RosBREED Strawberry Phenotyping Protocol

Recording Sites:

USDA-ARS/Oregon State University

Michigan State University

Driscoll's Strawberry Associates California

University of New Hampshire

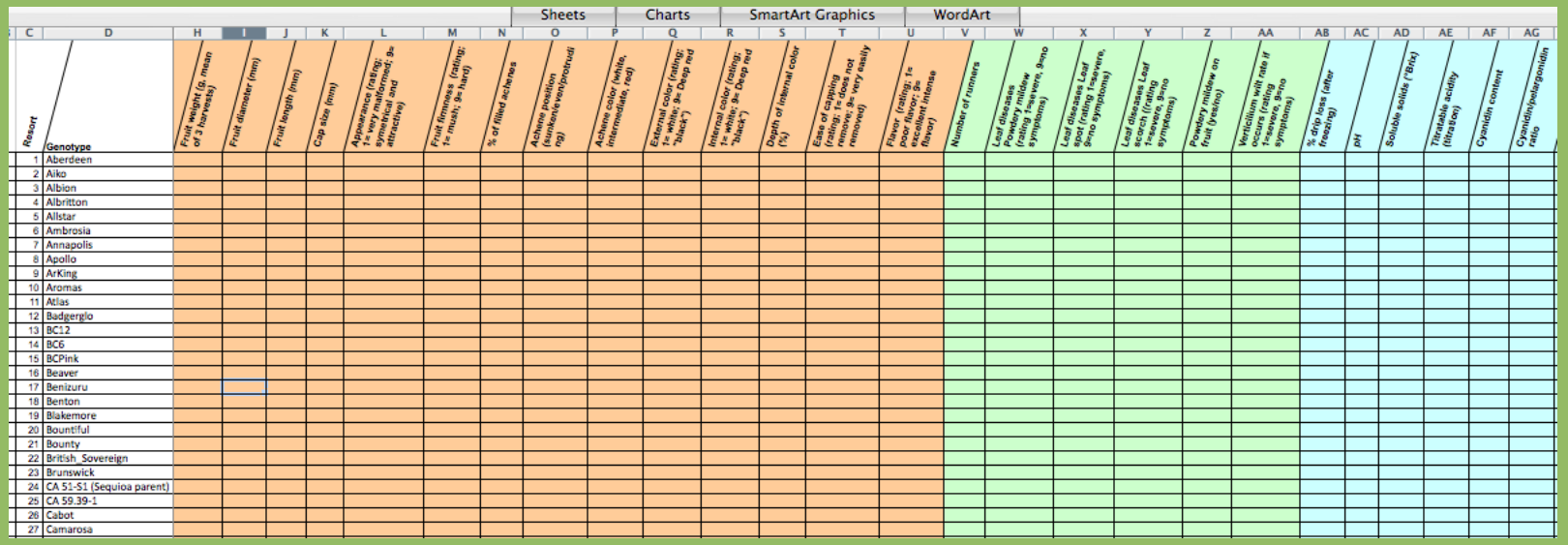
University of Florida

Data Recording

- Phenotype data should be entered in Excel in the format presented.

-This will help us remain organized and keep consistency throughout our locations!

Data Sheet



	Resort	Genotype	Fruit weight (g, mean of 3 harvests)	Fruit diameter (mm)	Fruit length (mm)	Cap size (mm)	Appearance (rating; 1= very misformed, 9= symetrical and attractive)	Fruit firmness (rating; 1= mush, 9= hard)	% of filled achenes	Achene position (sunken/even/protrudi ng)	Achene color (white, intermediate, red)	External color (rating; 1= white, 9= Deep red "black")	Internal color (rating; 1= white, 9= Deep red "black")	Depth of internal color (%)	Ease of capping (rating; 1= does not remove, 9= very easily removed)	Flavor (rating; 1= poor flavor, 9= excellent intense flavor)	Number of runners	Leaf diseases Powdery mildew (rating 1=severe, 9=no symptoms)	Leaf diseases Leaf spot (rating 1=severe, 9=no symptoms)	Leaf disease Leaf scorch (rating 1=severe, 9=no symptoms)	Powdery mildew on fruit (yes/no)	Verticilium wilt rate if occurs (rating 1=severe, 9=no symptoms)	% drip loss (after freezing)	pH	Soluble solids (°Brix)	Titratable acidity (titration)	Cyanidin content	Cyanidin/pelargonidin ratio
1		Aberdeen																										
2		Aiko																										
3		Albion																										
4		Albritton																										
5		Allstar																										
6		Ambrosia																										
7		Annapolis																										
8		Apollo																										
9		ArKing																										
10		Aromas																										
11		Atlas																										
12		Badgerglo																										
13		BC12																										
14		BC6																										
15		BCPink																										
16		Beaver																										
17		Benizuru																										
18		Benton																										
19		Blakemore																										
20		Bountiful																										
21		Bounty																										
22		British_Sovereign																										
23		Brunswick																										
24		CA 51-51 (Sequioa parent)																										
25		CA 59.39-1																										
26		Cabot																										
27		Camarosa																										

