

## **1st Annual PIP-CAP Meeting**

### Agenda:

- 1. Introduction Project and Slack groups (Mark)
- 2. Program Coordinator and budget overview (Lizeth)
- 2: Groups (Group Leaders)
- 3. CA Trip Summary (Mark and Ricardo)
- 4. Student and Postdoc Presentations

Sam Humphrey: Rooting plug plants from tips of different sizes Pooja Tripathy: Effect of artificial chilling treatment on vegetative growth and runnering of 'Albion' and 'Fronteras' strawberry propagation transplants

growth and runnening of Albion and Fronteras strawberry propagation transp

Xi Luo: Progress in identifying DNA variants associated with

runnering and flowering traits in strawberries

- 5. Adjourn and invite people to stay for the virtual meet and greet for students
- 6. Meet & Greet for Students, staff, post-docs and PIs (not mandatory)



## Development and Integration of Next Generation Propagation Strategies to Increase the Resilience of The US Strawberry Supply Chain

USDA-NIFA Award: 2021-51181-35857 Budget: \$5,294,195



## Mission:

## Provide the industry with controlled environment protocols to <u>propagate</u> <u>strawberries</u>





Outcomes:

- Technology transfer: CE protocols to propagate strawberries.
- Fundamental knowledge on physiology, genetics, economics and supply chain.
- Building networks



#### Phase 1: Acquiring fundamental knowledge

Phase 2: Performance and technology transfer





## Today:

- Introduction of groups
- A short summary of our stakeholder visit in June 2022
- Brief research updates
- Student/Post-Doc meet and greet













## **Questions?**

- Can we develop similar practices for the US?
- Can we move things into completely enclosed environments?
- Chilling/Storing/Conditioning?









SLACK:

- Q&A Group
- Sharing science (interesting pubs/talks/videos)
- Share your strawberry swag (you want others to see/talk about your research?)
- Hobbies & Random



## https://strawberries-pip.cals.ncsu.edu/



# **Thank You**

mark.hoffmann@ncsu.edu

## **Program Coordinator for PIP-CAP**



## **Education History**

BS in Education from New Mexico State MS in Administration from Concordia University-Portland

## Work History

#### Public Education for 14 Years

- High school teacher
- IEP Coordinator

NC State University with Dr. Mark Hoffmann



## K. Lizeth Vigil

kvigil@ncsu.edu 575-993-8212



# **Budget Overview**



Account	Name	Total Sub. Amount	1st Distribution Amount	PTD Avtivity	Balance Available
500023	US Davis	\$210,000	\$125,253	\$0.00	\$125,253
500024	Univ. of Florida	\$377,839	\$234,389	\$736.16	\$233,653
500034	Ohio	\$447,504	\$252,660	\$34,893.75	\$217,766
562798	Virginia	\$79,237	\$30,561	\$2,099.32	\$28,462
568230	Univ. of California	\$138,921	\$80,570	\$0.00	\$80,570
569154	Univ. of Maryland	\$500,000.00	\$306,122.00	\$36,186.97	\$269,935.03
569164	Cal Poly	\$217,517	\$153,660	\$0.00	\$153,660
569176	Cornell	\$179,990	\$105,123	\$6,611.58	\$98,511
569201	Rutgers	\$335,000.00	\$207,859.00	\$12,452.61	\$195,406.39
569206	USDA	\$304,050	\$241,368	\$0.00	\$241,368
573704	Hoffmann		468,241.00	\$86,036.53	382,204.47
569148	Tregeagle		\$156,291	\$13,696.81	\$142,594
569149	Schweizer		169,063.00	\$15,029.07	154,033.93
569150	Hernandez		342,431.00	34,033.83	308,397.17
569151	Fernandez		138,986.00	\$4,170.77	134,815.23
569152	Devel-Jackson		139,901.00	\$2,164.44	137,736.56
	Total			\$248,111.84	\$2,904,366



# Team Leads Presentations



Objective 1. Characterization of mother plant physiological responses to the environment.



