Updates on Berry Fruit Research; Finding Ways to Mitigate Heat and Disease Stress

Kalpalatha Melmaiee, Ph.D.

Associate Professor

Department of Agriculture and Natural Resources,
Delaware State University,
Dover, DE 19901

Research Interests

- High temperature stress in blueberries

- Resistance to Fruit rot diseases

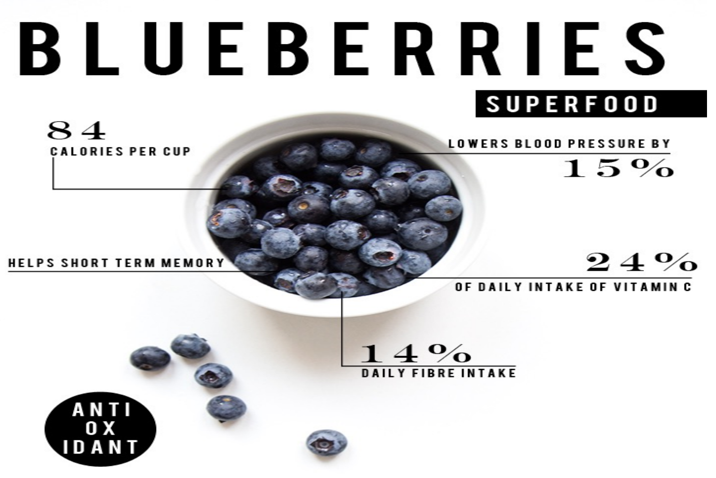
Blueberries: one of the "superfoods"

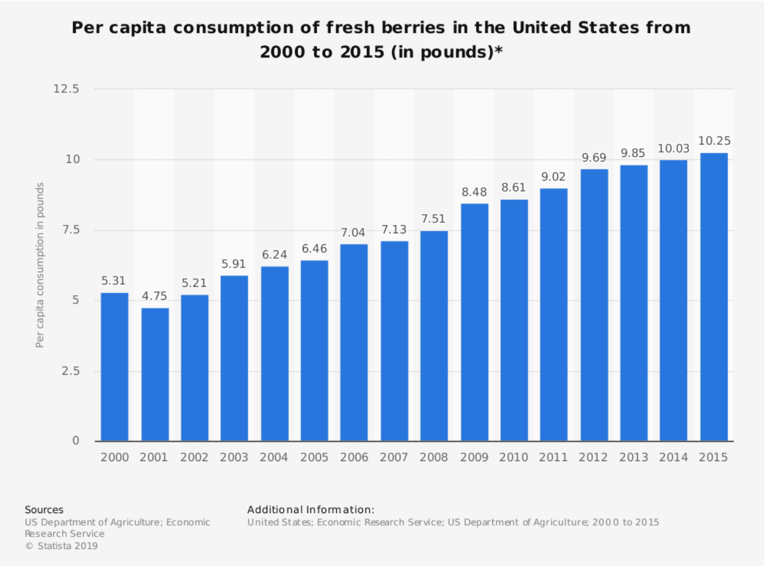
- Rich source of phyto-compounds such as anthocyanins, phenolic acids, flavonoids, stilbenes, and tannins.
- Possess a good amount of nutritive compounds including vitamins, carotenoids, and minerals
- A high-value crop, produced in the US, Canada, Europe, Australia, New Zealand, Chile, and Argentina
- The US is the largest blueberry producer with a market value of $904 million in the year 2020.
- Global production of blueberries: 655 thousand tins per year.
- **Production increased 20 folds in 20 years.**

INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION
2021, VOL. 72, NO. 5, 650-652
https://doi.org/10.1080/09637486.2020.1852192
Taylor & Francis
Taylor & Francis Group

Blueberry benefits to cognitive function across the lifespan

Lynne Bell and Claire M. Williams
School of Psychology and Clinical Language Sciences, Reading, UK





High Temperature Stress Effects

- The global mean temperature continues to increase at a rapid rate. Heat waves are projected to become more intense & increase in frequency
- The estimated optimal temperature is 25/20 °C to 30/20 °C day/night
- Unusual warm spring/ Warm spring waves : early floral development
- Unusual hot summers/ heat waves: fruit of most cultivars being too soft for extended storage
- **High Temp. Symptoms:** Necrosis, spotting, shriveling/wrinkling and poor coloration on berries

Yang et al. 2019. HortScience, 54(12), 2231-2239.

How to address

Screening existing plant types/genotypes

Developing new plant types/

Controlling growing environments

Genetic diversity analysis

Blueberry (*Vaccinium* L. sect. Cyanococcus)




Need for new genetic sources

- Wild populations have high genetic diversity
- Lately domesticated; highly depended on phenotypic observations
- Present cultivars derived from a very **narrow genetic base**
- **Underlying genetic diversity is not fully captured**
- **Inbreeding depression** (crosses from closely related sps.) can be a serious issue in future breeding programs
- Consequences: reduced fruit set, smaller berries, later-maturing berries; reduced seedling survival, and vigor

J. Amer. Soc. Hort. Sci. 133(3):427–437. 2008.

Impact of Wide Hybridization on Highbush Blueberry Breeding

Patricio A. Brevis[1,2]
Department of Horticulture, Michigan State University, A316 Plant and Soil Sciences Building, East Lansing, MI 48824

38 southern highbush blueberry cultivars

Collection Sites

Alpine meadows and exposed, rocky sites (Vb).

