

# College of Food, Agricultural, and Environmental Sciences

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2022 Annual Research Conference

**CFAES**



**THE OHIO STATE UNIVERSITY**

COLLEGE OF FOOD, AGRICULTURAL,  
AND ENVIRONMENTAL SCIENCES

# 2022 Annual Research Conference

- Directors Welcome and Remarks
- Recap of Faculty Research Awards
- Undergraduate Student Poster Awards
- Graduate and Professional Poster Awards



# CFAES Strategic Alignment: Goals

- Student-First Philosophy
- Innovative Scholarship to Sustain Life
- Capacity Building of Our People and Communities
- Partner of Choice
- Resource Stewardship in a One College Model



# CFAES Strategic Alignment: Grand Challenges

- **Sustainability** – simultaneously ensuring viable ag production, food security and safety, and environmental and ecosystem sustainability
- **One health** – Studying the intersections or interactions among human, animal, and environmental health
- **Rural-urban interface** – exploring the tensions and opportunities created in the communities, industries, policies, economies, and communications among urban and rural residents
- **Leadership** – preparing the next generation of scientists and leaders





**CFAES**

# CFAES MASTER PLAN

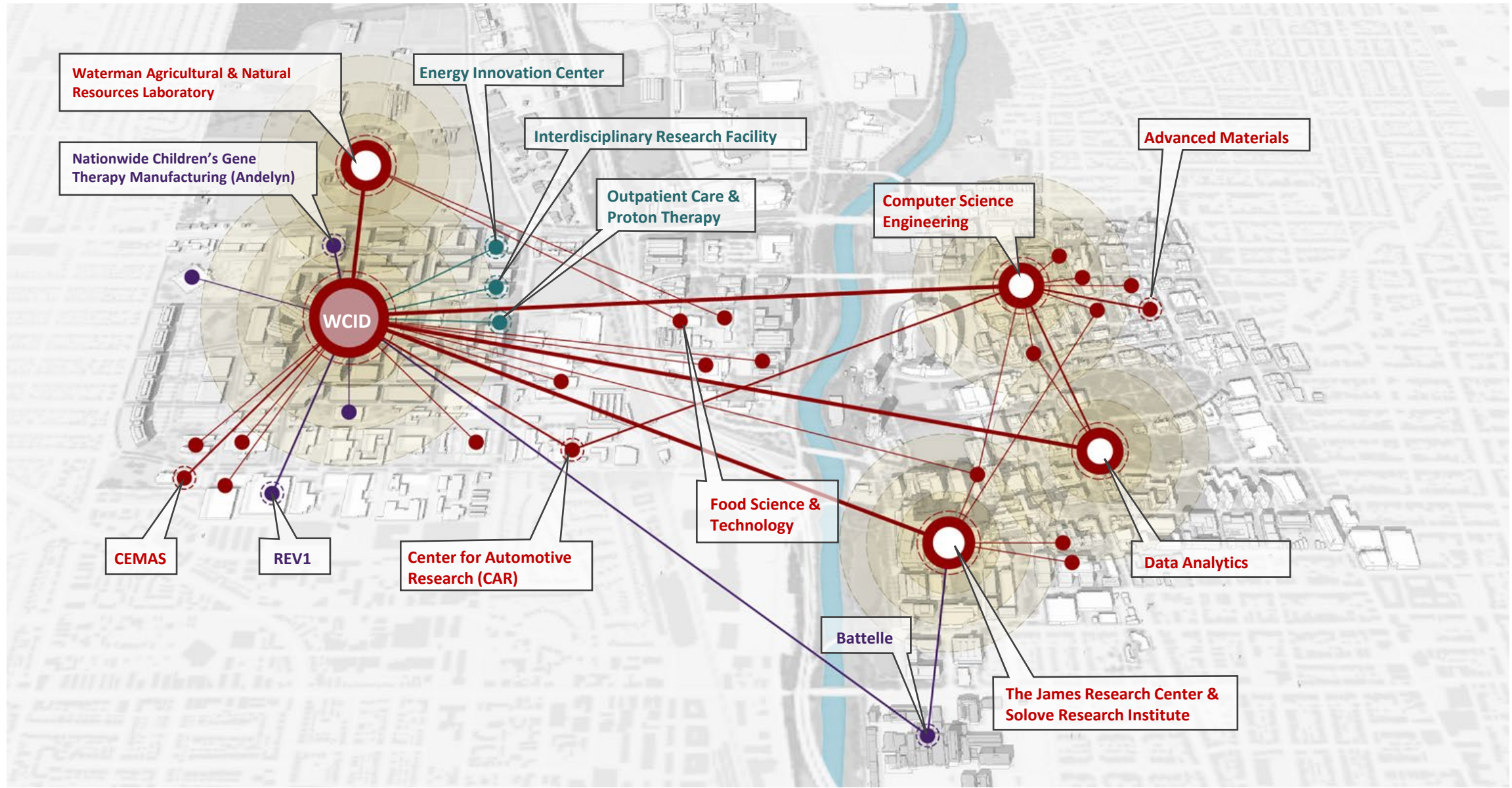
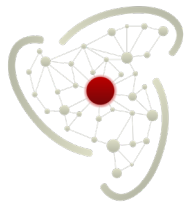
AUGUST 2021



  
**THE OHIO STATE  
UNIVERSITY**  
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AND ENVIRONMENTAL SCIENCES



# WCID | THE MOMENTUM



# West Campus Innovation District Interdisciplinary Research Facility



The Interdisciplinary Research Facility will catalyze convergence research in life sciences and biotechnology while providing new opportunities for the community and industry to engage Ohio State researchers.



