Phenotyping in Sweet Cherry

By Blessing Athanson
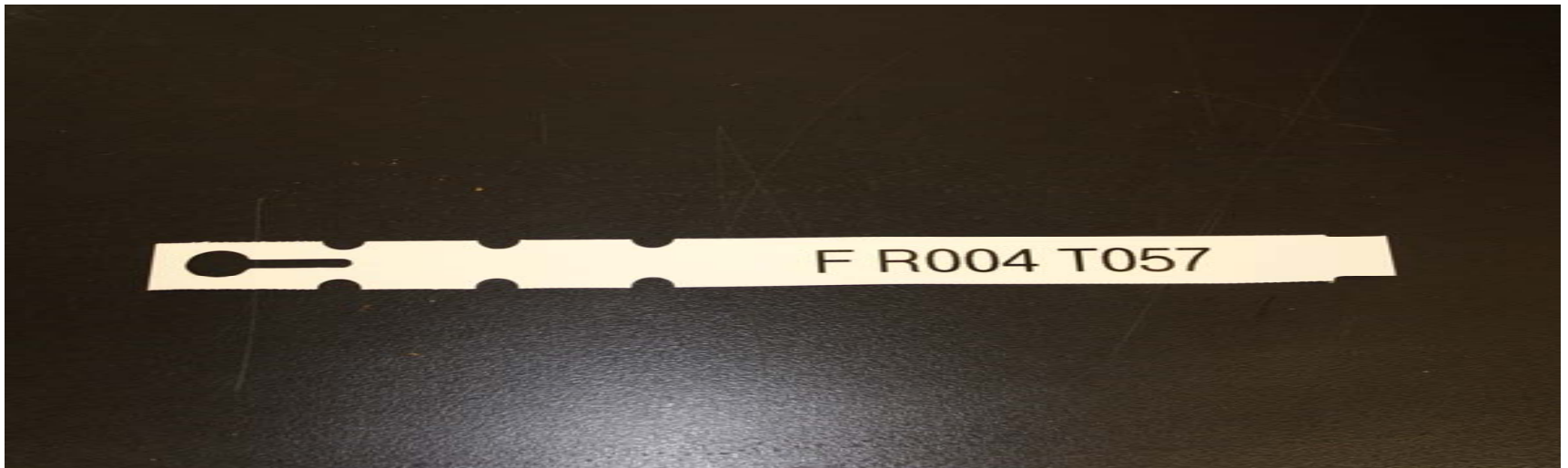**Associate in-Research**
&
Addie Dahl
Associate in-Research

Nnadozie Oraguzie – **PD**

Amy Iezzoni and Cameron Peace-**Collaborators**

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.

R OO4 TO57
7/14/10
Pick #1

R OO4 TO57
7/19/10
Pick #2
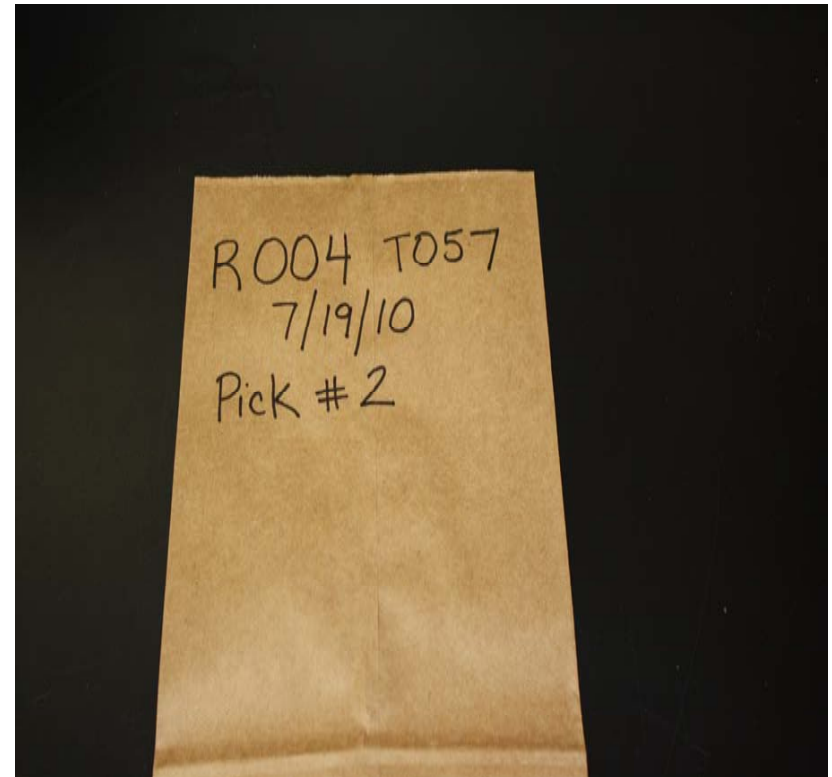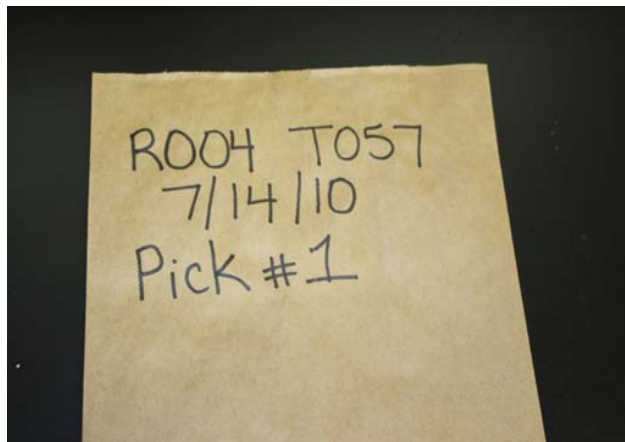
Bloom