# Characterization of mother plant physiological responses to the environment –shoot

- <u>Current Activities</u>: CO<sub>2</sub> and Light intensity chamber set up (Sam), Transplant rooting capacity experiment completion (Sam). Arrival of new PhD student (Moein).
- <u>Upcoming research activities</u>: Execution of CO<sub>2</sub> and Light experiment, set up and experiment for light distribution, light quality, and photoperiod.



Sam Humphrey

**Ricardo Hernandez** 



Moein Moosavi-Nezhd

# Characterization of mother plant physiological responses to the environment – root zone

- <u>Current Activities</u>: Completed initial substrate formulations and first round of characterization. Container modeling (substrate physical properties) is ongoing.
- <u>Next Immediate Steps</u>: Coordinating with Hernandez, Kubota, and Boldt on selecting a "common" substrate for all lab groups to use in testing and trials. Mixing/preparing that product and distribution to lab groups.
- <u>Next Fiscal Year</u>: Complete substrate characterization and container modeling. Conduct and complete strawberry plant growth trials in experimental mixes, beyond the one used for/across all groups.



Brian Jackson



**Brandan Shur** 

## JSDA A

Agricultural Research Service

Current activities:

1. Evaluate  $NO_3^{-}:NH_4^+$  on runner production and daughte r plant quantity and quality (July – Nov 2022)

1.2a Nutrient optimization of mother plants (USDA-ARS)

2. Test viability of multiple hydroponics setups for future nutrient studies (Aug – Oct 2022)

Upcoming research activities:

- Repeat NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> study in new indoor space (fall/winter 2022-2023)
- 2. Impact of EC on mother plant and runner production (greenhouse; winter/spring 2023)

#### Current personnel:





Jennifer Boldt, Pl Erin Yafuso, Post-doc



Transplant day! (Mona-Lisa Banks, technician)



Objective 2. Development of environmental protocols for transplant establishment, conditioning and longterm storage.

Plant Physiology



Objective 2 Environmental protocols for transplant establishment, conditioning (runnering/flowering), and long-term storage

#### UPDATE

Chieri Kubota (Ohio State Univ.) Edward Durner (Rutgers Univ.) Celina Gomez (Purdue Univ.) Mark Hoffman (NC State Univ.)

## Obj. 2 Environmental protocols for transplant establishment, conditioning (runnering/flowering), and long-term storage Team members:









Chieri Kubota The Ohio State University Edward Durner Rutgers University Celina Gomez Purdue University Mark Hoffmann NC State University

Stage	Objective	Research	Outcome				
<b>OBJ. 2</b> Environmental protocols for transplant establishment, conditioning (runnering/flowering), and long-term storage.							
Propagation Transplants	<b>OBJ. 2.1</b> Environ. strategies to condition Propagation transplants for optim- ized propagation behavior.	Conditioning Treatments Chilling Nitrogen Rates	Outcome: Plant material with multiple microscopic crowns with high runnering capacity				
Plug Plants	<b>OBJ. 2.2</b> Environ. strategies to condition plug plants for a predetermined flowering behavior.	Conditioning Treatments	Conditioned transplants to have early and higher fruit production yield				
Unrooted and rooted daughter plants	<b>OBJ. 2.3</b> Environ. recipes to maintain unrooted + rooted daughter plant in storage with minimum impact on plant quality	Low temp. and storage days, Low temp. + light combin- ations to reach photo- synthesis = respiration compensation point and long storage time	High quality plant material (plug plant) coming from storage for either fruit or daughter plant production				

Obj. 2.1 – Conditioning plants for propagation, update



- Bare root plants were received for three cultivars "Albion, Monterey and Fronteras" in November 2021
- Greenhouse was set up for runnering
- A grad student (Pooja Tripathi) joined in January 2022
- The first experiment began in April 2022 using two cultivars (Albion and Fronteras) to test artificial chilling to improve the vigor of propagation transplants.