# Controlled Environment Food Production Research Complex (CEFPRC) – 50,000-SF

## Location

- Waterman

## Scope

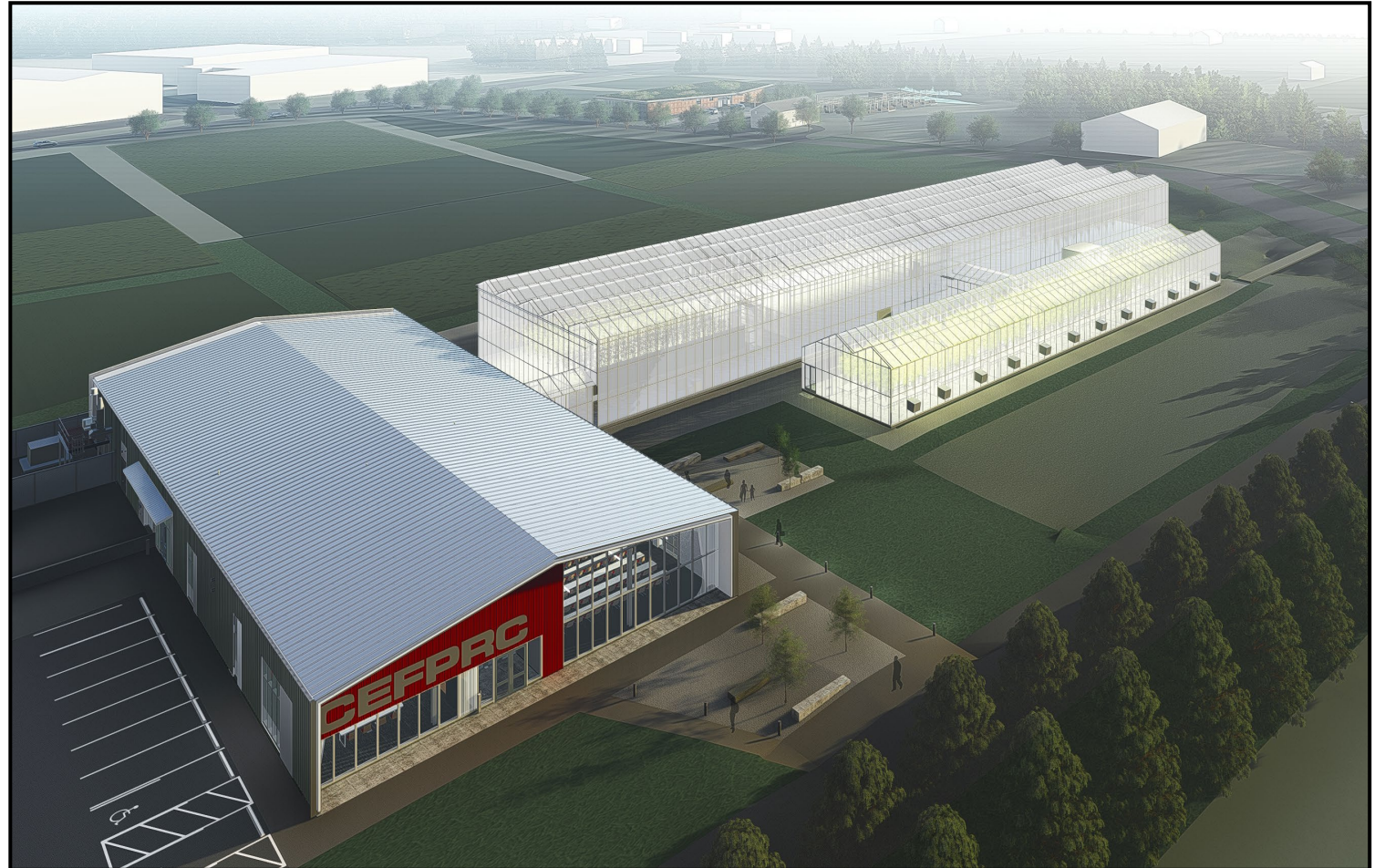
- Production Greenhouse
- Research Greenhouse
- Indoor/Outdoor Learning
- Headhouse & Offices
- Sustainable Features

## Budget: \$35.8M

- Philanthropy
- CFAES

## Completion

- Fall 2022







# CEFPRC March 2022

Open Fall 2022



# George Washington Carver Science Park

Nanoracks: \$140 M from NASA

OSU: Projected \$14 M

CFAES and COE

HCS, FABE, PP



# GWC Science Park Founding Partners



**Voyager Space**  
Strategy Lead

Advisory Board

**Nanoracks**  
Owner and Operator

Management Team

**Lockheed Martin**  
Spacecraft Integration Lead



**DreamUp**  
STEM Education Advisor

- Coordinate youth and college STEM education efforts



**Ohio State University**  
University Consortium

- Coordinate university research
- Manage terrestrial research analog facilities
- Provide inputs into lab development efforts.



**Universities Space  
Research Association**  
Director, GWC Park

- Manage the GWC Science Park
- Oversee the scientific operations of the lab



**International Association  
of Science Parks**  
Global Engagement

- Foster a global network of academic and commercial researchers
- Build research pipeline to grow GWCSP utilization



**ZIN Technologies**  
Component Development

- Overall lab design, component upgrades, and overall architecture
- Develops key lab subcomponents

**Industry expertise spanning decades of NASA projects,  
commercial development, and worldwide academic research**



We do controlled environment!



