RosBREED Apple Phenotyping Protocol

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

Overview

- Apple fruits from individuals to be evaluated will be harvested at a defined stage of maturity based on starch pattern index and evaluated for various at 3 times:
  1. Harvest
  2. 10 weeks postharvest cold storage + 1 week room temp
  3. 20 weeks postharvest cold storage + 1 week room temp

Crop Load and Thinning

- After any chemical thinning (if done), trees should be hand-thinned (beginning 5-6 weeks after bloom) to **retain 20-25 fruit per tree for younger trees**, or **4 fruit per foot of branch length for older trees** (not including current season's extension growth) and no more than 2 fruit per spur or cluster.

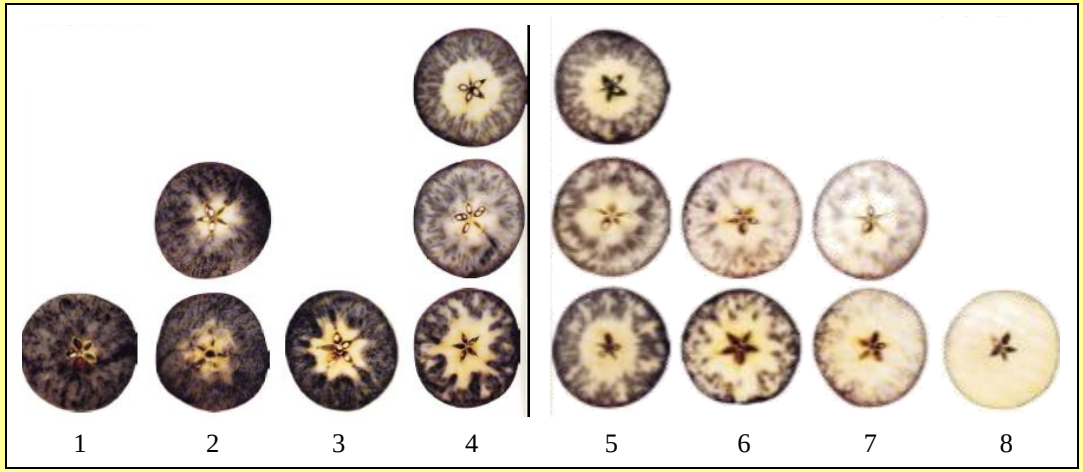
- 15 fruit is minimum needed to cover all evaluation stages (5 fruit x 3 evaluation times), and extra fruit provides some backup in case of fruit rot in storage. We would like to harvest a minimum of 10 fruit per tree to allow for evaluations at harvest and after 10 weeks of storage.

Fruit Maturity and Harvest

- Check trees weekly for fruit color changes that indicate onset of fruit maturation – changes in background color (from green to cream or light yellow) and brightening in red blush color development.

- When the tree looks like it may be ready for the first harvest sampling, select 1-2 fruit with the most advanced maturity appearance and conduct a starch-iodine test. **Aim for a maturity indicator of SPI** 3-5 on the 1-8 Cornell (Blanpied) Chart below (available at http://ecommons.library.cornell.edu/bitstream/1813/3299/2/Predicting%20Harvest%20Date%20Window%20for%20Apples.pdf) or **1.5-2.5 on a 0-6 starch pattern chart as a generic harvesting 'go date'**. This is equivalent to commercial harvest, and is a good intermediate range where it is unlikely for any trees to be too immature or over-mature.

- If the SPI indicates onset of maturation for a tree, visually evaluate the remaining fruit on the tree for their range in maturity. If ~50 % of the fruit have a 'harvest-mature' appearance (roughly similar to the SPI-tested fruit), take the sample of 15-40 fruit selectively picked to be at the correct harvest maturity. 15 fruit is minimum to cover all evaluation stages (5 fruit x 3 evaluation times), and extra fruit provides some backup in case of fruit rot in storage.



Fruit Maturity and Harvest

- Fruit should be harvested wherever possible from well exposed areas of the tree but avoiding the top and bottom of the canopy. Avoid sunburned fruit, cracked fruit, etc. if possible. Careful harvest is also required because fruit can be readily damaged by stem pulls, finger bruising, etc.

- Harvest into tray-lined plastic lugs for storage, labeled with following information:
  - **Row No.** and **Tree No.** (i.e., tree location)
  - **Designation of cultivar/selection/seedling** (e.g. “Honeycrisp”, or “WSU47”, or “RGxBB #23”)
  - **Harvest date**
  - **Number of fruit**

- Transfer fruit to cold storage as soon as possible.

Data at Harvest

- Record data for the following traits at harvest
  - Harvest date
    - When maturity indicator of SPI 1.5-2.5 on a 0-6 starch pattern chart or 3-5 on the 1-8 Cornell Chart
  - Crop load (rating) (note: with thinning advised above, this should usually be medium or less)
    - 1=v.light
    - 2=light
    - 3=medium
    - 4=heavy
    - 5=v.heavy
  - Preharvest dropping (rating)
    - 1=none
    - 2=light (<5%)
    - 3=medium (5-30%)
    - 4=heavy (30-60%)
    - 5=v.heavy (>60%)

Other Traits

- Disease traits may also be recorded from field plots at harvest if conditions permit
  - Diseases (rated 0=none, 1=slight, 2=medium 3=severe)
    - Apple scab severity (rating)
    - Fireblight severity (rating)
    - Powdery mildew (rating)
- These cropping traits can be derived from annual harvest data
  - Annual bearing (based on annual crop load rating)
  - Precocity (yr to bearing)

Traits Evaluated Using Aggregate Rating on Five Whole Fruit

The following traits are rated based on an aggregate sample five fruit at harvest and/or after 10 or 20 weeks of storage:

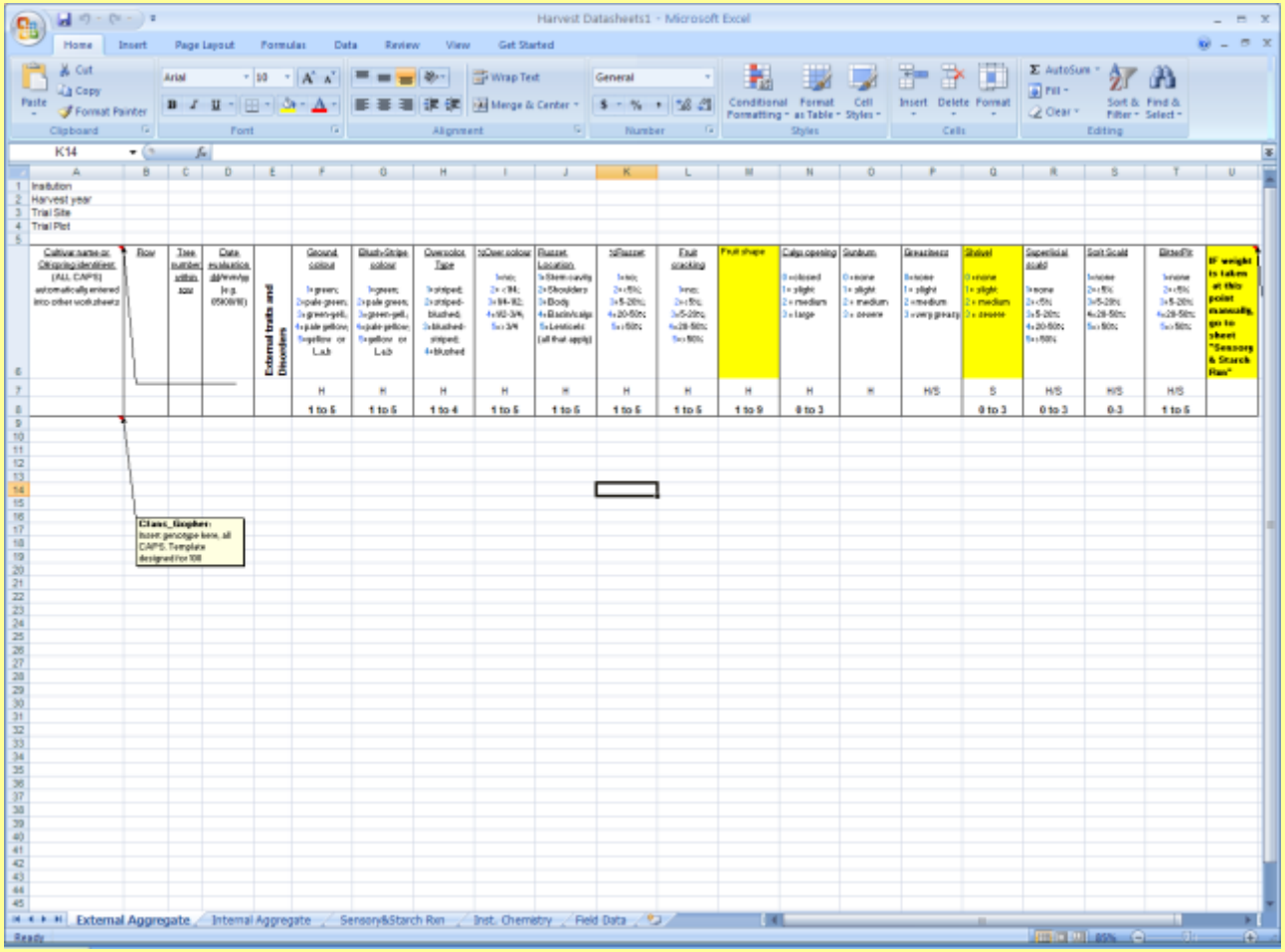
Harvest only

- Ground color
- Blush/strip color
- Overcolor type
- Red/blush stripe coverage
- Russet location
- Russet coverage
- Fruit cracking
- Fruit shape
- Calyx opening
- Sunburn

Harvest and Storage

- Greasiness (not common at harvest)
- Shrivel (likely only after storage)
- Bitter pit
- Scald (not common at harvest)

Data sheets



Excel worksheets that can be used for data entry are available at [link]

Ground color

When: Harvest only

1 – Green
2 – Pale Green
3 – Green-Yellow
4 – Pale yellow
5 – Yellow

1

http://www.shop.globalfayre.com/images/McIntosh.jpg

2

http://www.seasonalchef.com/051406f.jpg

3

http://www.isons.com/images/apple_yellow_delicious_sm.JPG

4

http://www.haneysappledalefarm.com/images/apples/grimesgolden2.jpg

5

Blush-Stripe Color

When: Harvest only

1 – Orange
2 – Orange Red
3 – Pink Red
4 – Red
5 – Dark Red

http://www.orchardworld.co.uk/graphics/content/fruit/tentation.jpg

http://fruitforum.files.wordpress.com/2010/03/pinklady2.jpg

http://www.boyernurseries.com/images/apple_close_ups/Rome.jpg

http://www.plant.photos.net/images/thumb/8/81/Apple_williams_pride.jpg/180px-Apple_williams_pride.jpg

Over Color Type

When: Harvest only

1 – Striped
2 – Striped-Blush
3 – Blush-Striped
4 – Blushed

http://www.seasonalchef.com/051406f.jpg

%Red blush/stripe coverage

When: Harvest only

Coverage of the fruit with

1 – None
2 – <25%
3 – 25-50%
4 – 50-75%
5 – >75%

Russet Location (all that apply)

When: Harvest only

1 – Stem cavity
2 – Shoulders
3 – Body Basin/Calyx
4 – Lenticels

% Russet

When: Harvest only

1 – No russet (rare but can occur)
2 – stem cavity russet but not extending over shoulders or on body of fruit
3 – Slight – one to 2 fruit with russet extending over shoulders or on body of fruit
4 – Severe – three or more fruit with russet extending over shoulders or on body of fruit

Fruit Cracking

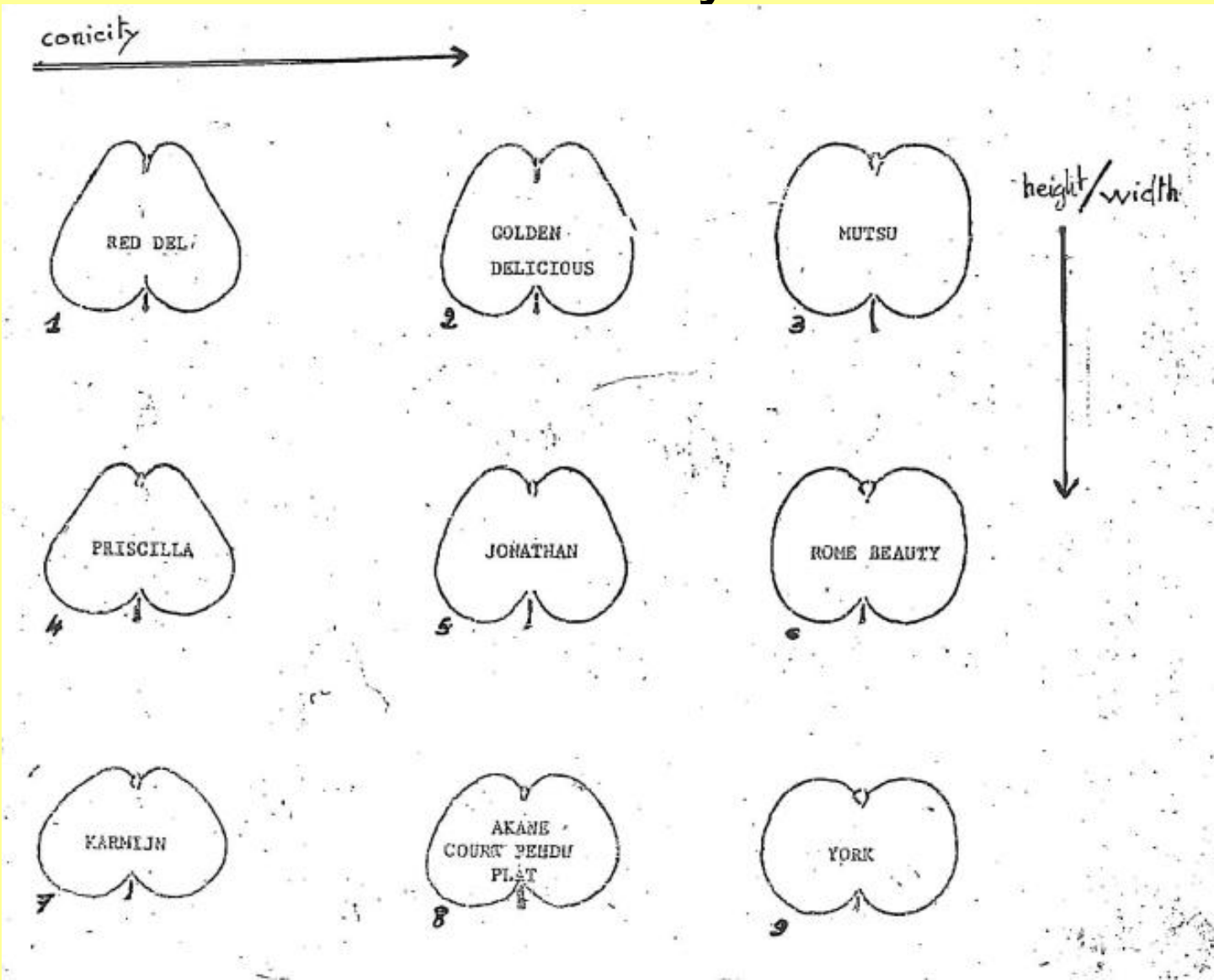
When: Harvest only

This will usually occur at the stem or calyx end of the fruit
1 – None
2 – Slight – one fruit affected
3 – Medium – 2 or 3 fruit affected
4 – Severe – 4 or 5 fruit affected

Fruit Shape

When: Harvest only

A scale developed by INRA (France) is used. Shape is rated from 1 to 9 as shown based on amount of conicity and height/width ratio.



