The Biology of Pomegranates: All about Flowers, Fruit and Arils

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Pomegranate

- *Punica granatum* L.
- Cultivated since antiquity.
- Rich history in food, medicine, art, religion, culture.

Ancient Egyptian wall painting

A detail from Botticelli's *Madonna of the Pomegranate* (c.1487)

Pomegranate

- Fruit have a leathery rind
- Valued for its juicy arils.
- Marketed as whole fruit, juice, and many products.

Why the recent public interest in consumption of pomegranate products?

- Solid science-based research verifying health benefits.
- Aggressive advertising to promote public awareness and consumption.
- Interest of the business sector to develop and market new products.

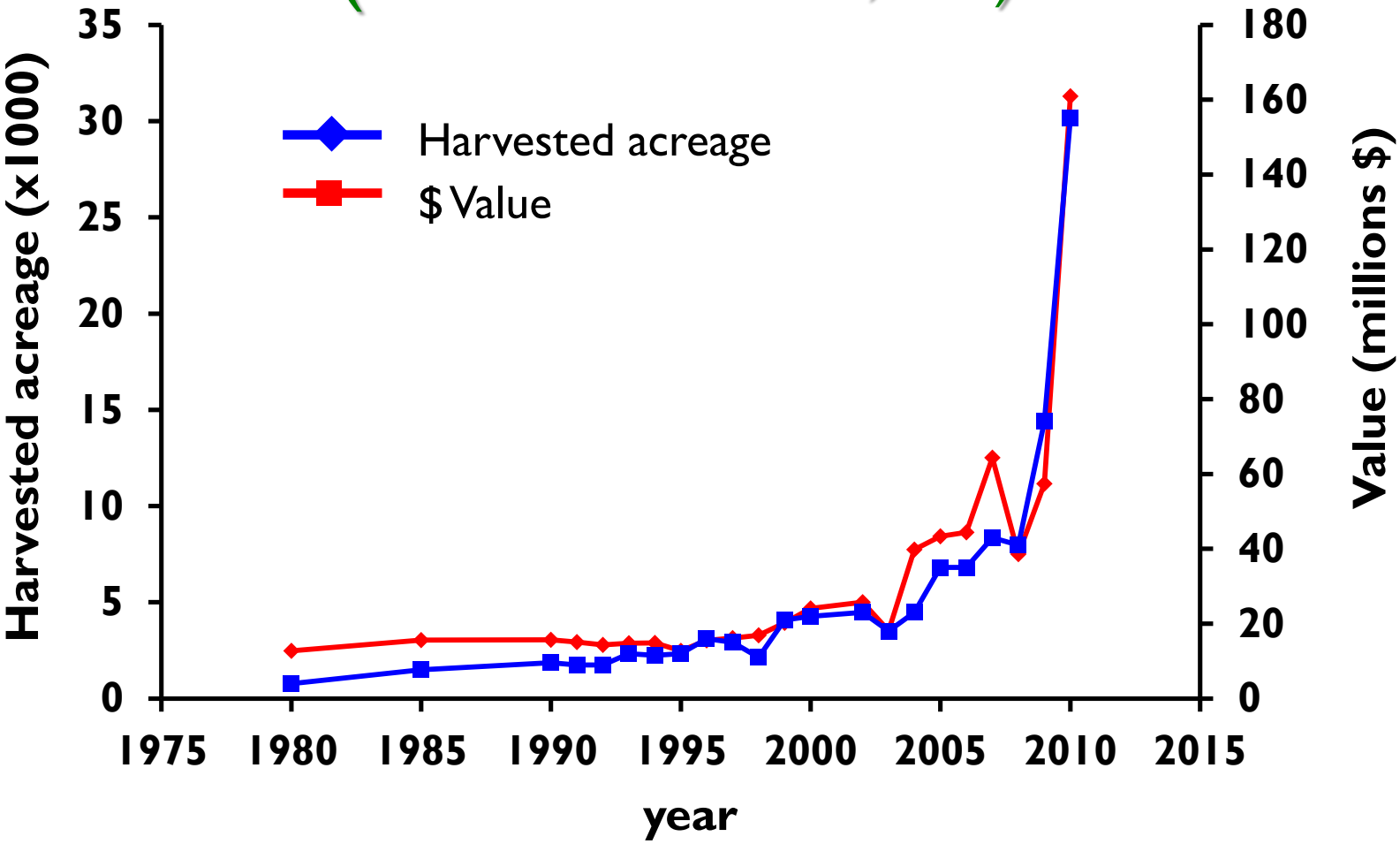
Est. world pomegranate production

Country	Planted area (ha)	Production (t)
1. Iran	65,000	600,000
2. India	54,750	500,000
3. China	Unknown	260,000
4. United States	6,070	110,000
5. Turkey	7,600	90,000
6. Spain	2,400	37,000
7. Tunisia	2,600	25,000
8. Israel	1,500	17,000
9. Other: Egypt, Morocco, Chile, Argentina, Australia	Unknown	Unknown

From: Holland and Bar-Ya'akov (2008) Chronica Horticulturae 48:12-15

Changes in pomegranate production

(Data for California, U.S.)



Historical data from CA Agric. Commission

Collaborative Research with California Growers

- Electrostatic pollination
- Fungicide effects on pollination
- Flower receptivity
- Seedless fruit
- Almond, pistachio, apple, citrus.

- Paramount Farming Co.
- Paramount Citrus Co.
- POM Wonderful

The big question with POMS:

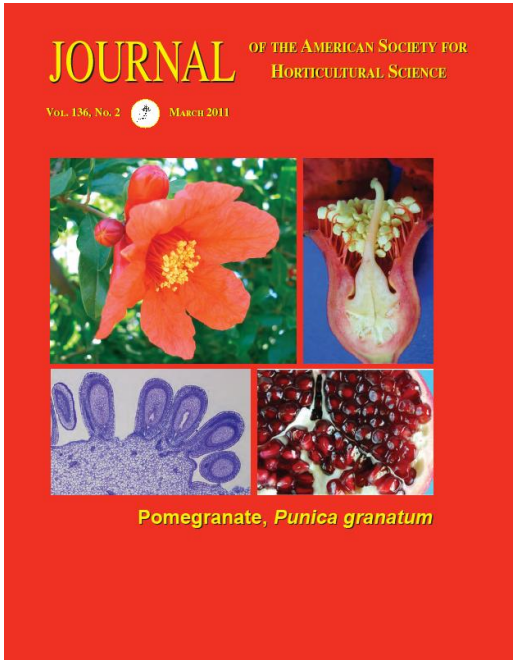
- Although cultivated since antiquity, literature on reproductive biology is limited.
- What strategies can we use to increase production and quality?
  - Larger desirable fruit
  - Higher fruitset
  - Improved fruit for processing and storage
- Important missing issues:
  - Flower development
  - Pollination
  - Fruit development
  - Postharvest

A need to characterize flowering in pomegranate.

Studies on flower morphology and histology

Wetzstein, Ravid, Wilkins, Martinelli, 2011.

J. Amer. Soc. Hort Sci. 136:83-92.



Key Questions.

- What is the timing and process of flowering, pollination and fertilization in pomegranate?

  Information on flower development and receptivity can be useful in developing production strategies.

- How do bisexual and male flowers differ in structure and function?

  Only bisexual flowers set fruit. Under production conditions, high numbers of male flowers can result in decreased yields.

Study site

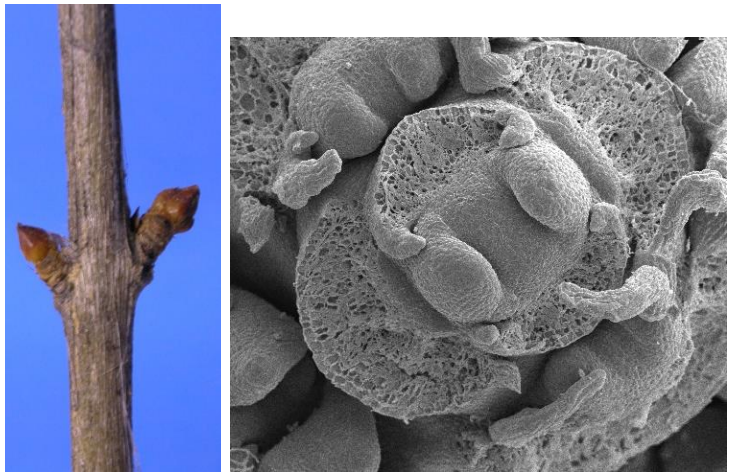
- Paramount Farming Co. orchards near Delano, CA.

Microscopy Methods

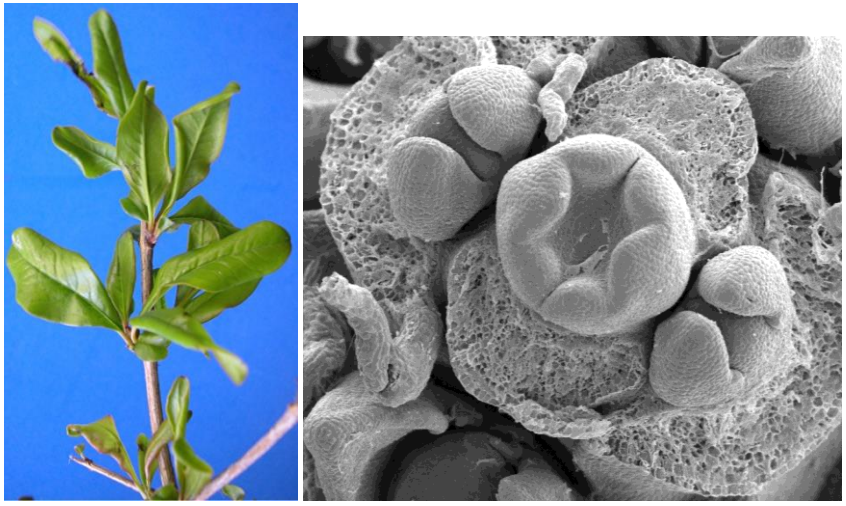
- Light Microscopy
  - Fixed in HistoChoice; embeded in JB-4 Resin.
  - Serially sectioned and stained.
- Scanning Electron Microscopy
  - Glutaraldehyde fixation, dehydation, CPD.
  - Mounted and sputter coated with gold.
- Fresh Sections
  - Using a Vibrating Microtome.
- Pollen Germination
  - In vitro germination assays

Vegetative versus Reproductive Growth

- Fruiting requires vegetative meristems to become reproductive.
- Flower initiation is associated with early spring growth.