(not meant to be read)

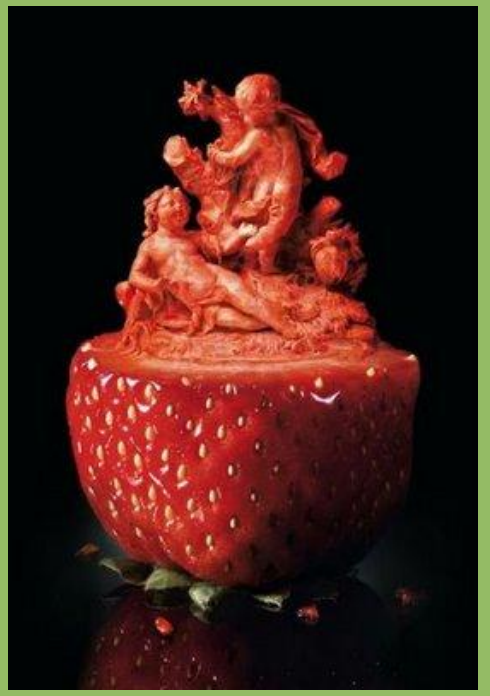
Orange = Fruit Traits

Green = Plant/Leaf Traits

Blue = Lab Procedures

Fruit Traits

- Fruit weight (g)
- Cap size
- Soluble solids (°Brix)
- Acidity (titration)
- pH
- % drip loss (after freezing and thawing)
- Appearance (rating; 1= very malformed; 9= symmetrical and attractive)
- Fruit firmness (rating; 1= mush; 9= hard)
- Skin Toughness (rating; 1=soft; 9=tough)
- % of filled achenes
- Monkey face (yes/no)



http://photojunction.blogspot.com/2008_0_01_archive.html

Fruit Traits Continued

- External color (rating; 1= white; 9= Deep red "black”)
- Gloss (rating; 1=dull; 9=shiny)
- Internal Color (rating; 1= white; 9= Deep red "black”)
- Depth of internal color (%)
- Ease of capping (rating; 1= does not remove; 9= very easily removed)
- Flavor (rating; 1= poor flavor; 9= excellent intense flavor)
- Achene position (sunken/even/protruding)
- Achene color (white, intermediate, red)
- Crop estimate (rating 1=no fruit; 9=over-cropped)
- Cyanidin content (Only NH)
- Cyanidin/pelargonidin ratio (Only NH)
- Anthocyanins total spectrophotometrically