# RESEARCH IN REVIEW

FISCAL YEAR 2021

## AWARDS & SUBMISSIONS

**\$50 M**

**637** Proposals Submitted  
**344** Total Awards

## RESEARCH EXPENDITURES

**\$44 M**

Sponsored Programs  
**>1,000** Active Projects

## FEDERAL CAPACITY FUNDING

**\$8.7 M**

Federal support allocated to each state as a land-grant institution.

## INTELLECTUAL PROPERTY

**25** New Innovators  
**18** Patent Filings  
**7** Active Startups  
**38** Invention Disclosures



## SPONSORED PROGRAM FUNDING SOURCES

Federal	25%
Industry	20%
Colleges/Universities	19%
Private Agency	17%
State	12%
Other Non-Federal	7%



## ACTIVE PROTOCOLS

IACUC **170**



IBC **119**



IRB Exempt **1130**



IRB Approved **160**



## NEW INVENTION DISCLOSURES BY TYPE

**18** Plant Variety



**4** Research Tools



**16** Technology



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## CONTACT US

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Website: [research.cfaes.ohio-state.edu](http://research.cfaes.ohio-state.edu)



# Additional Research Highlights

- Number of proposals submitted increasing
- 10 proposals requesting \$5 M+
  - 2 funded, some still pending
  - 2 funded @ \$1 M+, 18 @ \$500k - \$1 M
- Graduate enrollment increasing
- Inaugural The Ohio Program Fellowship





# Additional Research Highlights

- CFAES only college to maintain SROP summer 2021
- Embedded mental health counselor CFAES Wooster
- Stone Lab under CFAES umbrella
- Inaugural cohort: STARS





# Looking Ahead

- Continued emphasis on faculty development
- Energy Advancement and Innovation Center
- Emerging topics: AI, microbiome science, controlled environment food production, etc.
- New faculty hires
- Kellogg Chair & AMP



# Research Summary

- Strategic Alignment and Master Plan provide path forward
- Graduate programs and funding in good standing
- IRF and CEFPRC will add much needed capacity
- Research enterprise continues to evolve



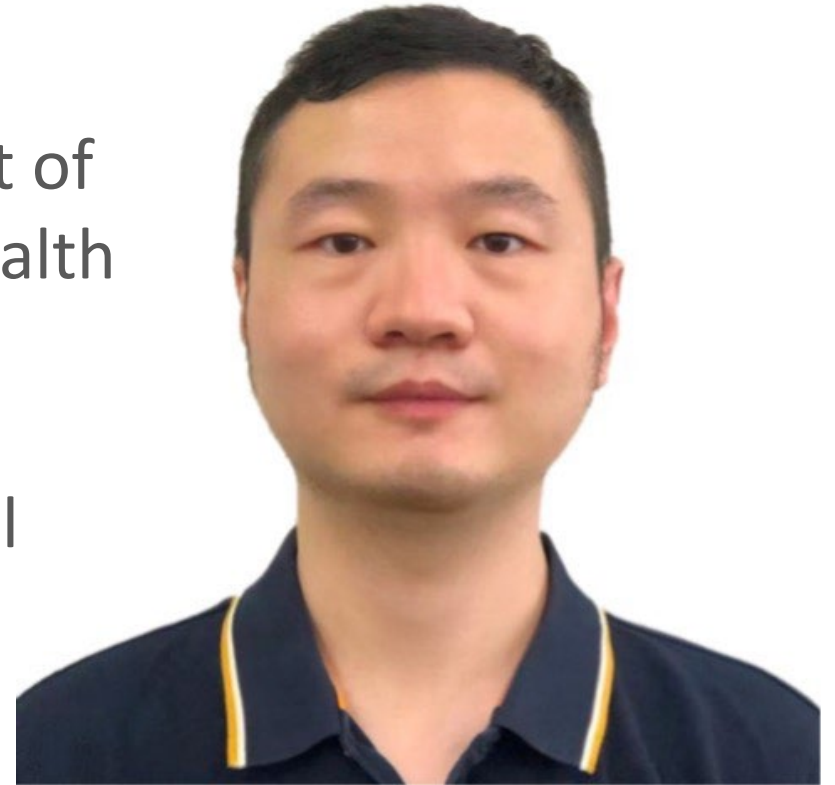
# **William E. Krauss Director's Award *for Excellence in Graduate Research***

## **Yusheng Guo**

Graduate Research Associate in the Department of  
Animal Sciences and Center for Food Animal Health

### **Advised by Anastasia Vlasova**

Assistant Professor in the Department of Animal  
Sciences and Center for Food Animal Health





# 2022 CFAES Faculty Awards

## *Graduate Mentor Award*

**Monica Giusti**

Professor and Graduate  
Studies Chair in the  
Department of Food Science  
and Technology



# 2022 CFAES Faculty Awards

*Distinguished International Research and Engagement Award*

## Clay Sneller

Professor in the Department of  
Horticulture and Crop Science



# 2022 CFAES Faculty Awards

## *Distinguished Junior Faculty Research Award*

**Laura Lindsey**

Associate Professor in the Department  
of Horticulture and Crop Sciences





# **2022 CFAES Faculty Awards**

## ***Distinguished Senior Faculty Research Award***

### **Gireesh Rajashekara**

Professor in the Department of Animal Sciences and Interim Head of the Center for Food Animal Health

