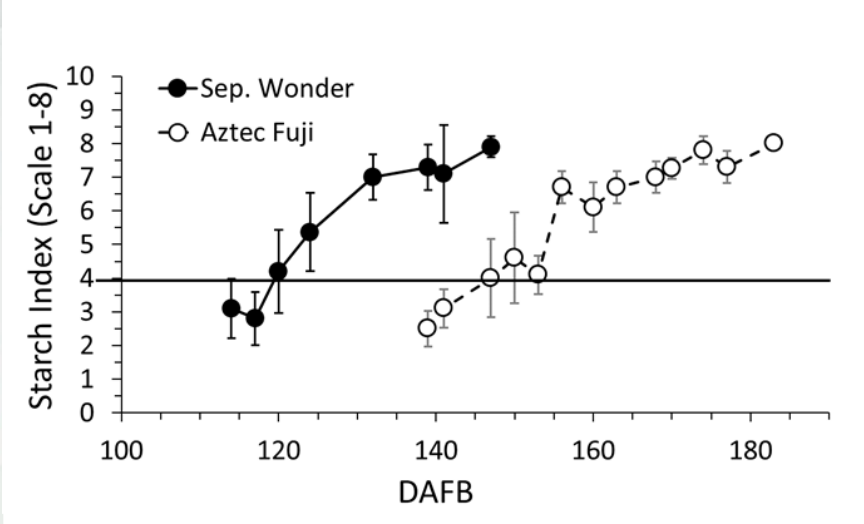




Questions

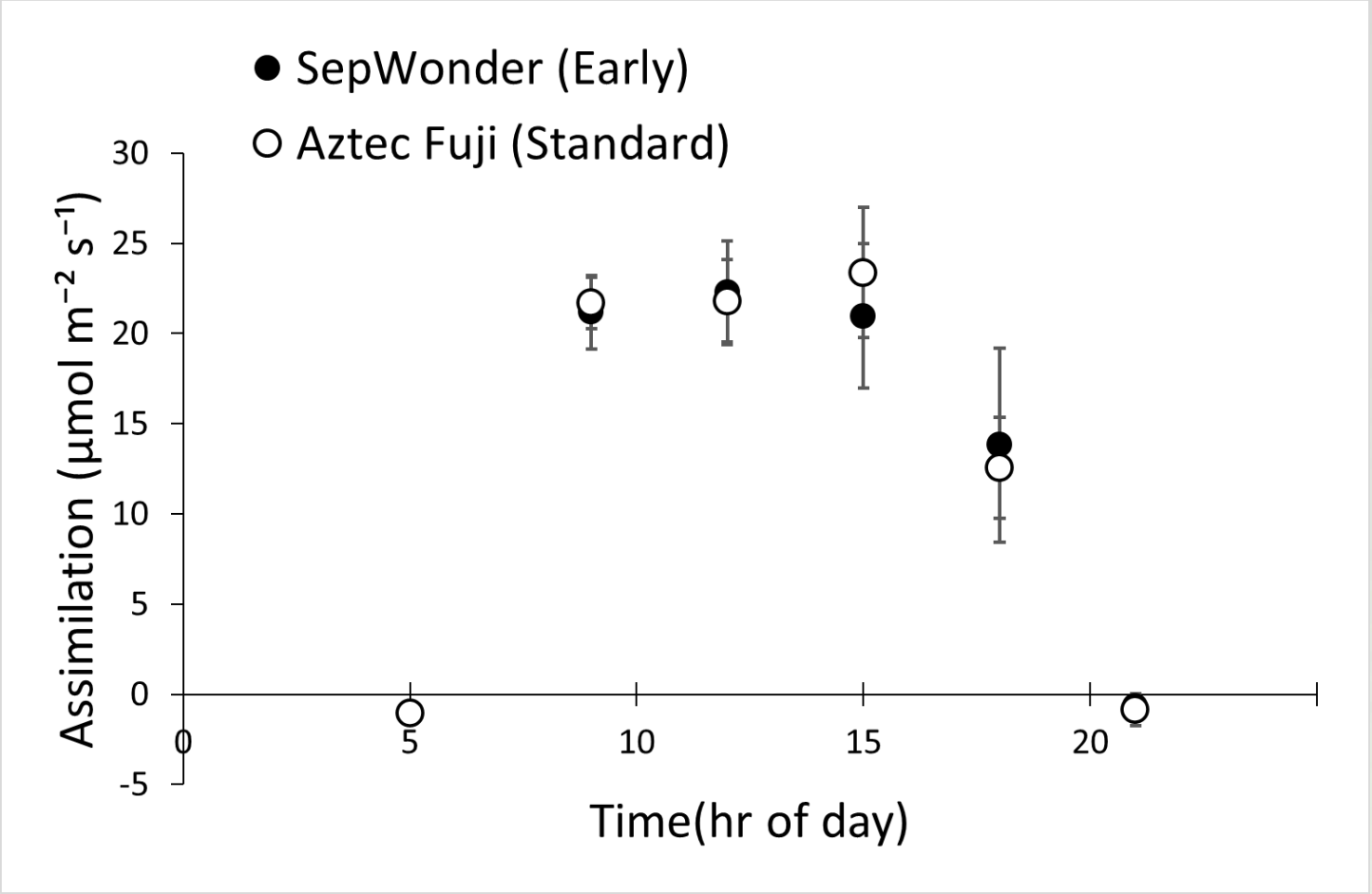
1. Do earlier harvesting sports exhibit a pause in maturation before ripening, or do they display a shorter development?
2. Is leaf photosynthetic rate tied to developmental rate and/or harvest date determination?
3. Which genetic mutation caused early or delayed maturation in our selected bud sports?