# Obj. 2.2 – Conditioning plants for fruit production, update



- Bare root plants were received for three cultivars "Albion, Chandler and Fronteras" in November 2021.
  - Fronteras did not establish well in the greenhouse
  - 'Ruby June' was added as alternative material
- Started working towards "protocols development" to develop high quality plug/tray plants and their flower mapping data
  - Photoperiodic lighting quality (red, far-red, and blue light)
  - Nitrogen pulse treatment
  - Correlating flower mapping data with flower and fruit production in greenhouse, high tunnel, and open field
- Mark Hoffmann and Michael Palmer (PhD Student) are joining this Objective: Impact of chilling on floral development and plant performance

Obj. 2.3 – Low-temperature storage of unrooted or rooted runner tips, update

- Project site moved to Purdue University
  - Experimental design will be updated based on the facility availability at Purdue
- Project starting date will be January, 2023
- Graduate student joined the lab in August 2022
- Need to arrange plants (and select cultivars) this fall
- Seeking collaborations with commercial nurseries for getting their runner tips or plugs to use in storage experiments



Celina Gómez Purdue University



Plant Genetics

## **Objective 3.**

## Development of a genetic matrix, based on phenotypic responses to environmental treatments.

## **Genetics Team**



USDA-SCRI Project: Development and Integration of Next-Generation Propagation Strategies to Increase the Resilience of the US Strawberry Supply Chain

https://strawberries-pip.cals.ncsu.edu/



## **Team Members**





Dr. Zhongchi Liu Professor



Ms. Christina Ippoliti PhD student

## Advisors to the team

Dr. Gina Fernandez NCSU

> Dr. Courtney Weber Cornell
### **Project summary**

Done DNA extraction and <u>W</u> hole <u>G</u> enome <u>S</u> equencing		On-going	Next
		Variant Calling	Correlate variants with phenotype
<u>Short-</u> Day	<u>Day</u> neutral	Genes known to regulate runnering and flowering In <i>Fragaria vesca</i>	Develop a list of phenotypes
Brilliance	Albion	• CO	<ul> <li>Runner proliferatively or net</li> </ul>
<ul> <li>Camarosa</li> </ul>	Cabrillo	• FI (FI1, FI2, FI3)	nol Lowering time
Chandler	<ul> <li>Monterey</li> </ul>	• 5001	<ul> <li>Flowering time</li> <li>Viger (number of leaves?)</li> </ul>
Fronteras	• Moxie	• GA20ox4	<ul> <li>Refined day length</li> </ul>
Radiance	Portola	• <i>RGA1</i>	<ul> <li>Chilling hour requirement?</li> </ul>
Ruby June	• Finn	• DAM	



Industry Economics

Determine expected economic costs/returns to industry of adopting developed techniques, and estimate the economic impact of adoption on the US strawberry supply chain.

# **Economics Team**





**Rachael Goodhue** 



**Daniel Tregeagle** 

### **Completed and Ongoing**

- Review literature on strawberry and specialty crop supply chains
- Description of current "conventional" supply chain
- Identify nursery business characteristics
- Develop framework for CA strawberry nursery production cost

## Planned for 2022-23

- Design of summer 2023 interview / focus group discussion
- Seek and receive IRB approval
- Conduct interviews / focus groups to quantify supply chain and production costs

# Economics is about understanding the aggregate outcomes of tradeoffs made by individuals

- More "vigorous" plants vs. higher production costs? [quality]
- Do invest in building tabletops now, later, or never? [time]
- Should we produce more fresh bare root or frigo plants? [form]

Quantifying the production costs and supply chain allows us to analyze these (and other) tradeoffs



Plant Performance

# **Objective 5.**

# Translation and integration of new propagation systems with industry partners.



# <u>Objective 5:</u> Translation and integration of new propagation systems with industry partners

5.1: Validation and scale-up of PIP and Greenhouse Protocols5.2: Development and of field-based propagation protocols5.3: Nationwide transplant evaluation