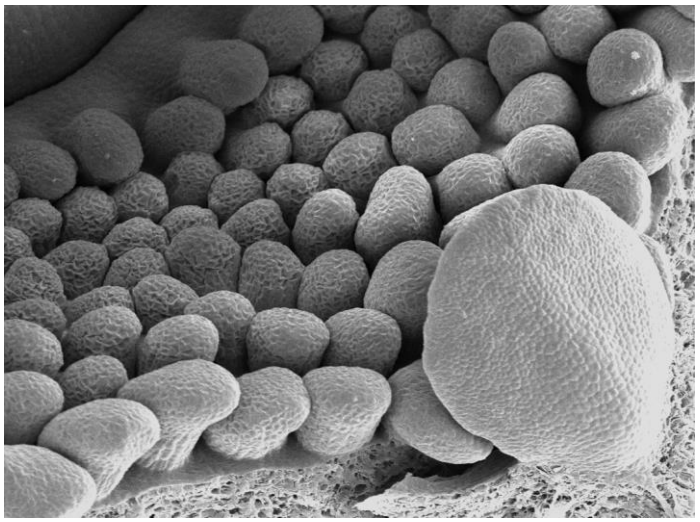


Dormant bud with vegetative apex

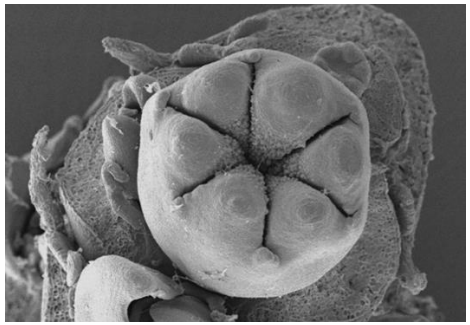


Expanding shoot with reproductive apex

Floral Organogenesis



Anther and petal primordia



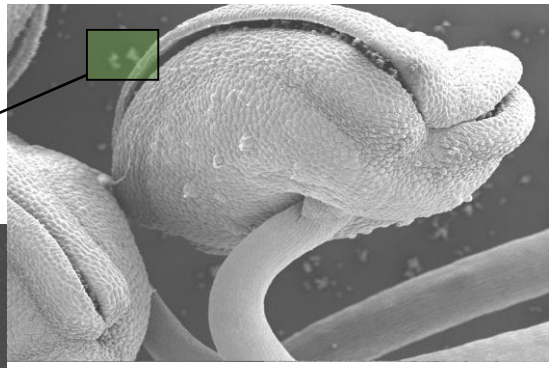
Sepals enclose apex

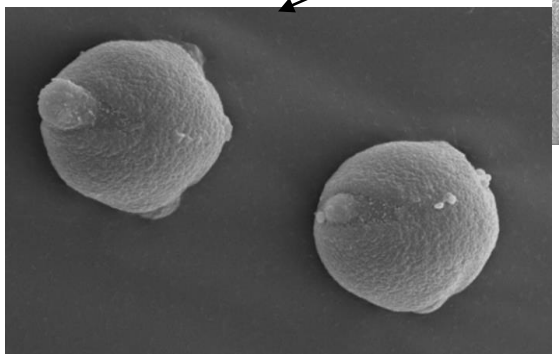
Gynoecium within

6 mm long bud

- Sepal, petal, anther, and gynoecium development.
- Well differentiated in small buds.

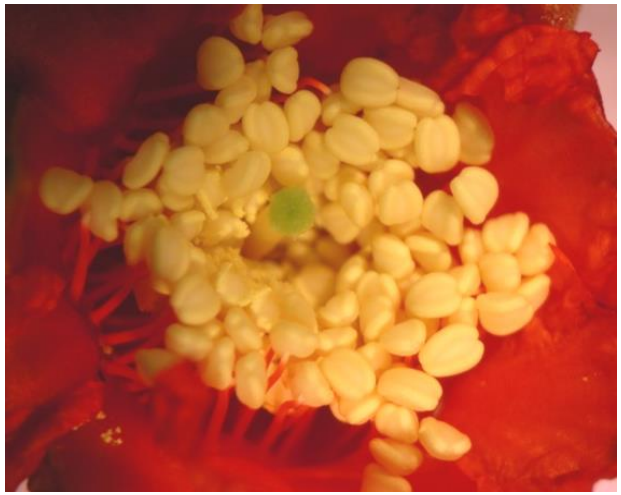
Pomegranate Flowering

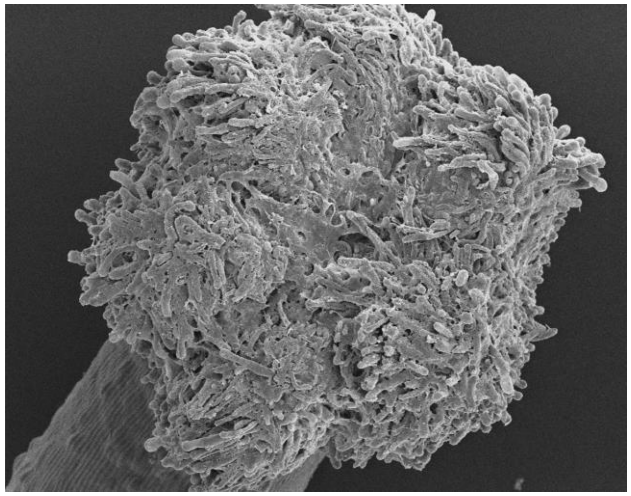


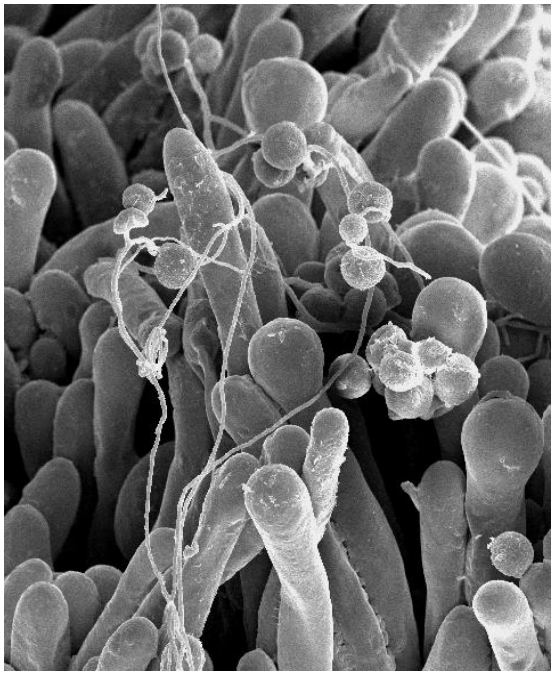


- Showy petals
- Anthers (150+ per flower)

Pollen capture and germination

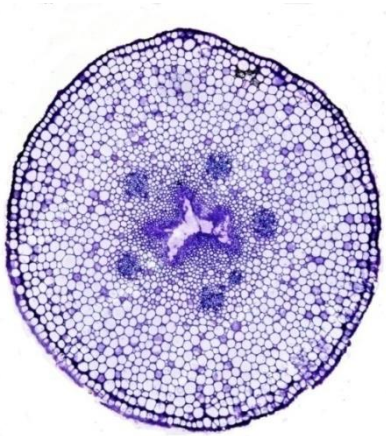


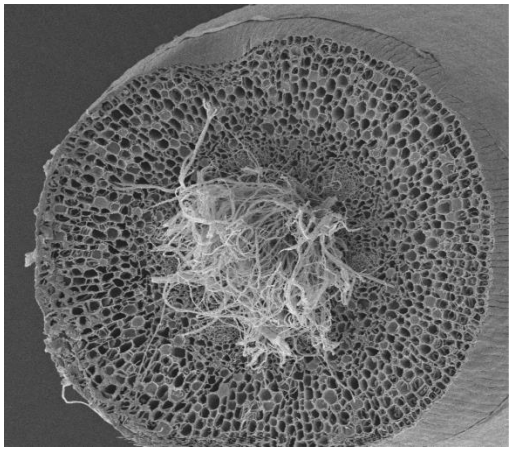


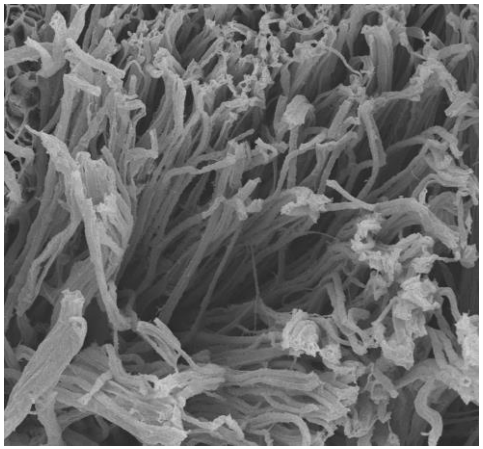


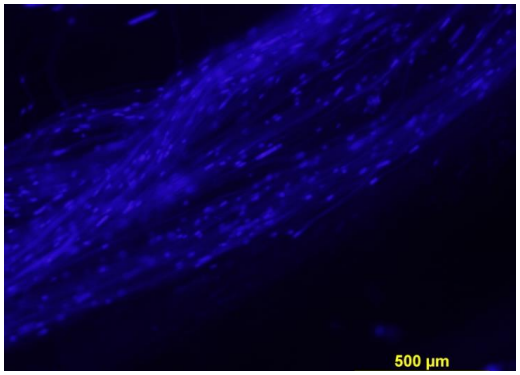
Disc-shaped stigma with elongate papillae.