Fruit Maturity and Harvest

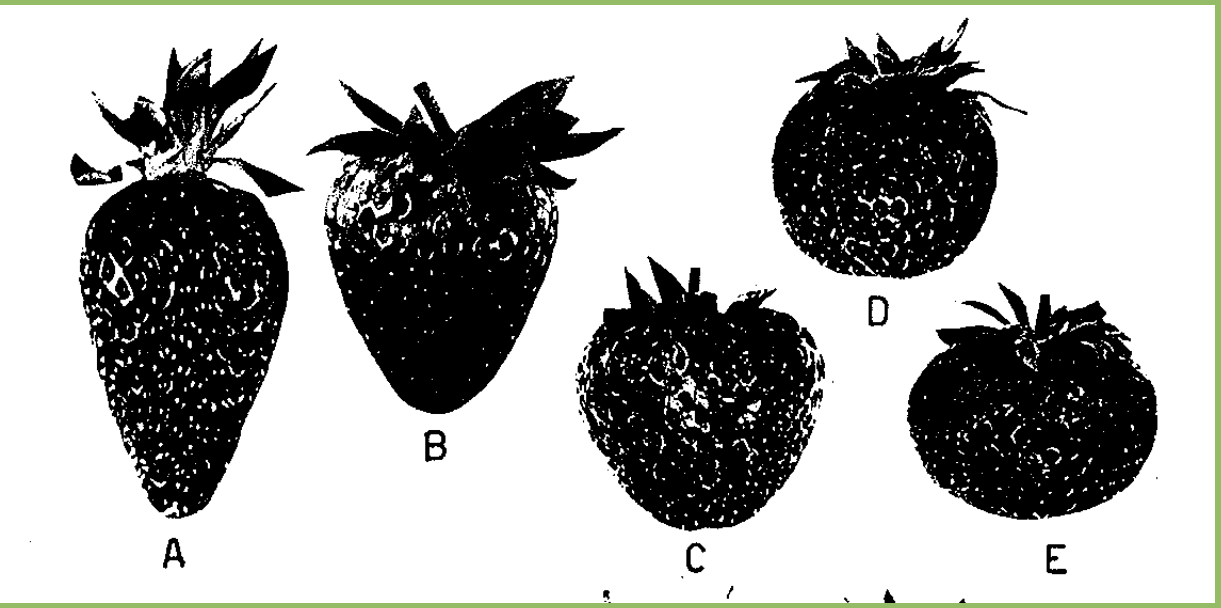
- Fruit should be harvested when fruit color (red, yellow, white) is fully developed (50% of fruit on plant is ripe) allowing evaluation of 10 primary fruit
- Record harvest date on data sheet
- Fruit should be harvested as uniform as possible (same time each day).

Fruit weight (g, mean of 1 harvests)

- 5 fruit from each genotype should be weighed and their mean recorded on the data sheet
- Fruit can be weighed back in lab to speed harvest

Fruit Shape

- Rate fruit 1-9 accordingly,
- round conic to round



9 **7** **5** **3** **1**

Neck Line

- Rating of 1-5 (1 sunken to 5 raised, ratings of 2 and 4 for fruit that is in between)

1 sunken neck

3 Flat neck

5 raised neck

Note: Pictures are more drastic than would normally be observed

Fruit Measurement (cap size)

Cap < width of fruit

Cap ~ = width of fruit

Cap > width of fruit

Appearance

(rating; 1= very malformed;
9= symmetrical and attractive)

General across bulk sample

8

8-9

Percentage of filled achenes

- Average rating of 5 fruit
- Record percentage of achenes that filled
- Example below is a “monkey face” distortion with 10-15% achenes unfilled. Please denote on data sheet “yes” if fruit distortion is monkey face.
- Fruit with perfect shape but only a few achenes should be recorded as the % of their achenes and checked “no” for not “monkey face”

http://www.omafra.gov.on.ca/IPM/english/strawbrries/insects/tarnished-plant-bug.html

Fruit Firmness

- Fruit firmness (rating; 1= mush; 9= hard)

measured by pressing on fruit. Test 1-2 fruit.

- To keep data uniform this should be performed by ideally the same person (at each site) weekly

Toughness of Skin

- Skin toughness is tested by rubbing skin of fruit with thumb. (rating; 1=soft; 9=tough). Test 1-2 fruit
- To keep data uniform this should be performed by ideally the same person (at each site) weekly