Borders of lakes, streams sandy swamps (Vc)

Forests and in shrubby areas (Vt)

Pine forests, Pine flatwoods; sand hills (Vd, Vm)

Blueberry Voyage

Philip E. Marucci Center for Blueberry and Cranberry
Research and Extension
125A Lake Oswego Road
Chatsworth, New Jersey 08019

Genetic and Phenotypic Differentiation Analysis

V. darrowii

2x

81 accessions

Alabama, Florida, Mississippi

V. tenellum

2x

38 accessions

North Carolina, Georgia, Virginia

V. boreale

2x

14 accessions

Cape Brenton, Nova Scotia

V. myrsinities

4x

29 accessions

Munson Alabama,

Polk County Florida

V. corymbosum

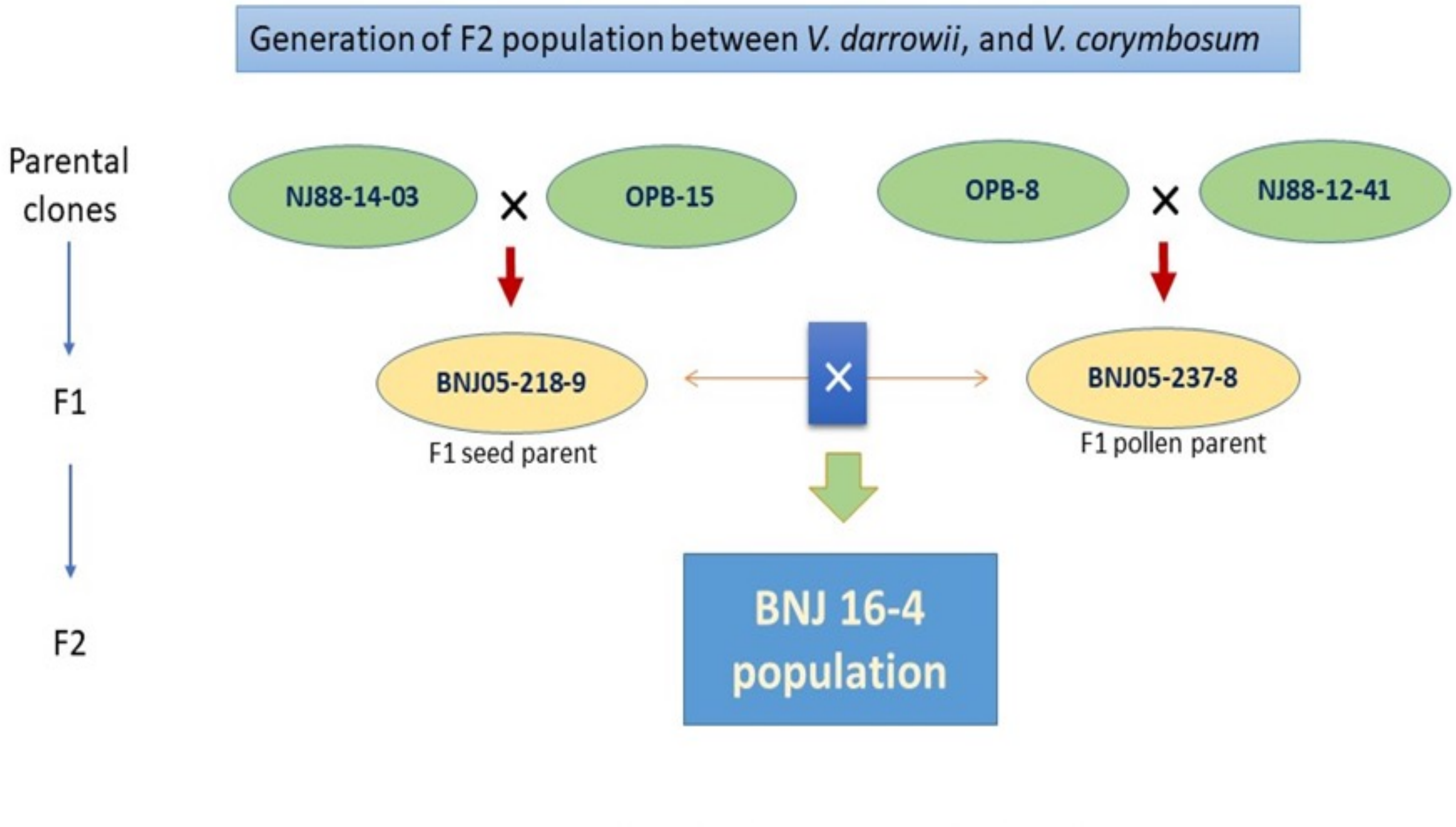
2x & 4x

33 accessions

195 accessions

The new population development

An interspecific *pseudo*-F2 population comprising 260 plants was used



F1 and F2 plant material used in the study

For **BNJ16-5** population, **BNJ05-237-8** was seed parent, whereas **BNJ05-218-9** was pollen parent

The new population Analysis

Phenotypic distribution of the HTS traits

- Blueberry F_2 plants showing phenotype differences after 4 days of high-temperature stress.
- The upper half showing plants with little or no leaf scorching, whereas the below half showing plants with high leaf scorching symptoms.
- Leaf scorch occurs when plants are transpiring rapidly during periods of high temperatures