Path of pollen tubes

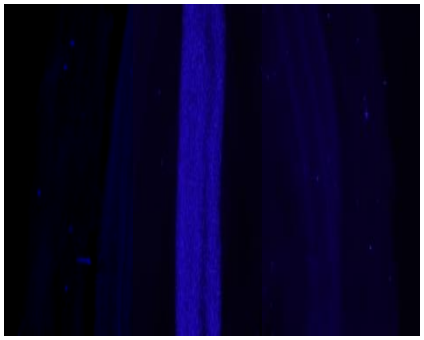








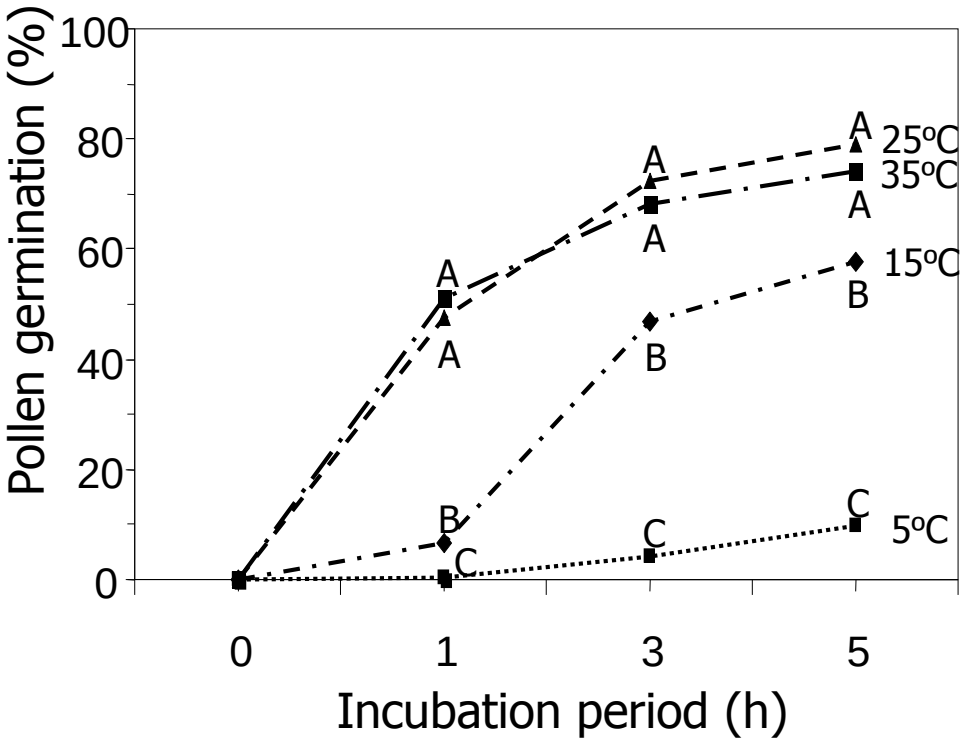
Callose plugs



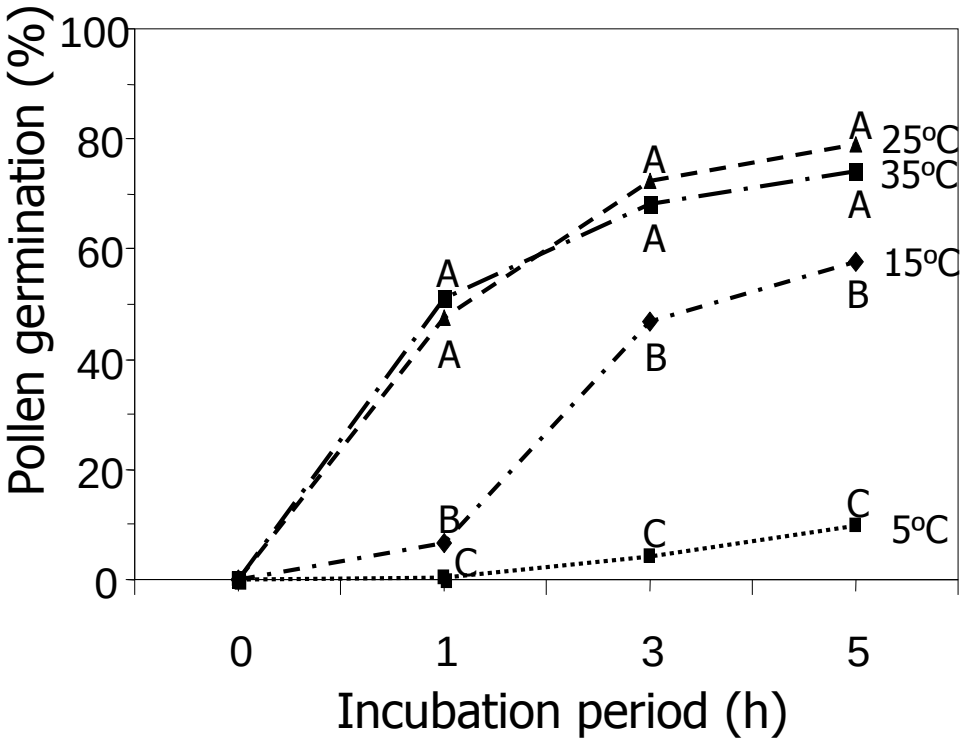
Analine blue staining
Transmitted light (top)
UV light (bottom)

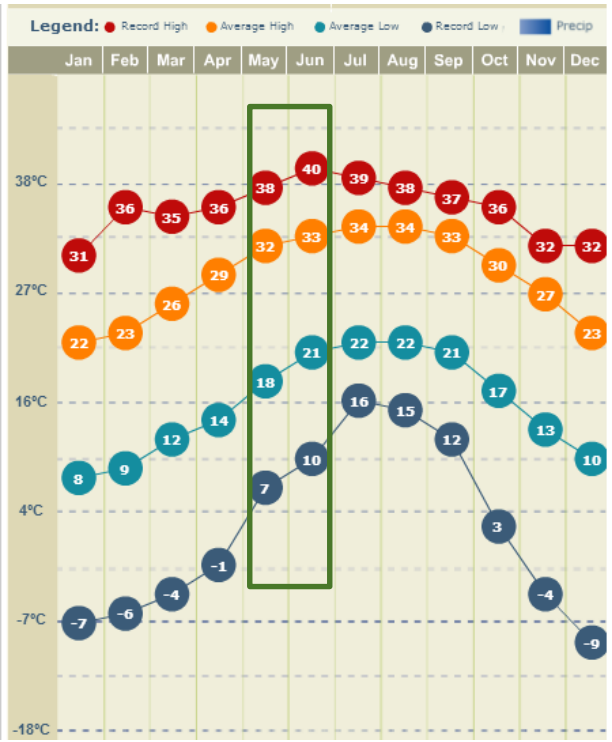
- Grow within a central stylar canal.
- Tubes reach the base of the style within 24 hr.

Pollen viability and temperature effects on germination



Pollen viability and temperature effects on germination

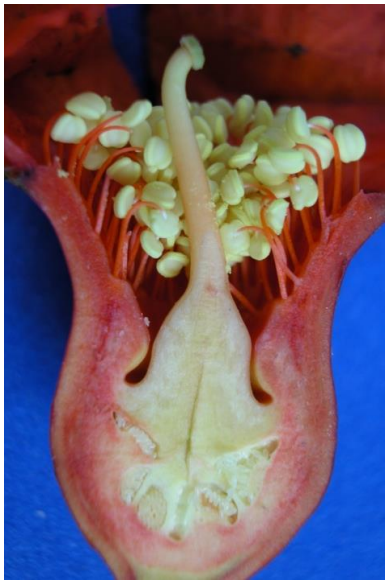


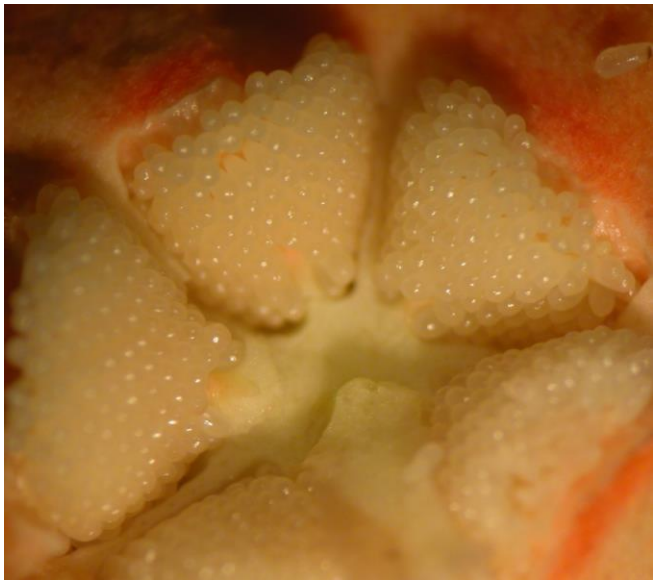


www.weather.com

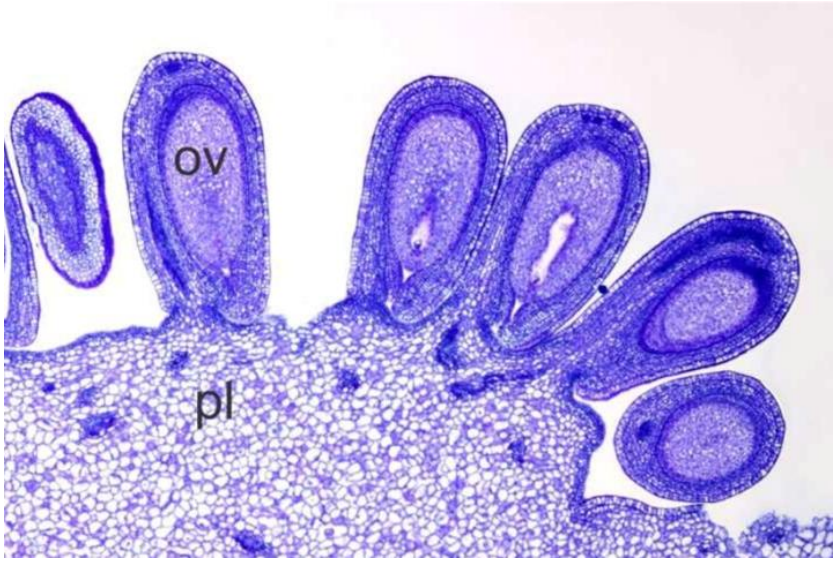
Avg Hi, Low and Record Temps
Lake Alfred, Fl

Fertilized ovules give rise to arils

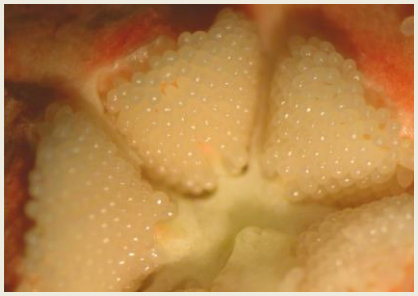


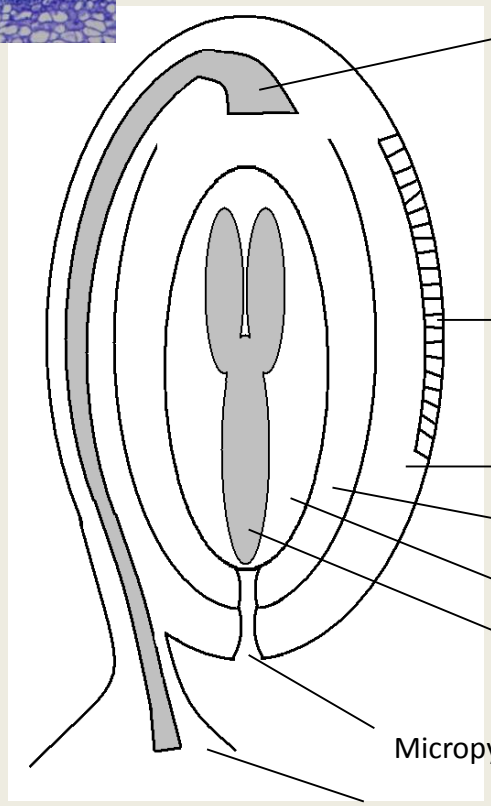


Each aril results from an independent fertilization event.



From ovule to aril

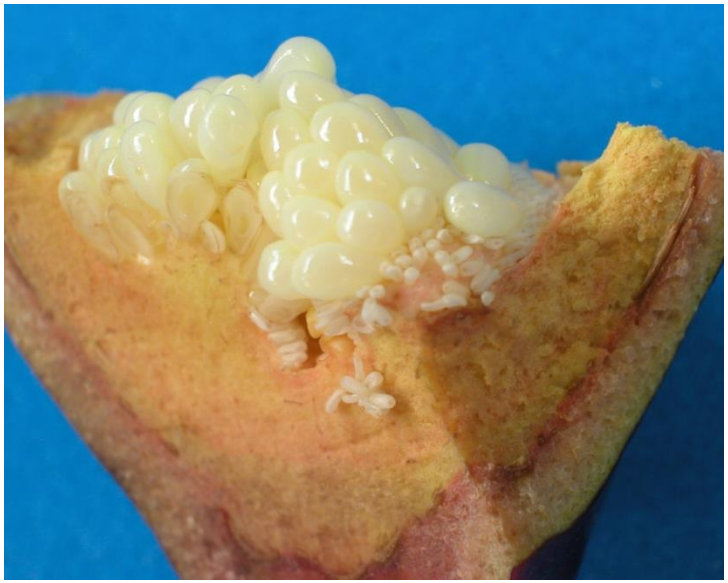


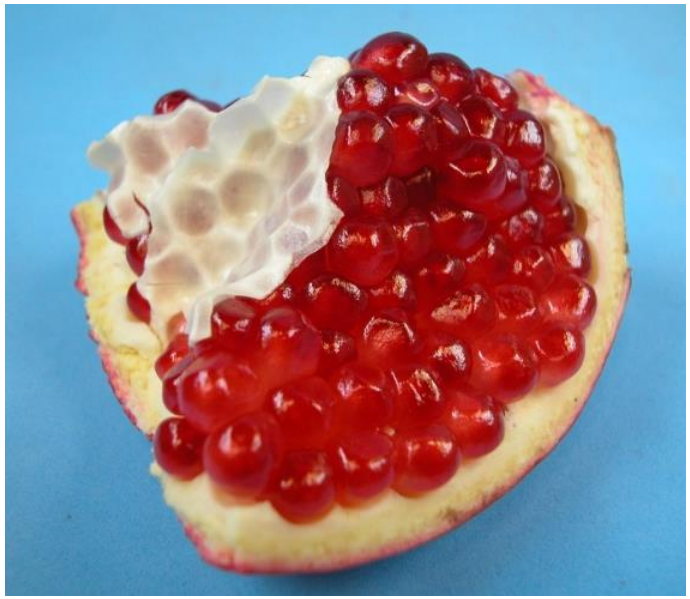


Requirements for fruit development

- Pollination with viable pollen
- Functional pistil
- Fertilization

Mature fruit commonly contain 300 to >1,000 arils.