#### PIP-CAP: Objective 5



**Gerald Holmes** Director, California Strawberry Center



Shinsuke Agehara Assistant Professor, UF



**Courtney Weber** Assoc. Professor Cornell University







) Cornell University



**Oleg Daugovich** Field Advisor, Ventura Co.



**Giuliano Galdi** Field Advisor, Siskiyou Co.



Mark Hoffmann Small Fruits Extension Specialist, NCSU



Gina Fernandez Distinguished Professor NCSU

### NC STATE UNIVERSITY

#### **UNIVERSITY OF CALIFORNIA** Agriculture and Natural Resources

# **Students and Staff**

Emma Volk

Research Assistant MS Student Greenhouse Nursery Operations

### **NC STATE** UNIVERSITY



**Michael Palmer** PhD-Student Transplant Evaluation and Optimization

#### **NC STATE** UNIVERSITY



Samantha Simard MS-Student Transplant Evaluation





# 2022-2023: Trails



Evaluate optimal planting date for rooted tips

**NC STATE** UNIVERSITY

Evaluate field performance of PIP rooted tips Develop optimal chilling protocols for tray plant production (in collaboration with Kubota & Durner)

UNIVERSITY OF CALIFORNIA Agriculture and Natural Resources

Evaluate optimal row-cover use in field nurseries



Technology Transfer

# **Objective 6.**

# Development of extension and outreach services and products for industry and public stakeholders.

# **Extension & Outreach Team Members**

- Peter Nitzsche
  - Agriculture & Natural Resources Agent, Rutgers NJAES Cooperative Extension of Morris County
- Mark Hoffmann
  - Small Fruits Extension Specialist, North Carolina State Extension, NCSU.
- Oleg Daugovich
  - Strawberry Vegetable Crop Advisor, Cooperative Extension Ventura County, UC ANR
- Shinsuke Agehara
  - Assistant Professor of Horticulture, Institute of Food & Agricultural Sciences, UF
- Jayesh Samtani
  - Small Fruit Extension Specialist, Virginia Agriculture Experiment Station, VT
- Giuliano Galdi

- Agronomy and Crops Advisor, Cooperative Extension Siskiyou County, UC ANR

## Current Activities Year Plan

Website Development

### (<u>https://strawberries-</u> pip.cals.ncsu.edu/)

#### STRAWBERRY PIP-CAP SCRI



## Next Fiscal

- Blog / Newsletter Development
- Video on CA Strawberry Nursery Industry
- Work with other teams to document their research
- Student exchange



### Stakeholder Visits California Jun 26 – Jul 1, 2022



Students & Staff

Emma Volk, Rocco Schiavone, Sam Humphrey, Yue Shan, Jung Hoon Han, Christina Ippoliti

• Pls

Mark Hoffmann, Ricardo Hernández, Peter Nitzsche, Zhongchi Liu, Heidi Schweizer

## Planning/Management Lizeth Vigil

### Who did we visit?



- California nursery system
- California strawberry production system
- Issues and expectations

#### **NC STATE UNIVERSITYCA NURSERY Systems: Summer before planting**



### **NC STATE UNIVERSITY** CA Nursery Systems: June after planting



### NC STATE UNIVERSITY CA Nursery Systems: Spring-Summer after planting



### **NC STATE UNIVERSITY** CA Nursery Systems: Spring-Summer after planting





### **NC STATE UNIVERSITY** CA Nursery Systems: Shortly before harvest





### CA Nursery Systems: Harvest (Sep-Nov)



Video Clip



### **NC STATE UNIVERSITY** CA Nursery Systems: Shipping/Storing (Sep-May)





### **CA** Production



2022 California fresh volume of 5,708,083 trays is below the projected total of 6,421,381 trays for this week.

2022 volume projections are calculated using this year's acreage estimates multiplied by the 4-year average yields per acre, per district.