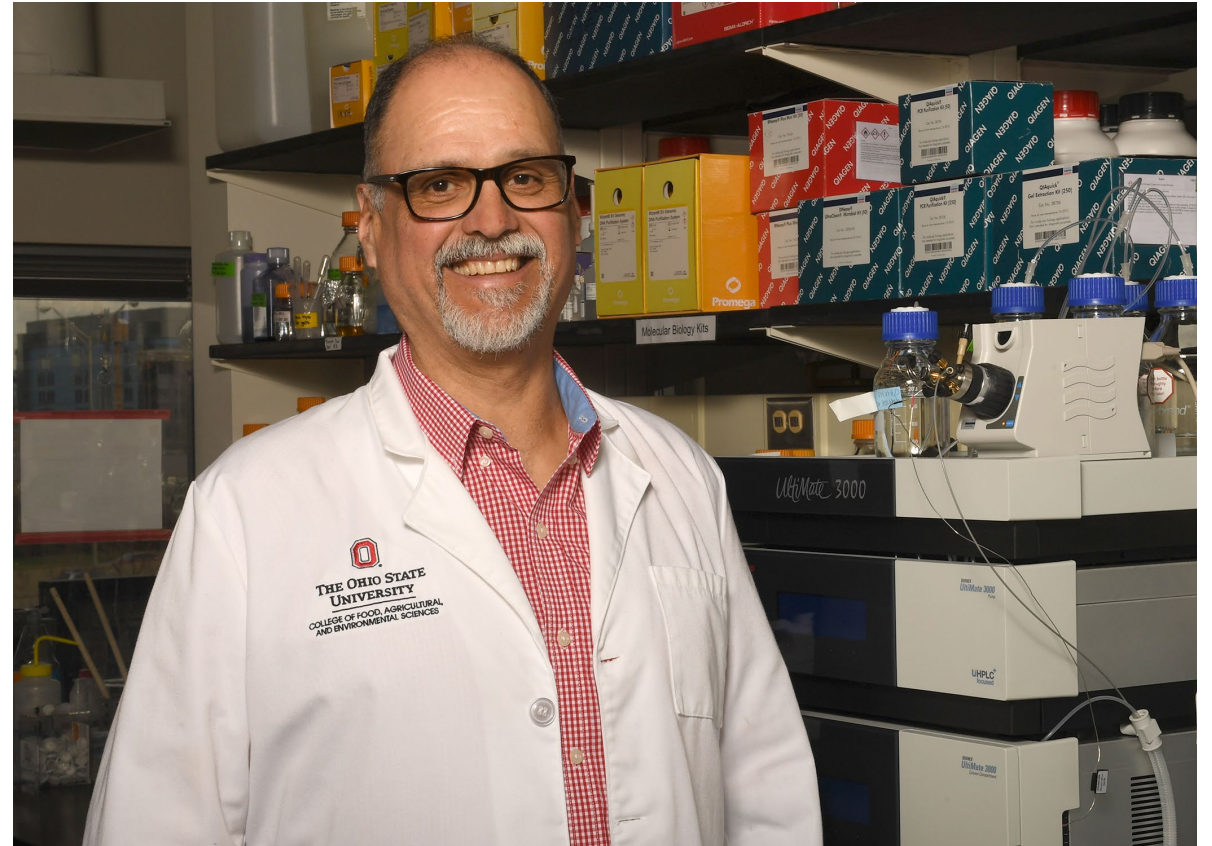


# 2022 CFAES Faculty Awards

## *Innovator of the Year Award*

**Rafael Jimenez-Flores**

Professor in the Department of  
Food Science and Technology





# **Congratulations Faculty & Krauss Award Winners!**

# Graduate School Awards

## Leadership Award (GALA)

**Ellia La, Ph.D. Candidate**

Department of Food Science &  
Technology

**Adrian Pekarcik, Ph.D. Candidate**

Department of Entomology  
CFAES - Wooster Campus

## Teaching Award (GATA)

**Jai Tiarks, M.S.**

School of Environmental and Natural  
Resources



# CFAES Presidential Fellows 2022

**Rafael Quijada Landaverde**

Agricultural Communication, Education, and Leadership

Advisor: Mary Rodriguez

**Taylor Klass**

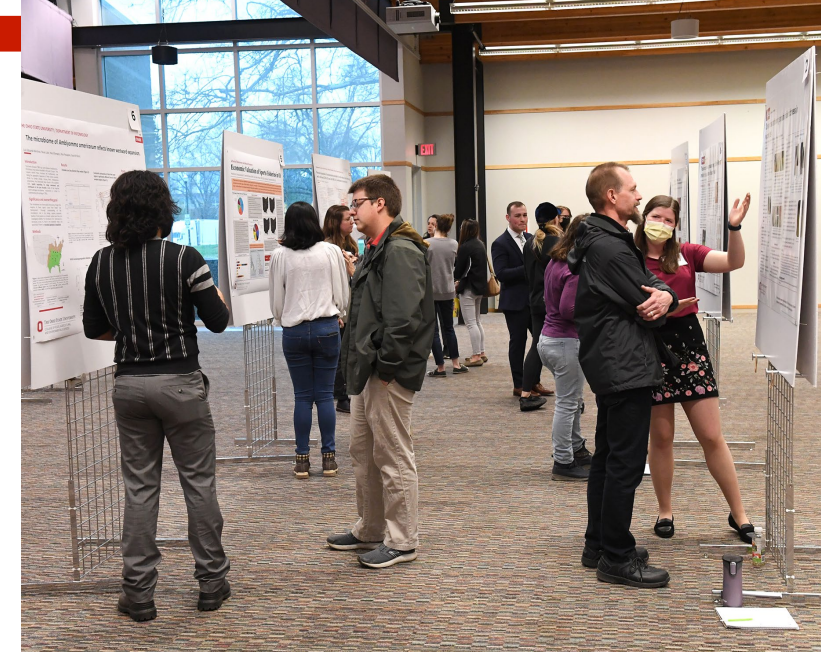
Plant Pathology

Advisor: Jonathan Jacobs



# Congratulations Graduate Award Winners!







# Undergraduate Competition Winners

# Animal Sciences - Health

## First

**Madison Pinkerton** *advised by Dr. Jessica Pempek*

## Second

**Elizabeth Ohl** *advised by Dr. Alvaro Garcia Guerra*

## Third

**Hailey Main** *advised by Dr. Christopher Hadad and Dr. Christopher Callam*

# Animal Sciences - Nutrition

First

**Sophia Kienzle** *advised by Dr. Jeffrey Firkins*

Second

**Elizabeth Due** *advised by Dr. Sheila Jacobi*



# Entomology/Environmental Sciences

First

**Aleacia Laird** *advised by Dr. Francesca Hand*

Second

**Isabel Nazarian** *advised by Dr. Reed Johnson*

Third

**Nicole Sammons** *advised by Dr. Reed Johnson*

# Food Science & Technology

First

**Talia Katz** *advised by Aishwarya Badiger*

Second

**Cameron McCurdy** *advised by Dr. Kichoon Lee*

Third

**Thania Ortiz Santiago** *advised by Dr. Monica Giusti*

# Social Science

## First

**Shanvanth Arnipalli** *advised by Dr. Feng*

## Second

**Maddie Allman** *advised by Dr. Ken Martin & Dr. Joe Campbell*

## Third

**Kiley Holbrook, Callee Aviles, Haley Schmersal** *advised by Erica Summerfield*



# Master's Competition Winners

# 2022 CFAES Poster Competition Master's Category, Third Place

CFAES

## Brandon Shannon Entomology & Environmental Sciences Advisor: Dr. Reed Johnson

Department of Entomology

### Toxicity of Spray Adjuvants and Tank Mix Combinations To Adult Honey Bees

Brandon Shannon (Shannon.325@OSU.edu) / Emily Walker / Reed Johnson