Flowering in Pomegranate

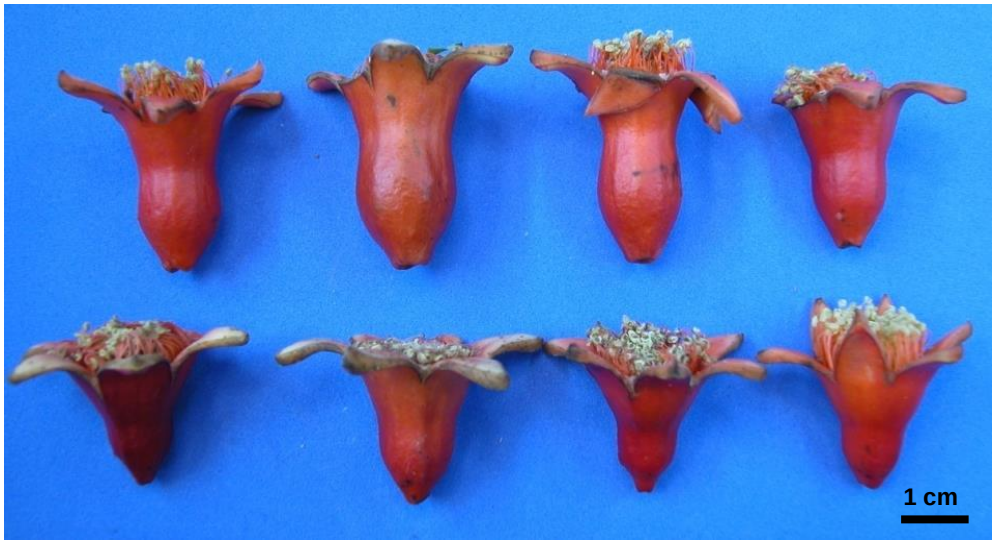
- Andromonoecy
  - Bisexual
  - Male
- Extends over a prolonged period of time with overlapping stages of flower development.
- Information on flower development is limited.

Distinguishing the flower types

Closed bud

Not so apparent at the bud stage or in open flowers

Flower Type	Style Length	Ovary Shape
Bisexual	**Long**	**Urn**
Male	**Short**	**Vase**



Bisexual vs. male flowers

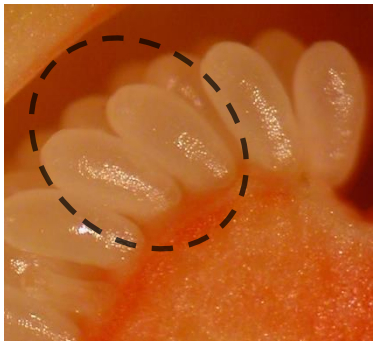
- Male flowers do not set fruit.
- The ratio of male: female flowers varies with season.

Bisexual

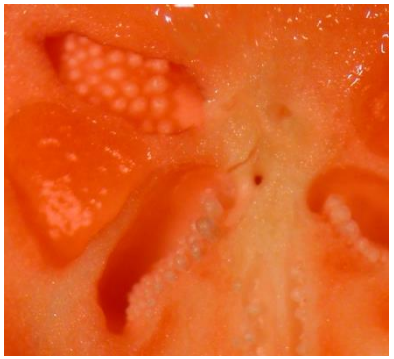
Male

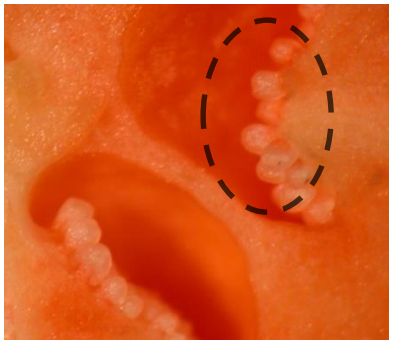
- Male flowers have rudimentary pistils.
- Degenerate ovules.

Bisexual



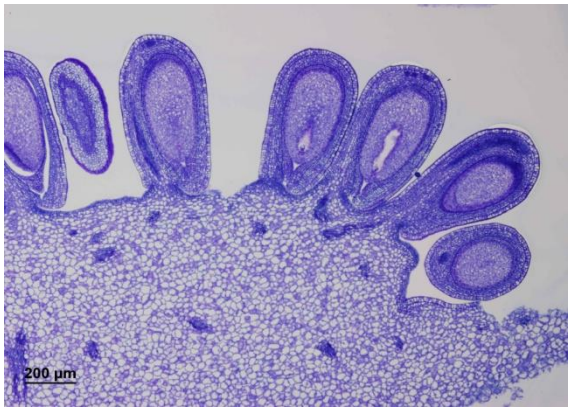
Male

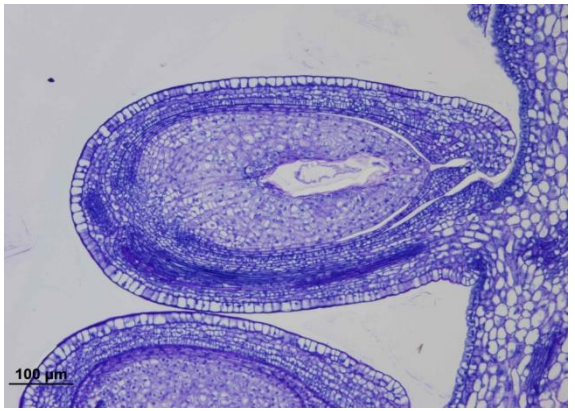




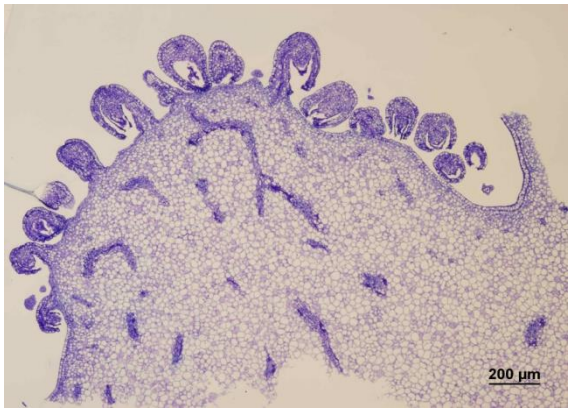
Pomegranate Ovules

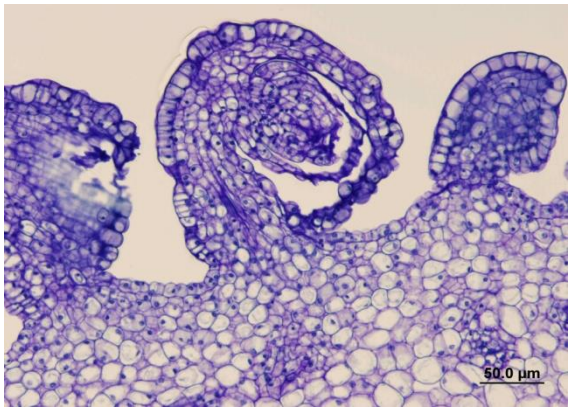
Bisexual



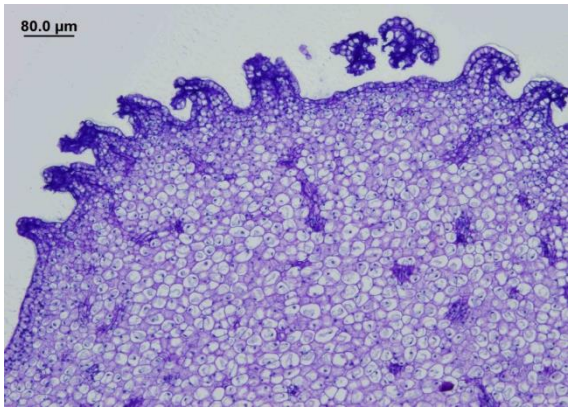


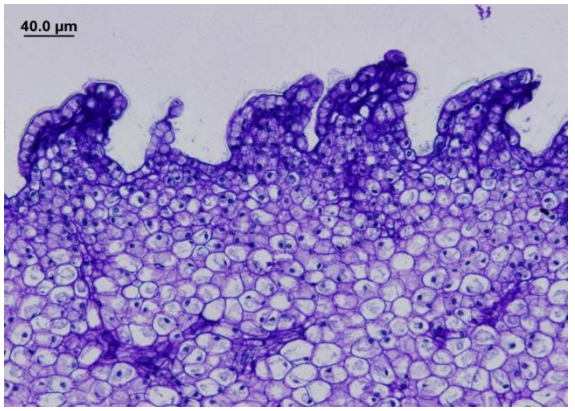
Male





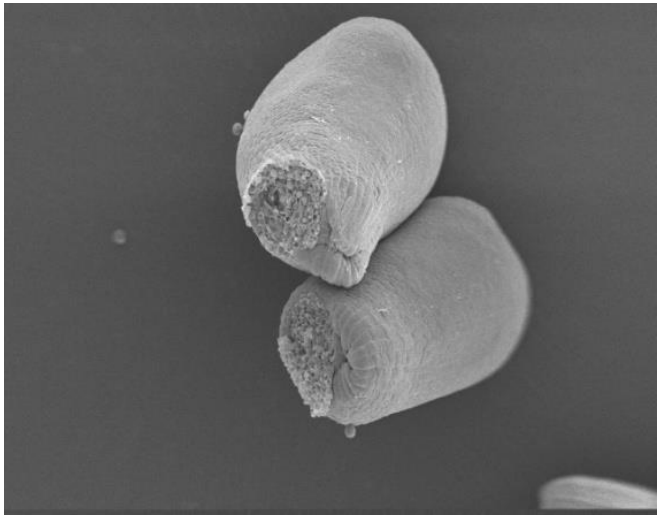
Male

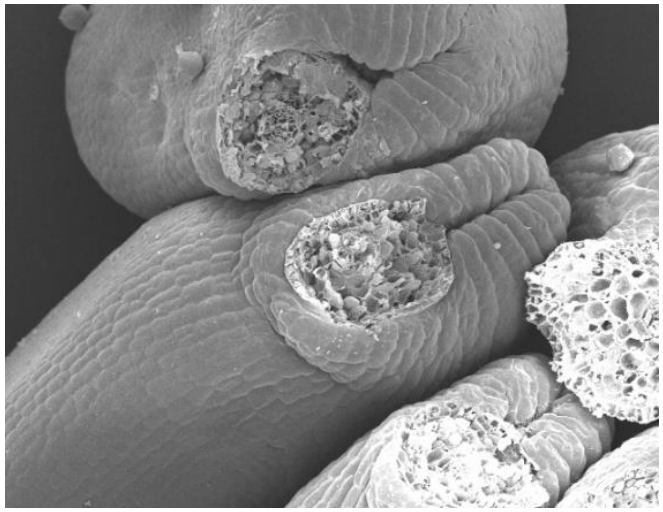




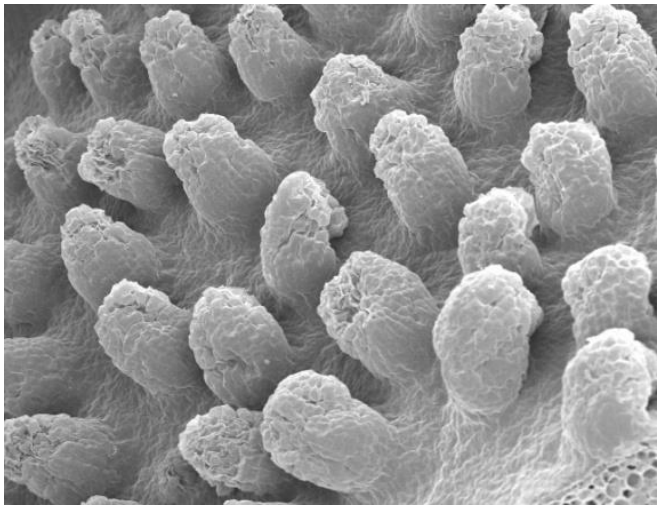
Ovules in bisexual and male flowers

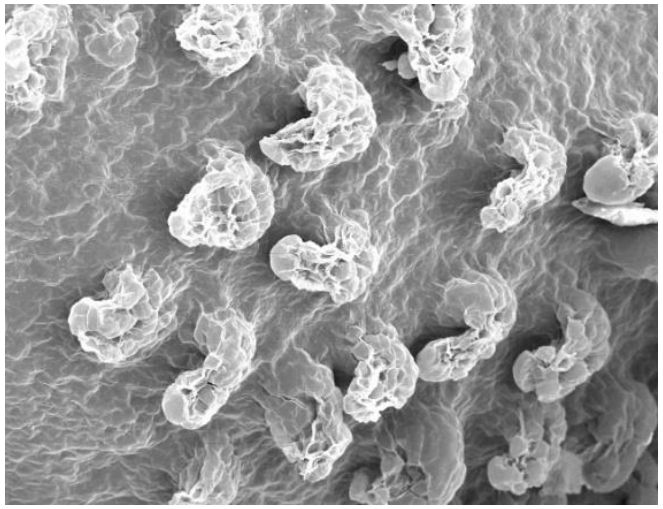
Bisexual Flowers





Male Flowers





Flower receptivity

- Question: How does flower age affect pollination, fruitset, and fruit quality?
- Approach: Flowers were pollinated at different ages. Fruitset, and fruit attributes evaluated.