### **CA** Production

Сгор	Area	2022 Acreage	% change to 2021
<b>Fall Planting</b> Winter, Spring, Summer Production	Oxnard/Santa Maria Watsonville/Salinas	30,383	+6.4%
<b>Summer Planting</b> Fall – Winter Production	Oxnard/Santa Maria Watsonville/Salinas	7,643	-15.1%
Mexico	Central Mexico Baja	40,900	+27%
Florida	Hillsborough Co.	12,169	+1.4%

- Fundamental knowledge on plant physiology and technology transfer two key expectations
- CA industry tightly connected to Mexico industry
- Key definitions are lacking (e.g. high quality plant)



# **Thank You**

<u>mark.hoffmann@ncsu.edu</u> <u>ricardo\_hernandez@ncsu.edu</u>



# EST. 1870

Effect of artificial chilling treatment on vegetative growth and runnering of 'Albion' and 'Fronteras' strawberry propagation transplants

Pooja Tripathi PhD student Kubota Lab Department of Horticulture and Crop Science



COLLEGE OF FOOD, AGRICULTURAL, AND ENVIRONMENTAL SCIENCES **Objective 2:** Development of environmental protocols for transplant establishment, conditioning and long-term storage.

### **Specific objective 2.1:**

Environmental strategies to condition young plants for optimized propagation behavior.

### **Objective:**

To examine the effects of various levels of artificial chilling treatments on vegetative growth and runnering capacity of two strawberry cultivars.

### Hypothesis:

- Chilling treatment will promote vegetative growth, runnering and increase the number of daughter plants.
- Increase in chilling hours will make the plants more vegetative.

#### Chilling requirements of strawberry cultivars in conventional propagation.

- For Albion, 10-18 days of supplemental chilling is recommended, depending on how much in field chill the plants got. If the plants get 600 hours of in-field chill, 10 days of supplemental chilling is recommended before transplanting.
- Likewise, for Fronteras, a short-day cultivar, 4-7 days supplemental chill with at least 250 hours in-field chill is recommended prior to transplanting. (Source: I.A Rainwater, Strawberry Licensing Field Representative, UC Davis, personal communication)

#### Table 1: Calculation of chilling treatments

71

Cultivars	Low end of minimum	Low end of minimum + 50% increase
Albion	600+240 = 840 h	840+420 = 1260 h
Fronteras	250+96 = 346 h	346+173 = 519 h

THE OHIO STATE UNIVERSITY COLLEGE OF FOOD, AGRICULTURAL, AND ENVIRONMENTAL SCIENCES



### Treatments

#### Table 2: Chilling treatments for Albion

Factors	Levels	Values
Chilling	3	0 h, 800 h, 1200 h

#### Table 3: Chilling treatments for Fronteras

Factors	Levels	Values
Chilling	3	0 h, 350 h, 500 h
#### Methodolo

Harvestin g daughter plants

Note: Photosyn photoperiod du





Date of transplant: 8/11/2022









#### Data collection

#### Weekly

- Number of runners
- Length of runners
- Number of daughter plants
- Number of flower trusses removed

#### Bi-Weekly

- Petiole length of mother plant
- Number of leaves of mother plant
- Number of crowns in mother plant

Two times measurement of leaf area index and photosynthesis of mother and daughter plants

#### End of the experiment:

- Crown diameter of daughter plants
- Weight and number of daughter plants (FW and DW) per mother plant
- Weight of the stolon and mother plant
- Rooting capacity of daughter plants

#### Before transplanting



Plant architectural analysis (microscopic flower and runner mapping) of mother plants

#### Thank you!



#### Acknowledgement

Advisor: Dr. Kubota Kubota lab members Mark Kroggel Jason Hollick John Ertle Jeffrey Bates

USDA NIFA Specialty Crop Research Initiative (SCRI)

USDA National Institute of Food and Agriculture

This presentation is supported by Specialty Crop Research Initiative [grant no. 2021-51181-35857] from the USDA National Institute of Food and Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.'