- 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 1st 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.

- Maturity date is usually determined subjectively using a combination of skin color, taste and firmness for mahogany type cherries but this is based on a brix value of 16-17% for blushed varieties.

# Fruit Collection Procedure for Fruit and Pit Phenotyping

- Collection of fruit twice per season, first when fruit deemed mature 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)

...

1   2   3   4   5   6   7   22   23   24   25
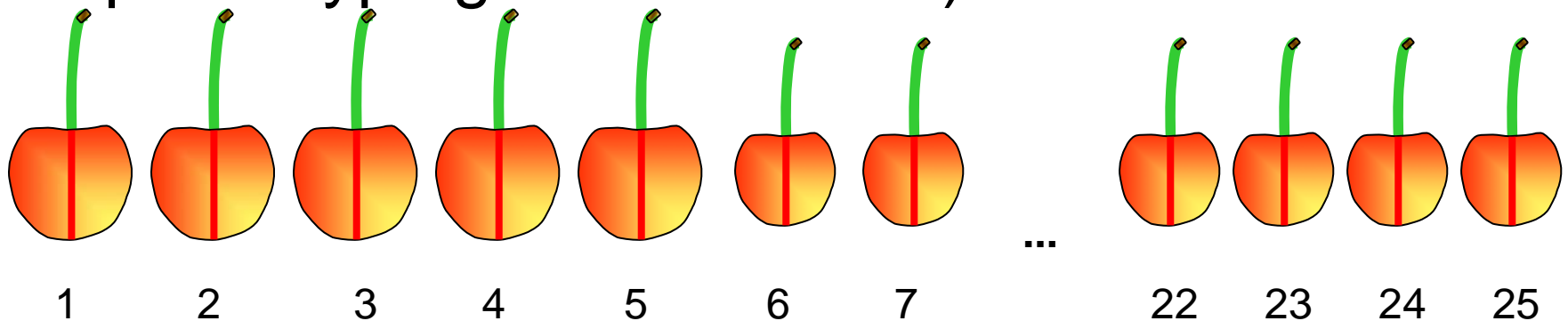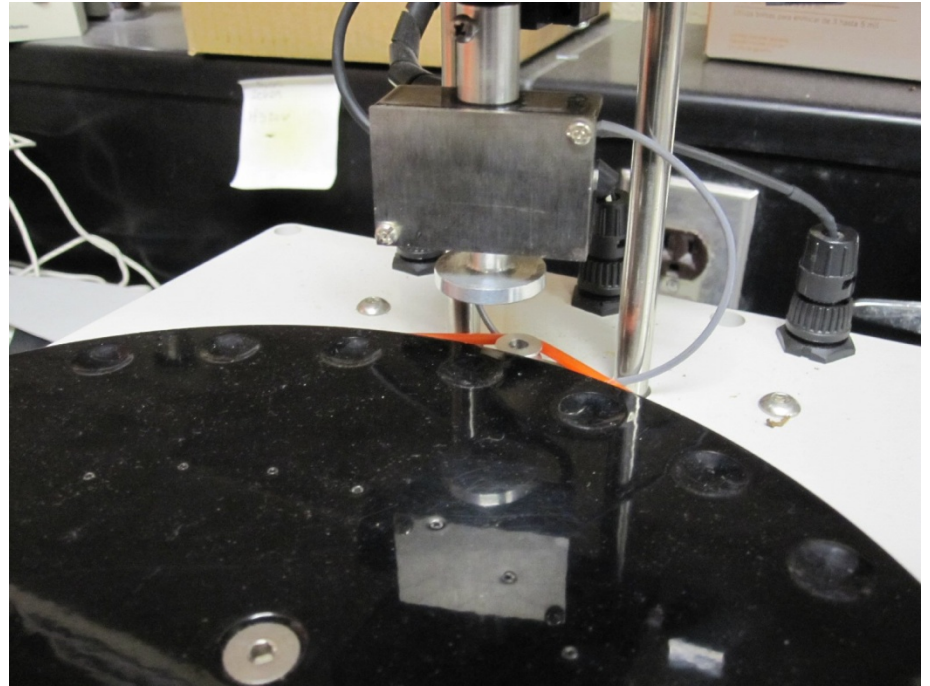
Fruit Fate Flow Chart
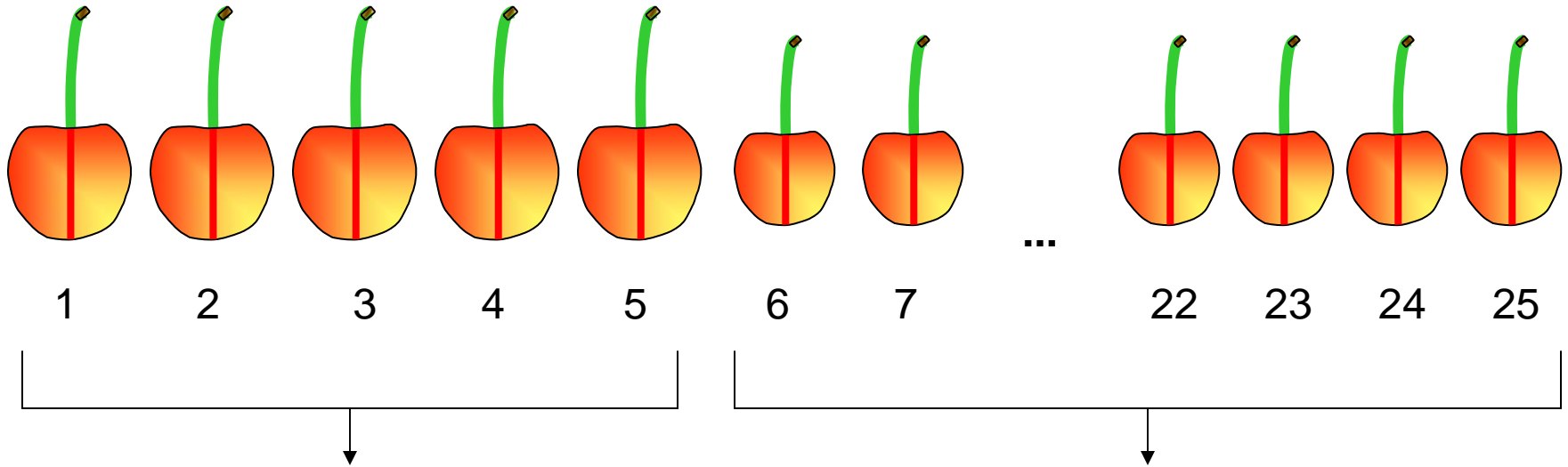
- Fruit Firmness
- 12 additional Phenotypic Traits

