
• No good developmental data was available
• Valencia is good overall example since it is grown world-wide
• PURPOSE: Develop detailed information on changes in citrus fruit (orange) during its development
MATERIALS AND METHODS

• Valencia fruitlets collected from bloom until maturity
• Physical, chemical and anatomical measurements taken throughout development
• Australia – six month shift for northern hemisphere
RESULTS
Changes in component parts during early development of Valencia orange.

Ovary (fruit) size at bloom.
Fruit growth does not start at bloom!

Fig. 1.—Fruit growth in the 1954 season, showing the three stages in development distinguished on a calendar basis.
Oct. = April
Dec. = June

Fig. 2.—Developmental changes found in the fruit during Stage I from blossom to mid December.
Segment locule & seed
Fig. 3.—Respiration rate on a fresh weight basis throughout fruit development (measured at 20°C).
Fig. 4.—Respiration rate per fruit throughout development (measured at 20°C).
Fig. 5.—Developmental changes found in the fruit during Stage II from mid December to mid July.
Fig. 6.—Percentage moisture content in the fruit, the peel, and the pulp throughout development.
Med. Climate

Fig. 7.—Percentage sugar and acid content in juice of maturing fruit.
Nitrogen changes during Stage II of fruit development

Fig. 8.—Changes in nitrogen content and dry weight during Stage II from mid December to mid July.
Fig. 11.—Developmental changes found in the fruit during Stage III from mid July to maturity.
FIG. 2.7. ALBEDO CELLS OF ORANGE WITH LARGE INTERCELLULAR SPACES (is) AND BRANCHING TUBE-SHAPED CELLS (C, D) are shown as well as the torn end of a vascular bundle (vb) (C). Small protuberances (pr) occur on the cells, and strands of pectin (pe) from cell separation are also present (C, D).
Nitrogen changes during Stage III of fruit development

Total N from 140 to 240, or N was 60 % of total at end of Stage II

Recommend 2/3rds of total N be applied by end of Spring
Fig. 10.—Changes in moisture content and protein nitrogen level throughout fruit development.

1 to 1 for whole fruit
$\frac{64}{106}=60\%$
accumulated by
end of second
growth period

Fig. 9.—Changes in soluble and protein nitrogen content throughout fruit development.
DISCUSSION AND CONCLUSIONS

• Cell enlargement follows cell division as main means of growth (transition, 6 to 8 weeks, exception is epidermis).
• Major part of nitrogen accumulation by summer
• Early development (albedo), middle development (juice vesicles), later development (soluble solids and flavor compounds)

• Review article of fruit development and senescence
MATERIALS AND METHODS

• No new data
• Information from Holtzhausen, Bain, etc
RESULTS
Parts of mature fruit (A) and relative development of main parts of citrus fruit over time (B).
Relative cross-sectional area (A) of main fruit parts during development and change in cell size of albedo (B) with time.
DISCUSSION AND CONCLUSIONS

• Overall development – cell division & enlargement

• Component parts relative importance at different stages of growth
  – Cuticle develops after 2 cm diameter
  – Albedo becomes much smaller part at maturity

• Senescence measured in peel keeping quality
  – Decay resistance lost
  – Delayed by GA3
  – Fruit drop increases (2, 4-D can reduce)

- General chapter reviewing Bain’s and other work.
- Good reference for constituents and their levels at different stages of growth or maturity.
Plants grow - collections of semiautonomous interacting organs that compete for resources.

Carbohydrate are transported from supply regions (sources) to the demand regions (sinks) through the phloem.

PURPOSE: To demonstrate that the capacity of the vascular system in citrus is principally limited and may restrict fruit growth.
MATERIALS AND TREATMENTS

* THEY USED TREES OF MARSH GRAPEFRUIT (SEEDLESS), 20 YEARS OLD, IN A COMMERCIAL GROVE IN THE CENTRAL COASTAL PLAIN OF ISRAEL.

* 1992, SIX TREES WERE HARVESTED, EXCEPT FOR 40 FRUITS PER TREE.
   THINNED AND GIRDLED (T&G).
   THINNED (T).
   GIRDLED (G).
   CONTROL (C).

* 1993, FIVE TREES WERE CHOSEN AND TWO UNIFORM MAJOR BRANCHES WERE TAGGED ON EACH TREE. TEN FRUITS WERE MEASURED, ONE BRANCH WAS GIRDLED, THEY REMOVED ALL THE FRUIT THAT WERE NOT MEASURED, ADDITIONAL MEASUREMENTS OF THE PEDICEL DIAMETER WERE DONE.
* PEDICEL THICKENING SLOWED DOWN ABOUT 60 d AFTER ANTHESIS, WHILE FRUIT GROWTH RATE WAS JUST ABOUT TO BEGIN ITS LINEAR PHASE.