Calyx Opening

When: Harvest only

1 – All Closed
2 – Mixed
3 – All open

Open

Closed

Sunburn

When: Harvest only

Use attached photos as guides:

1 – None (Grade 0)
2 – Slight (Grade 1-2)
3 – Medium (Grade 3)
4 – Severe (Grade 4-5)

Greasiness (Tackiness)

When: Harvest and After Storage

Greasiness is detected by trying to slide the thumb or finger firmly across the surface of the fruit

1 – None – finger slides with no resistance, may make audible squeak if great pressure is applied
2 – Slight – slight resistance and tackiness
3 – Medium – considerable resistance and persistent tackiness that lingers on finger
4 – Very Greasy – extreme force is needed to slide finger,

Shrivel

When: Harvest and After Storage

Note shriveled skin indicating water loss in portions of fruit with sound skin (ignore areas damaged by disease or mechanical contact)

1 – None
2 – Slight – one fruit affected
3 – Medium – two or three fruit affected
4 – Severe – four or five fruit affected

Bitter Pit

When: Harvest and After Storage

1 – None
2 – Slight – at least one pit observed on one fruit
3 – Medium – 2 or 3 fruits have pits
4 – Severe – 4 or 5 fruits have pits

Scald

When: Harvest and After Storage

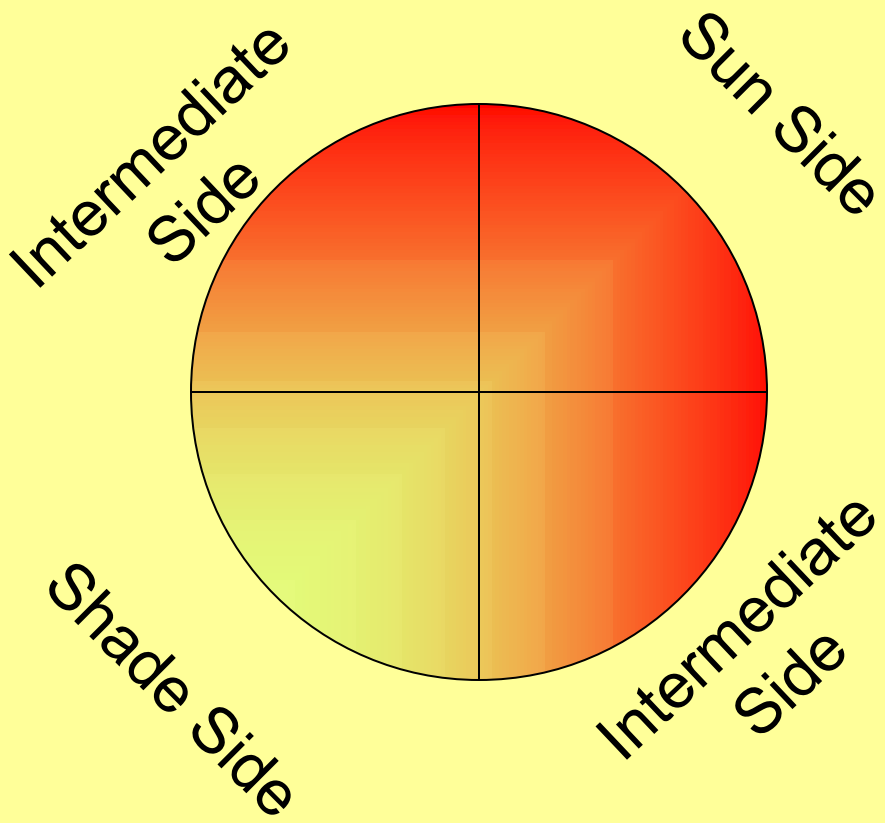
Discoloration and may have a epoxy glue-like flavor
1 – None
2 – Slight – one fruit affected
3 – Medium – 2 or 3 fruits affected
4 – Severe – 4 or 5 fruits affected

Courtesy of Dr. Dave Rudell, USDA-ARS

Marking and Cutting Fruit

After aggregate traits are rated, each of the five fruit should be numbered and marked to indicate sun, shade, and intermediate sides of fruit.

Identify sun and shade sides for instrumental assays.



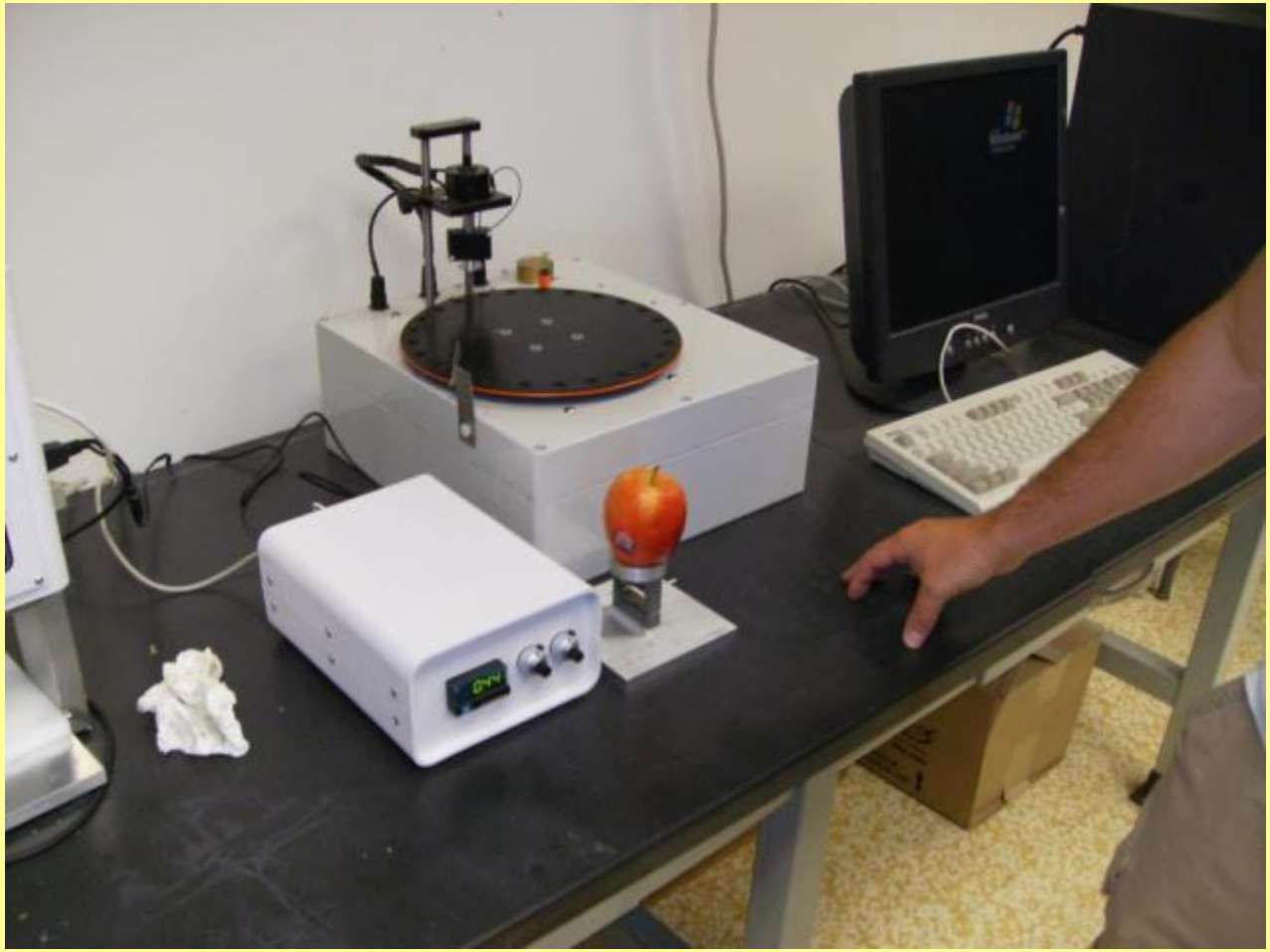
Prior to instrumental analyses, each fruit should be marked with waterproof, marker with a number from 1 through 5 and quarters of the fruit should be marked to indicate sun, shade, and intermediate sides of fruit.

Make long longitudinal marks to differentiate the two intermediate quarters.