#### Introduction

##### Honey Bee Almond Pollination

- 73% of total U.S. honey bee population (1.9 million colonies) is transported to California to pollinate almonds [1]
- Deaths of adult honey bees have been reported by beekeepers during almond pollination (Feb. 15 – Mar. 15) [2]

##### Spray Adjuvants and Pesticide Tank Mixtures

- Pesticides, such as fungicides and insecticides, are combined with spray adjuvants into tank mixtures, which are commonly sprayed during almond bloom and may contribute to honey bee mortality
- Spray adjuvants contain one or more 'principal functioning agents', some of which are known to harm bees [3]
- Our hypotheses were that adjuvant sprays are toxic to honey bees

- When applied alone
- When applied in tank mix combinations with pesticides

##### Adjuvant, Insecticide, and Fungicide Usage in Almond Orchards

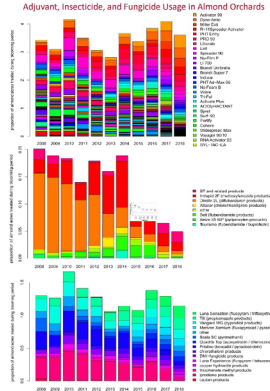


Figure 1. Proportion of bearing almond acres (1,011,455 acres in 2018) to which spray adjuvants (A) insecticides (B) and fungicides (C) were applied during almond bloom (Feb. 15 – Mar. 15) in California.

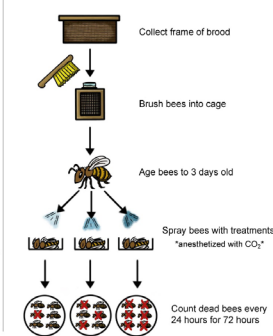
#### Materials and Methods

##### Pesticide Matrix

- Pesticide use data were obtained from the California Pesticide Information Portal (<https://calpi.cdpr.ca.gov/main.cfm>)
- Spray adjuvant, insecticide and fungicide use from 2008-2018 were summarized (Figure 1) and testing was conducted on commonly used formulated pesticide adjuvants, insecticides and fungicides
- Treatment concentrations with formulated products were determined based on multiples of the maximum application rate listed for almonds on the product label

##### Spray Treatment

- Frames of brood were collected from hives and aged to 3 days old in an incubator (34°C, 80% RH and continuous darkness)
- Adult bees were sprayed with formulated products or combinations in DI water using a Potter Spray Tower at 1X to 30X the label rate
- Water alone was the negative control and Mustang Max (active ingredient zeta-cypermethrin) the positive control
- Data were analyzed by fitting a two-parameter logit model to each treatment in R. The fit of the dose-response relationship was evaluated and LC50s were estimated [6]. Significant differences between treatments were determined with a ratio test [5].