Wetzstein, Yi, Ravid, and Wilkins. (2011) HortScience 46(9):S148 Abst. (ASHS Hawaii)

Emasculation and bagging

- 720 flowers at the closed petal stage were tagged.
- 600 emasculated and bagged to prevent open pollination.
- 120 control tagged only to define floral characters.

Controlled pollinations

- Subsets of flowers hand pollinated.
- Days 0, 1, 2, 3, or 4.

Day 0

Day 1

Day 2

Day 3

Day 4

Assessments

- Fruitset
- At maturity, fruit were harvested, and next-day shipped to UGA.

- Fresh weight
- Width
- Height

Fruit Characteristics

Fruit were separated into component parts.

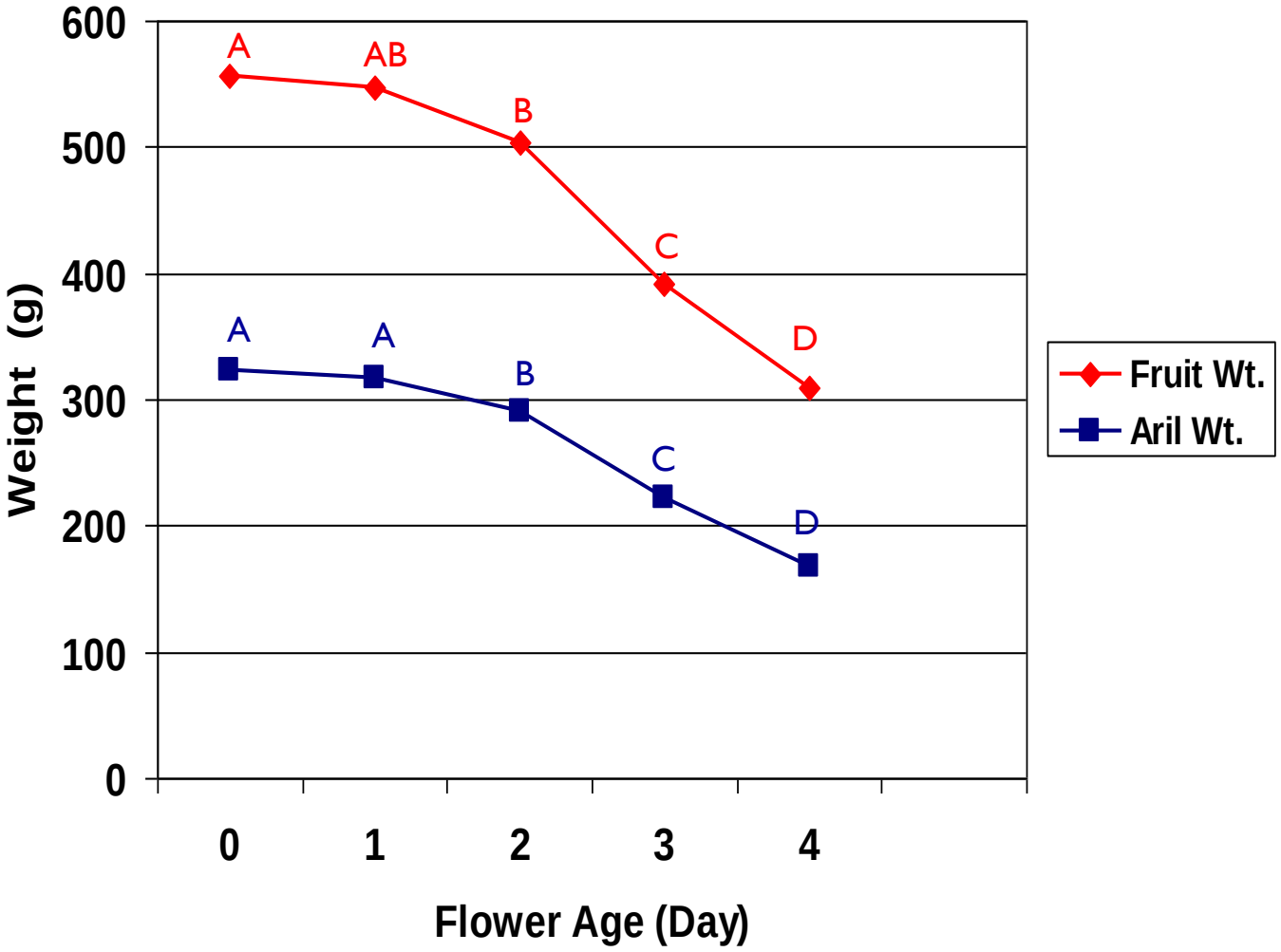
- Total aril wt.
- Total non-aril wt.
- No. arils per fruit

Effects of flower age at pollination on fruit characteristics at harvest.

Flower age	No. fruit	Height (cm)	Width (cm)	Fresh wt. (g)	No. arils	Fruit set (%)
Day 0	74	9.3 A	10.6 A	557 A	941 A	79
Day 1	71	9.2 AB	10.5 A	547 AB	933 A	72
Day 2	60	8.9 B	10.2 A	504 B	843 B	63
Day 3	52	8.1 C	9.3 B	392 C	641 C	58
Day 4	44	7.6 D	8.6 C	309 D	480 D	50

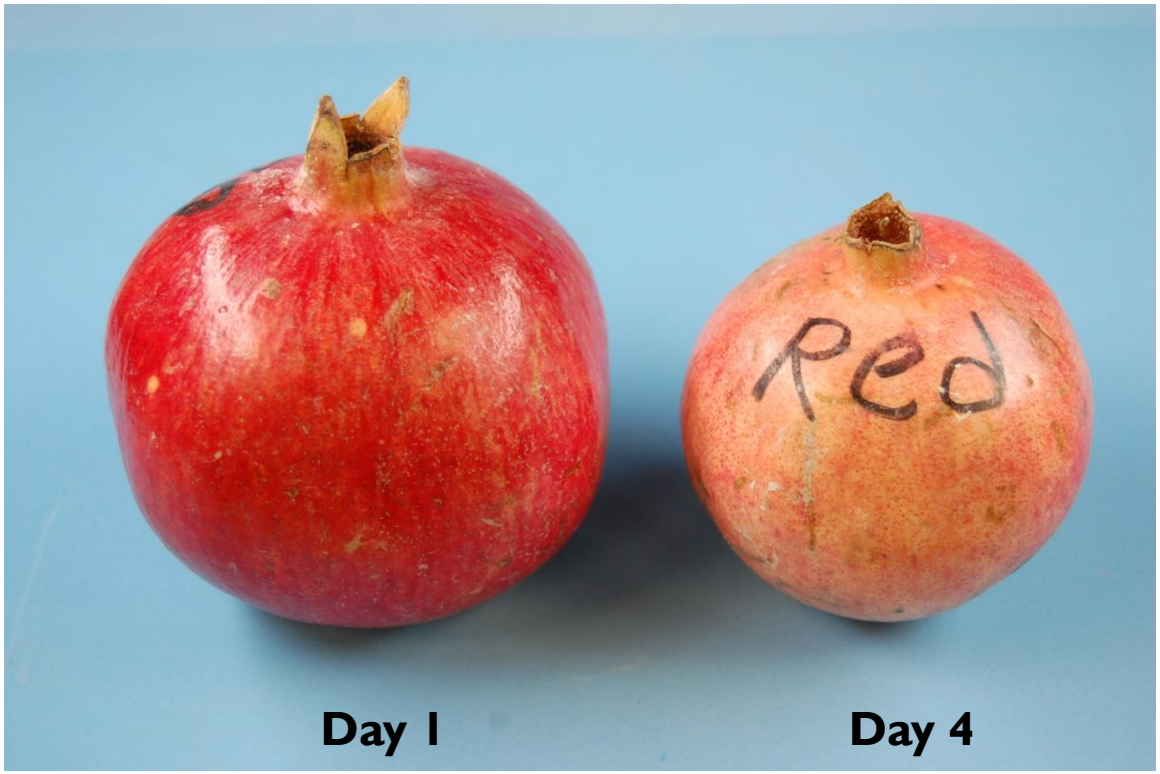
Means in columns followed by the same letter are not significantly different at P≤ 0.05 using Duncan's multiple range test.

Flower age at pollination is critical



Mean separations within fruit or aril wt groupings at P≤ 0.05.

Fruit from flowers pollinated at Day 1 versus Day 4



Selected fruits represent the mean fresh weight for the treatment.

Bigger fruit are produced if flowers are pollinated early.

Day 4 fruit are 56% smaller.

Also fruitset factor: drop from 79% to 50%

What characters determine fruit size in pomegranate?

- What makes a big fruit big, and a small fruit small?
- Do peel:arils ratios change with fruit size?
- What the best fruit for juicing? Extracted arils?
- What is more important, more arils or bigger arils?

- Production strategies to optimize factors are quite different.

Characterization of Attributes Related to Fruit Size

- Wetzstein, Zhang, Ravid and Wetzstein 2011. HortScience 46:908-912.

Developing a Fruit Quality Matrix

- Flowers at the same stage were tagged.
- Fruit were harvested at maturity.
- A range of fruit of different sizes were obtained.
- Fruit, aril and juice characteristics were determined.