Intermediate sides to be assayed by Digi-Test and sensory panelists

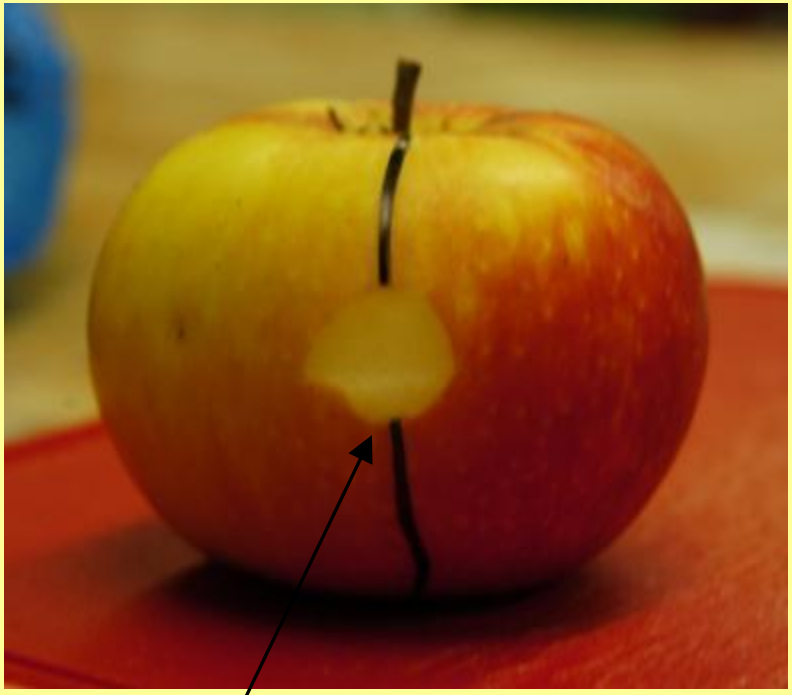
Weigh Fruit

(When: Harvest and after storage)



After marking, the weight of each fruit is recorded in grams

One Intermediate side will be assayed via Mohr Digi-Tester.

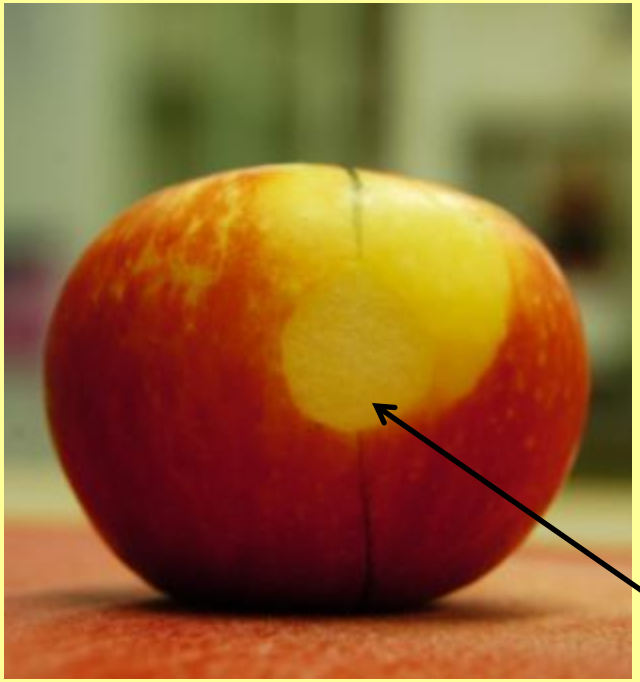


Assume that this mark is the site of the Digi-Test assay. Remove skin from this portion.

Mohr DigiTest Assessment

When: Harvest and After Storage

The Mohr DigiTest (MDT) is used to estimate fruit diameter, flesh firmness and flesh crispness. The skin is removed prior to each MDT probe insertion.



- Remove skin from one intermediate side of the fruit before MDT testing.
- We use a mandoline slicer to shave off just a couple mm.
- This where the probe will be inserted.

Mohr DigiTest Assessment

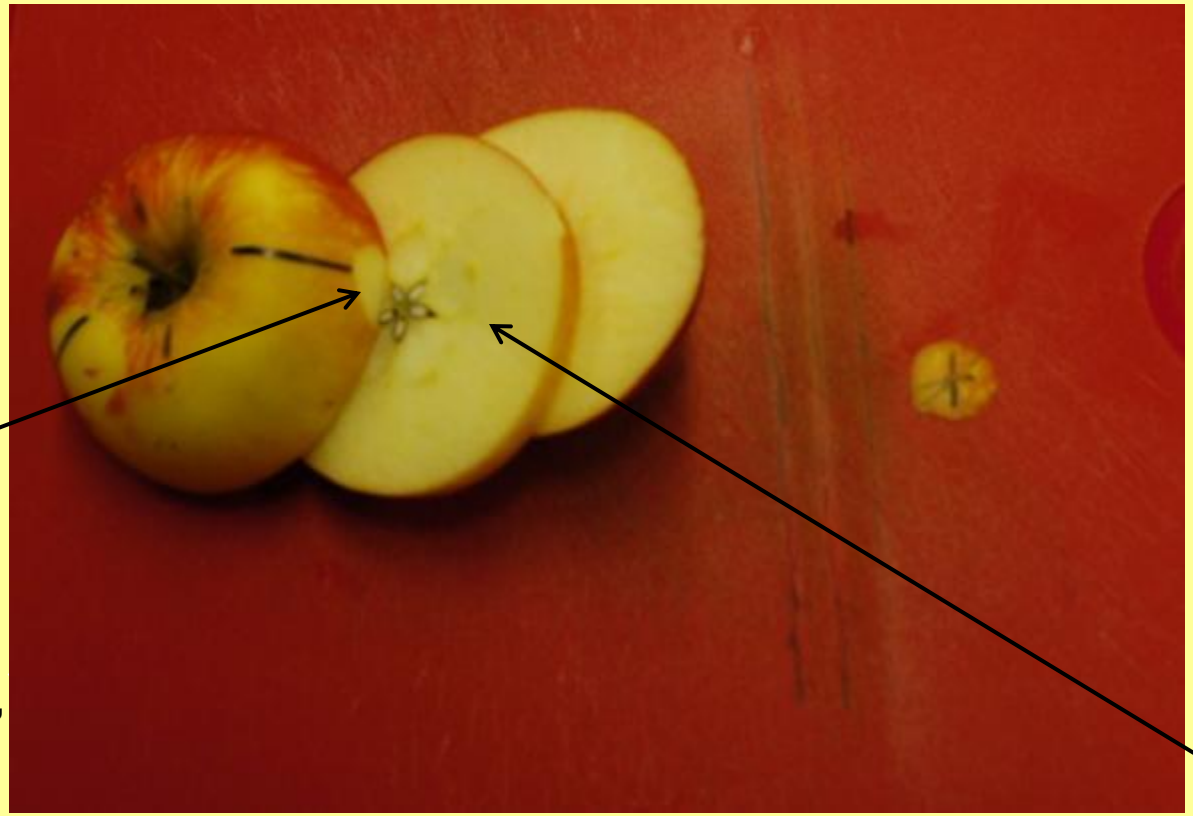
When: Harvest and After Storage

Test one intermediate side in the Digi-Test machine. Use the green button (2 on keypad) for each fruit and it will only give one value per ID.

Segmenting fruit for starch iodine test (harvest only) and evaluation of sensory and internal traits

- After the MDT evaluation, each fruit is sliced into appropriate segments to evaluate the following traits:
  - **Starch pattern index** (Harvest only)
  - **Water core** (Harvest and after storage)
  - **Moldy core** (Harvest and after storage)
  - **Core size (carpels)** (Harvest only)
  - **Core area (vascular bundles)** (Harvest only)
  - **Core opening** (Harvest only)

Remove an equatorial slice for starch-iodine assay (only for at-harvest samples). Thinness does not matter, other than that the slice must maintain structural integrity.