- Fruit Firmness
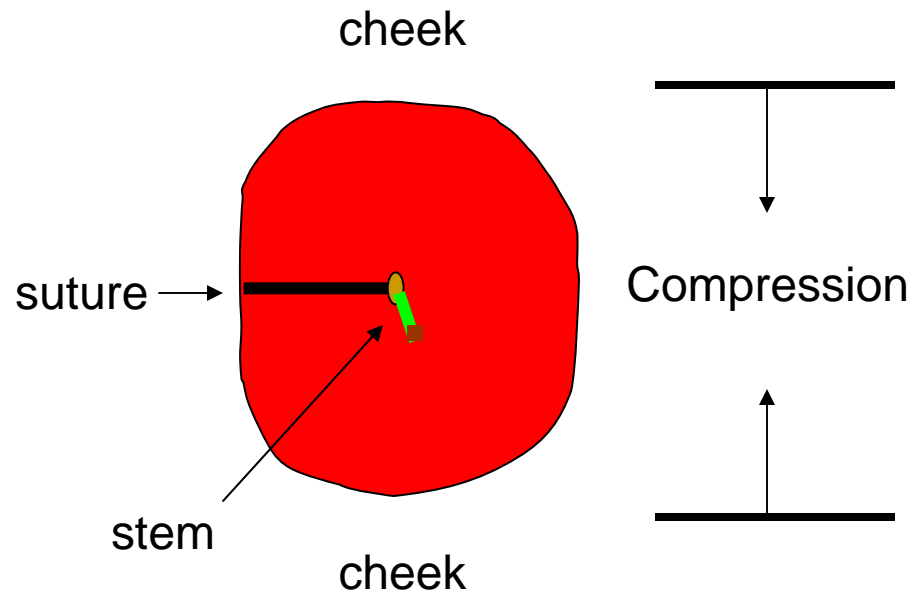- Bulk Fruit Weight
- Discard

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

cheek

suture →

stem

cheek

Compression

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

1 2 3 4 5
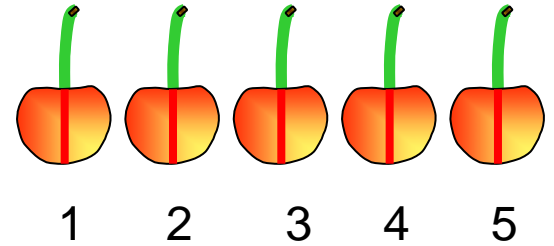
- ## Sample Data Sheet for the First 5 Fruit

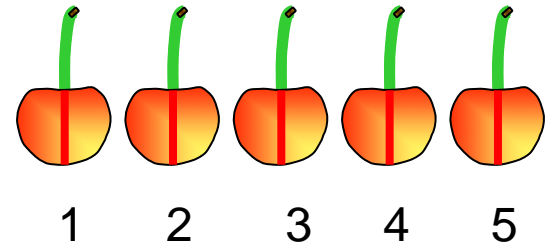| Harv date					Pull Force	Fruit Weight	Fruit Length	Fruit Width	SSC	FS	Pit Weight	Pit Length	Pit Width
				1									
				2									
Field	Row	Tree		3									
				4									
				5									
SK		FL											

SK = Skin color

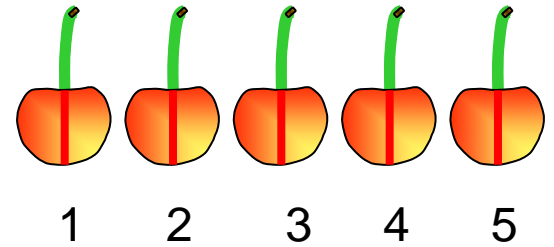FL = Flesh color

SSC = Soluble Solids Content

FS = Freestone

Pull Force (grams)

1 2 3 4 5

- 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
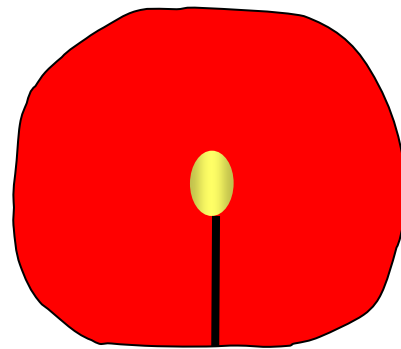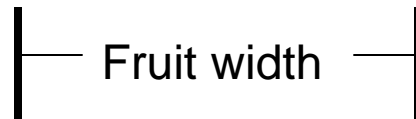
Individual Fruit Weight (g)