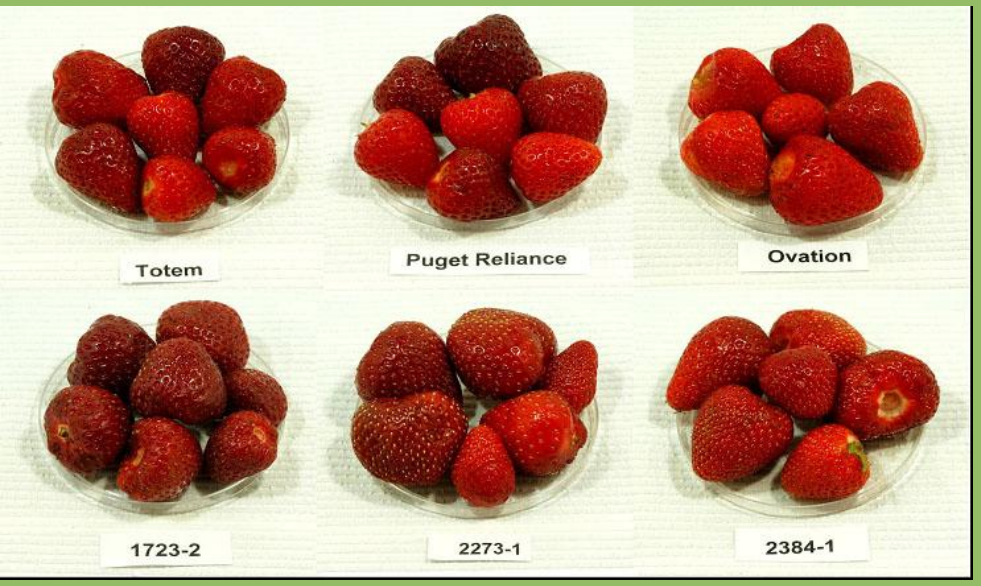
1

External Fruit Color

- External color

(rating; 1= white; 9= Deep red "black”)

8 7 6



8-9 8 7

9

Gloss

- Gloss (rating; 1=dull; 9=shiny)

Internal Fruit Color

- Internal color (rating; 1= white; 9= Deep red "black”) Would do fresh, unfortunately my only pics are after frozen and thawed. Basically a 1 on the left and a 7-8 on the right.

Depth of Internal Color

- Depth of internal color (% of red at intervals of 10) of 2 fruit, if both of these fruit appear to be different cut a third and so-on.
- 0% red to 100% red

Flavor

- If enough fruit are available.
- rating; 1= poor flavor; 9= excellent intense flavor

Ease of Capping

- rating; 1= does not remove; 9= very easily removed

Achene Position

- Select from drop down list:
- sunken/even/protruding

Achene Color

- Rate achene color on a 1-9 scale from Dark(brown-red) to Yellow

Crop estimate

- (rating 1=no fruit; 9=overcropped)


High drip loss

% drip loss (after freezing)

1.) Weigh 5-10 fruit from each genotype. Record the weight to the nearest 0.01g. AVOID LONG EXPOSURE TO THE AIR BEFORE WEIGHING (to minimize condensation).

2.) Record room temp. Transfer samples to a stainless steel mesh above a plate/bowl to catch liquid. Allow samples to sit for 3 hrs. or until they reach room temp.

3.) After thawing transfer berries back to a tarred beaker and weigh, AVOID squeezing to release more juice.


Low drip loss

http://blog.calgarypubliclibrary.com/blogs/food/archive/2009/04.aspx

Grinding Samples

After drip loss is measured, juice should be placed back with the sample and blended.

An Osterizer blender can be used to do this or samples can be crushed in Ziploc bag

pH, Titratable Acidity and Soluble Solids can be measured from this puree

pH

- pH is then measured from the blended fruit/juice with a pH meter.

Soluble solids (°Brix)

SS are measured by placing approximately 10g of puree onto the refractometer.

Acidity (titration)

- 5 grams of puree is mixed with 45 ml of CO_2 free water
- CO_2 free water is made by starting with DI water then boiled and allowed to cool or the DI can be sonicated
- Titrate with 0.1 normal NaOH

Total Anthocyanins

- Determined by measuring the change in absorbance at 2 different pH values of 1.0 and 4.5