* THIS INDICATES THAT THE INVESTMENT IN THE VASCULAR CONNECTION RECEIVES HIGH PRIORITY AT THE EARLY STAGES OF FRUIT DEVELOPMENT.
Results

- AN ADDITION OF 1 mm² TO PEDICEL CSA CONTRIBUTED ABOUT 32 g TO FINAL FRUIT FRESH WEIGHT, AT HARVEST.
- Contributed or Associated?
* An increase of fruit growth rate was the immediate response to branch girdling and fruit thinning. An increase of pedicel CSA occurred about 2 weeks later.
SPECIFIC MASS TRANSPORT INCREASED IMMEDIATELY AFTER TREATMENT AND REMAINED HIGHER THAN IN CONTROL FRUIT PEDICELS.

Therefore pedicel growth due to more available phloem substrate?
Differences in pedicel diameters for different fruit

No leaves above vs leaves below & multiple fruitlets on left vs one fruitlet on right

In multiple fruiting stems, not all fruit doing equally well
X section of pedicels at bloom, 30 days post bloom and 60 days post bloom.
X sections of pedicels of fruiting (A) – (B) vs leaf stem (C-D)

(E) Pedicel is round and developed (F) vs leaf in (G) – (I)
DISCUSSION

IT IS REASONABLE TO ASSUME THAT THE TRANSLOCATION SYSTEM MAY PUT LIMITS TO FULL EXPRESSION OF THE POTENTIAL SINK STRENGTH.

THE INCRASING DEMAND FOR SUBTANCES MADE BY SEVERAL GROWING FRUITS OFTEN EXCEEDS THE CAPACITY OF THE VASCULAR SYSTEM OF THE TWIG TO SUPPORT IT, IN SPITE OF ITS FAST DEVELOPMENT.

Chicken and the egg? Does position favor peduncle and fruit development or does peduncle come first?

- Review of several sources arguing that transport system capacity and Pn are not factors in determining fruit size or assimilate accumulation
- Experiment proposed and completed to verify this hypothesis.
Pedicel development accelerated from 50 days after anthesis until 120 days. Xylem development was highest from 60 to 80 DAA and phloem development followed the same pattern.
Fig. 3. Rate of dry matter accumulation (open symbols) and specific mass transfer in the phloem of the pedicel (closed symbols) in fruitlets of Marisol clementine. Data for dry matter accumulation are means of 200 fruits sampled at random. SMT is calculated from the anatomical characteristics of the pedicel of 15 fruits of average size. Bars represent s.e. and are shown when larger than the symbol.
SMT and DM accumulation

- Two peaks, 40 to 80 days and 90 to 130 days
- First coincides with pedicel development and second after.
Fig. 4. Daily absolute (fruitlets d$^{-1}$; closed symbols) and relative (% of surviving fruitlets; open symbols) abscission rates in Marisol clementine. Absolute abscission rate is the mean of three trees ± s.e.
Fig. 5. Dry matter (g d$^{-1}$) accumulated in the developing fruit in a tree of Marisol clementine. Values are calculated from the rate of accumulation of dry matter in the fruit (Fig. 3) and the number of developing fruitlets. Values are mean of three trees ± s. e.
Fig. 9. Specific mass transfer in the pedicel of fruits from untreated control (open circles), 2,4-D-treated (closed circles) and 2,4-DP-treated (triangles) trees. Values calculated from the rate of accumulation of dry matter in a random sample of 100 fruits from 20 trees, and the anatomical characteristics of 15 pedicels from average-sized fruits. Vertical bars show the lowest significant difference ($P \leq 0.05$) on each date.
Summary

- Authors claim pedicel development follows carbohydrate accumulation.
- Data suggests that it slightly precedes or is coincident with fruit up swings.
- Some justification on both sides?

- Little work done on cuticle development of citrus (1980s)
- Electron microscopy methodology, plus chemistry available to study
- PURPOSE: Review of several studies by Albrigo, et al.; nature of cuticle and wax development
MATERIALS AND METHODS

• Surfaces prepared for SEM
• Waxes solubilized and weighed over time of development
• Weight loss determined in relationship to surface wax coating
• Many cultivars and fruit types studied
Changes in Valencia fruit surface area and waxes over time of development
Exposure of stomata on fruit surface during early fruit development
Development of wax platelets on surface of Valencia oranges during second half of development
### General differences in wax covering on fruit of different fruit types