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

Fruit Width (mm)
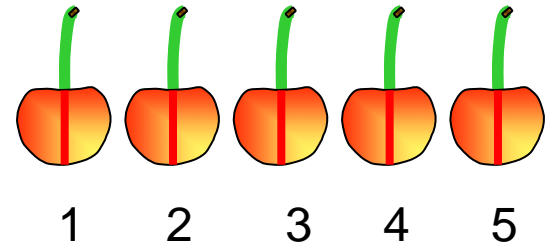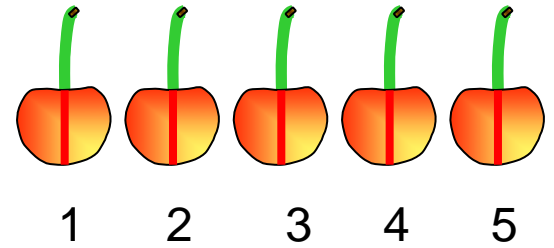
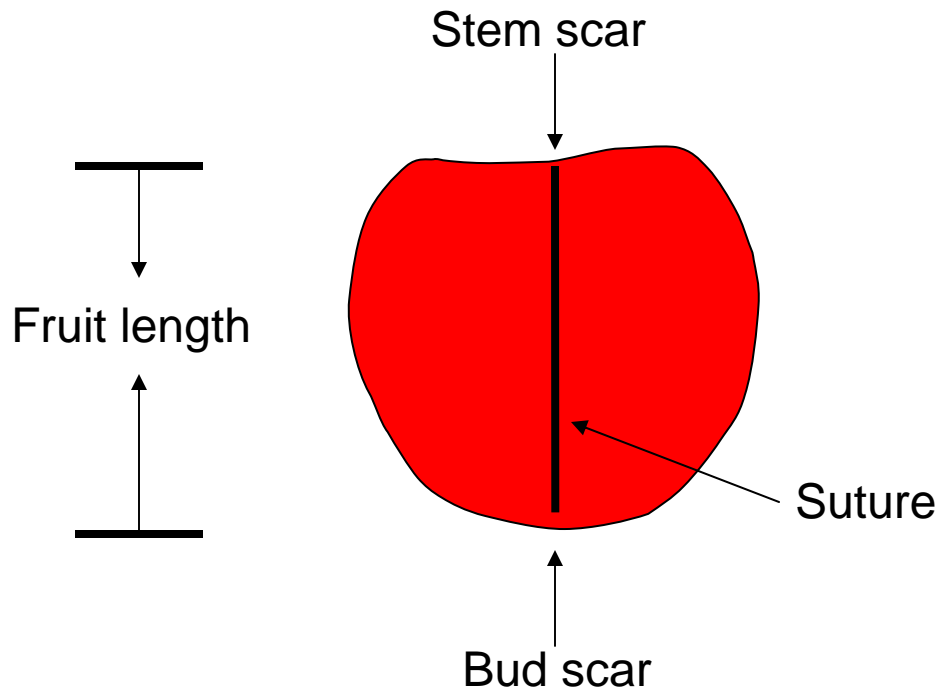- Using a caliper, measure the diameter of each fruit from cheek to cheek

Fruit width

suture

Fruit Length (mm)

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

Stem scar
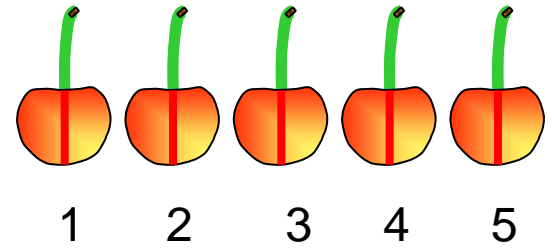
Fruit length

Suture

Bud scar

Skin Color (L, a, b)

1   2   3   4   5

- 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.

Take reading here
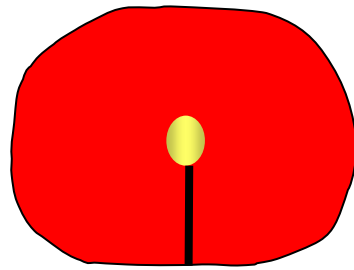
suture

Skin Color (visual rating)

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

1

2

3

4

5

6

7

8
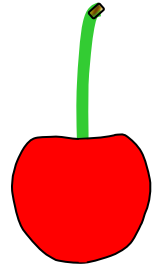
Free Stone
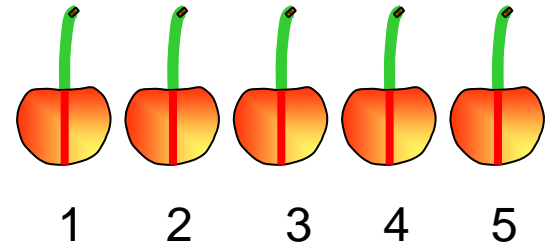
- Using a razorblade, carefully cut along the suture of the fruit all the way around and gently twist the two halves of the fruit apart

- Free stone is rated by how easily the pit comes loose of the flesh.

