# Phenotyping in Tart Cherry

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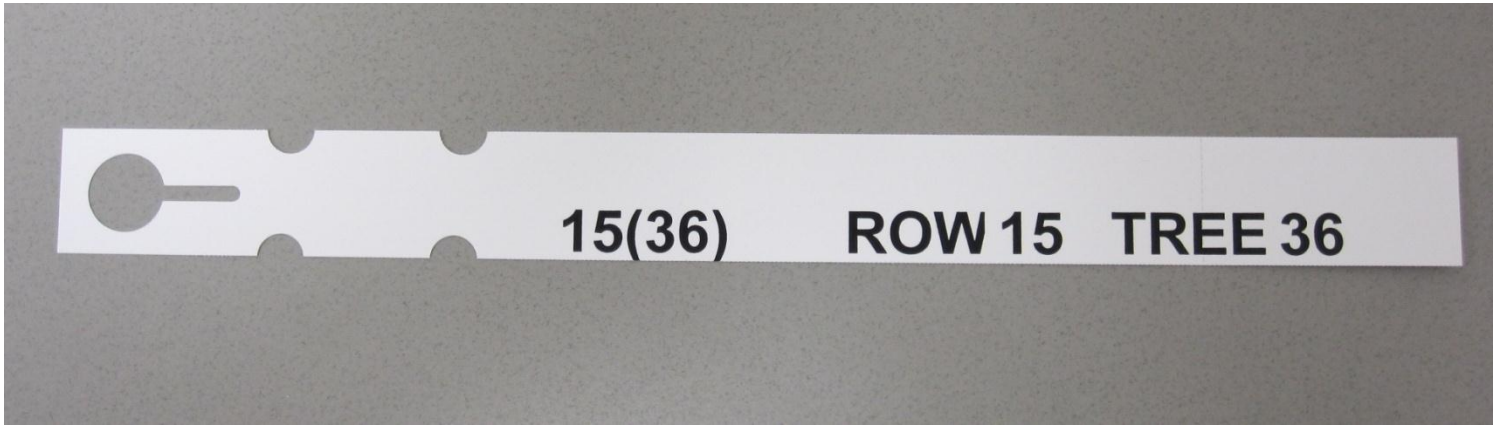
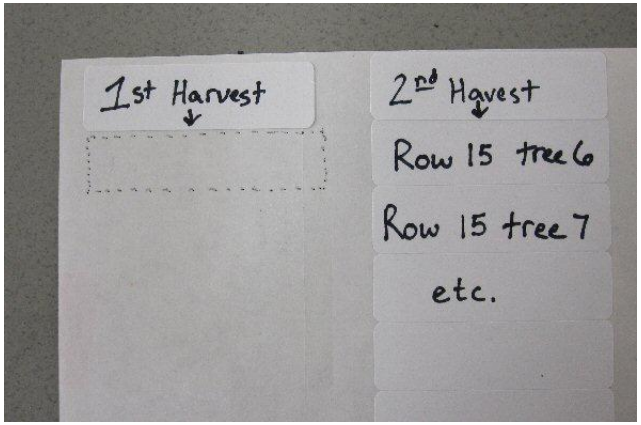
# Tree Labels

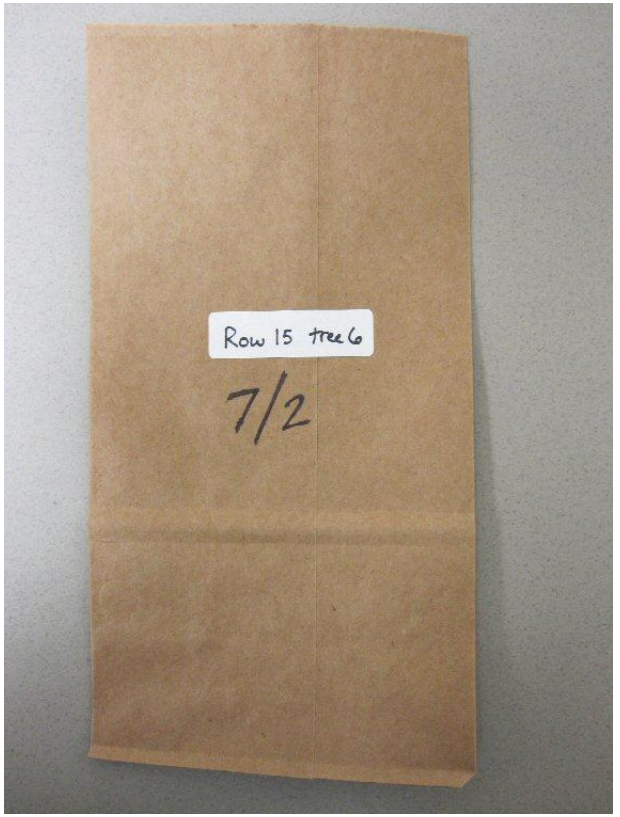
- It is important that trees be individually labeled prior to field season for easy identification and to reduce mistakes



# Bag Labels for Fruit Collection

- Pre-written/printed labels can be used to quickly label bags when collecting fruit. This also minimizes writing errors in the field. If two harvests are done, two labels can be printed.