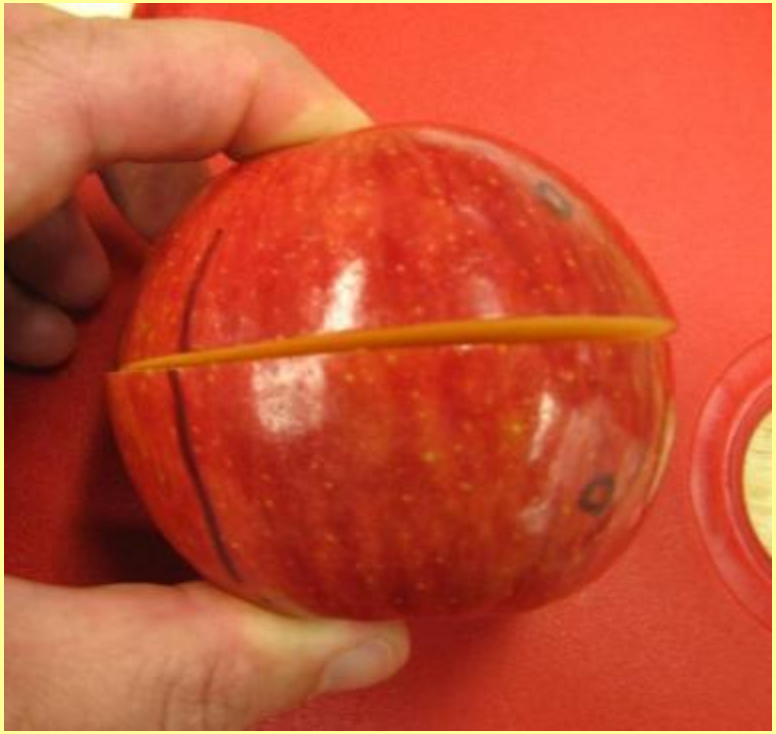


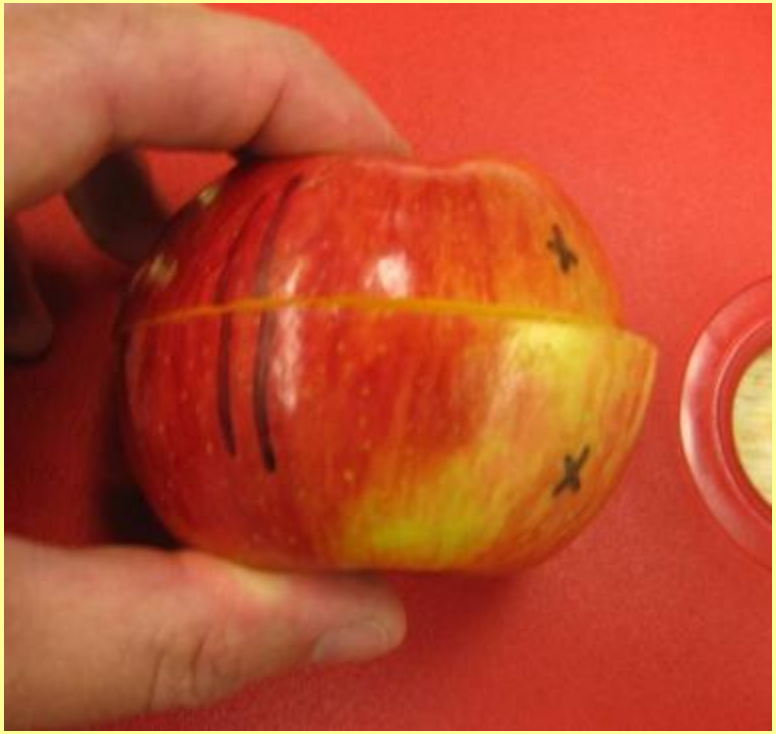
Note that after firmness and crispness measurements, there will be a hole punched here on one intermediate side

Taken for starch test and other evaluation

(at harvest only – not stored fruit)

Align separated tops and bottom pieces using the vertical marks.



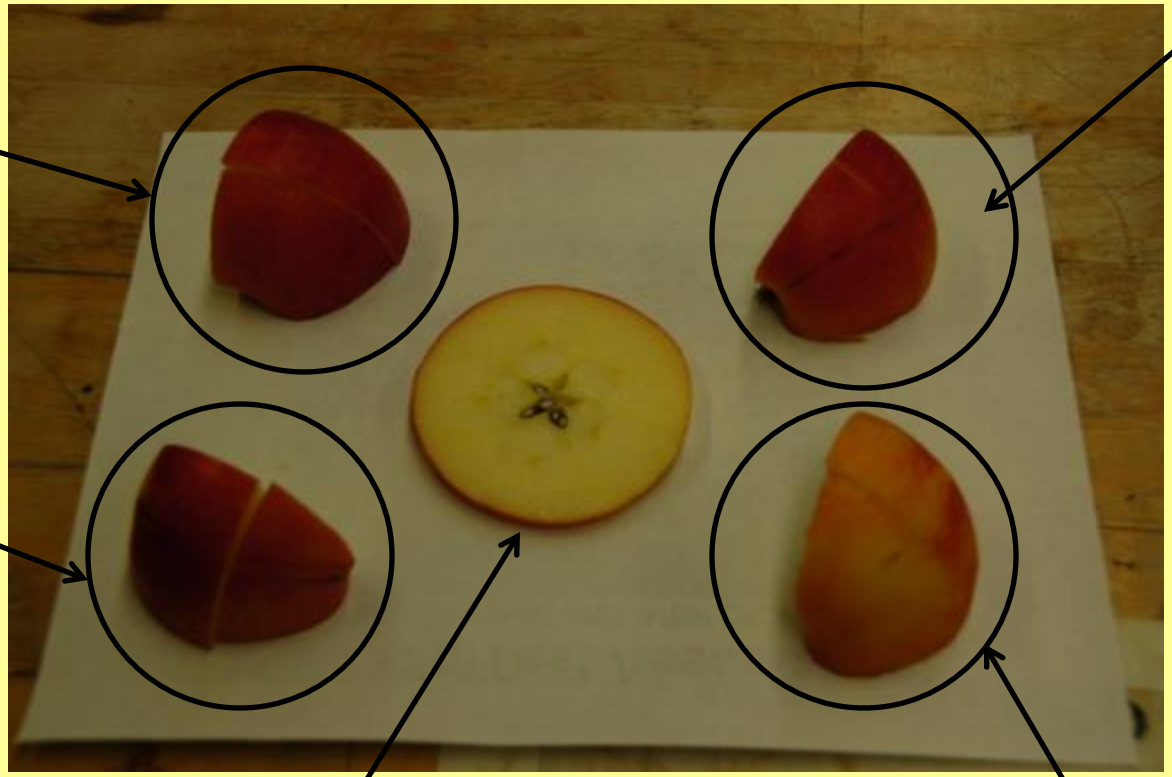


Cut remaining tissue into longitudinal quarters. Ensure that corresponding calyx and pedicel sections stay paired.

This is what you should have. The starch-iodine and juice samples should be placed into pre-labeled zipper lock plastic bags and set aside, but only until the sensory evaluation step is complete for the day.

Can be used for juicing if needed .

Only need one intermediate quarter segment for sensory evaluation for 4 of the 5 fruit.

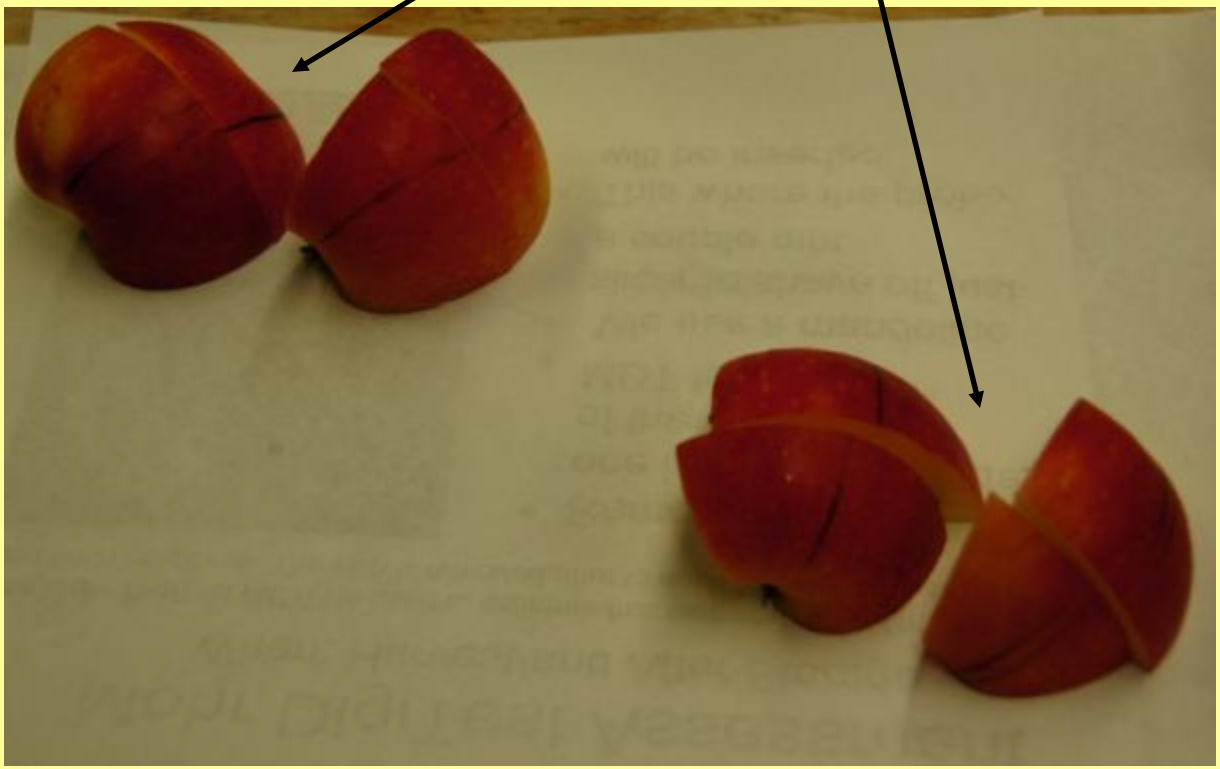


One intermediate quarter already utilized in MDT rating – can be used for juicing if needed.