Fruit Characteristics

1) Whole fruit fresh weight
2) Fruit width (avg. of 2 values) and height
3) Fruit volume (est. as a sphere: 4/3 Pi r^3)
4) Total aril weight per fruit
5) Total non-aril weight per fruit = (1)-(4)
6) Total number of arils per fruit
7) Avg. weight for one aril = (4)/(6)
8) 48 fruit used for matrix

Aril and seed characteristics

Whole arils

Seed

- From each of 48 fruit, 30 arils were randomly selected and manually depulped.
- Aril fresh weight
- Seed fresh weight
- Seed dry weight
- Juice + pulp weight
- % juice + pulp weight (juice +pulp weight/aril weight x 100)

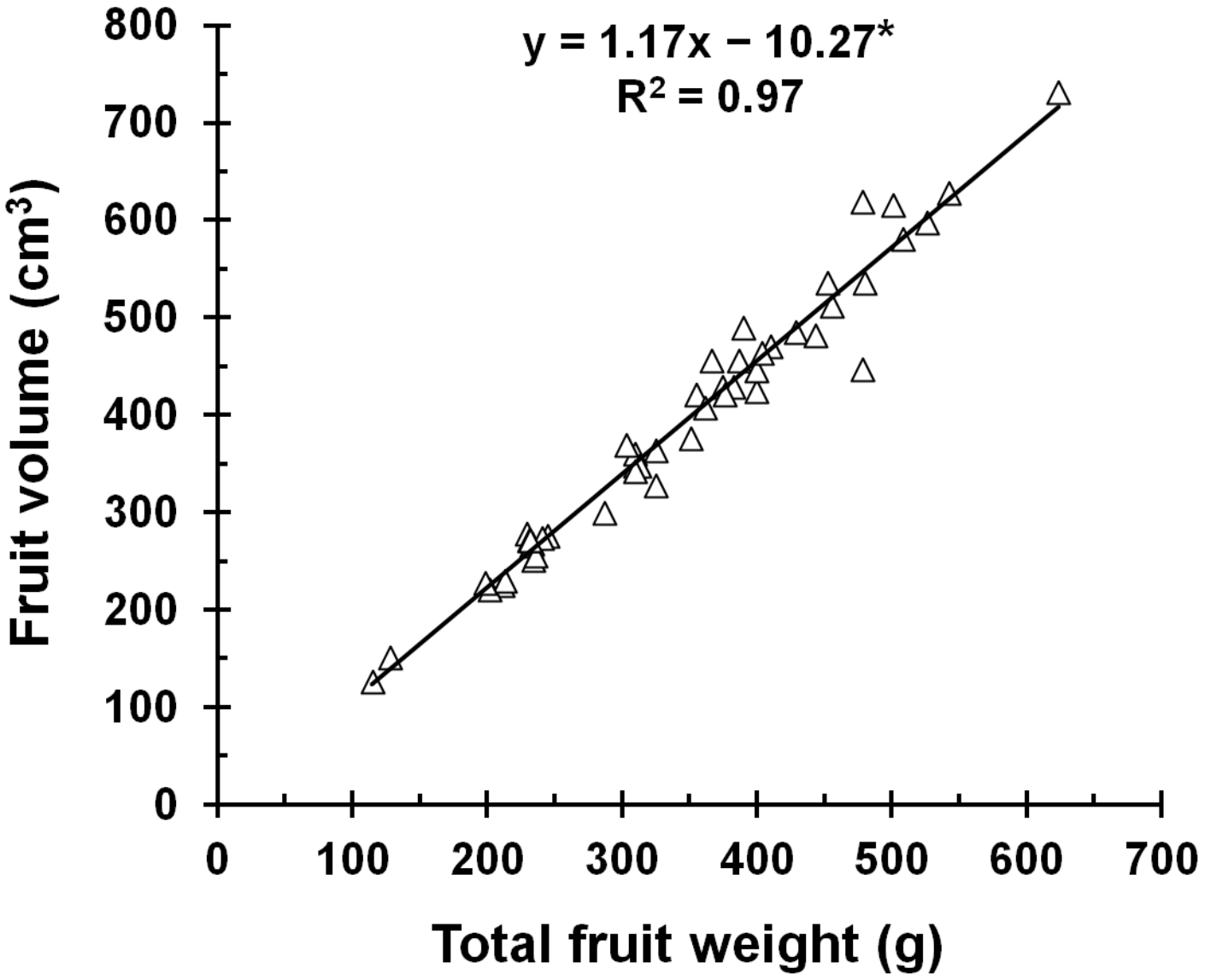
Fruit Quality Matrix - Summary Statistics

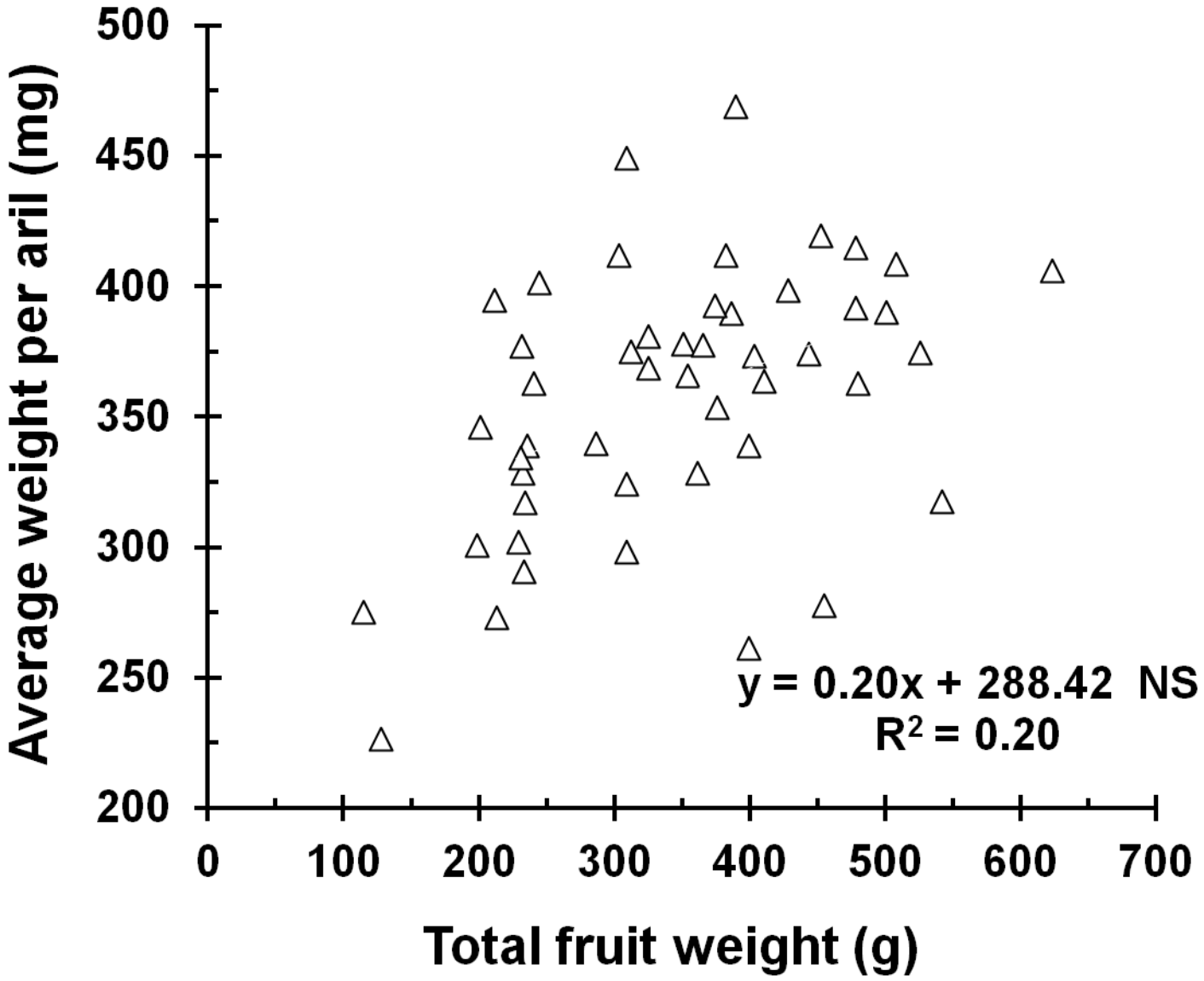
Fruit Characteristic	Mean ± SD	Minimum	Maximum
Fruit volume (cm^3)	391 ± 136	126	731
Total fruit wt. (g)	345 ± 114	114	623
Total aril wt. per fruit (g)	174 ± 62	55	313
Total no. arils per fruit	488 ± 167	201	985
Total non-aril wt. per fruit (g)	170 ± 56	60	334
% Aril wt. to total fruit wt.	50.4 ± 3.9	40.0	57.7
Avg. aril wt. (mg)	357 ± 51	226	469
Avg. seed fresh weight (mg)	33 ± 2.9	25	40
Avg. Seed dry wt. (mg)	23 ± 1.7	18	26
Avg. Juice + Pulp wt. per aril (mg)	324 ± 49	196	436
% Juice + pulp wt.	90.5 ± 1.4	86.6	92.9

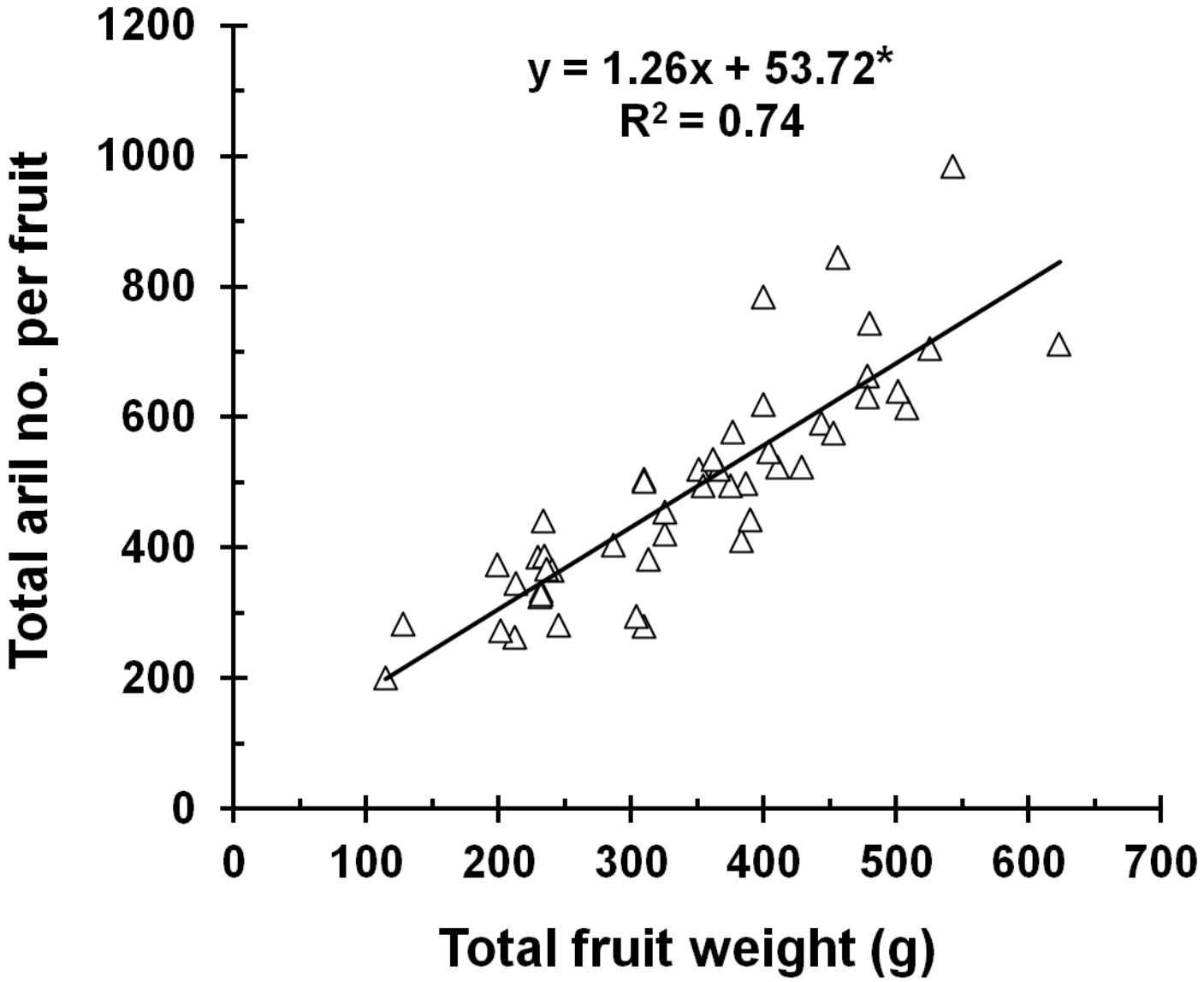
Pomegranate Fruit Correlation Matrix

Fruit Character	Fruit Vol.	Total Fruit Wt	Total Aril Wt. per Fruit	Total # Arils per Fruit	Total Non-aril Wt.	% Aril Wt. per Fruit	Avg. Aril Wt.	Avg. Seed Fresh Wt	Avg. Seed Dry Wt	Avg. Juice Pulp Wt.	% Juice Pulp Wt.
Fruit Volume	1.000										
Total Fruit Wt	0.983	1.000									
Total Aril Wt/Fruit	0.957	0.975	1.000								
Total # Arils/Fruit	0.830	0.863	0.914	1.000							
Total Non-aril Wt.	0.955	0.970	0.891	0.756	1.000						
% Aril wt./Fruit	0.170	0.175	0.379	0.453	-0.060	1.000					
Avg. Aril Wt.	0.474	0.452	0.384	0.008	0.501	-0.167	1.000				
Avg. Seed FW	0.178	0.150	0.060	-0.083	0.240	-0.394	0.421	1.000			
Avg. Seed DW	0.461	0.439	0.370	0.161	0.489	-0.241	0.666	0.741	1.000		
Avg. Juice/Pulp Wt.	0.475	0.454	0.389	0.013	0.498	-0.147	0.999	0.372	0.638	1.000	
% Juice+ Pulp Wt .	0.396	0.394	0.380	0.066	0.386	0.084	0.811	-0.163	0.306	0.839	1.000