# Bloom

- Bloom date is taken at the same time every day when approximately 50% of the flowers are open, and again when 100% of the flowers are open (full bloom).
- Bloom date will then be converted into Growing Degree Days (GDD) with accumulation beginning on Jan 1$^{st}$ and calculated using simple averages and a base of 4.4°C.

# Maturity Date

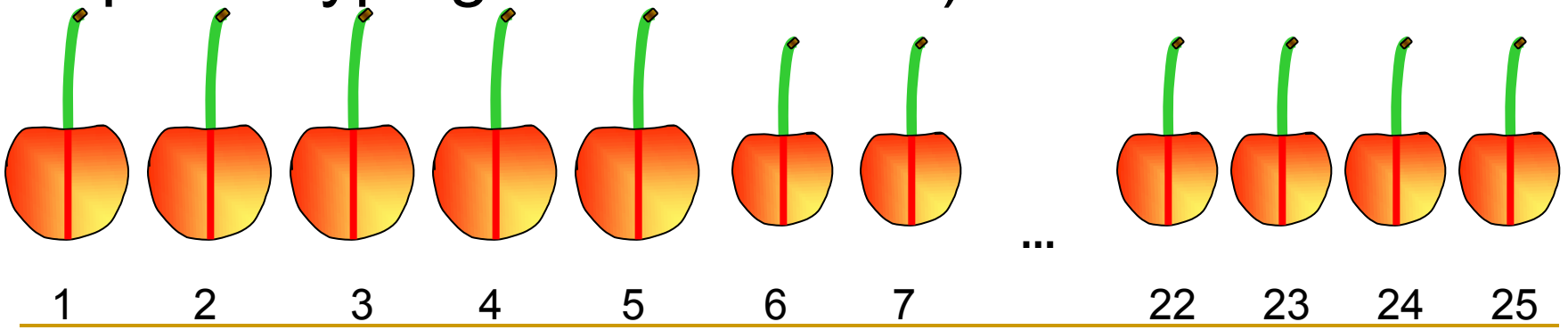
- Maturity date will be the recorded date when fruit are first harvested.
- Usually deemed mature when pull force reaches ~300g

# Fruit Collection Procedure for Fruit and Pit Phenotyping

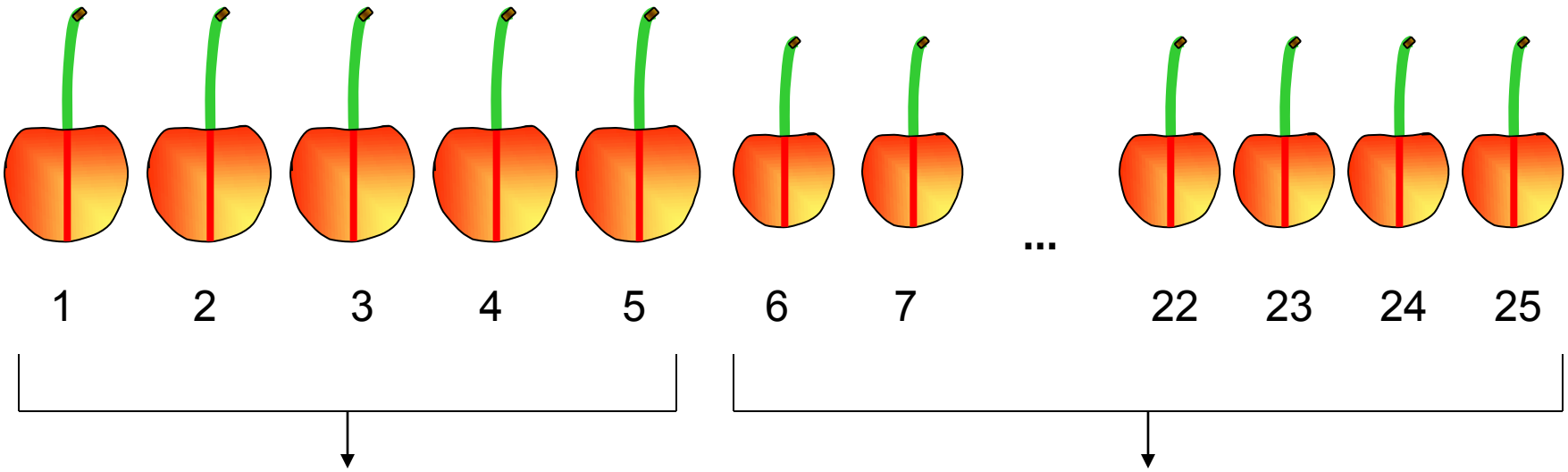
- Collection of fruit twice per season, first when fruit deemed mature (pull force ~300) and again 3-5 days later.
- Approximately **30** fruit harvested (**WITH** stems) each harvest date for fruit phenotyping, focusing on the larger ripe fruit (to realize genetic potential), plus an **ADDITIONAL 10** fruit without stems for titratable acidity.
- The 30 fruit with stems should be put in a paper bag labeled with the genotype and harvest date to allow fruit to “breathe” and dry out if there is excess moisture from dew/rain
- The additional 10 stemless fruit can be put in a plastic bag labeled with the date and genotype, and can be frozen and analyzed later (see Titratable Acidity)