##### Performing Potter Spray Tower Applications



#### Results

##### Pesticides Alone

- Of the four fungicides tested (Pristine, Tilt, Vanguard, and Luna Sensation), none had a significant dose-response curve when applied without an adjuvant.
- Of the two insecticides tested (Altorac and Intrepid), neither had a significant dose response curve when applied without an adjuvant.

##### Adjuvants Alone

- Of the twelve adjuvants tested, seven showed an LC50 below the maximum tested rate of 30X (Figure 2).

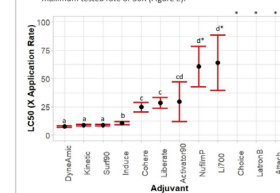


Figure 2. Estimated LC50 of adjuvants when applied alone, expressed as multiples of the application rate. Error bars indicate 95% confidence intervals. An asterisk indicates an estimated LC50 above the maximum application rate tested. Letters indicate a significant difference in the LC50 estimates using a ratio test (p-value < 0.05) [5,6].

##### Adjuvant-Pesticide Combinations

- Seven adjuvants (Dyne-amic, Kinetic, Surfact, Induce, Cohere, L700, and Nufin P) were tested for toxicity in tank mix combinations with pesticides. Of these seven, three showed significantly increased toxicity in tank mix combinations.

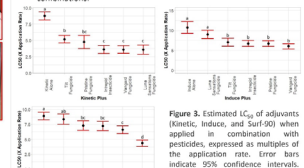


Figure 3. Estimated LC50 of adjuvants (Kinetic, Induce, and Surfact) when applied in combination with pesticides, expressed as multiples of the application rate. Error bars indicate 95% confidence intervals. Letters indicate a significant difference in the LC50 estimates using a ratio test (p-value < 0.05) [5,6].

#### Discussion

- Some adjuvants are inherently toxic to honey bees and can have increased toxicity when combined in tank mixes
- All adjuvants are not equivalent in their toxicity to bees or potential for interacting with pesticides.
- Toxicity is increased for adjuvants with a higher labeled application rate, shown with a Spearman's Correlation ( $R = -0.64$ ,  $p = 0.024$ )
- The seven adjuvants with an estimated LC50 below the maximum application rate tested were applied on nearly 250,000 acres of almond orchards and affect about 500,000 colonies
- The Almond Board of California recommends against adjuvant use
- Future work will test adjuvant 'principal functioning agents' to determine the mode of action of toxicity to honey bees.

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- Mullin, C.A. Effects of 'toxic' ingredients on bees. Current Opinion in Insect Science. 2015;10:291-295. doi:10.1016/j.cis.2015.01.005.
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#### Acknowledgements

We are grateful to Celeste Welby, Joe Reed, and Larry Phelan for providing pesticides used. We are also grateful to Dylan Riecke for their help in bee wrangling, counting, and feeding. The work reported on this poster was supported by state and federal appropriations to the Ohio Agricultural Research and Development Center (OH001277) and the Almond Board of California (POLL17).

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WOOSTER

# 2022 CFAES Poster Competition

## Master's Category, Second Place

**Camila Gutierrez Manriquez**  
Department of Horticulture and Crop Sciences  
Advisor: Dr. Jonathan Fresno Ramirez

### CFAES/Horticulture and Crop Science

## Identifying genetic markers for self-compatibility and herbicide tolerance in rubber dandelion (*Taraxacum kok-saghyz*)

Camila Gutiérrez Manriquez<sup>1</sup> and Jonathan Fresno Ramirez<sup>1</sup>

<sup>1</sup> The Ohio State University, Department of Horticulture and Crop Sciences.

### Introduction

#### Natural rubber (NR) a global critical biomaterial

NR is a global key biopolymer used to manufacture more than 50,000 products, from the soles on our shoes to airplane tires. For the nonce, NR production has been entirely based on a single species, the rubber tree (*Hevea brasiliensis*) which grows in tropical regions, such as Amazon, Southeast Asia and West Africa. Whereas the USA produces zero NR, is the third biggest consumer globally, 1 million tons/year, all of which is currently imported<sup>1</sup>.

Nowadays global supply of NR is threatened by an augmenting consumption and demand, diseases that risks its production and vulnerable trade chains. Besides, it is worth to point out that artificial rubber would not cover all the uses of gum, because has not the same quality than NR in compression and impact resistance. Thus, the concern about a NR shortage has led to exploring new sources of this biomaterial.

#### *Taraxacum kok-saghyz* (TK) as a new NR source

Rubber dandelion (*Taraxacum kok-saghyz*), native from Kazakhstan, Uzbekistan, and China, has been identified as a promising alternative source of NR thanks to the high molecular weight of the biopolymer accumulated in its roots<sup>2</sup>, and moreover, it is adapted to grow in the USA. The current principal disadvantage is that rubber dandelion continue to be undomesticated. In addition, it is an obligate outcrossing species, which precludes gaining homogeneity in the germplasm quickly and then producing stable cultivars. Besides, it is outcompeted by weeds in outdoor settings. To turn this wild species into a competitive and profitable crop for agriculture is imperative to improve its germplasm, incorporating and enhancing features that improve performance on the field. Thus, this study seeks to improve two agronomic crucial traits such as self-compatibility and herbicide tolerance.