- Rated from 1 (very clingy) to 5 (completely free of 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 5

Rating of 1

# 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)
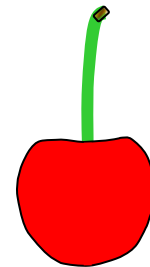
- Using color cards, give an overall flesh color.

1
2
3
4
5

WSU flesh color index card

Soluble Solids

1   2   3   4   5
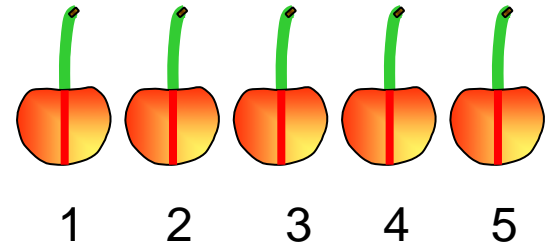
- Using a refractometer, squeeze several drops of juice onto the reader and record the soluble solids of each of the 5 fruit.

- After the reading has been taken, the fruit flesh can be discarded.

Pit Weight (grams)

1   2   3   4   5
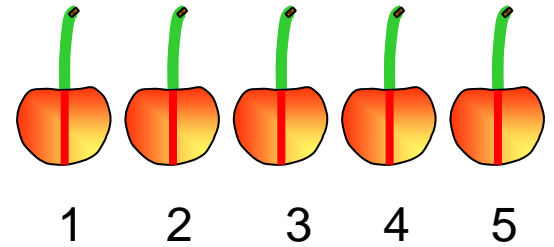
- Clean the pits of any remaining fruit flesh

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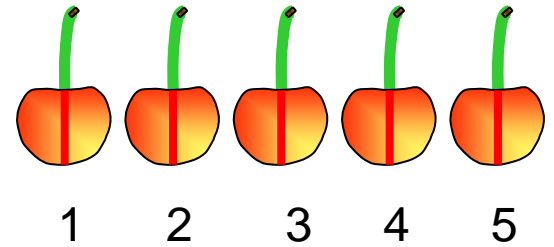
- ■ This can be calculated for each of the 5 fruit by subtracting the pit weight from the weight of the stem free fruit.

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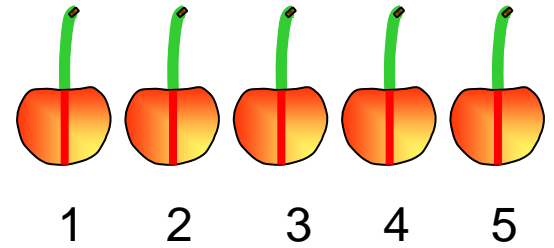
- Using a caliper, measure the pit length (along the seam of the pit, from tip, to tip)

Pit length

Seam

Pit Width (mm)

1 2 3 4 5
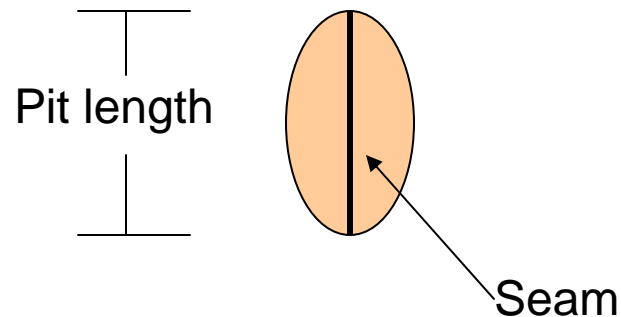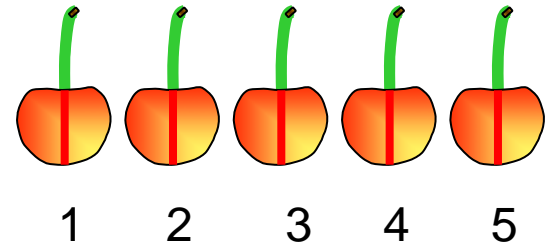
- Using a caliper, measure the pit perpendicular to the seam.

- Pit can then be discarded.