# Fruit Phenotyping

- Of the 30 fruit (WITH stems), discard any fruit with blemishes or defects, saving 25 fruit for further analysis
- The 5 largest fruit should be singled out (those will be used for the majority of the phenotyping after firmness)



# Fruit Fate Flow Chart

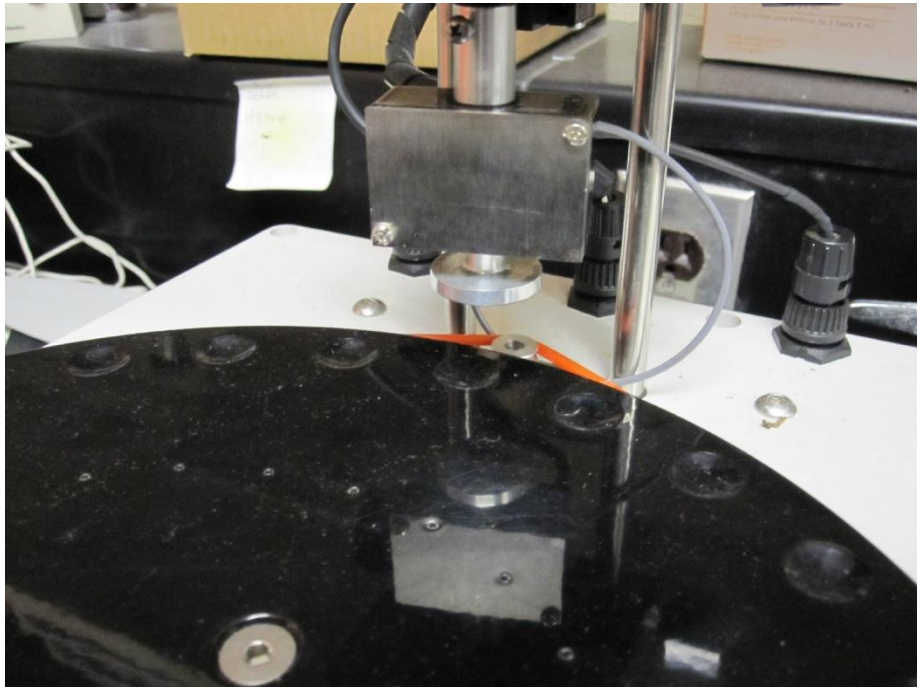



- Fruit Firmness
- 12 additional Phenotypic Traits

- Fruit Firmness
- Bulk Fruit Weight
- Discard

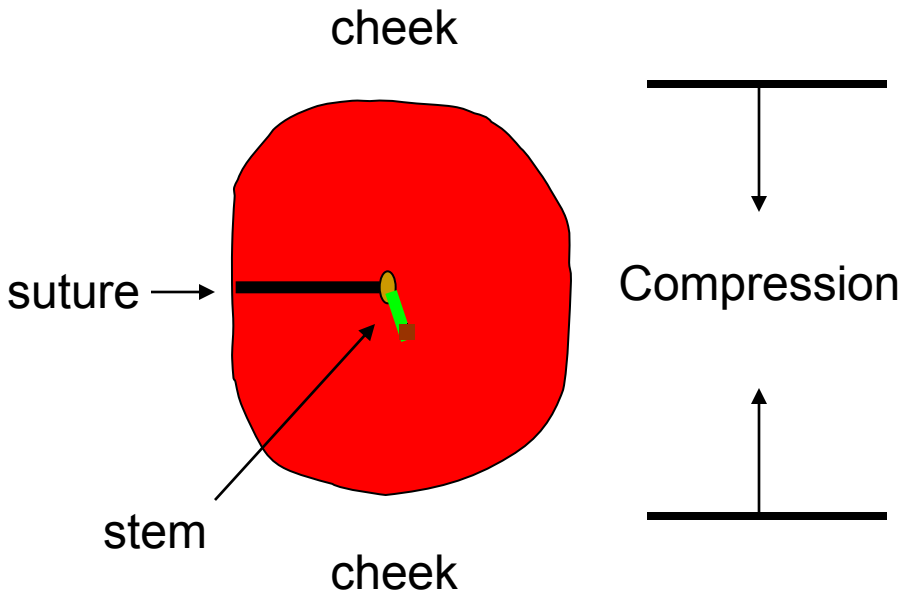
# Firmness

- Use a Firmtech 2 firmness tester
- Data collected on 25 fruit, keeping first 5 fruit in order
- Fruit should contain stems
- Place stems inward, and compression should happen from cheek to cheek (see next slide)



# Firmness continued

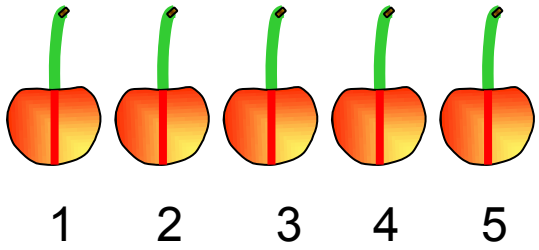
- Compression should take place from cheek to cheek on the firmtech 2



# Bulk Fruit Weight (grams)

- Fruits 1-5 (your largest fruits) are set aside **in order**
- Fruits 6-25 can be weighed in bulk with the stems removed, averaging the fruit weight from the number of fruit bulked