Note lower wax covering on mandarin types and more on later maturing cultivars; mandarins have highest weight loss.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Location</th>
<th>Soft wax</th>
<th>Total wax</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Calamondin</td>
<td>Central Florida</td>
<td></td>
<td>37 a</td>
<td>(32–53)</td>
</tr>
<tr>
<td>Dancy tangerine</td>
<td>Central Florida</td>
<td></td>
<td>48 a</td>
<td>(40–53)</td>
</tr>
<tr>
<td>Orlando tangelo</td>
<td>Central Florida</td>
<td></td>
<td>57 a</td>
<td>(53–59)</td>
</tr>
<tr>
<td>Hamlin orange</td>
<td>Central Florida</td>
<td></td>
<td>79 b</td>
<td>(61–92)</td>
</tr>
<tr>
<td>Pineapple orange</td>
<td>Coastal Florida</td>
<td></td>
<td>83 bc</td>
<td>(69–95)</td>
</tr>
<tr>
<td>Pineapple orange</td>
<td>Central Florida</td>
<td></td>
<td>100 cd</td>
<td>(74–114)</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>Coastal Florida</td>
<td></td>
<td>116 de</td>
<td>(107–125)</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>Central Florida</td>
<td></td>
<td>117 de</td>
<td>(103–131)</td>
</tr>
<tr>
<td>Valencia orange</td>
<td>Central Florida</td>
<td></td>
<td>122 e</td>
<td>(92–154)</td>
</tr>
<tr>
<td>Valencia orange</td>
<td>Central California</td>
<td></td>
<td>178 f</td>
<td>(174–186)</td>
</tr>
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</table>
Relationship of surface wax to weight loss for round oranges

Each data point a grove sample

- VALENCIA $r^2 = .89$
- PINEAPPLE $r^2 = .37$
Number of plugged or partially plugged stomata on orange surface
Florida climate fruit vs California fruit

Albrigo, 1972
DISCUSSION AND CONCLUSIONS

- Waxes on surface are important to minimize water loss through cuticle.
- Differ by cultivar and grove source.
- Change during fruit development (increase).
- More wax on fruit developed in hot-dry climate than in humid climate.

- Juice vesicles are most important part of citrus fruit (peel for perfumes, marmalade)
- Understanding their development is important to understanding juice and sugar accumulation as well as acidity changes
- PURPOSE: Determine developmental sequence of juice vesicles
MATERIALS AND METHODS

• Star Ruby grapefruit collected throughout development and prepared for TEM and SEM
RESULTS
Fig. 1  Fruit fresh weight, length, and diameter of cv Star Ruby grapefruit from anthesis to 300 d of fruit development. Data plotted are the means of at least six fruitlets or fruit, with error bars indicating standard deviation.
Ovule - seed

+ or − 2 days of bloom

Ovule-seed
Juice vesicle development 10 to 21 days post bloom
4 to 10 weeks post bloom

Central cavity development on left and cellular and lipid development on right
Further development of central oil core on left and epidermal, hyperdermal cell wall plus juice vesicle & juice cells
Central core in mature juice vesicle

Osmophylic and fibrillar materials
DISCUSSION AND CONCLUSIONS

- As with all parts of fruit, early development by cell division and later development by cell enlargement
- Development comes from outside wall and nearby lateral walls (near vascular bundles, but without vascular system in juice vesicles (all transport is cell to cell by diffusion or active cytoplasmic transport)

- Juice vesicles stay in clump when segment wall removed
- Certain conditions lead to vesicle groups falling apart
- PURPOSE: Study nature of adhesion between vesicles and their surface development
MATERIALS AND METHODS

• Segments and vesicles treated in various ways, prepared for SEM or TEM and examined.
RESULTS
Surface waxes on vesicles
Typical cuticle development with waxes acting as bonding agent
Surface waxes at early stages of vesicle development
Waxes on surface of vesicles
Waxes at later stages of development
DISCUSSION AND CONCLUSIONS

• Juice vesicles have cuticle and epicuticular waxes just like surface of fruit.
• Solvents, heat, etc. can dissolve or melt waxes and loosen adhesion of vesicles to each other.
  – Liquid N2 can result in wax layer, weakest point, separating to leave vesicles in individual units (used in processing slurry of vesicles (10% juice).

- Recognized that not all fruit on a tree are equal.
- Systematic sampling to characterize on tree location characteristics.
- Important for proper sampling with payments being made on juice quality – lbs solids per box (% juice x % brix x 90 lbs)
Percentage juice by tree position

Highest values on top and outside
Tree top down view of SS distribution in fruit. Outside higher than inside. West & South higher than E & N.
SS or Brix higher on outside and top of tree
Graphic representation of SS distribution by canopy position of fruit on tree - Valencia

Increasing with tree height reduced shade?
Citric acid highest in middle of canopy – SS accumulation versus heat effect?
Brix to Acid ratio higher top and outside
Conclusions

- Better sugar and higher ratio on outside and top of tree
- North to South effects values
- Representative sample requires taking fruit around tree at arms reach
- Could take advantage of this for spot picking
- Why are values different around tree – evaluate more papers
- Weakness – Lack of statistics
The enzymatic activity of juice vesicles may be way to assess stage of development or senescence.