BNJ16-4-06

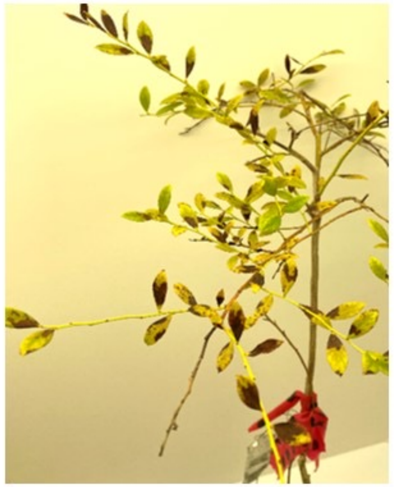
BNJ16-4-09

BNJ16-4-13

BNJ16-4-15

BNJ16-4-35

BNJ16-4-19

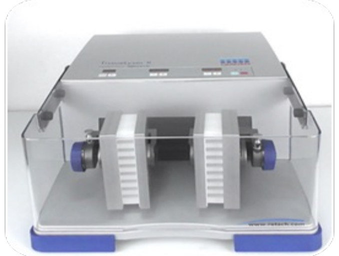


BNJ16-4-21

BNJ16-4-22

Library preparation, DNA sequencing, & Analysis

Tissue collection



Leaf tissue disruption using TissueLyzer II

DNA isolation Qiagen

DNA quantification Nanodrop

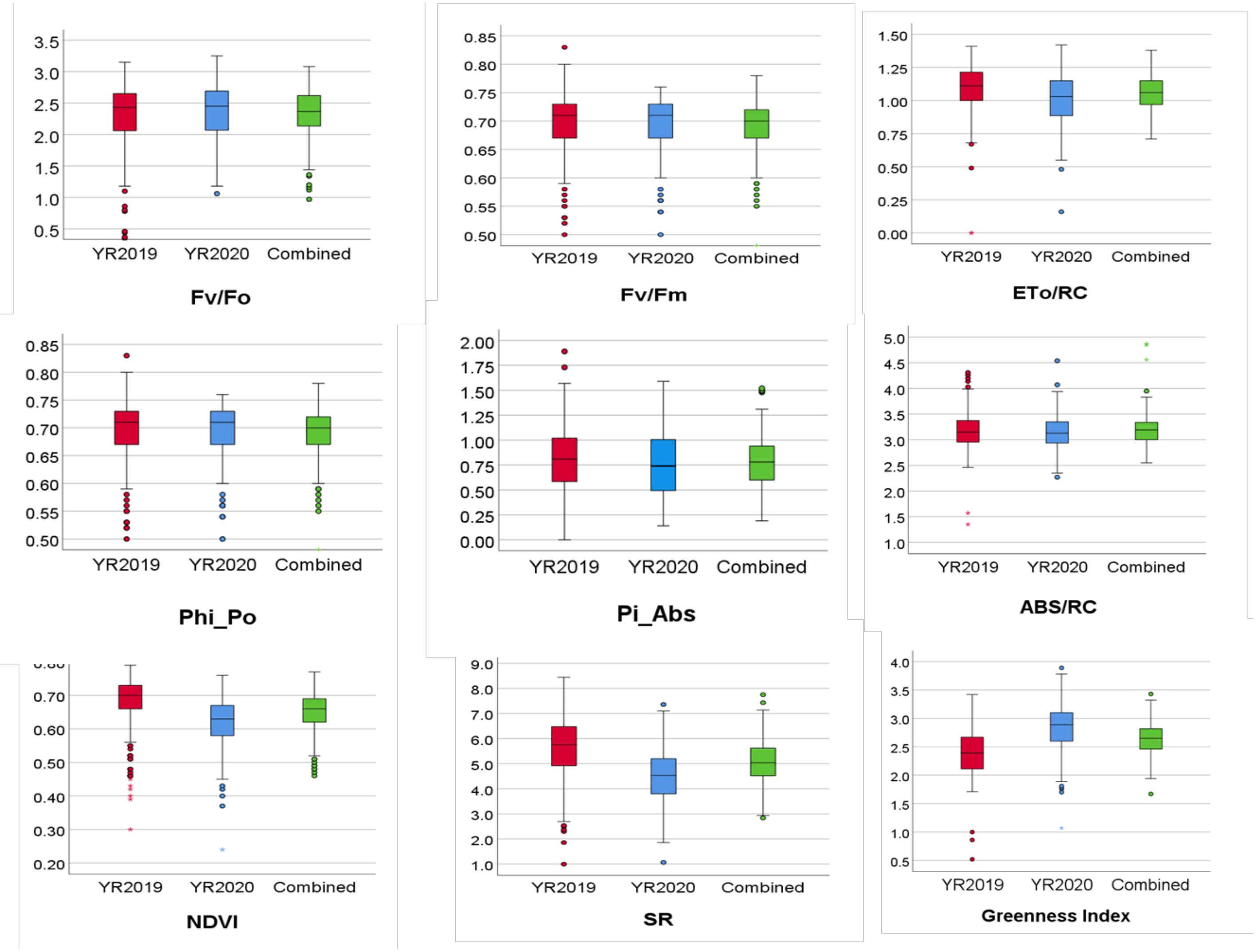
ds-DNA quantification Qubit

Umesh K. Reddy, Ph.D.
Professor of Genetics and Genomics Department of Biology
West Virginia State University 141 Hamblin Hall
Institute, WV 25112-1000
Phone: (304) 766-3066
ureddy@wvstateu.edu

Alignment with reference genome

- *Vaccinium corymbosum* cv. Draper v1.0 genome sequence
- Tetraploid genome
- Reads were mapped to the first 12 scaffolds

Phenotypic distribution of the HTS traits



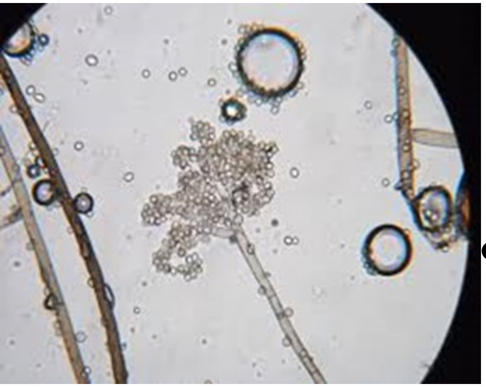
Box plots for QY displaying the range of phenotype differences among the F_2 progenies subjected to high-temperature stress.

Gray mold

Typical *botrytis* infection (gray mold) symptoms on strawberries. A. Gray mold lesions on calyces of developing fruit, B. Early-stage lesions on fruits, C. Advanced stage symptoms; excessive sporulation can be seen

Botrytis cinerea

- Necrotrophic fungus
- Produces mycelium and conidia
- Spores thrives in wet, warm, humid conditions
- Symptoms: plant rot, brown lesions on leaves and fruit, gray conidia form.
- Common name: Gray mold



(Petrasch et al., 2019)

Botrytis cinerea

- Infects over 200 plant species
- Remains dormant for a substantial period
- Causes 80 percent loss of flower and fruit
- Causes a 10-100billion USD loss in revenue
- Common fungicide for botrytis: Greencure Fungicide

(Petrasch et al., 2019)

Gray Mold in other fruits/vegetables

- ❖ **Develop resistant varieties**
  - ❖ **Try for new fungicides and alternative methods- why?**
  - ❖ **Overuse of fungicides and development of chemical resistance in fungus**

Potassium Silicate

Use of Potassium Silicate as an Alternative to Chemical fungicide in Rice and other cereal crops