### Rooting Efficacy of Different Size Strawberry (*Fragaria ananassa*) Tips in a Controlled Environment Propagation System

Samson Humphrey, Ricardo Hernández, & Mark Hoffmann

North Carolina State University Department of Horticulture SCRI PIP-CAP Annual Meeting, 2022



Three areas of optimization:

Three areas of optimization:



Mothers produce daughters (Photo courtesy: Xiaonan Shi)

#### Three areas of optimization:



Mothers produce daughters (Photo courtesy: Xiaonan Shi)



Daughters root and grow









\*Shi, et al. 2021: Timing of Stolon Removal Alters Daughter Plant Production and Quality in the Ever-bearing Strawberry 'Albion', HortScience \*\*Xu & Hernandez 2021: The Effect of Light Intensity on Vegetative Propagation Efficacy, Growth, and Morphology of "Albion" Strawberry Plants in a Precision Indoor Propagation System, MDPI Applied Sciences



\*Shi, et al. 2021: Timing of Stolon Removal Alters Daughter Plant Production and Quality in the Ever-bearing Strawberry 'Albion', HortScience \*\*Xu & Hernandez 2021: The Effect of Light Intensity on Vegetative Propagation Efficacy, Growth, and Morphology of "Albion" Strawberry Plants in a Precision Indoor Propagation System, MDPI Applied Sciences

#### **Objectives:**

- Determine if daughter plant size affects rooting success
- Determine rooting differences between daughter plant sizes

#### **Hypothesis:**

Daughter plant size will affect root development but will not rooting success rate

- Crown diameter
- Root number
- Leaf number
- Fresh mass
- Position on the stolon
- Leaf size (area or length)
- Stolon diameter
- "Greenness"



#### Crown diameter

- Root number
- Leaf number
- Fresh mass
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- Leaf size (area or length)
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- Crown diameter
- Root number
- Leaf number
- Fresh mass
- Position on the stolon
- Leaf size (area or length)
- Stolon diameter
- "Greenness"





Very Small (VSM)



Small (SM)



Medium (M)



Large (L)











- Cultivar: Monterey
- Randomized complete block design
- n = 12, where n = 1 plant

Variable	Value
Duration	28 days
Temperature	24.2 ± 1.3 °C
Relative Humidity	99.3±2.2%, lowered to 91.6±3.5%
Vapor Pressure Deficit	0.02±0.06 kPa, raised to 0.24±0.09 kPa
Photoperiod	18h
Light intensity (PPFD)	80.3±0.9 μmol m <sup>-2</sup> s <sup>-1</sup> (30B:70R)
CO2 concentration	470±73
рН	5.8
EC	2.1
Nutrients	Custom nutrient solution

https://docs.google.com/spreadsheets/d/1A0JTtL bf3jGzdnU8Ry34rKbMjaoYEe5VcsEpXJxoKAI/edit? usp=sharing

	Growing Conditions ☆ 団 ⊘     File Edit View Insert Format Data Tools Extensions Help Last edit was seconds ago						
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D26	D26 - fx						
	A	В	С	D	E		
1			Young plants	Propagative	nother plants		
2	Parameter	Units	Humphrey tip size experiment, Monterey plug plants	Humphrey stock plants, Monterey and Fronteras	Humphrey CO2 experi Monterey and Fronte		
3	Light intensity (canopy level)	PPF, µmol mol-1 s-1	80	400	200 or 400		
4	Photoperiod	hours	18	18	To be decided		
5	Light spectrum	RGB %	30B:70R LEDs	8B:15G:77R (Arize Lynk2 PKR)	25B:38G:37R (Arize Lynk2 B		
6	Temperature (air)	degrees C (day/night)	25 setpoint (24.2 ± 1.3)	25/25	To be decided		
7	Temperature (liquid culture)	degrees C	N/A	N/A	N/A		
8	Relative Humidity	%	99%, lowered to 90% on day 20	~50%	To be decided		
9	Carbon dioxide	µmol mol-1 (=ppm)	400 setpoint (470 ± 73)	400	400 or 700 or 1200		
10	Air velocity	m s-1	<0.1	0.5 to 1	To be decided		
11	Watering	L	As needed, watered over top (depend	As needed to maintain soil moisture	As needed to maintain soil me		
12	pН	pH	5.8	5.5 to 6	5.5 to 6		
13	EC	Sm-1	2.1	<1	<1		
14	Nutrition (solid media)	mol kg-1 (dry)	N/A	N/A	N/A		
15	Nutrition (liquid culture)	mmol L-1	Custom mix based on tomato	Custom mix based on tomato for the f	To be decided		
16	Substrate type		70:30 coconut coir:perlite by volume	Sungro MetroMix	To be decided		
17	Container volume	L	~76 mL	~3L	To be decided		
18	Room/chamber specifications	Floor area and model	2.7x1.2 m custom chamber	2.7x1.2 m custom chamber	1.3x2.8 m chamber		
19	Barrier beneath lamps		>3 mm thick transparent acrylic	None	None		
20	Airflow	up, down, or horizontal	Horizontal	Down	To be decided		
21	Notes		NO misting (unlike common strawberry rooting systems), instead we maintained very high humidity; duration of this experiment was 28 days		Treatments for light intensity CO2		
22							