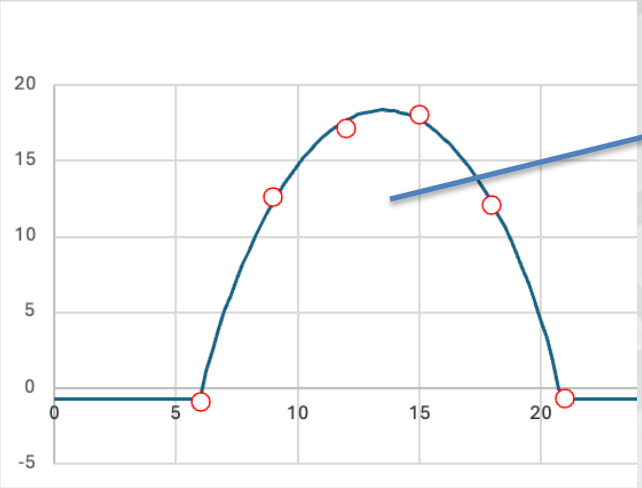
Photosynthesis – Methods

- 2 leaves per tree
- First leaf of bourse shoot
- 5 trees per cultivar
- 6 measurements throughout day
- 5 data collections during the season per cultivar
- Licor6800-Walker Lab

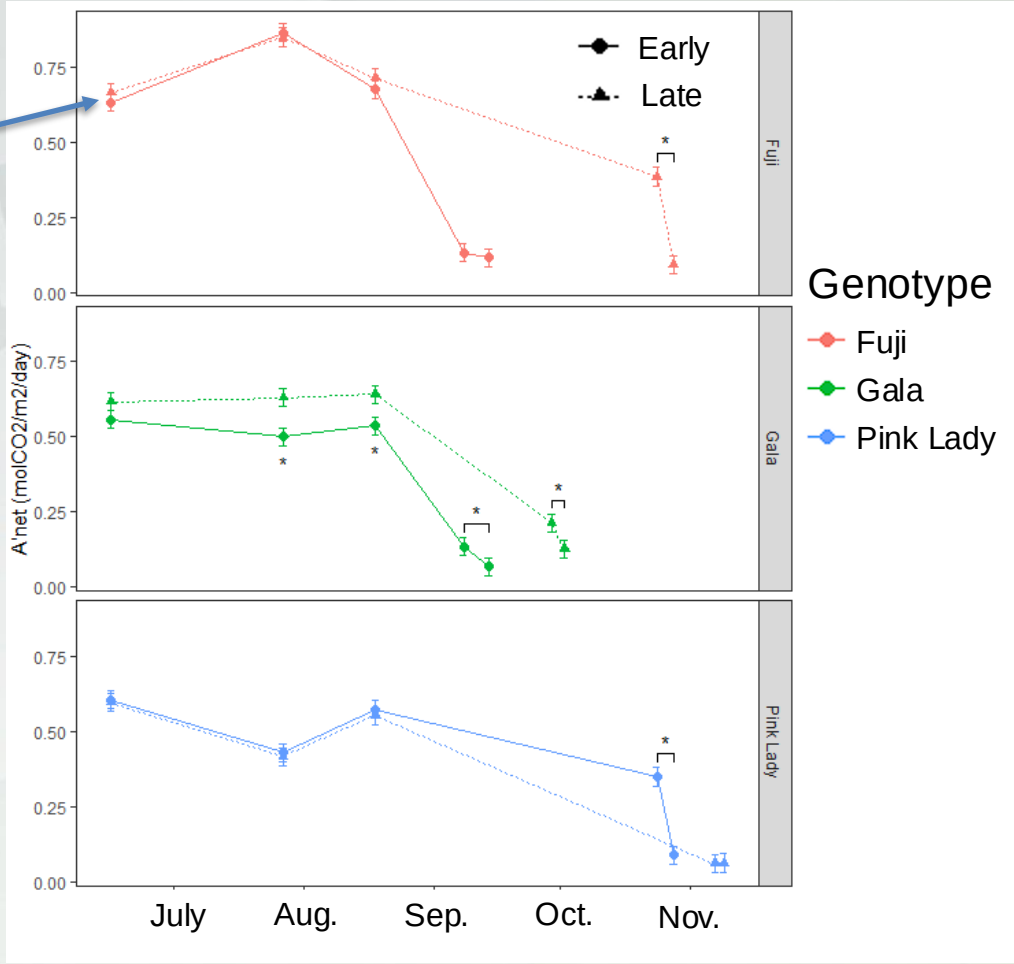
Dr. Berkley Walker

Results


SepWonder (Early)
Aztec Fuji (Standard)
Assimilation (µmol m⁻² s⁻¹)
30
25
20
15
10
5
0
-5
0
5
10
15
20
Time(hr of day)



Dr. Mauricio Diego Tejera Nieves
-Postdoc in Walker lab



Photosynthesis Conclusions