Test its absorption and efficacy in strawberries

Foliar Application

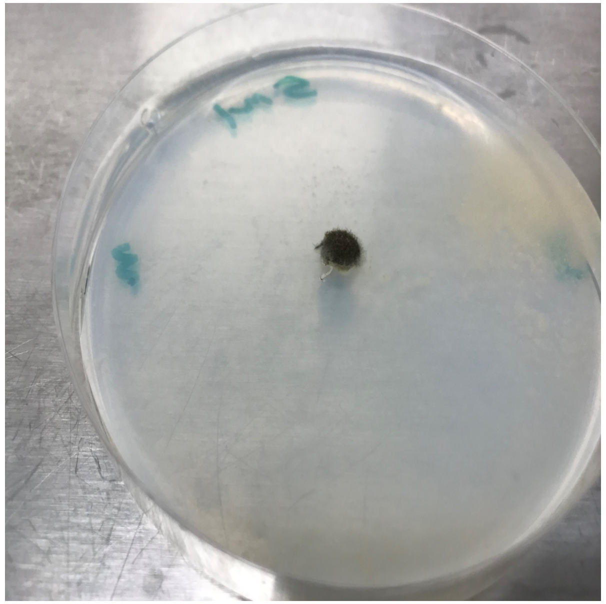
Dilution treatments

1. 0ml silicate solution/Gallon of water
2. 2ml/ga
3. 3ml/ga
4. 4ml/ga

Antifungal activity of potassium silica against *B. cinerea*

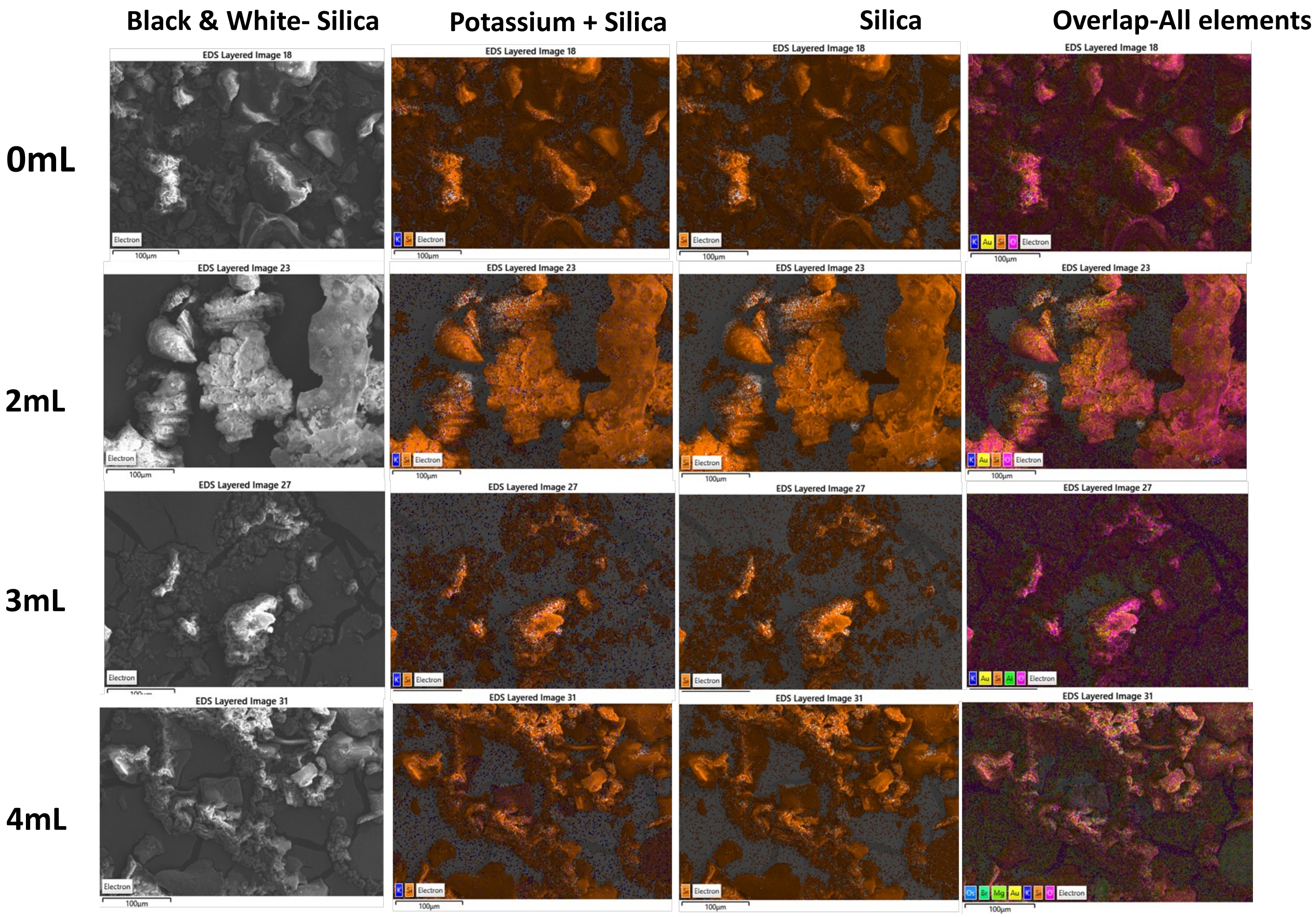
Potato Dextrose Agar (PDA) → Prepare plates → Measure mycelium growth

Treatments	PDA + streptomycin + silica solution
0 mL	250 mL + 1 mL
1 mL	250 mL + 1 mL
2 mL	250 mL + 1 mL
3 mL	250 mL + 1 mL
4 mL	250 mL + 1 mL
5 ml	250 mL + 1 mL