Values are correlation coefficients


y = 1.17x − 10.27*
R² = 0.97
Fruit volume (cm³)
800
700
600
500
400
300
200
100
0
0
100
200
300
400
500
600
700
Total fruit weight (g)


Average weight per aril (mg)
500
450
400
350
300
250
200
0
100
200
300
400
500
600
700
Total fruit weight (g)
y = 0.20x + 288.42 NS
R2 = 0.20


y = 1.26x + 53.72*
R2 = 0.74
Total aril no. per fruit
1200
1000
800
600
400
200
0
0
100
200
300
400
500
600
700
Total fruit weight (g)

Aril number defines fruit size

- High correlations
  - Volume
  - Weight
  - Aril number
- ***Fruit size*** is determined by the ***number of arils*** in a fruit, not aril size.
- **Bigger fruit have more arils.**
- Pollination and fertilization are critical.

300 g
480 arils

550 g
930 arils

Critical questions about flower quality and fruit size

- Is fruit size limited by the quality of flowers?
- Does flower type and position influence fruit productions?
- Is aril number limited by ovule number?
- How variable are flowers?

Flower Vigor Studies: Effect of Flower Position and Size

Wetzstein, Yi, Porter, Ravid (2013) J. Amer. Soc. Hort. Sci. 138(3):1-8

Evaluation of bisexual flower vigor

Single flower

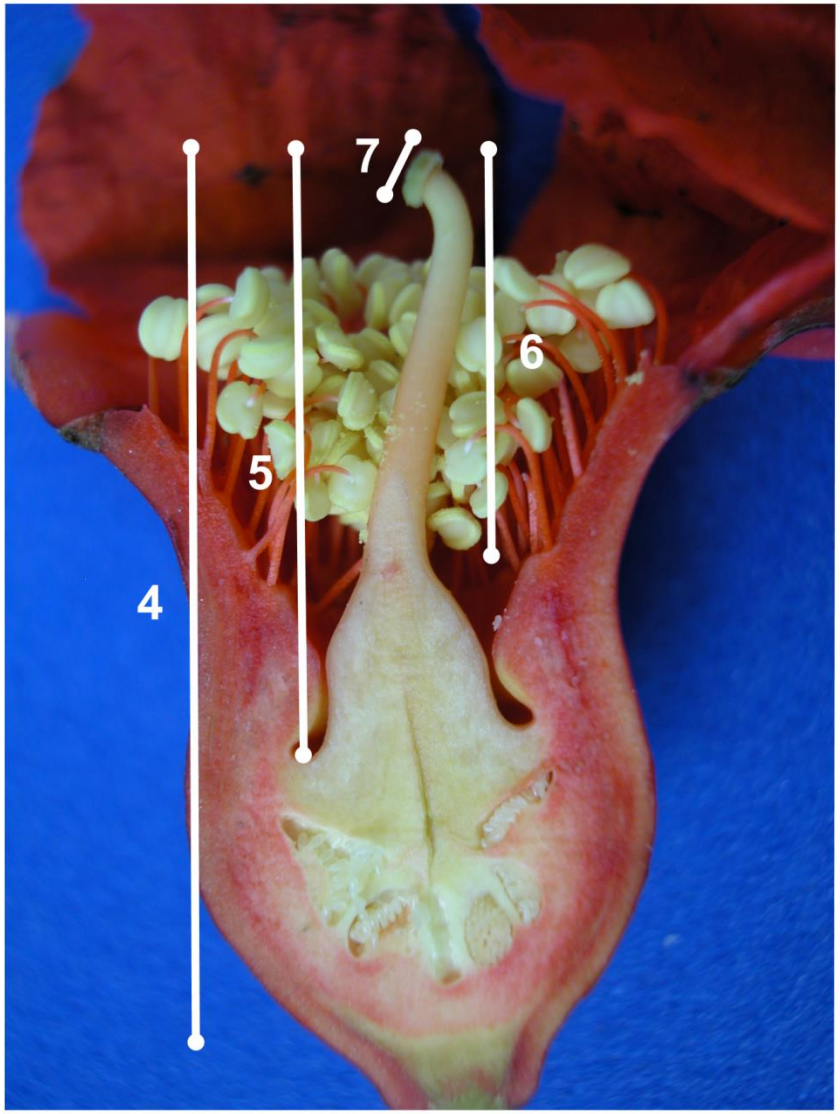
- A population of bisexual flowers at the open petal stage were collected.
  - Single flower
  - Terminal flower on a cluster
  - Lateral flower on a cluster

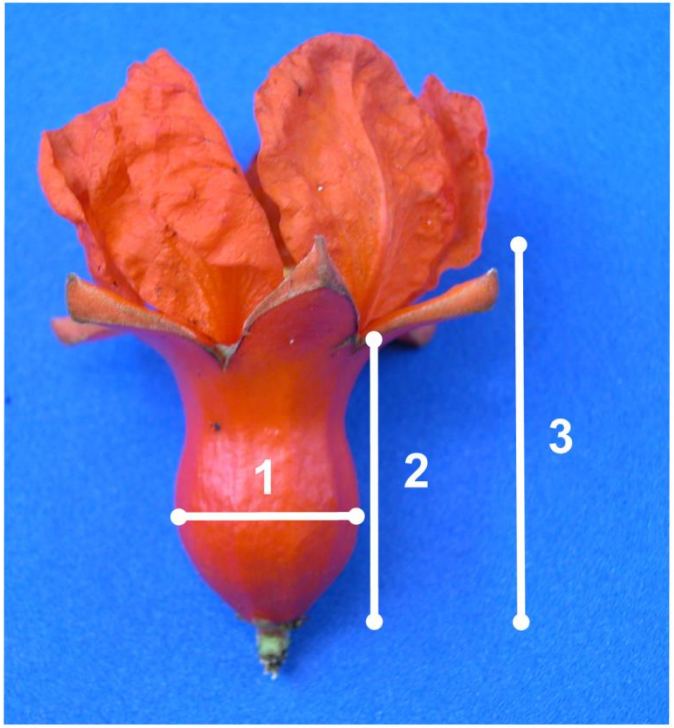
Terminal flower

Lateral flower

Flower vigor measurements

- Individual flowers were numbered and flower parts measured.
- 300 flowers (100 of each type)
- The basal portion of the flower including the ovary and ovules were placed in fixative for later analysis.





Flower characteristics measured

1. Ovary width
2. Base to sepal notch
3. Base to tip of sepals
4. Total pistil length
5. Stigma + style + stylopodium
6. Stigma + style
7. Stigma diameter

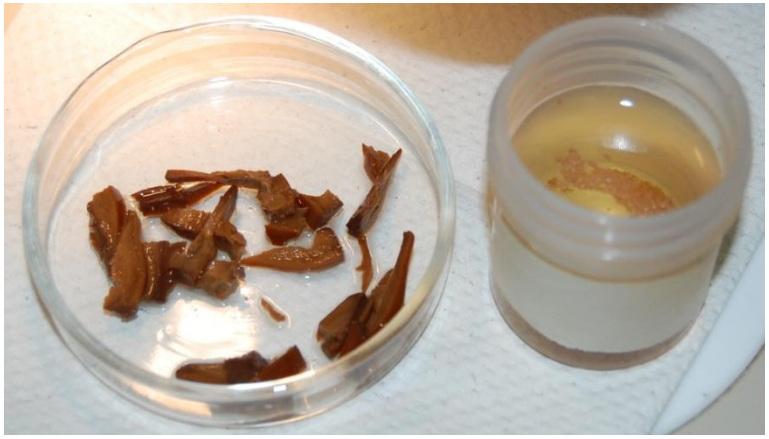
Flowers are not created equal

Table 1. Size Measurements for Bisexual Flower at Different Postions

Measurement (mm)	Terminal		Single		Lateral	
	mean	range	mean	range	mean	range
1. Ovary width	**14.5 A**	10.4 - 22.0	14.6 A	11.7 - 18.2	**13.6 B**	**10.4 - 16.6**
2. Base to sepal notch	**30.9 A**	19.7 - 40.5	30.9 A	22.3 - 36.9	**25.1 B**	**16.5 - 33.1**
3. Base to sepal tip	**42.0 A**	31.3 - 50.8	41.1 A	31.4 - 48.8	**35.1 B**	**25.0 - 46.2**
4. Pistil length	**30.6 A**	21.4 - 40.0	31.2 A	23.2 - 37.1	**26.1 B**	**15.8 - 37.1**
5. Stigma + style + stylopodium	**20.1 A**	15.7 - 31.5	20.2 A	15.4 - 28.3	**17.6 B**	**8.6 - 23.3**
6. Stigma + style	**12.4 A**	7.5 - 16.5	12.7 A	9.0 - 16.9	**10.9 B**	**5.1 - 14.5**
% collected flowers that were bisexual	**93%**		**90%**		**51%**	

Means within a row followed by different letters are significantly different at P≤0.05.

Methods for ovule counting



- Ovules were separated from other ovary tissue using a dissecting microscope and fine probes.
- 53 flowers, representing flowers of different sizes.
- Flowers were categorized into four size quartiles.

Methods for Ovule Counts

- Dissected ovules were portioned into culture plate wells.
- Individual wells were photographed.
- Image analysis (APS Assess) was used to determine ovule number.