- The data do not display a tie between net leaf carbon assimilation and fruit developmental rate.

Questions

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2. Is leaf photosynthetic rate tied to developmental rate and/or harvest date determination?
3. Which genetic mutation caused early or delayed maturation in our selected bud sports?

Which genetic mutation caused early or delayed maturation in our selected bud sports?

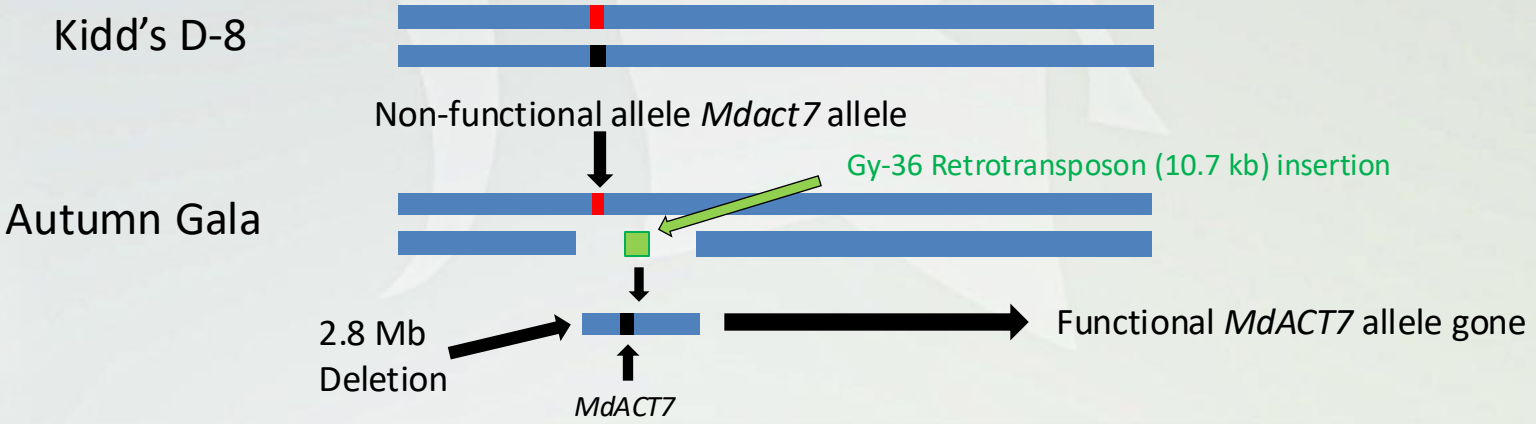
Christopher Gottschalk, Ph.D.