Digital caliper

Si Accumulation in Leaf Samples upon Treatment 1

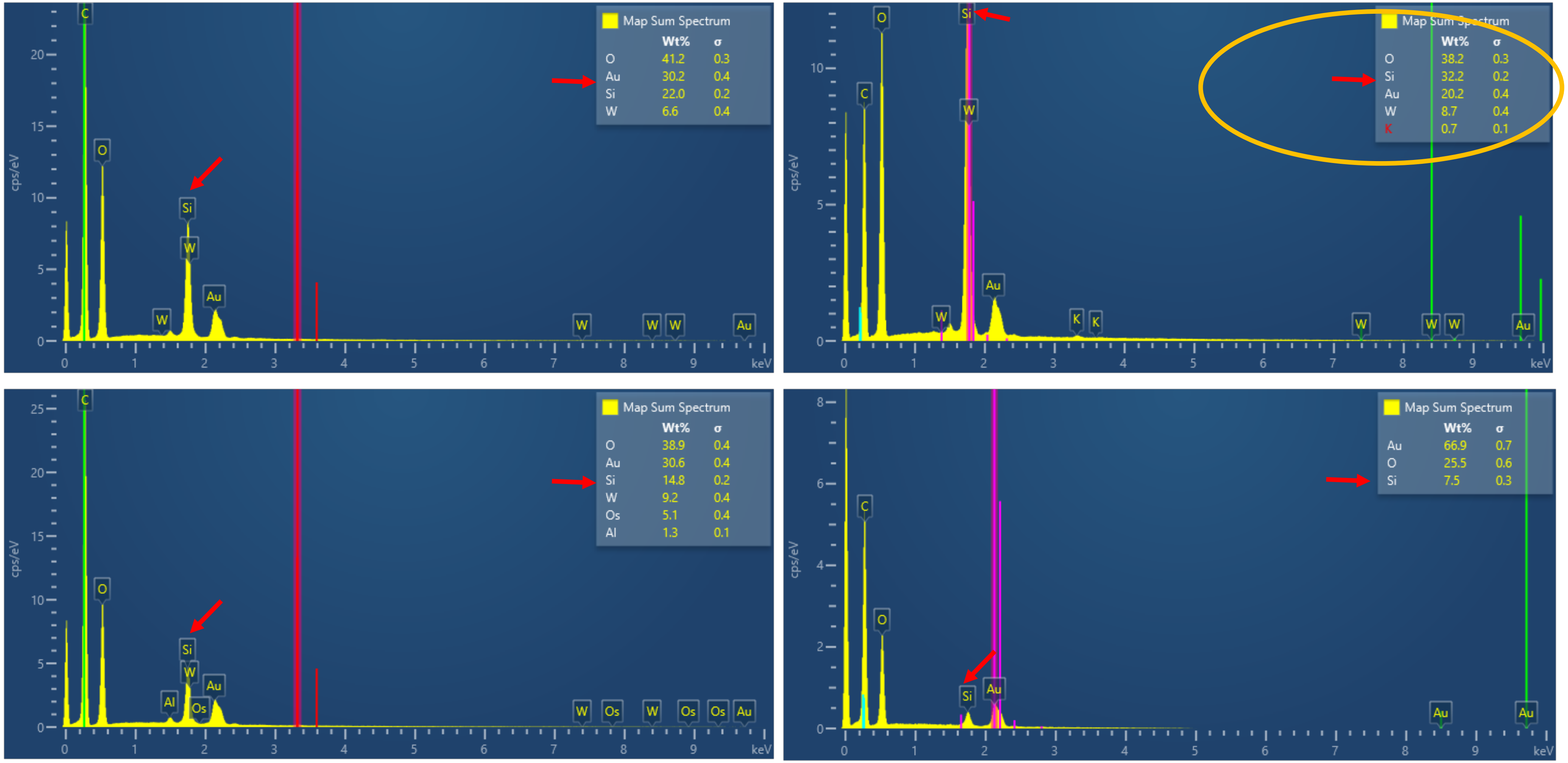


Semi quantification of elements using Scanning Electron Microscopy images coupled with Energy Dispersive X-ray Spectrometry (SEM- EDS) analysis maps in strawberry leaf samples

EDS Analysis – Silica Weight % in Strawberry Leaf Sample upon

0mL

2mL



3mL

4mL

Si treatment effect on strawberry canopy growth

0mL

2 mL

3 mL

4 mL

Plants treated with 2mL exceeded the growth size than 3mL, 4mL treatments and control

Effect of Potassium Silica Treatments on Strawberry Agronomic Traits Under High Tunnel Conditions

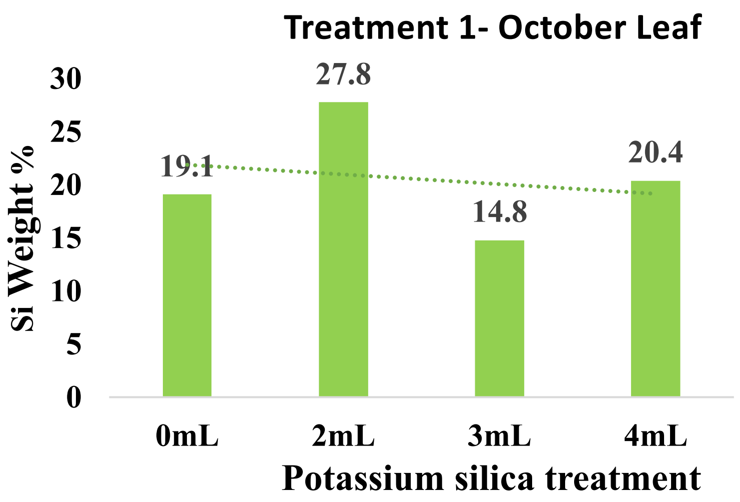
Treatment	Marketable yield (g)		Non-marketable yield (g)		Height (cm)		Width (cm)	
	Flavor	RS	Flavor	RS	Flavor	RS	Flavor	RS
0mL	33.00^{b}	31.57^{b}	13.20^{bc}	9.35^{c}	20.22^{ab}	19.55^{ab}	150.02^{b}	143.21^{b}
2mL	80.20^{a}	82.80^{a}	28.40^{a}	24.00^{ab}	22.83^{a}	19.41^{ab}	183.16^{a}	152.81^{b}
3mL	88.33^{a}	40.00^{ab}	13.60^{abc}	13.60^{abc}	19.91^{ab}	17.75^{b}	156.73^{b}	111.99^{c}
4mL	57.80^{ab}	47.20^{ab}	19.80^{abc}	16.60^{abc}	19.83^{ab}	22.91^{a}	152.55^{b}	158.83^{a}
Treatment (T)	**		*		NS		***	
Genotype (G)	NS		NS		NS		**	
G*T	NS		NS		NS		**	

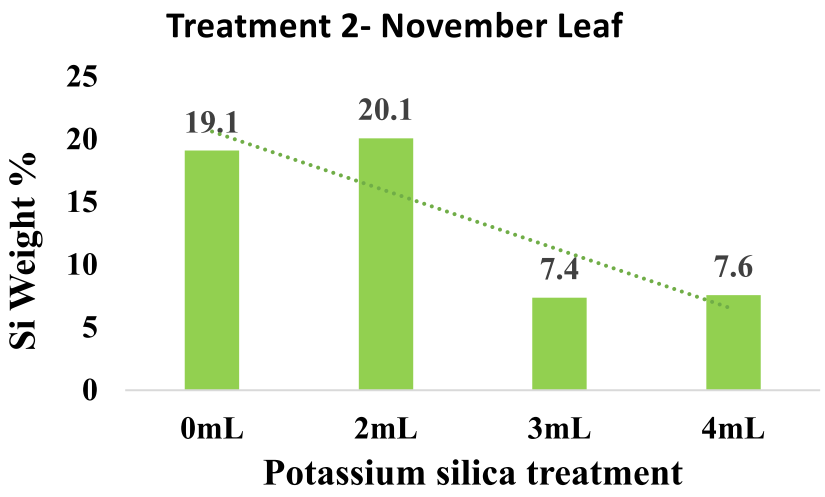
Flavor: Flavorfest, RS: Rutgers Scarlett's