- **DO NOT** remove the stems from the first 5 fruit, pull force will be done on those fruit

# Sample Data Sheet




- Sample Data Sheet for the First 5 Fruit

Harv date _____

Field _____ Row _____ Tree _____

SK _____ FL _____

| | Pull Force | Fruit Weight | Fruit Length | Fruit Width | SSC | FS | Pit Weight | Pit Length | Pit Width |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | |
| 2 | | | | | | | | | |
| 3 | | | | | | | | | |
| 4 | | | | | | | | | |
| 5 | | | | | | | | | |

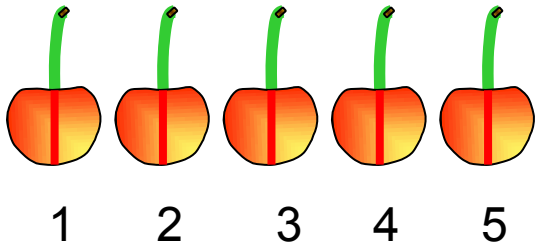
SK = Skin color

FL = Flesh color

SSC = Soluble Solids Content

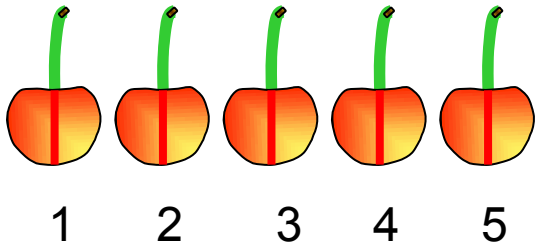
FS = Freestone

# Pull Force (grams)



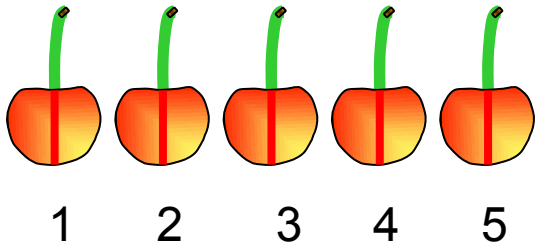

- Using a pull force meter, pull firmly and evenly on the stem until it comes loose.
- Record the pull force (grams) and reset the meter for the next fruit
- Make sure to keep the 5 fruit in order so all data can be traced back to a single fruit