Pit width

Seam
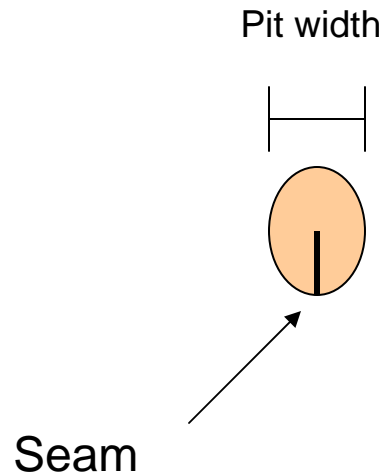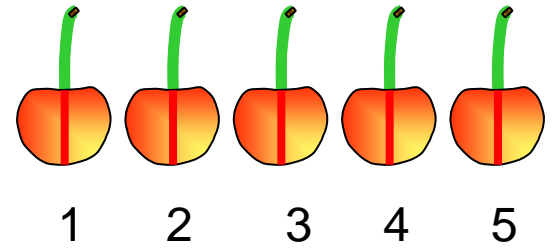
Mesocarp Length

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

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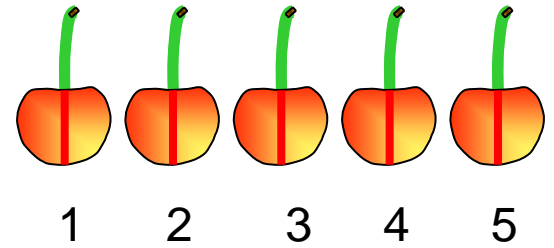
- Can be calculated by subtracting the pit width from the fruit width.

Titratable Acidity–Using the Metrohm Auto-Titrator
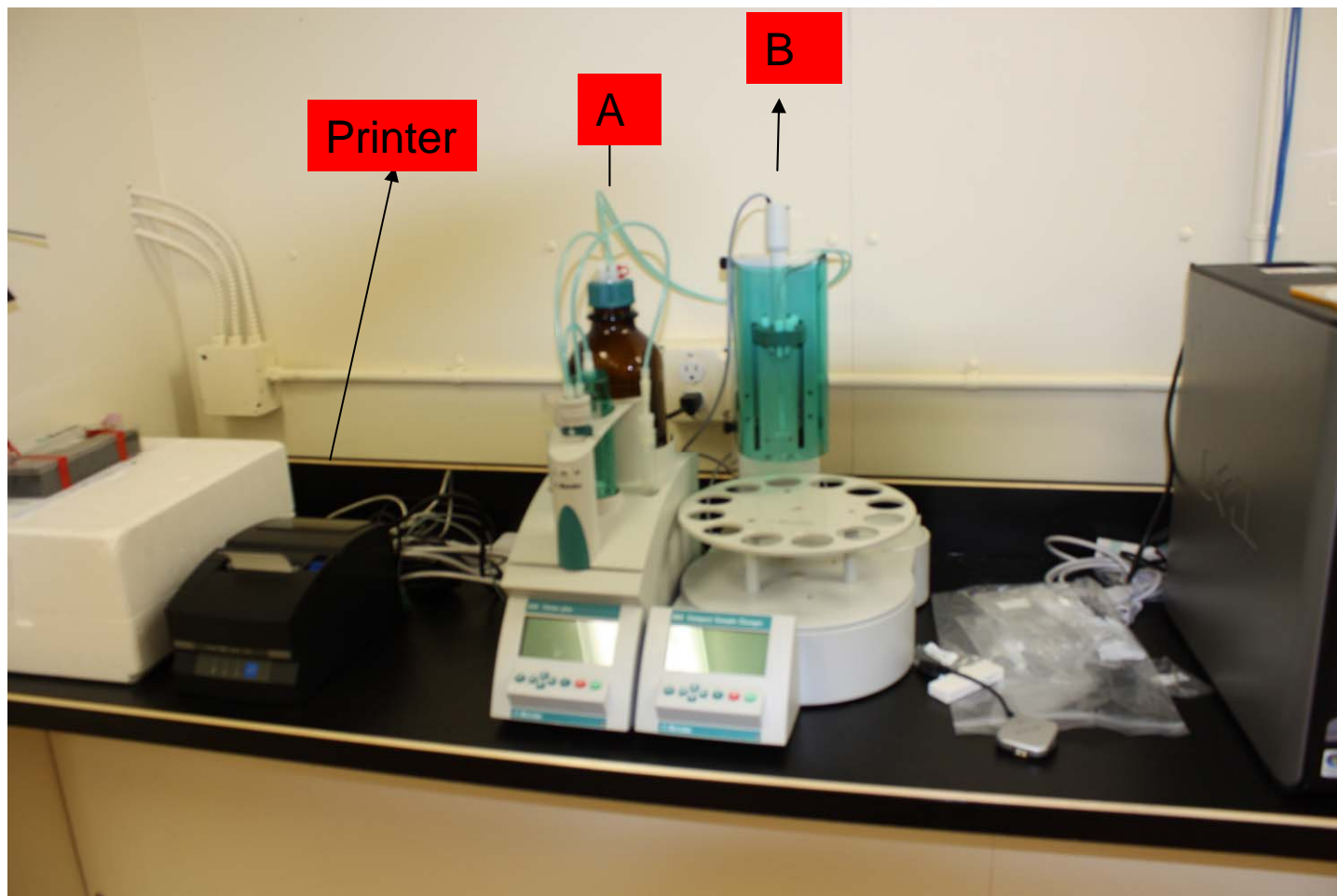
Calibrating the Auto-titrator