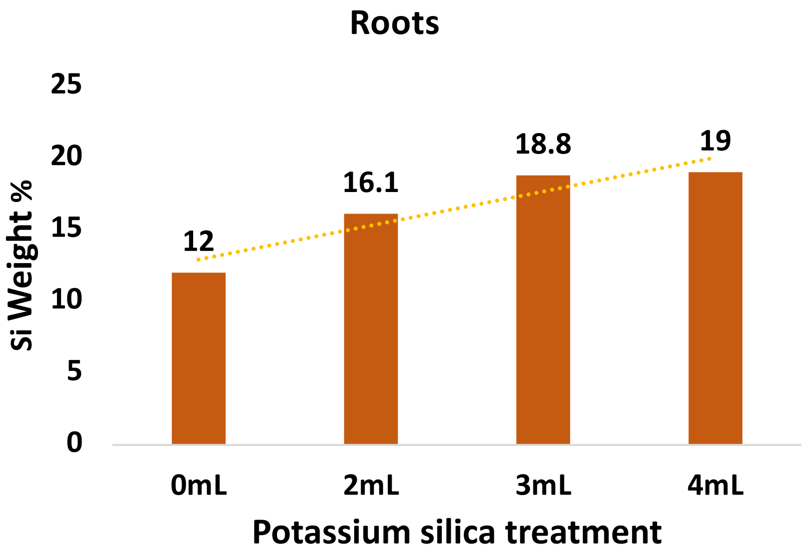
*, **, *** P< 0.05, P< 0.01, P< 0.001, significance levels, respectively, NS- Non-Significant

Si Concentrations in Strawberry Plant Samples

SEM-EDS analysis







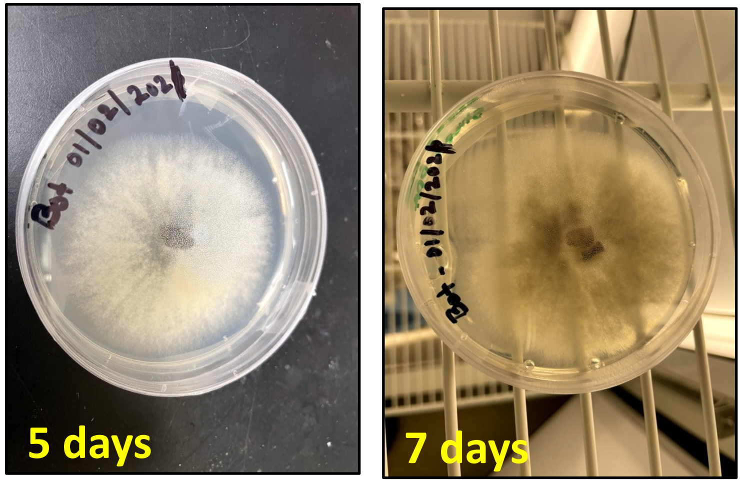
- **Strawberry leaf samples accumulated higher concentrations of silica at 2mL treatment compared to other treatments.**
- **However the increasing trend of silica accumulation in 2mL was not observed in the root samples.**

Summary

- The 2mL/g treatment was very effective under high tunnel conditions for strawberry cultivation and significantly improved agronomical traits such as growth and yield
- Spec and microscopic analysis showed the higher accumulation of silica upon 2mL/g treatment in leaf samples compared to other treatments
- The 2mL/g concentration was effective in reducing the *B. cinerea* growth under *invitro* conditions

Project 2: Evaluation of strawberry genotypes for gray mold disease resistance

Botrytis cenerea



B. cenerea **growing on PDA media**

Disc method

Vegetative

Flower

Fruit

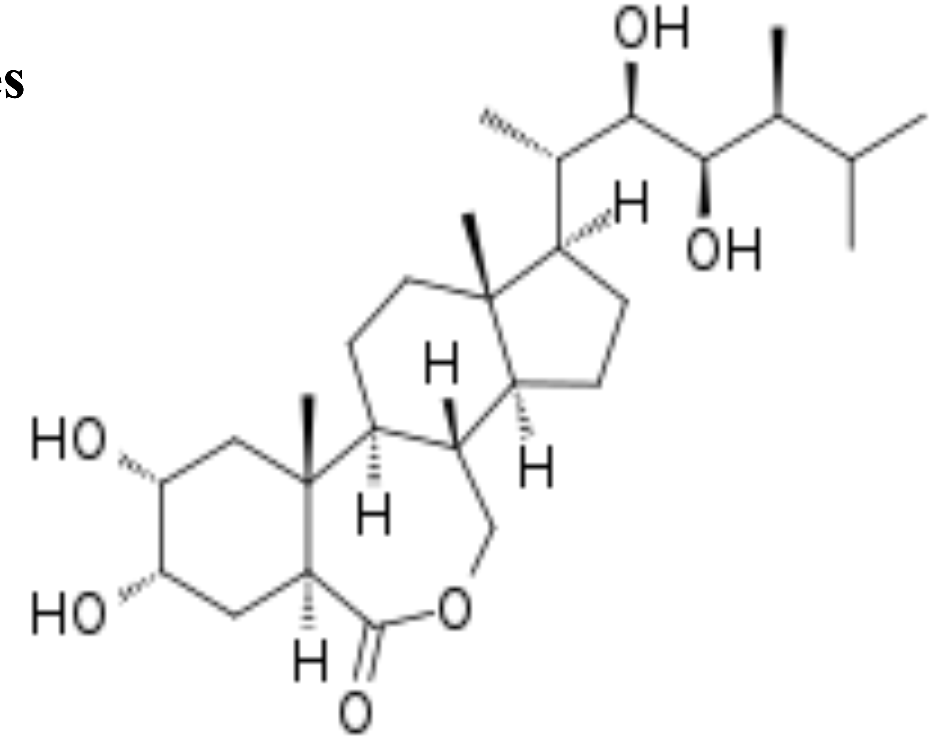
Control

Infected

Department of Agriculture & Natural Resources: Plant Molecular Breeding Lab

Brassinosteroid (BR)

- Synthesized from basic hormone; campesterol
- Discovered in *Brassica nupus* (Rapeseed/ Canola)
- Plant hormone structurally similar to animal steroid hormones
- BES1 key gene in transduction pathway
- Plays role in:
  - Root growth
  - Reproduction
  - Cell elongation/division
  - Immunity
- Mediate plant response to stress:
  - Freezing, Drought, Salinity, Heat/ nutrient deficiency



(Planas-Riverola et al., 2019).