# Individual Fruit Weight (g)

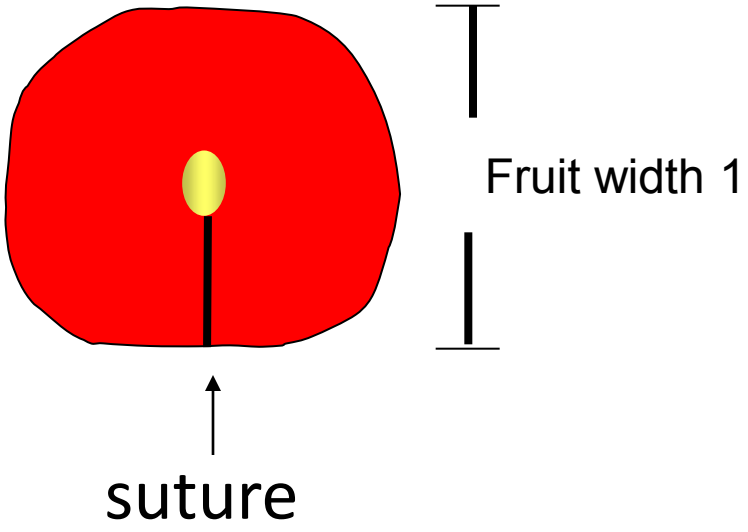



- After pull force has been recorded, weigh each fruit making sure to keep the order of the fruit separate.

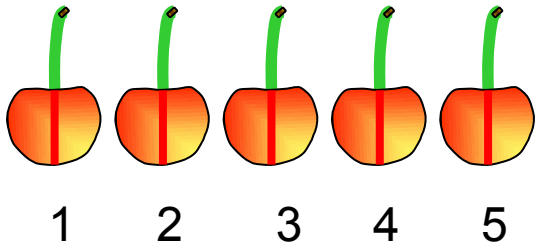
# Fruit Width 1 (mm)



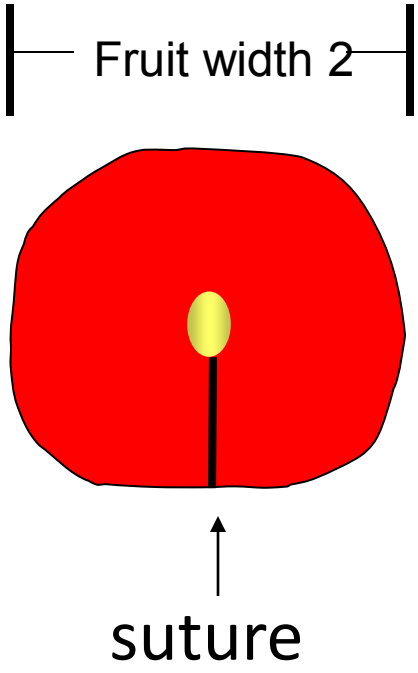

- Using a caliper, measure the diameter of each fruit from cheek to cheek



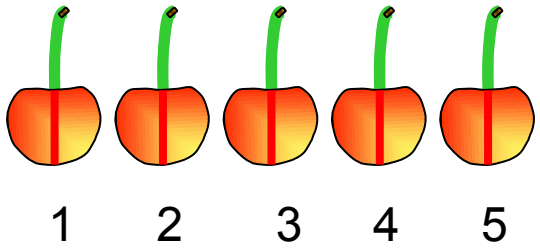
# Fruit Width 2 (mm)