Starch-Iodine, core measurements, etc.

One quarter reserved for juicing (or more for smaller fruit)

These intermediate segments for sensory evaluation.



Two different fruit chosen for each panelist.

Each fruit represented by one intermediate quarter

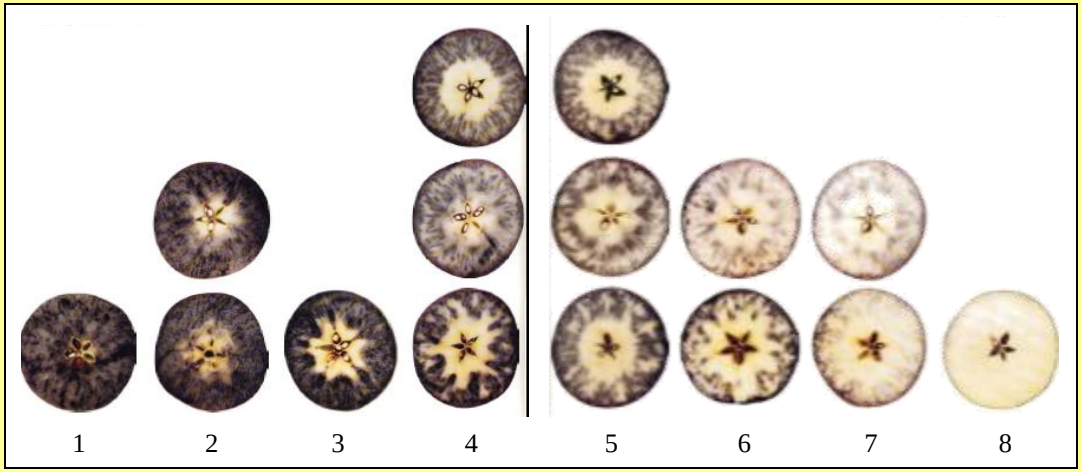
Starch testing

When: Harvest

Arrange slices for starch testing

Apply iodine

Use the Blanpied chart (Cornell)



Samples are expected to be in the 3-5 range, as this is what we harvest at.

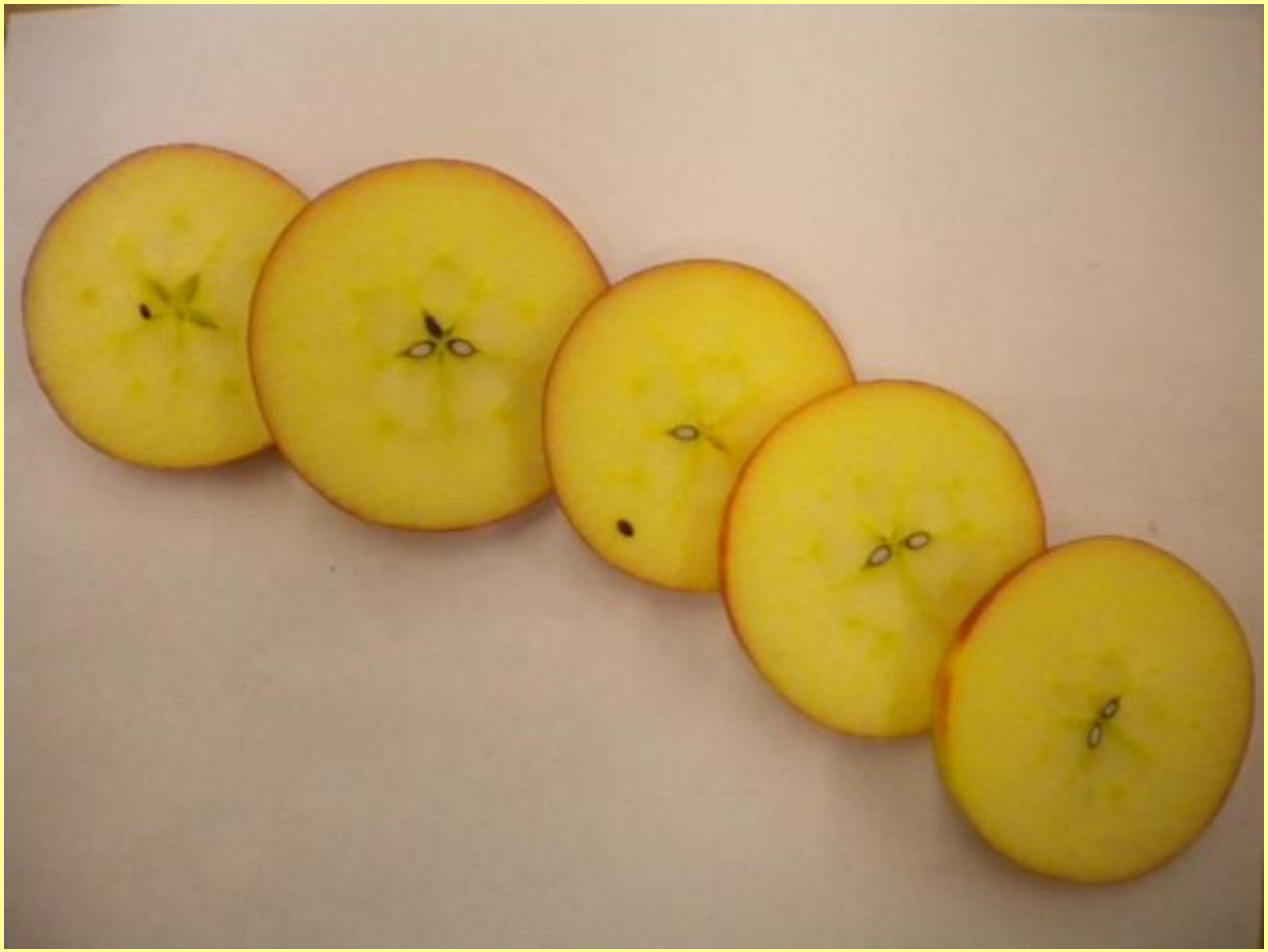
Starch Reaction

All 5 fruit will be tested and a record is made for each fruit on the 1 – 8 Blanpied scale.