- Cultivar: Monterey
- Randomized complete block design
- n = 12, where n = 1 plant

Variable	
Duration	28 days
Temperature	24.2 ± 1.3 °C
Relative Humidity	99.3±2.2%, lowered to 91.6±3.5%
Vapor Pressure Deficit	0.02±0.06 kPa, raised to 0.24±0.09 kPa
Photoperiod	18h
Light intensity (PPFD)	80.3±0.9 μmol m <sup>-2</sup> s <sup>-1</sup> (30B:70R)
CO2 concentration	470±73
рН	5.8
	2.1
Nutrients	Custom nutrient solution

- Crown diameter
- Visual root assessment
- Shoot and root fresh and dry mass
- Water and nitrogen consumption
- Number of leaves
- Longest leaf length
- Plant height
- SPAD chlorophyll content
- Leaf area
- Gas exchange and transpiration



- Crown diameter
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#### Rooting Success Rate (%)

No roots developed
 Roots developed





# Rooting Success Rate (%) No roots developed Roots developed





#### Rooting Success Rate (%)

No roots developedRoots developed





Hypothesis: Initial size will not affect rooting success

#### Rooting Success Rate (%)

No roots developed
 Roots developed





Hypothesis: Initial size will not affect rooting success

Finding #1: We can root all sizes of daughter plants in controlled environments

### **Results: Growth Differences**



## **Results: Growth Differences**



Т

Root Rating (1-3)

## **Results: Growth Differences**



Т

Root Rating (1-3)





Finding #2: Larger size before rooting is correlated with larger size after rooting

#### **Results: Relative Growth Rate**



#### **Results: Relative Growth Rate**
### **NC STATE UNIVERSITY**

(final – initial)

initial

100





### **NC STATE UNIVERSITY**

(final – initial)

initial

100

### **Results: Relative Growth Rate**



### **NC STATE UNIVERSITY**

(final – initial)

initial

100

### **Results: Relative Growth Rate**



Finding #3: Growth rate varies depending on initial plant size

### **Main Conclusions**

#1: We can root all sizes of daughter plants#2: Final size is correlated with initial size#3: We can predict growth rate based on initial size



- Expected results:
  - Potentially similar plant growth in the field, despite plug plant size (Hokanson, 2002; Bish, 2000)

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  - Potentially similar plant growth in the field, despite plug plant size (Hokanson, 2002; Bish, 2000)
  - Early yield: Larger plug plants may have greater early yield (Bish, 1997; Takeda, 2001; Rice, 1986) however, this may be cultivar dependent (Rice, 1986).

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  - Early yield: Larger plug plants may have greater early yield (Bish, 1997; Takeda, 2001; Rice, 1986) however, this may be cultivar dependent (Rice, 1986).
  - Late yield: Unaffected by size, regardless of cultivar (Rice, 1986; Bish, 2002)

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  - Late yield: Unaffected by size, regardless of cultivar (Rice, 1986; Bish, 2002)

# Can we use small daughters to grow the same quality of fruiting plants?

### Acknowledgements

Dr. Ricardo Hernández Dr. Mark Hoffmann Cristian Collado Xiaonan Shi Dr. Eshwar Ravishankar Partin Thompson Jerry Yu Emma Volk This project was funded by the USDA-NIFA specialty crop research initiative, award nr: 2021-51181-35857