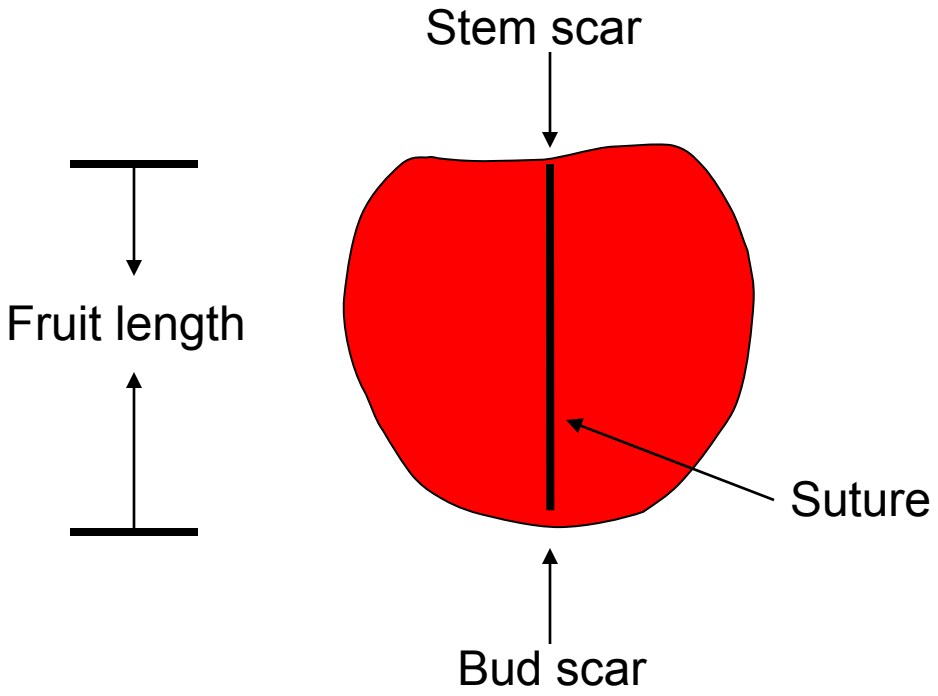

- Using a caliper, measure the diameter of each fruit from cheek to cheek



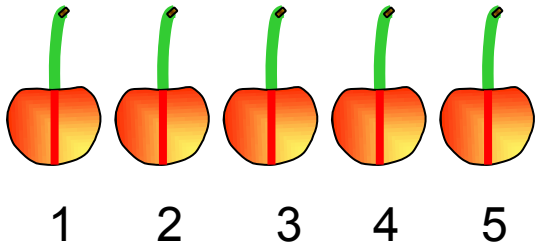
# Fruit Length (mm)



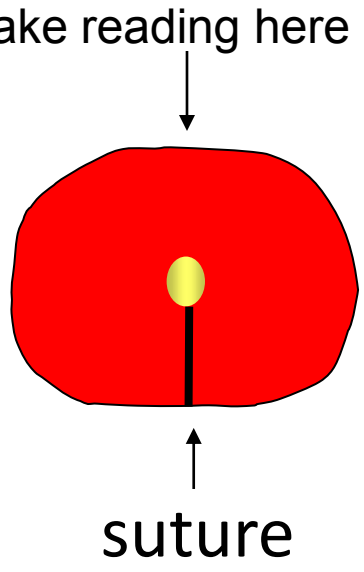

- Using a caliper, measure the fruit length of each fruit from stem scar to bud scar.



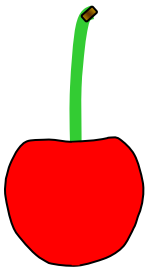
# Skin Color (L, a, b)




- Using a spectrophotometer, take a reading of the skin color of each 5 fruit giving the darkness/lightness (L*), red/green (a*), and blue/yellow (b*) color data.
- Take the reading on the side of the fruit opposite the suture.

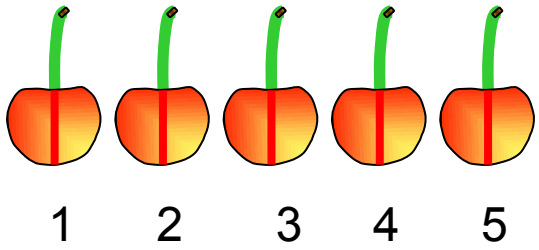


# Skin Color (visual rating)



- Using color cards, give an overall fruit color of each genotype, looking at the face opposite the suture.

# Free Stone



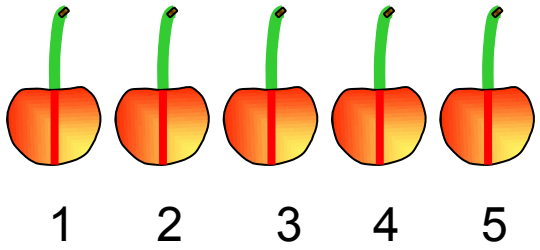

- Using a razorblade, carefully cut along the suture of the fruit all the way around and gently twist the two halves of the fruit apart
- Free stone is rated by how easily the pit comes loose of the flesh.
- Rated from 1 (Completely free of flesh) to 5 (very clingy flesh)
- KEEP the pits once they are removed for further analysis, making sure to keep the pits in order so they correspond to the fruits they were removed from!!

# Free Stone continued

Rating of 1

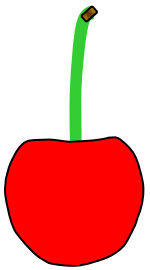
Rating of 5

# Flesh Color (L, a, b)




- Using a spectrophotometer, take a reading of the skin color of each 5 fruit giving the darkness/lightness (L*), red/green (a*), and blue/yellow (b*) color data.
- Take the reading from the flesh of the opened fruit.
- Make sure to clean off juice from spectrophotometer after each use.

# Flesh Color (visual rating)



- Using color cards, give an overall flesh color.