#### Natural rubber global trade

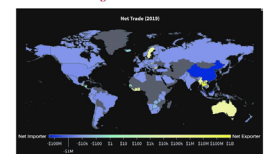


Figure 1. Natural rubber global trade, including production, self-sufficiency and imports. Source: The Observatory of Economic Complexity, 2021. Retrieved March 15, 2022. <https://www.economico.com/en/visualizations/2021/03/15/natural-rubber-global-trade/>

### Aim

This study pursue to interrogate, enhance, and combine self-compatibility and herbicide tolerant in novel rubber dandelion germplasm.

### Objectives

- Generate segregating germplasm and identify single nucleotide polymorphism markers.
- Enable quantitative trait loci mapping to determine genetic components involved in the target traits.

### Materials

Germplasm previously identified and developed within PENRA.

Self-seeders "candidate" lines:

- OH-1004: 9<sup>th</sup> generation self-seeding
- OH-0061: 6<sup>th</sup> generation self-seeding
- OH-0205: 2<sup>nd</sup> generation self-seeding
- Herbicide-tolerant accessions
- Tolerant to ALS (Imazethapyr)
- Tolerant to PPO (Sulfentrazone & Flumioxazin)

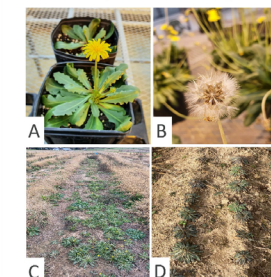
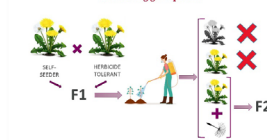


Figure 2. A. Rubber dandelion (TK) growing in the field. B. Rubber dandelion (TK) growing in the field. C. Rubber dandelion (TK) growing in the field. D. Rubber dandelion (TK) growing in the field.

### Methods

#### A. Building germplasm



#### B. Building a genetic map

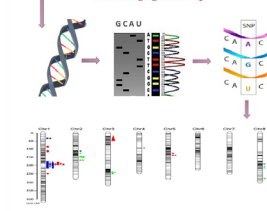


Figure 3. Methods flow diagram. A. Germplasm from self-compatible "candidate" lines and flowering clones are crossed, with 10 grown in greenhouse under controlled weather conditions, and growing chambers to early reveal selection responses and promote flowering. B. For the next generation, a selfing cross is made between self-compatible and herbicide-tolerant accessions to generate F1. After selfing, F1 and F2 offspring that will segregate for both traits independently and together. C. From developed families, F1 will use for selfing to identify single nucleotide polymorphism markers to perform a linkage map that will enable QTL mapping to determine genetic components involved in self-compatibility and herbicide tolerance.

### Expected results

It is expected to develop novel and relevant germplasm with self-compatibility and herbicide tolerance combined, as well as identification of regions on the rubber dandelion genome governing those targeted traits, which will finally enable identifying genomic polymorphisms for implementation of marker-assisted selection to expedite the domestication of TK.

### CFAES

### Rubber dandelion for seed and root production



Figure 4. "TK" rubber dandelion growing in greenhouse for seed and root production.

### Pitfalls

The most significant pitfall would be the loss of plants due to neglected management in the greenhouses and growing chambers. Periodic visits will be needed to appropriate monitoring and caring. Besides, summer temperature had caused problems in flowering, making crosses more challenging to achieve. Therefore, I will avoid these activities during the hottest periods; instead, I will put plants to vernalize into the growing chambers during summer season.

RNA extractions with proper quality can be complex due to concentrations of polysaccharides in leaves, considering previous experiences in our team, which may delay sample preparation for genetic analysis and sequencing. It makes imperative to follow the protocols with extreme meticulousness.

### Bibliography

1. Cornish, K. (2017). Alternative Natural Rubber Crops: Why Should We Care? Technology & Innovation, 18(4), 244-255. <https://doi.org/10.21300/18.4.2017.245>
2. Leading natural rubber consuming countries 2020 | Statista. (n.d.). Retrieved March 22, 2022, from <https://www.statista.com/statistics/275392/top-10-consumers-of-natural-rubber/>
3. Hodgson-Krasky, K. J. M., Stoffyn, O. M., & Wilyun, D. J. (2017). Recurrent Selection for Rubber Yield in Russian Camellia. Journal of the American Society for Horticultural Science, 142(6), 470-475. <https://doi.org/10.21273/ASH50425-17>

### Acknowledgements

I thank Mr. Brandon Wheeler for the training in greenhouses related activities. Funding was provided by the PENRA Consortium, DARDC, and USDA NIFA AFRI Project Number 2020-07013-20576.



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WOOSTER



# 2022 CFAES Poster Competition

## Master's Category, First Place

**Yuan Li**  
Entomology  
Advisor: Dr. Peter Piermarini

Department of Entomology

### A Putative Role of Renal (Malpighian) tubules in Regulating Calcium Homeostasis in the *Aedes aegypti* mosquito

Yuan Li and Dr. Peter Piermarini

CFAES

#### Introduction

##### Background

- Calcium ( $\text{Ca}^{2+}$ ) plays important roles in insect physiology.<sup>1-3</sup>
  - Dietary regulation
  - Signal transduction
  - Egg development
- Malpighian tubules (MTs) are a major site for  $\text{Ca}^{2+}$  homeostasis.
  - $\text{Ca}^{2+}$  concentrations are detected in MTs of various insects (Fig. 1A-B).<sup>4</sup>
  - In flies, MTs sequester excess  $\text{Ca}^{2+}$  when fed on high  $\text{Ca}^{2+}$  diet.<sup>4</sup>
  - How MTs of mosquitoes (Fig. 1C) contribute to  $\text{Ca}^{2+}$  homeostasis remains unknown.
- Given that *Aedes aegypti* is an important disease vector, revealing how MTs regulate mosquito  $\text{Ca}^{2+}$  homeostasis can strengthen our understanding of vector biology and reveal potential target.