Development of enzymes based on DNA to RNA to produce protein.

PURPOSE: Determine pattern of enzyme development as fruit develop as means to assess juice vesicle maturity.
MATERIALS AND METHODS

• Juice vesicles collected prepared and examined for DNA and some key enzymes related to metabolism
RESULTS
Days after Anthesis

JUNE  JUNE  JULY  AUG.  SEPT.  OCT.
Acid metabolism enzyme changes with development

? Where is acid formed and used
Respiration changes over time (X)
### Table 1: Changes in enzyme activities in juice vesicle tissue during development of Satsuma mandarin fruit

<table>
<thead>
<tr>
<th>Date</th>
<th>Days after anthesis</th>
<th>Enzyme activities (µmoles/min/fruit)</th>
<th>Malic enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PEPC</td>
<td>MDH</td>
</tr>
<tr>
<td>10/VI-12/VI</td>
<td>23-25</td>
<td>0.119</td>
<td>9.27</td>
</tr>
<tr>
<td>29/VI-1/VII</td>
<td>41-44</td>
<td>1.61</td>
<td>38.7</td>
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<tr>
<td>13/VII-14/VII</td>
<td>56-57</td>
<td>3.11</td>
<td>108</td>
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<tr>
<td>27/VII-28/VII</td>
<td>71-72</td>
<td>5.64</td>
<td>107</td>
</tr>
<tr>
<td>16/VIII-17/VIII</td>
<td>90-91</td>
<td>6.90</td>
<td>60.4</td>
</tr>
<tr>
<td>30/IX-1/X</td>
<td>135-136</td>
<td>6.35</td>
<td>473</td>
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<tr>
<td>29/X-30/X</td>
<td>164-165</td>
<td>5.07</td>
<td>557</td>
</tr>
</tbody>
</table>

4 ½ months development, 3 ½ months to major increases
Table 2: Changes in enzyme activities in juice vesicle tissue during fruit development of sweet lime fruit

<table>
<thead>
<tr>
<th>Date</th>
<th>Days after anthesis</th>
<th>PEPC</th>
<th>MDH</th>
<th>Citrate synthetase</th>
<th>Aconitase</th>
<th>NAD-IDH</th>
<th>Malic enzyme</th>
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</thead>
<tbody>
<tr>
<td>5/VI-6/VII</td>
<td>42-43</td>
<td>0.23</td>
<td>12.8</td>
<td>0.135</td>
<td>0.083</td>
<td>0.0547</td>
<td>—</td>
</tr>
<tr>
<td>22/VII-23/VII</td>
<td>58-59</td>
<td>2.51</td>
<td>52.4</td>
<td>0.950</td>
<td>3.73</td>
<td>0.947</td>
<td>5.98</td>
</tr>
<tr>
<td>24/VIII-25/VIII</td>
<td>91-92</td>
<td>10.4</td>
<td>330</td>
<td>3.33</td>
<td>4.36</td>
<td>1.59</td>
<td>29.9</td>
</tr>
<tr>
<td>19/X-20/X</td>
<td>147-148</td>
<td>10.4</td>
<td>550</td>
<td>14.9</td>
<td>3.39</td>
<td>5.62</td>
<td>36.8</td>
</tr>
</tbody>
</table>

3 months
DISCUSSION AND CONCLUSIONS

• Most of activity at 100 to 120 days post bloom (3 months)
• Fruit acidity decline started just after that?
• Sugar accumulation earlier start
  – Transport issues

• Little work on exact nature of sugar transport and accumulation
• Review of general localization of sugars in fruit (Reitz and Sites)
• PURPOSE: General overview of transport of photosynthates to fruit and important changes in sugar and acid metabolism of fruits
MATERIALS AND METHODS

• Review of work by Koch; H.J. Reitz, et al and others
RESULTS
Nearest leaf effect on sugar accumulation (25% to closest sink)

Major point for ingress of photosynthates into juice vesicles
Soluble Solids accumulation in fruit in different parts of tree
Acid accumulation in fruit in different parts of tree

Somewhat similar to SS
Sugar to Acid ratio in fruit in different parts of tree
Important sugar transport and metabolism for fruit energy and general metabolism.
Source/sink versus utilization and general climatic effects.
DISCUSSION AND CONCLUSIONS

- Sugars for fruit metabolism and growth come first from nearby leaves.
- Climate effects Pn and transport into different parts of tree and therefore fruit.
- In fruit metabolism a balance between energy needs and storage.
- Overall, fruit is one sink competing for Pn.