1

2

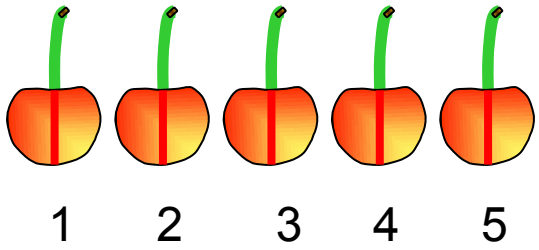
3

4

5

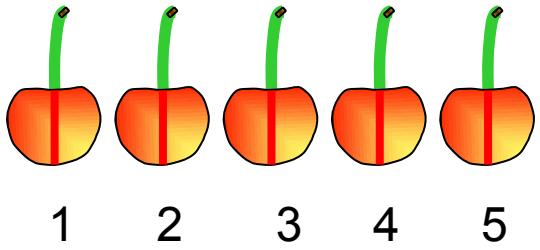
WSU flesh color index card

# Soluble Solids



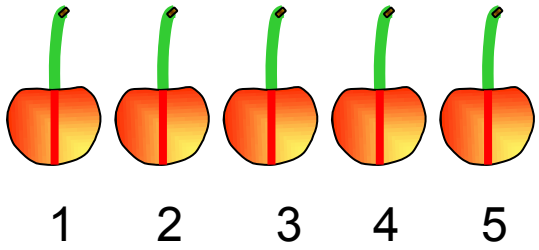

- Using a refractometer, squeeze several drops of juice onto the reader and record the soluble solids of each of the 5 fruit.
- After the reading has been taken, the fruit flesh can be discarded.

# Pit Weight (grams)



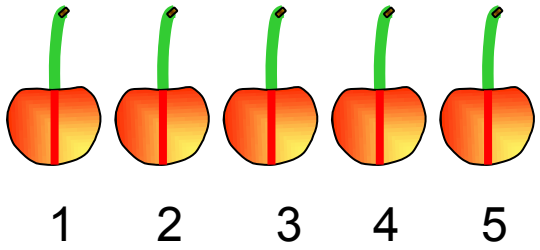

- Clean the pits of any remaining fruit flesh
- Weigh each pit, recording the data to three decimal points
- Make sure the pit values obtained correspond to the fruit the pit was taken from (important to calculate mesocarp weight and mesocarp size)

# Flesh Weight (grams)

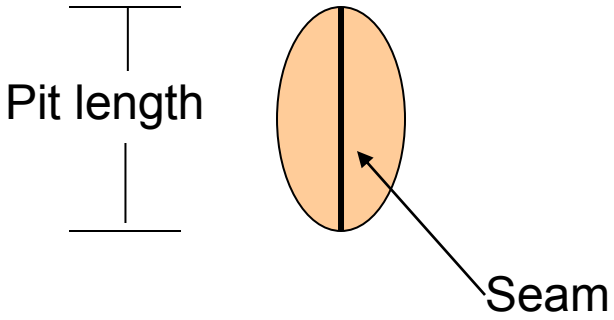



- This can be calculated for each of the 5 fruit by subtracting the pit weight from the weight of the stem free fruit.

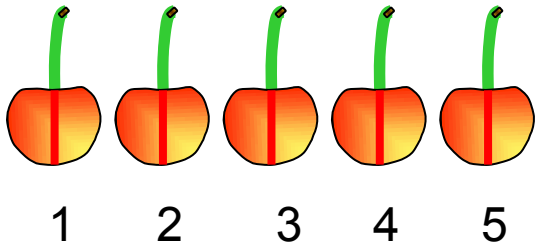
# Pit Length (mm)



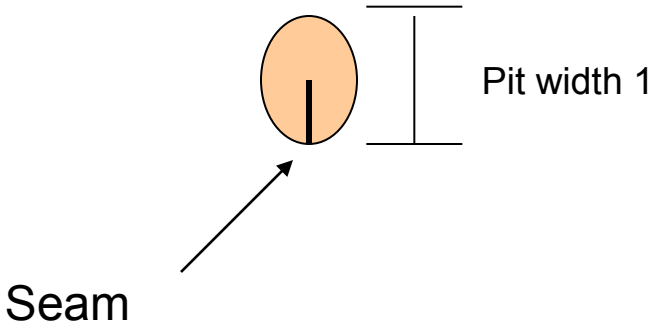

- Using a caliper, measure the pit length (along the seam of the pit, from tip, to tip)



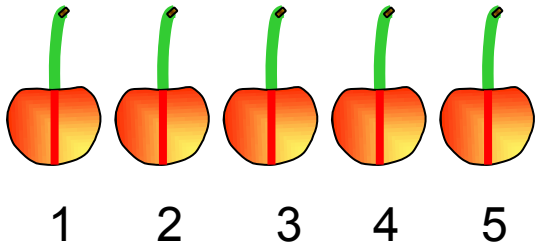
# Pit Width #1 (mm)



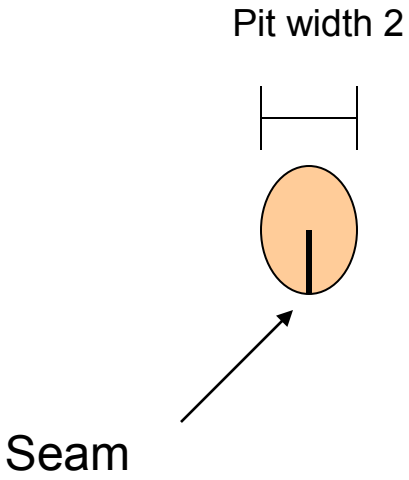

- Using a caliper, measure the pit from the seam to the back of the pit.
- Pit can then be discarded.