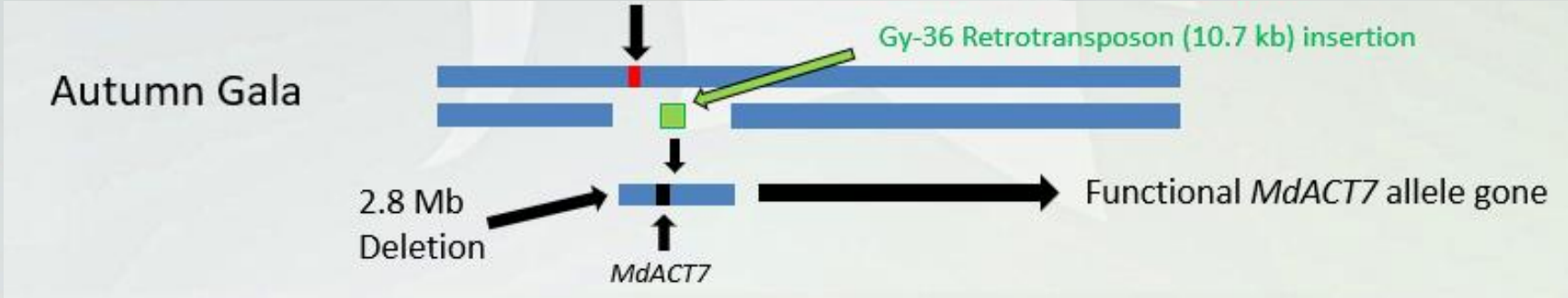
- Genomic DNA from one tree of each cultivar was sequenced
  - 150 bp Paired End Illumina sequencing
- Genomes were mapped to ‘Gala’ haploid
- Variants (SNPs and In/Dels) between cultivars and genome were identified and characterized
- A subset of variants that disrupt genes was identified
- Comparisons between those variants in early & late cultivars were made to ID candidates for causative mutations

Stephanie Hickey, Ph.D.

Preliminary Genomic Results

- Preliminary analysis in ‘Gala’ agrees with Ban et al. (2022)
  - They found a 2.8 Mb deletion in CHR 6 of ‘Autumn Gala’ and replaced by 10.7 Kb retrotransposon
  - We found that all SNPs in this region in ‘Autumn Gala’ were called homozygous when called heterozygous for ‘Kidd’s D-8’





- 238 genes were in this 2.8 Mb deletion region
- 167 of them are differentially expressed genes (DEGs)
- 167 DEGs may have massive repercussions!
  - Not just development! –may also affect storability, firmness, sugars, color, aroma, and other postharvest qualities

What about reversion?

- "Autumn Gala will not revert"
  - Personal communication with Dr. Ning Jiang (MSU Horticulture-heavy research in gene expression and transposon (jumping gene) activity).
- Bud sport reversion depends on the nature of the original mutation
- There is value here!
- Barnsby Cripps Pink may revert less often (based on subjective observation)
- Sequenced 'Barnsby', 'Maslin', and 'Cripps Pink' (parent and original Pink Lady)

Other comparison's results

- 'Fuji' and 'Pink Lady' comparisons show more variants than 'Gala', due in part to mapping to 'Gala' haploid and not a 'Fuji' or 'Pink Lady' genome
- 120 variants flagged in 'Fuji' comparison
- 75 variants flagged in 'Pink Lady' comparison

Conclusions of the study

- Maturity sports exhibit differences in development early in season, not during their developmental ripening phase
- Net photosynthetic activity did not tie with fruit developmental rate
- Genes involved in maturity and photosynthesis likely not related to maturation time.
- Preliminary analysis of ‘Gala’ genomic data is in agreement with study done by Ban et al. (2022)
- ‘Autumn Gala’ will not revert…what may we learn about other bud sports?

Thank you! Questions?

MICHIGAN APPLES

Project GREEEN