Brassinosteroid

- **Cross talk with Salicylic acid (SA), and Jasmonic acid (JA) during plant stress**
- **BR regulates ROS production in plants under stress**
  - **Unstable ROS leads to multiple adverse effect on plants**
- **BR enhances plant tolerance to biotic and abiotic**
  - **By activating BZR1/BES1 transcription factors**
- **Application of BR at low concentrations significantly improves:**
  - **growth and yield**
  - **increases resistance to viral and fungal pathogens**

(Planas-Riverola et al., 2019).

Objectives

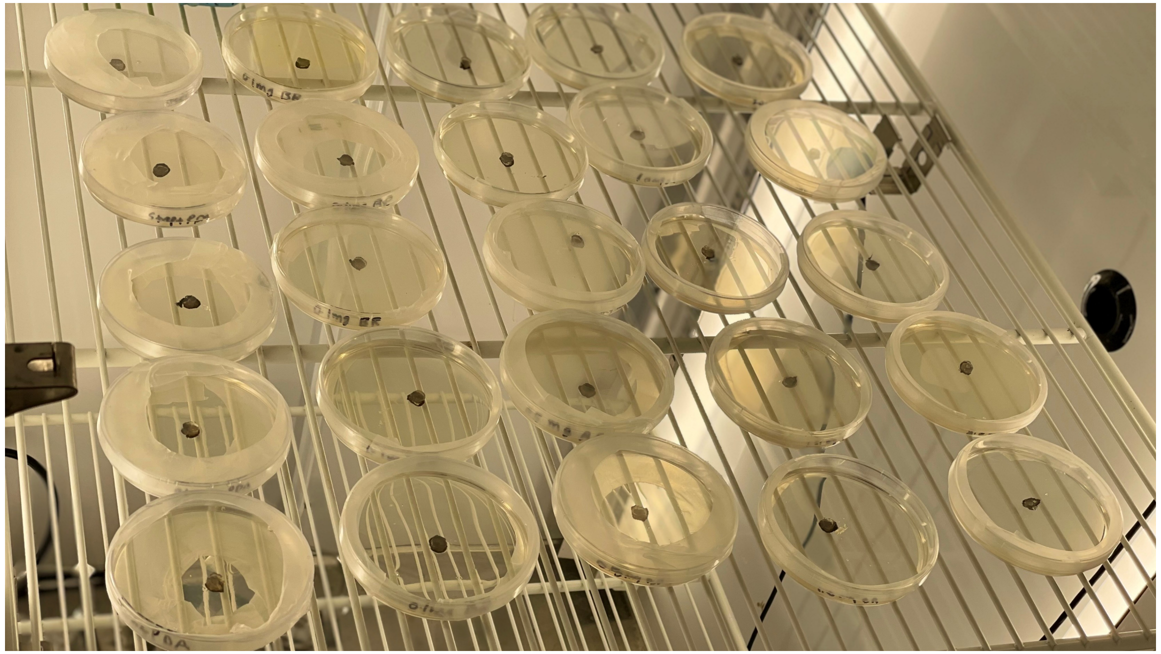
1. **Find optimal dose of BR to alleviate the infection of *B. Cinerea on Strawberries***
2. **Observing Physiological characterization of strawberry plants in response to botrytis infection upon BR application.**
3. ***In vitro* evaluation of brassinosteroid effect on *B. cinerea* mycelium growth.**
4. **Evaluate the role of BR in the enhancement of plant growth.**

invitro Evaluation of EP24 on Fungal Growth

1 day after inoculation of of B. cinerea on Epibrassinosteriod PDA

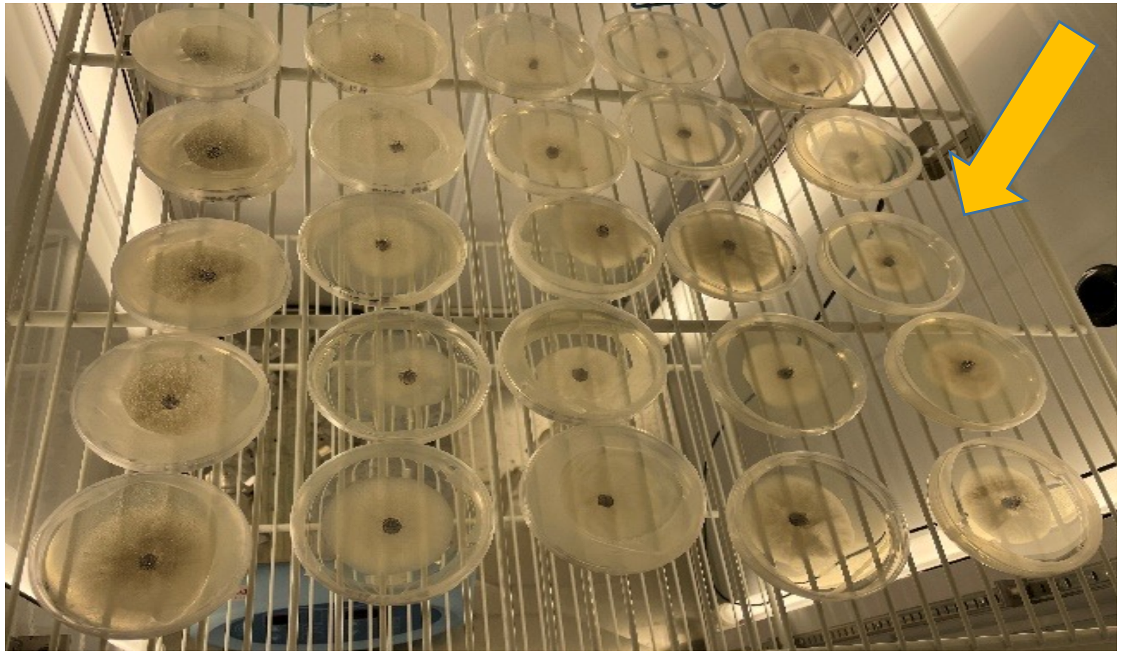
4 days after Inoculation of B. cinerea on Epibrassinosteriod PDA

A.