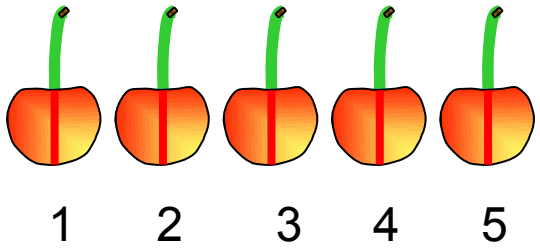
# Pit Width #2 (mm)




- Using a caliper, measure the pit perpendicular to the seam.
- Pit can then be discarded.

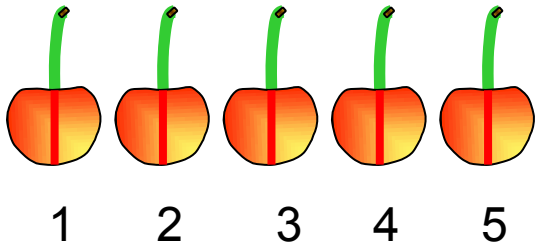


# Mesocarp Length




- Can be calculated by subtracting the pit length from the fruit length.

# Mesocarp Width




- Can be calculated by subtracting the pit width from the fruit width.

# Titratable Acidity

- The machine used to measure titratable acidity is the Schott TW alpha plus http://www.alphachemika.com/TW%20alpha%20plus.htm.

1. Place 10 frozen, pitted fruit in a potato rice masher, using a Kim wipe to strain the juice
2. Record mls of juice to be measured. Samples were titrated to pH 8.2 with 0.1 N NaOH.
3. For % Titratable Acidity, a column can be added to the excel worksheet with the following formula: [(mls NaOH*0.1*0.067)/gms]*100

| Genotype | mls of juice* | mls of NaOH | Percent Ta |
|---|---|---|---|
| 33 05 (11) | 6 | 6.42 | 0.63% |
| 33 02 (10) | 6 | 7.58 | 0.53% |
| 33 05 (17) | 6 | 8.51 | 0.47% |
| 33 02 (21) | 1 | 1.33 | 0.50% |
| 33 01 (10) | 5 | 6.76 | 0.50% |
| 33 06 (18) | 1.5 | 2.19 | 0.46% |

*Limited volume due to attempts to develop an easy method of extracting juice from the fruit.

# Cherry Leaf Spot (CLS)

- Only to be evaluated on populations where there is at least one resistant (or tolerant) parent.
- Rating will be made on a scale of 1 (totally resistant, no signs of infection) to 5 (highly susceptible, leaf covered with CLS and senescence will soon occur).

# Cherry Leaf Spot continued

← Top branch = 1

← Bottom branch = 5

# Data Quality Control

- After all of the data has been entered into excel, the following steps will be conducted to find mistakes due to human error. These steps will not necessarily guarantee a data file that is error free, but will catch the errors that may have occurred and may then be corrected.

# Data Quality Control continued

1. Each genotype in Excel will be compared to harvest date/field location sheets

Possible error: You find a genotype in your Excel file that does not exist (typing error) or you have two harvest dates for a genotype while only one of the pre-printed labels has been used, indicating that it was only harvested once. (see Bag Labels for Fruit Collection slide)

Possible outcome: data would be deleted for that genotype if mistake can not be corrected

# Data Quality Control continued

2. If data is available, skin and flesh color for each genotype will be compared to those values that were previously generated.

Possible error: Fruit was collected from the wrong tree in one of the years

Possible outcome: Data will be deleted if it does not correspond if the correct color can not be elucidated.

Ex. Last year, the skin color was rated as an 8, but this year it's a 5.

# Data Quality Control continued

3. Skin and flesh color will also be compared across harvest dates and deleted if not a match.

Possible error: Harvest dates 7/2 purple skin color, 7/6 red skin color.

Possible outcome: If still possible, fruit would be collected again, deleting the data that didn't correspond to the correct skin color.

# Data Quality Control continued

4. Each trait column will be sorted. Minimum and maximum values will be compared to what is a reasonable value for this trait. Data that is outside this range will be deleted if is not elucidated how the error occurred.

Possible error: Fruit weight entered as 1.9 grams.

Possible outcome: After looking at the hardcopy data sheets, it was discovered that the data was entered incorrectly, it should be 7.9 grams.