United States Department of Agriculture National Institute of Food and Agriculture





USDA-SCRI Project: Development and Integration of Next-Generation Propagation Strategies to Increase the Resilience of the US Strawberry Supply Chain

https://strawberries-pip.cals.ncsu.edu/

### Progress in identifying DNA variants associated with runnering and flowering traits in strawberries

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# Cultivated strawberries ( $F. \times ananassa$ ) are propagated by runners





Guo et al. 2021, Current Opinions in Plant Biology

Photo credit: Ruud Morijn

### **Genetics is powerful**!

srl YW

One nucleotide change = runner or not



Caruana et al. 2018

# The genes regulate runner and flowering traits in *F. vesca* could be applied to cultivated strawberries

- GA20ox4
- RGA1
- CO
- FT (FT1, FT2, FT3)
- *SOC1*
- *TFL1*
- DAM



Edger et al. 2019

# Hypothesis

- 1. Genetic variations in the key genes in cultivated strawberries may be correlated with their flowering or runnering phenotypic response to the environment
- 2. By identifying those correlation, propagation capacities of cultivated strawberries could be predicted

## The 12 cultivars included in the analysis

# June bearing (seasonal flowering)

- Brilliance
- Camarosa
- Chandler
- Fronteras
- Radiance
- Ruby June

### **Everbearing (perpetual flowering)**

- Albion
- Cabrillo
- Monterey
- Moxie
- Portola
- Finn

## **Analysis pipeline**

Genomic DNA isolation and <u>Whole Genome S</u>equencing

Alignment and Variant calling

Identifying DNA Variants (SNPs and Indels)

Collecting Phenotypic data (traits: runner, flower, chilling hours, etc.) Identifying association between high impact DNA Variants and specific phenotypes

Developing molecular markers for breeding and phenotype prediction

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### Whole genome sequencing data summary

	Reads	Bases	
	(Million)	(Billion)	Q30 bases (%)
Albion	246.45	36.81	94.17%
Brilliance	205.10	30.62	93.87%
Cabrillo	222.13	33.14	93.75%
Camarosa	214.25	32.00	93.42%
Chandler	214.03	31.94	93.44%
Finn	225.94	33.72	93.61%
Fronteras	233.60	34.89	93.61%
Monterey	224.13	33.48	93.62%
Moxie	204.68	30.56	94.10%
Portola	210.50	31.41	93.44%
Radiance	223.81	33.44	94.13%
Ruby June	214.21	32.01	93.94%

- ~ 35 billion bases each cultivar
- High quality: Q30 bases >93%
- 145X coverage on average

Note: Q30 means the sequence error rate is 1/1000 bases

## Aligning sequences to reference genome

Sequencing reads are disorganized



Sequencing reads are matched or "aligned" to reference genome

Reference genome

### Synthetic reference genome

Our reference genome composed of all of our genes of interest from the known *Fragaria vesca* genome.



# Variant calling: discovering variants in genes from different cultivars

•••	IGV		
GCF_000009065.1	NC_003143.1 ○ IC_003143.1:1,272,532-1,272,586 Go	+	
Coordinates		-	
Vertilecete			
Coverage			1
Reads alignment			An example: 10 reads coverage
→ Ref. Sequence	A A A A G C A A T A T G A T C A A G C G A T C A C T G T T T T T C A G A G T T T T G T G A A A C A G T A T C K K Q Y D Q A I T V F Q S F V K Q Y ANY MOUS	P	
	A T-to-C SNP with 100% variant allele frequency		

### **Calculate variant allele frequency**

Variant allele frequency:

 $reads\ harboring\ a\ specific\ variant\ at\ the\ locus$ 

all reads aligned to the locus



An example of 4 variant types at one locus in four cultivars.



### Future directions:

- 1.Fully develop a list of phenotypes
- Runner proliferatively or not
- Flowering time
- \*Vigor
- \*Refined day length
- Chilling hour requirement

2. Identify association between high impact DNA variants and specific phenotypes

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