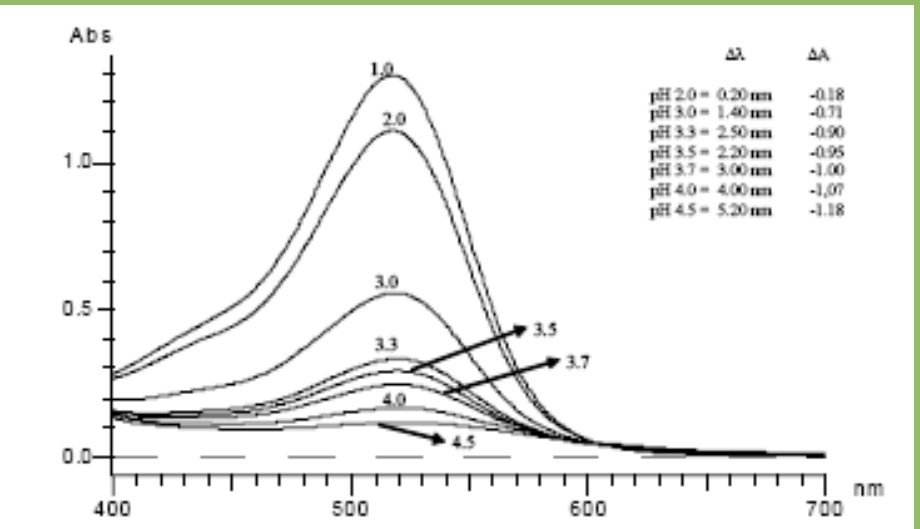


Figure 1 - Maximum absorption (Abs) wavelength of control solutions of anthocyanin Cabernet Sauvignon crude extract at different pH values (after 2 h) ($\Delta\lambda$ = wavelength changes; ΔA = absorbance values changes).

http://www.scielo.br/scielo.php?pid=S0103-90162010000100006&script=sci_arttext

Cyanidin/Pelargonidin

- Cyanidin content
- Cyanidin/pelargonidin ratio

- Will be done by NH

Plant Traits

- Period of flowering (evaluated weekly)
- Peduncle Length
- Genotype Sex
- Vigor (rating; 1=dead; 9= extremely vigorous)
- Number of runners
- Leaf diseases
  - Powdery mildew P*odosphaera aphanis* (formerly *Sphaerotheca macularis* f. sp. *fragariae*) (rating 1=severe, 9=no symptoms)
  - Leaf spot *Mycosphaerella fragariae* (Tul.) Lindau (rating 1=severe, 9=no symptoms)
  - Leaf scorch *Diplocarpon earlianum* (rating 1=severe, 9=no symptoms)
  - Leaf blight *Phomopsis obscurans* (rating 1=severe, 9=no symptoms)
  - Powdery mildew on fruit (yes/no) Can consider rating severity if occurs.
  - Verticillium wilt *Verticillium dahliae* and *V. albo-atrum* if occurs (rating 1=severe, 9=no symptoms)
  - While a fruit trait and I doubt we will see differences unless really good year for it… we should probably consider scoring grey mold/botrytis
  - Need to consult with Vance and Phil. I am guessing they will also want Colletotrichum (*Colletotrichum acutatum*; *C. gloeosporioides*)and Midwest or east might want Bacterial Angular Leaf Spot *Xanthomonas fragariae*

Period of flowering (evaluated weekly)

- Record blooming for each genotype weekly
- Record if flowering is Above canopy, below canopy or is buried (at 50% bloom)
- Record truss size (usually one of 3,5,7,9,11)
- Record fruit set
- Record peduncle length

http://www.sierrapotomac.org/W_Needham/Strawberry_050710.htm

Peduncle Length

Rate peduncle by above example of 5, 3 and 1 (allowing for the lengths in-between)

Ratings will be given by where the break is. A rating of 1 would be 90% after break, score of 3 would be 50% of and score of 5 would be 20%

Percent in 10% increments.

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Sex, Presence of Anthers

- Record for each genotype if anthers are present or not.
- Plants will be female if they have no anthers and bear fruit
- Male, if plant has anthers and no fruit
- Hermaphroditic, if plant has anthers and fruit

Vigor

(Record twice)

- (rating; 1=dead; 9= extremely vigorous)
- Record at bloom and in late summer/fall (during runner count)
- Spring vigor will determine winter hardiness
- Fall vigor will help determine virus/disease

Number of runners

- Record once in fall
- rating; 1= 0 runners; 9= hundreds of runners. (Ideal rating 4-5, consisting of 5-6 runners)

Leaf diseases

- Powdery mildew P*odosphaera aphanis* (formerly *Sphaerotheca macularis* f. sp. *fragariae*) (rating 1=severe, 9=no symptoms)
- Leaf spot *Mycosphaerella fragariae* (Tul.) Lindau (rating 1=severe, 9=no symptoms)
- Leaf scorch *Diplocarpon earlianum* (rating 1=severe, 9=no symptoms)
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Powdery mildew P*odosphaera aphanis*

Note the curled leaves and gray patches on underside of leaves. Also, powdery mildew may not show up white and the only symptom would be curdled leaves.