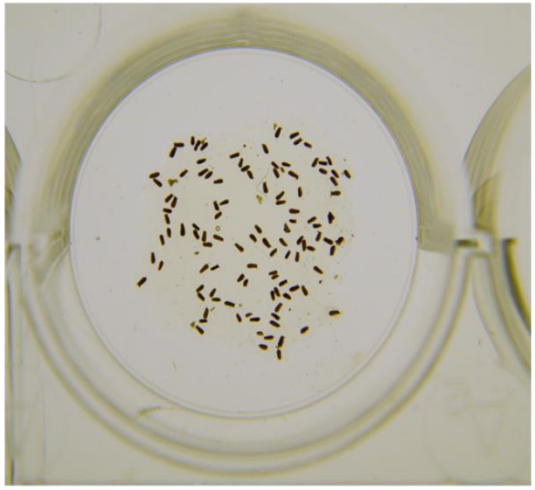
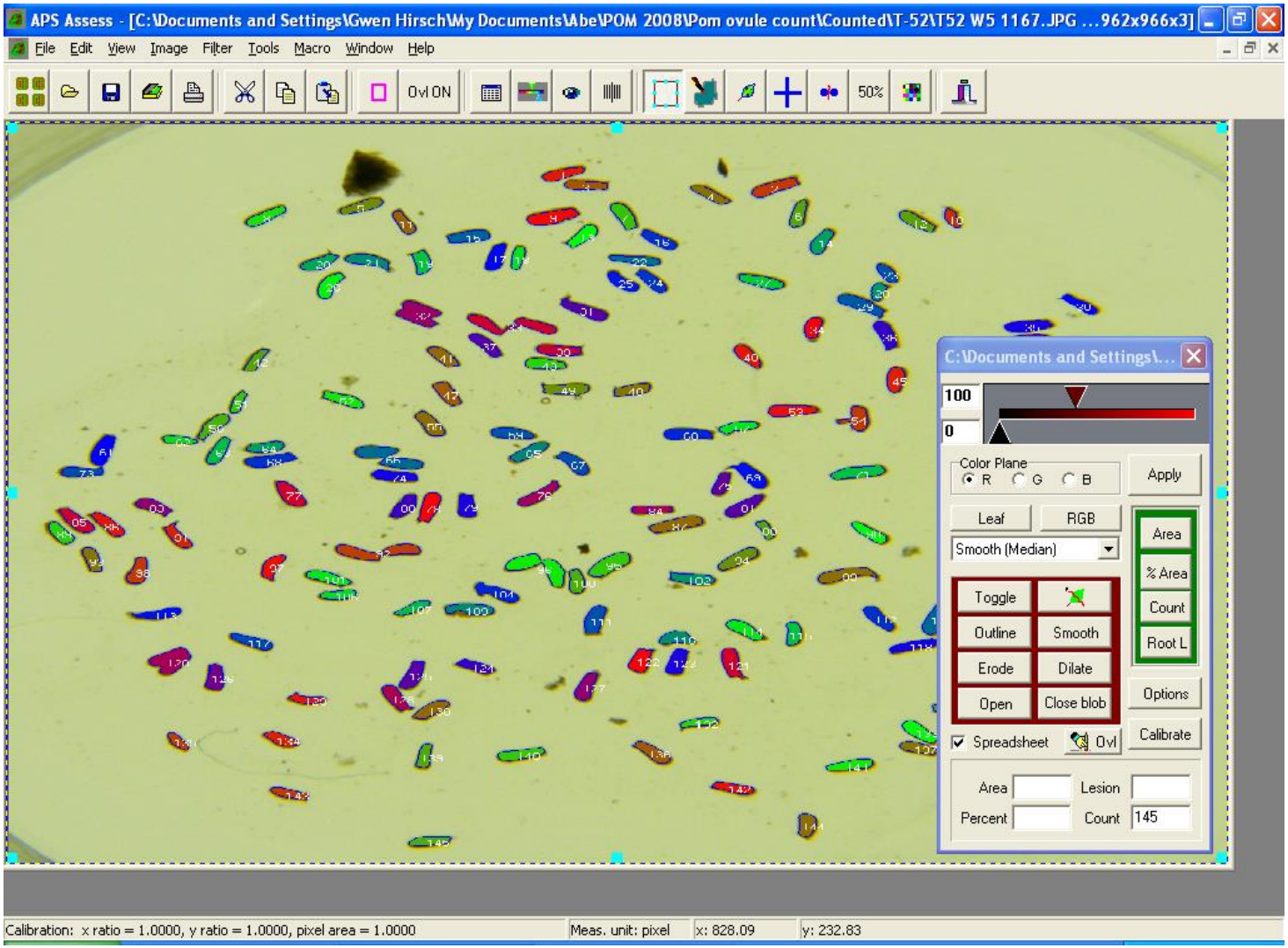


Image Analysis to Count Ovule Numbers



Larger flowers have more ovules

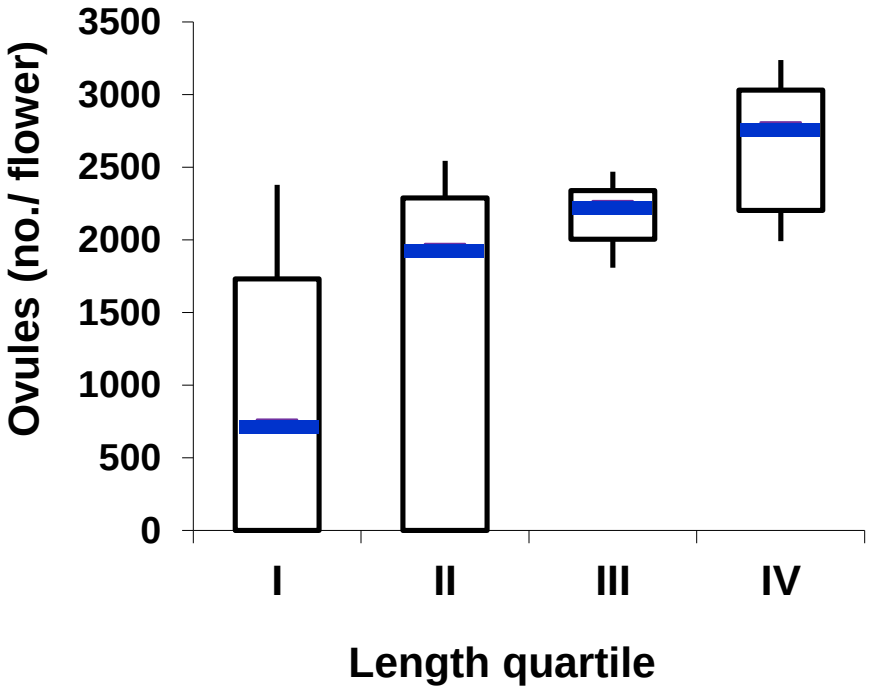
Ovule Numbers for Flowers in Different Length Quartiles

	Terminal flower			Single flower	
Size	# ovules	Range	Size	# ovules	Range
I	808 b	0-2379	I	1138 b	0-1950
II	835 b	0-2293	II	1915 ab	0-2544
III	2183 a	1809-2470	III	2204 ab	1990-2340
IV	2807 a	1991-3238	IV	2414 a	2032-2950

Size quartiles are for flower length from base to tip of sepals.
I = smallest quartile, IV = largest quartile.

Ovule numbers in different flower size groups

- Small flowers have fewer ovules and show greater variability.
- Large flowers consistently have high numbers of ovules.



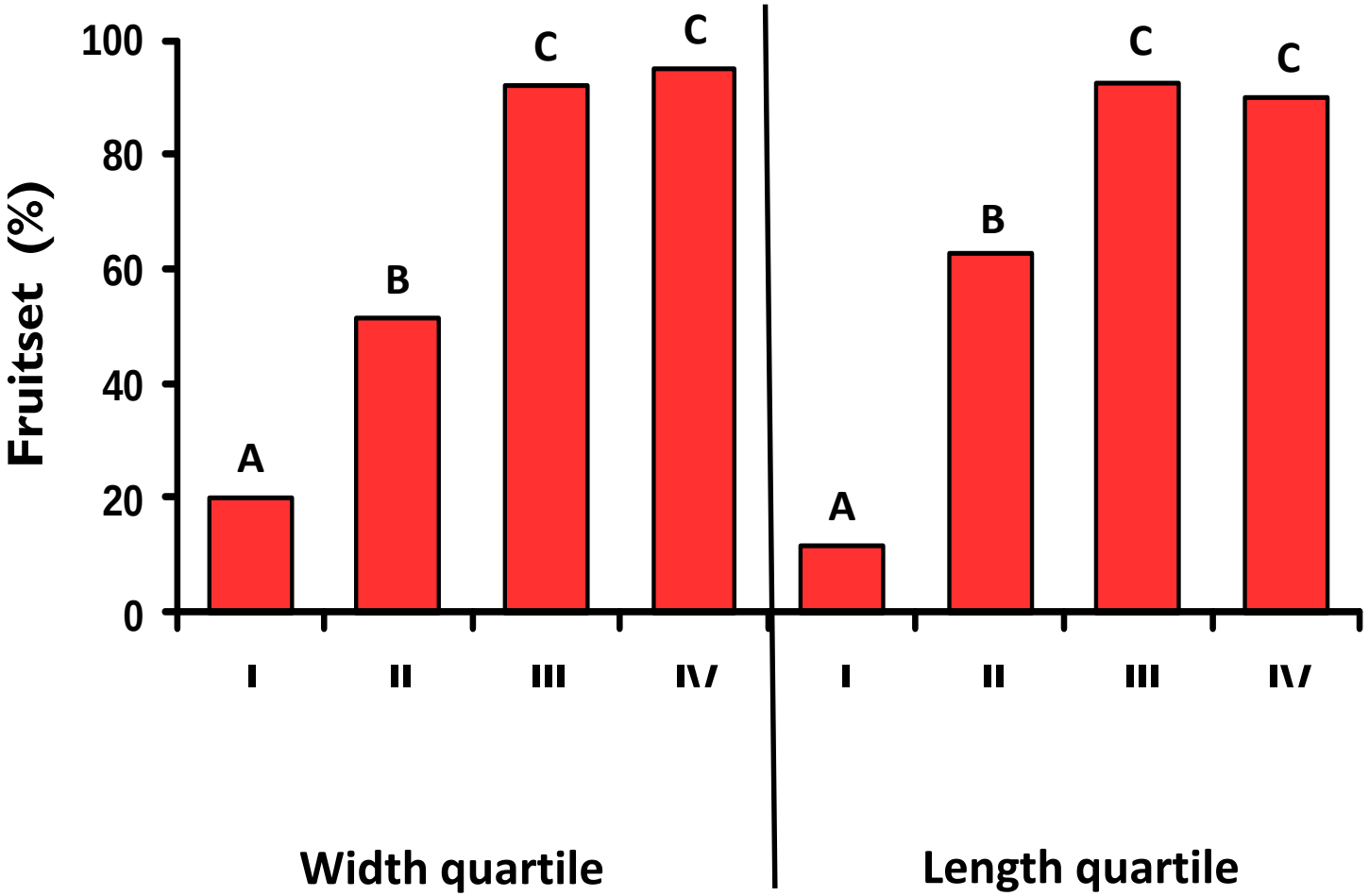
Does flower quality limit production?

- How does this translate under commercial production practices?
- Do better flowers produce better fruit?
- Or is flower development not a limiting factor?

Flowers were tagged and measured

- 535 flowers were tagged and numbered.
- Length and width measured.
- Hand pollinated.
- Fruitset and size at maturity.

Percent fruitset in controlled pollinations of flowers of different sizes



Characteristics of fruit obtained from flowers of different sizes

Size quartile	Mean fruit wt. (g)	No. fruit	Fruit in each size category (%)						
			US	42	36	30	22	16	≤30
I	-	4	-	-	-	-	-	-	-
II	387 C	48	0	4.2	37.5	43.8	14.6	0	58.4
III	435 B	223	1.4	2.2	20.6	33.2	42.2	0.5	75.9
IV	497 A	58	0	0	5.2	36.2	53.5	5.2	94.9

Quartiles based on flower width

Inferences from these studies

- Flower receptivity and vigor are important issues.
- Adequate pollination and fertilization of flowers is critical.
- Aril number is a key determinate to fruit size.
- Fruit size can be enhanced if
  - optimal pollination timing
  - adequate pollen loads
  - high flower vigor
- Farming for stronger flowers?

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Thank you for listening.

Any questions?