- Three standard solutions of pH 4, 7 and 10 (Metrohm Inc.) are used to calibrate the 848 Titrino Plus (see A in the picture).

- Load 20 ml aliquot of each of the pH standard solutions on the 869 sample changer (see B in the picture).

- On the Titrino Plus, go to **MENU**, **Sample table** and choose **Method.** Then select **CAL, PH.**

- Hit the **Start** button on the 869 sample changer and do the same on the Titrino plus.

Calibrations contd.

- Program the sample changer as follows:

  No of samples: 3 (this depends on the number of standard pH solutions).

  Next sample position: 1 (the turntable should have 12 pre-labeled positions at this stage). Set the sample changer to the position you want it to start reading from. The 12th position is normally for de-ionized water for electrode rinsing.

- Be sure to load the electrode with enough Hydrochloric acid (HCl).

- Both the 848 Titrino plus and the 869 sample changer should now be ready to read pH and %TA from experimental samples.

- % TA is calculated using the formula: [(mls NaOH*0.1*0.067)/gms]*100.

Titratable Acidity–Using the Auto-Titrator cont'd


```
&100UORAF

848 Titrino plus        04255  5.848.0012
2009-08-07 11:18:38
User name                           HCL

RESULT REPORT
Method            SET pH              TA
Determination time   2009-08-07 11:15:52
Duration                        162.4 s
Sample number                         4
ID1                          SUNSETBING
Sample size                    6.85 g

EP1    pH 8.118              9.5580 mL
       161.2 s
Regular stop
TA                             0.93 %
mLs                         9.56 mL
pH                             3.30
```

1. Place 10 (depending on the number of fruit harvested) fresh, pitted fruit in a juicer/cup (it's best to use a ceramic pestle with the cup).

2. Record volume of juice after straining with Kim wipe.

3. Juice can be stored in the freezer at -20 C until ready for use. The same juice can be used for determination of % soluble solids.

4. Sample volume is based on a 1:4 distilled water/juice ratio with the juice weight recorded.

5. A total sample volume of 50 ml including 10ml juice diluted with 40ml distilled water (weight of the 10ml juice recorded) is recommended for TA determination.

Titratable Acidity–Using the Auto-Titrator


```
&100U0RAF
848 Titrino plus          04255  5.848.0012
2009-08-07 11:13:58
User name                           HCL

RESULT REPORT
Method              SET pH              TA
Determination time  2009-08-07 11:10:08
Duration                         225.5 s
Sample number                          3
ID1                          SWEETHEART
ID2                            3202GA3
Sample size                     10.41 g

                               18.5440 mL
EP1    pH 8.112
       224.3 s
Regular stop
TA                                1.19 %
mLs                            18.54 mL
pH                                  2.97
```

6.   A total of 11 samples are loaded on the 869 sample changer connected to the 848 Titrino Plus.

7.   The 848 Titrino Plus auto-titrates the samples with 0.1N NaOH, recording the pH and %TA at the same time when the titration end-point (pH 8.2) is reached.

8.   A printer attached to the auto-titrator prints off the results which are then recorded in an excel spreadsheet.

Sweet cherry Powdery mildew (PM) infection

Foliar PM infection is scored as presence (1) or absence (0) at any time during the year in the greenhouses and in the field as from July following inoculum build up

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 7/14 purple skin color, 7/19 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.