Powdery mildew symptoms has a grayish-white appearance on underside of leaves.

http://plant-disease.ippc.orst.edu/disease.cfm?RecordID=1049

Leaf spot *Mycosphaerella fragariae* (Tul.) Lindau

http://www.forestryimages.org/browse/detail.cfm?imgnum=1436090

http://www.nysaes.cornell.edu/pp/extension/tfabp/lspsmf.shtml

Leaf scorch *Diplocarpon earlianum*

http://www.omafra.gov.on.ca/IPM/english/strawberries/diseases-and-disorders/leaf-scorch.html

http://www.extension.umn.edu/GardenInfo/diagnostics/fruit/strawberry/leavesspots.html

Leaf blight *Phomopsis obscurans*

http://strawberryplants.org/2010/05/strawberry-plant/

http://www.weedimages.org/browse/detail.cfm?imgnum=5385825

Powdery mildew on fruit

http://www.gov.mb.ca/agriculture/crops/fruit/blb01s08.html

Verticillium wilt *Verticillium dahliae* and *V. albo-atrum*

http://strawberryplants.org/2010/05/strawberry-plant/

grey mold/botrytis

http://www.inra.fr/hyp3/pathogene/6botci5.htm

http://www.gov.mb.ca/agriculture/crops/fruit/blb01s08.html

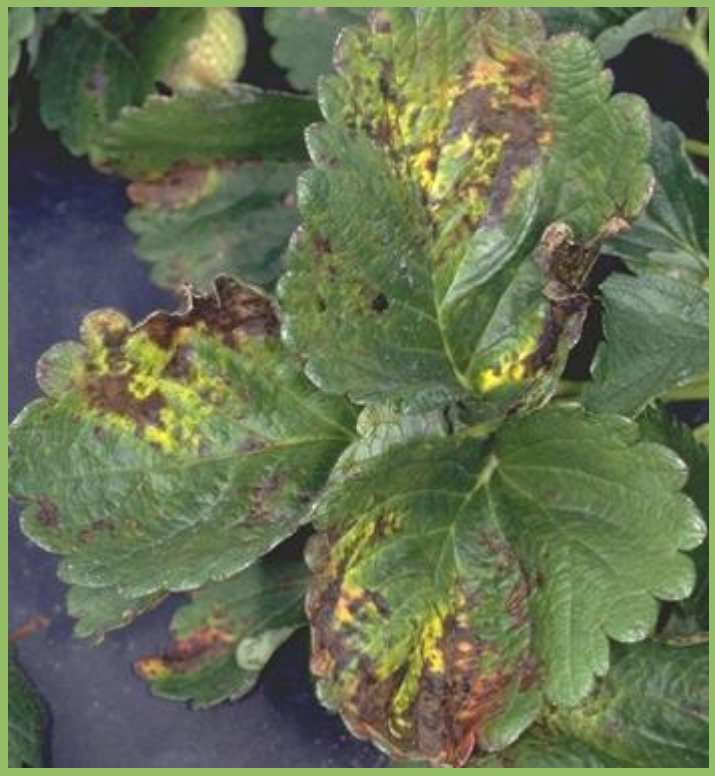
Colletotrichum acutatum; *C. gloeosporioides*

http://www.insectimages.org/browse/detail.cfm?imgnum=5407876

http://strawberry.ifas.ufl.edu/plantpathfiles/colletotcrownrot.htm

Bacterial Angular Leaf Spot
Xanthomonas fragariae

http://www.eppo.org/QUARANTINE/bacteria/Xanthomonas_fragariae/XANTFR_images.htm



http://strawberry.ifas.ufl.edu/plantpathfiles/diseasegallery6.htm

Ideal leaf size for a DNA extraction

Examples of newly emerging still folded leaves