http://www.theenglishappleman.com/journal_090904.asp

http://www.umass.edu/fruitadvisor/clements/articles/sitesttools.jpg

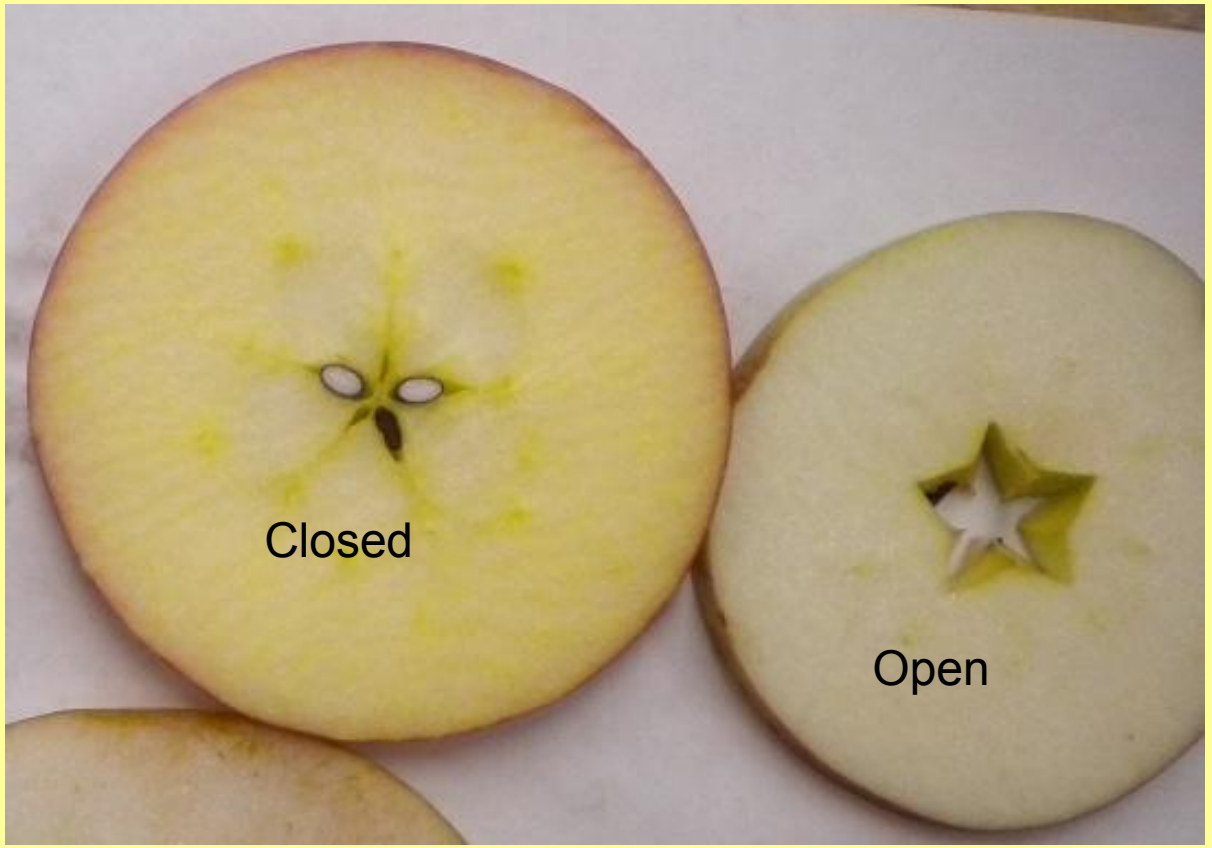
Internal Traits
(aggregate rating of 5 fruit)



Core opening

When: Harvest

0 - Open
1 – Closed



Water core

When: Harvest and After Storage

Using fruit cut for the starch test, indicate frequency of watercore

1 – None
2 – Slight – one fruit affected
3 – Medium – 2 or 3 fruits affected
4 – Severe – 4 or 5 fruits affected

http://www.ipt.us.com/wp-content/uploads/2009/11/Watercore-USDA.jpg

Internal Browning

When: Harvest and After Storage

Using cut fruit, indicate frequency of internal browning

1 – None
2 – Slight – one fruit affected
3 – Medium – 2 or 3 fruits affected
4 – Severe – 4 or 5 fruits affected

Moldy core

When: Harvest and After Storage

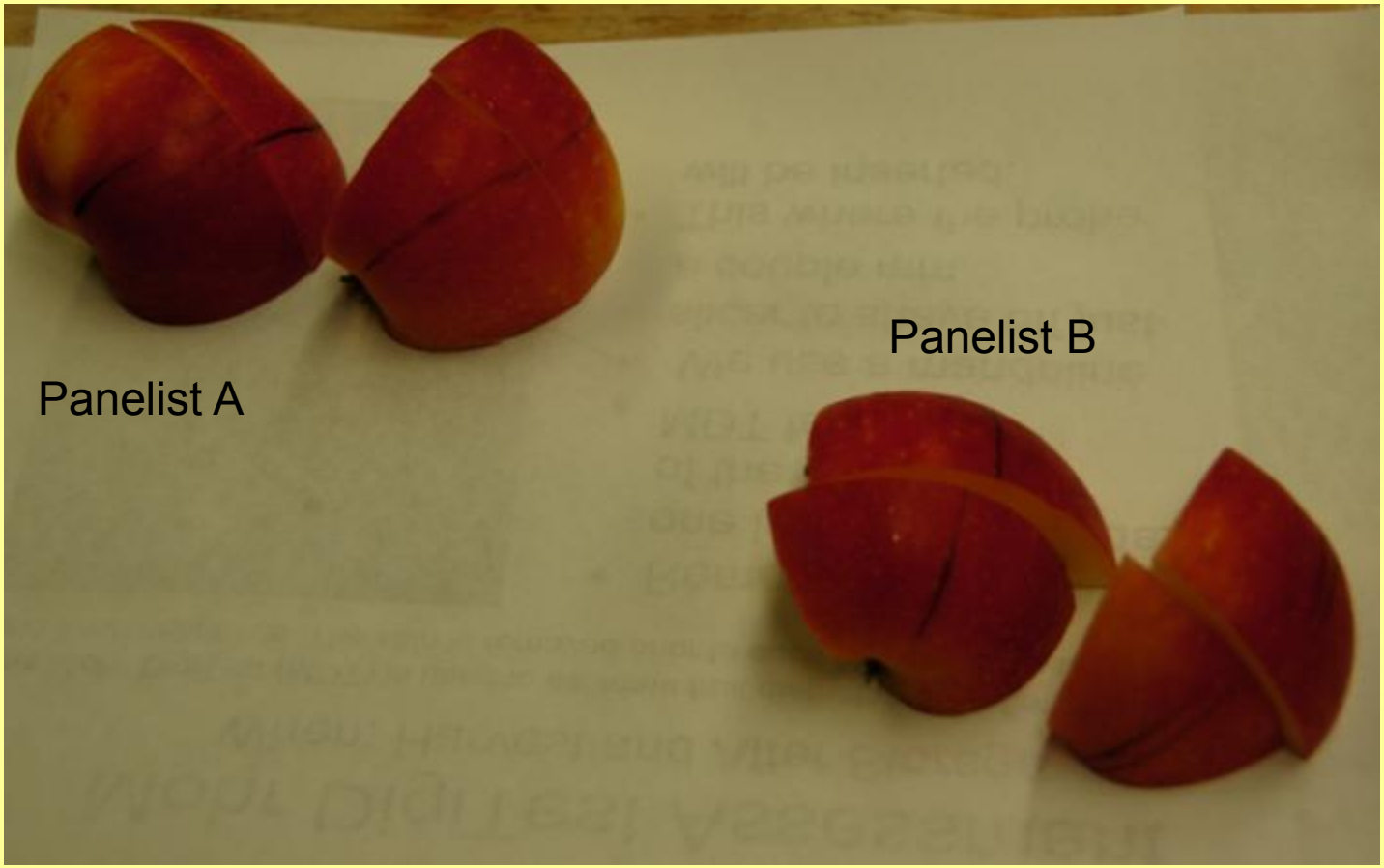
0 – None
1 – Present

Sensory Evaluation

When: Harvest and After Storage

- Sensory texture properties: Four fruit selected for evaluation per genotype. A minimum of two evaluators will each sample 2 different fruit. Two fruit segments from an intermediate side will be available to represent each fruit. Panelist should develop a single rating for each trait based on the 2 fruits s/he evaluates. Two panelists record scores independently and mean rating of panelists is used for FlexQTL.

2 Evaluators, 2 Fruit Each



Two panelists to independently record their scores

Sensory evaluation

Holding peel between thumb and forefinger, sensory panelists can bite for the crispness assay and then chew the fragment for firmness, juiciness and other assays.

Start with one fragment (calyx or pedicel section, doesn't matter which), then use the other fragment to confirm.