0mg 0.1mg 0.5mg 0.75mg 1.0mg

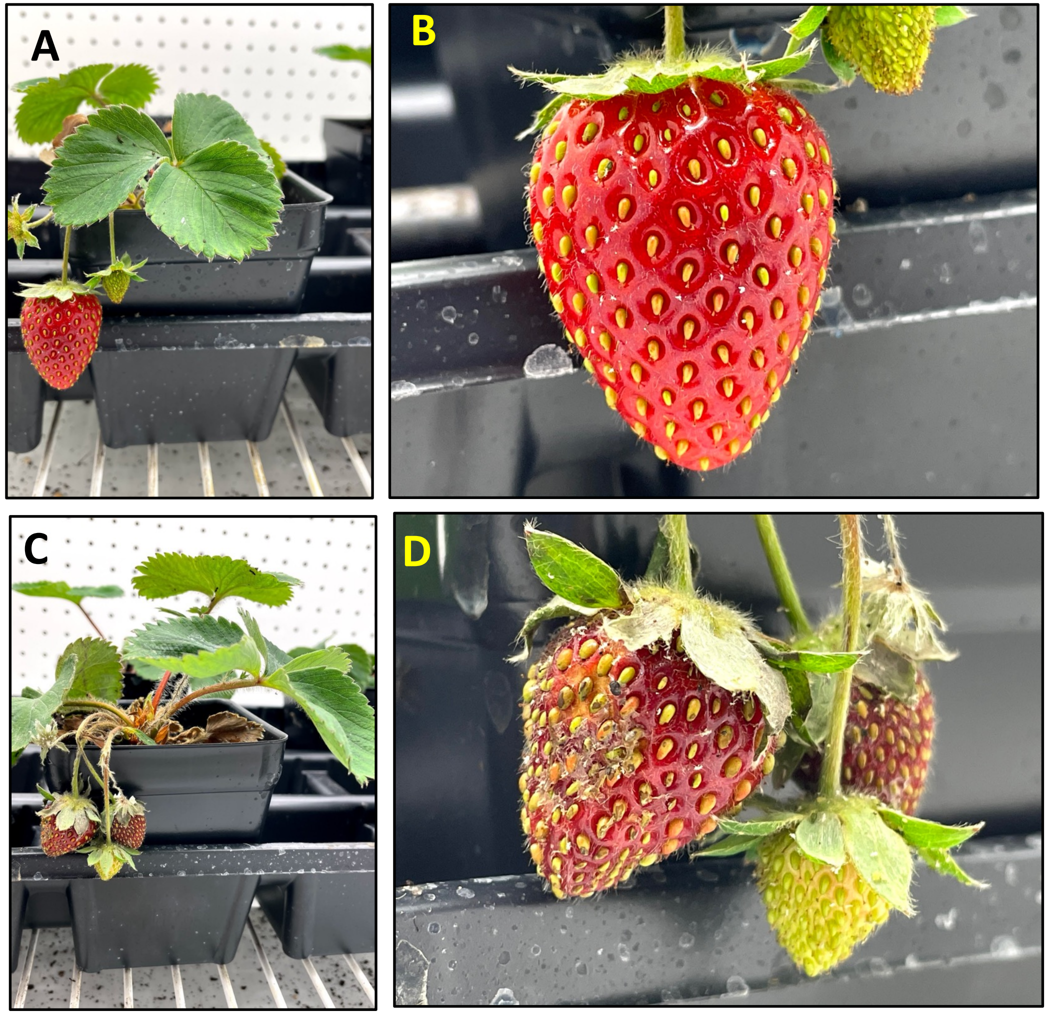
B.



0mg 0.1mg 0.5mg 0.75mg 1.0mg

In vitro evaluation of *Botrytis cinerea* treated with increasing EP24 concentrations. Columns of plates from left to right, control, EP24 BR doses 0.1mg, 0.5mg, 0.75mg, and 1.0 mg. **(A)** PDA plates of *B. cinerea* and EP24 doses at 0 DPI. **(B)** PDA plates of *B. cinerea* and EP24 doses 4 DPI.

Application of Epibrassinosteroid to Alleviate *Botrytis cinerea* Infection in Strawberry (*Fragaria x ananassa)*



A) Control- whole plant
B) B) control fruit

C) *B.cenerea* infected plants
D) *B.cenerea* infected fruits- Genotype- Flavorfest

Evaluation of *B. cinerea* on Epibrassinosteriod PDA

No epibrassinoid

Treatment	Measurements (mm)				
	0DPI	1DPI	2DPI	3DPI	4DPI
Control	1.6	3.6	45	55	70
0.1mg	1.6	4	27	35	66
0.5mg	1.6	4.3	34	48	67
1.0mg	1.6	3.8	29	53	60

Take Home Messages

- We need blueberry varieties which can tolerate erratic heatwaves and high temperature stress: we are making small steps towards reaching this goal
- Strawberries can absorb potassium silicate on foliar application
- 2.0 mL per gallon potassium silicate solution is effective in slowing down of gray mold fungal growth in high tunnel/ lab settings
- External application of Epibrassinosteriod (EP 24) slowed down the gray mold fungal growth on strawberry plants and fruits.

Acknowledgements

Dr. Nicholi Vorsa, Rutgers, The State University of New Jersey
Dr. Umesh K. Reddy, West Virginia State University
Dr. Sathya Elavarthi, Delaware State University
Dr. Kim Lewers, USDA-ARS, Beltsville, MD
Dr. Jayesh Samtani, Virginia Tech
Dr. Toktam Taghavi, Virginia State University
Dr. Massimo Iorizzo, North Carolina State University
DSU team

USDA

Capacity Building grants and AFRI grant

Acknowledgements

Lab Website: https://melmaieelab.github.io/index.html