Maturity

1 – Immature (starchy, granular)
2 – Mature (no detectable starch)
3 – Over mature (soft, lacks acid and contains overmature flavors)

Crispness

Front teeth bite – crack noise

1 – No noise
2 – Low noise
3 – Medium
4 – Noisy
5 – Very noisy

Firmness/Hardness

Three chews on molars – resistance

1 – Very soft
2 – Soft
3 – Medium
4 – Firm
5 – Very firm

Juiciness

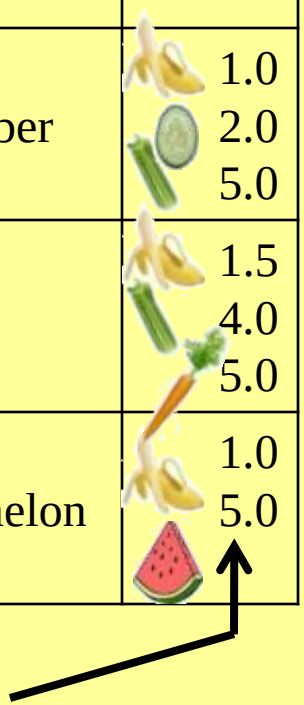
Three chews on molars – expressed juice

1 – Dry
2 – Low juiciness
3 – Medium
4 – Juicy
5 – Very juicy

Sensory traits are rated on a 1 to 5 scale. Panelists may use half points

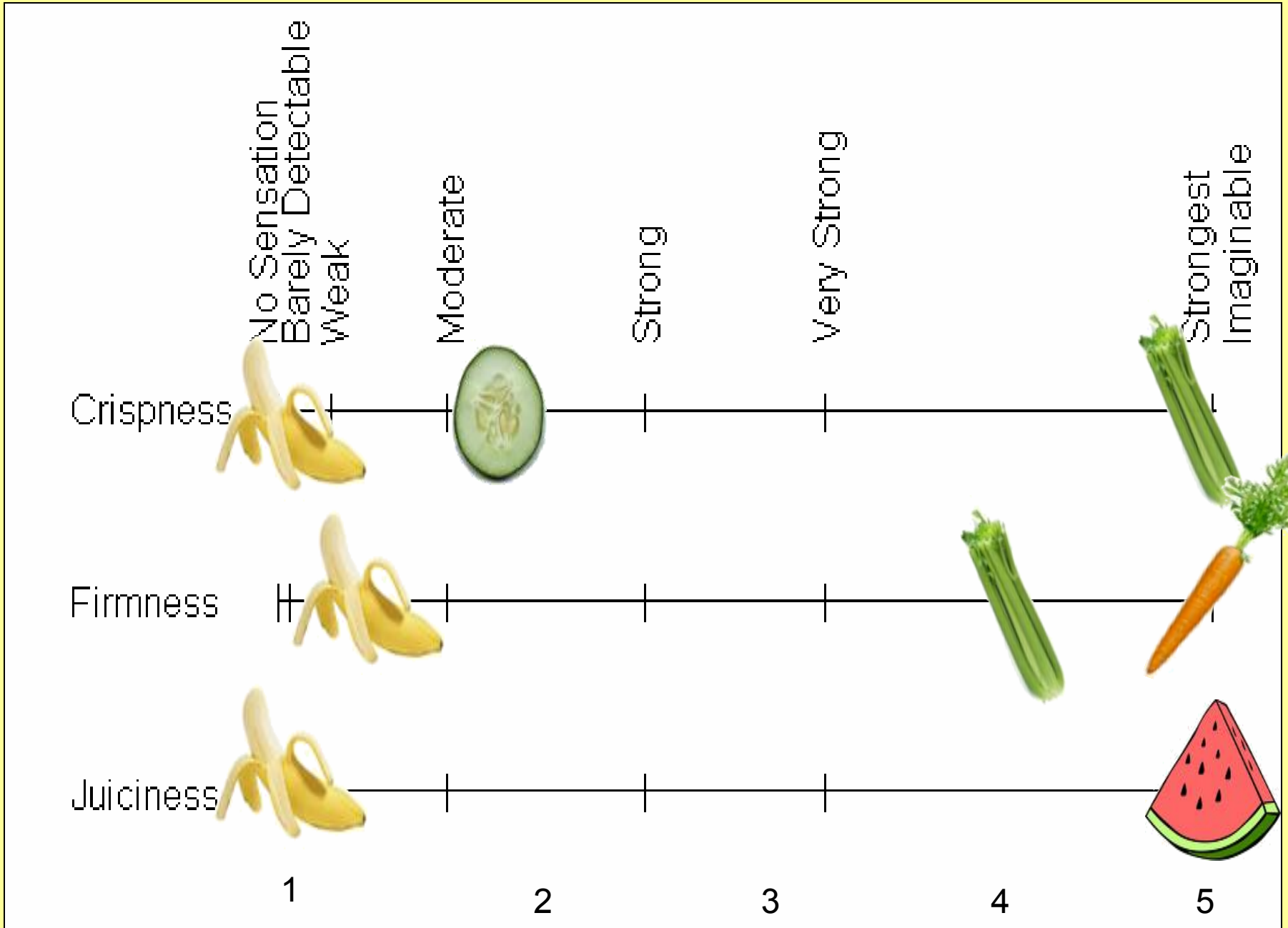
Description and Anchors for Texture Traits

Trait	Description	Anchors	Score
Crispness	The amount and pitch of sound when sample is first bitten with the front teeth. Place fruit piece between the front teeth and bite down evenly.	Banana Cucumber Celery	1.0 2.0 5.0
Firmness	The force required to bite through the sample. Place fruit piece between the molars and bite down evenly.	Banana Celery Carrot	1.5 4.0 5.0
Juiciness	The amount of juice released by the sample when chewing with the back teeth. Place fruit piece between the molars and chew.	Banana Watermelon	1.0 5.0



Where on the 1 to 5 scale the anchors sit.

This shows where the anchors lie.



Aroma/Flavor

1 – Very low
2 – Low
3 – Medium
4 – High
5 – Very high

Sweetness

1 – Very low
2 – Low
3 – Medium
4 – High
5 – Very high

Acidity

1 – Very low
2 – Low
3 – Medium
4 – High
5 – Very high

Sensory traits are rated on a 1 to 5 scale. Panelists may use half points

Samples for Juicing

Approximately equal portions from each of the five fruit should be placed into juicer for aggregate sample. This may be one quarter from each fruit or more depending on the size of apple.

Fruit segments for juicing can be saved and placed in labeled plastic bags to be saved for juicing. Place in refrigerator until juiced.

Preparing Juice Samples

Segments from all five fruit placed into juicer for aggregate sample.

2 aliquots (10-15 ml each) are saved from each aggregate sample in labeled vials.

Handling Juice Samples

Evaluate immediately for soluble solids or titratable acidity or store at -80C or lowest available temp.

Soluble Solids Concentration

- °Brix

Soluble solids concentration measured on a couple of ml juice, ^{0}Brix (digital refractometer or similar)

Titratable Acidity

- mg/L malic acid

Titratable acidity on 10 ml juice, mEq/L (manual or automatic titration), express as mg/L of malic acid

Storage of fruit for after-storage evaluations

Fruit not used for harvest evaluation should be stored as follows for after-storage evaluations:

- Place fruit into plastic lugs lined with perforated polythene carton liners (to prevent fruit shrivel) – one individual per lug. Each lug should be labeled clearly with the variety, harvest date, and number of fruit remaining.
- Pack lugs on pallets up to a maximum height of four layers to ensure sufficient air circulation – try to spread out even more when first stored so that fruit cool down as quickly as possible.
- Store fruit for 10 and 20 weeks at 0-1.0^{0}C from the harvest date.
- Periodically (once per 5 weeks will suffice) inspect each set of fruit in storage and remove any rotting fruit.

Evaluations after storage

- For each of the varieties after the two storage durations (10 weeks ±2 days, and 20 weeks ±2 days), **randomly select five sound fruit** and remove from storage. Place onto labeled trays in the fruit evaluation lab. Allow fruit to ripen at room temperature (20^0 C) for 7±1 days prior to evaluation. Number fruits 1-5 with food safe marker.

Evaluation at 10 weeks of storage, + 1 week at 20°C

- Evaluate the following traits as described above:

<u>External and instrumental traits</u>

- Greasiness
- Shrivel
- Bitter pit
- Scald
- Fruit weight
- Fruit diameter (MDT)
- Flesh firmness (MDT)
- Flesh crispness (MDT)
- Soluble solids (Brix)
- Titratable acidity

<u>Internal and sensory traits</u>

- Internal browning
- Water core
- Moldy core
- Sensory maturity
- Sensory crispness
- Sensory firmness
- Sensory juiciness
- Sensory sweetness
- Sensory acidity
- Sensory flavor aroma

Evaluation at 20 weeks of storage, + 1 week at 20°C

- Evaluate the following traits as described above:

<u>External and instrumental traits</u>

- Greasiness
- Shrivel
- Bitter pit
- Scald
- Fruit weight
- Fruit diameter (MDT)
- Flesh firmness (MDT)
- Flesh crispness (MDT)
- Soluble solids (Brix)
- Titratable acidity

<u>Internal and sensory traits</u>

- Internal browning
- Water core
- Moldy core
- Sensory maturity
- Sensory crispness
- Sensory firmness
- Sensory juiciness
- Sensory sweetness
- Sensory acidity
- Sensory flavor aroma