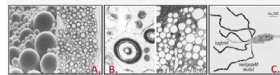


Figure 1. Light and electron micrographs of mineralized concretions in the Malpighian tubules of *Drosophila melanogaster* (A) and *Rhodnius prolixus* (B). Isolated elementary canal of adults female *Ae. aegypti* showing Malpighian tubules (C).<sup>1</sup>

##### Objective

**Aim #1.** To characterize how  $\text{Ca}^{2+}$  levels in MTs of adult female *Ae. aegypti* respond when fed on increasing dietary  $\text{Ca}^{2+}$  loads.

**Hypothesis #1.** As the  $\text{Ca}^{2+}$  concentration increases in mosquito diets, the MTs will be the major site of  $\text{Ca}^{2+}$  increase.

**Aim #2.** To investigate the molecular mechanism of renal  $\text{Ca}^{2+}$  regulation by quantifying the mRNA abundance of three putative  $\text{Ca}^{2+}$ -ATPases in mosquito MTs when fed on increasing dietary  $\text{Ca}^{2+}$  loads (Fig. 2).

- Plasma membrane  $\text{Ca}^{2+}$ -ATPase (PMCA)
- Sarco-/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA)
- Secretory pathway  $\text{Ca}^{2+}$ -ATPase (SPCA)

**Hypothesis #2.** ATPases that are the most important to  $\text{Ca}^{2+}$  regulation by MTs will show the greatest differential mRNA expression levels.

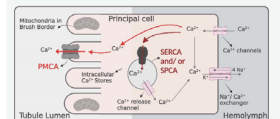


Figure 2. Hypothetical model of  $\text{Ca}^{2+}$  transport mechanisms in the principal cell of Malpighian tubules. The present study will focus on PMCA, SERCA, and SPCA.

#### Materials and Methods

##### Aim #1. Measurement of $\text{Ca}^{2+}$ Level

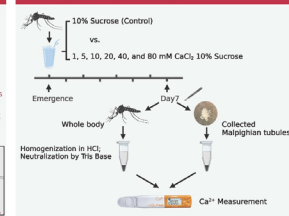


Figure 3. Workflow of  $\text{Ca}^{2+}$  level measurement in the Malpighian tubules and whole bodies of adult female *Ae. aegypti*. Malpighian tubules from 30-35 mosquitoes are pooled together in one microcentrifuge tube for measurement.

##### Aim #2. Measurement of Gene Expression

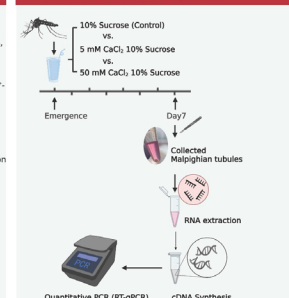


Figure 4. Workflow of gene expression quantification in the Malpighian tubules of adult female *Ae. aegypti*.

#### Results

##### A. Response of Whole Body/ MTs $\text{Ca}^{2+}$ Level to Dietary $\text{Ca}^{2+}$ Increase

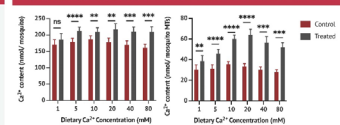


Figure 5. Effects of dietary  $\text{Ca}^{2+}$  treatments on  $\text{Ca}^{2+}$  levels of whole body (left) and Malpighian tubules (right) of adult female *Ae. aegypti*. Values are mean  $\pm$  SEM,  $n = 5-8$  replicates for each dietary  $\text{Ca}^{2+}$  concentration. Significant differences of  $\text{Ca}^{2+}$  level compared to control group are denoted based on the student's  $t$ -tests (\*\*\*  $p < 0.0001$ , \*\*  $p < 0.001$ , \*  $p < 0.01$ , \*  $p < 0.05$ ).

##### B. Percent Increase of $\text{Ca}^{2+}$ Level in response to Dietary $\text{Ca}^{2+}$ Increase

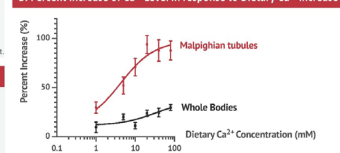


Figure 6. Percent increase of  $\text{Ca}^{2+}$  contents in mosquito whole body/ Malpighian tubules relative to the control groups in response to dietary  $\text{Ca}^{2+}$  treatments. Values are mean percent increase  $\pm$  SEM.

##### C. Impact of Increasing Dietary $\text{Ca}^{2+}$ on the mRNA Level of PMCA, SERCA, and SPCA in MTs (Preliminary Data)

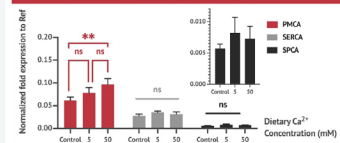


Figure 7. Impact of increasing dietary  $\text{Ca}^{2+}$  on the mRNA abundance of PMCA, SERCA, and SPCA in adult female *Ae. aegypti* Malpighian tubules. Values are mean  $\pm$  SEM. RM one-way ANOVA is performed separately for each gene of interest among different treatments. The differences are denoted based on Tukey HSD tests (\*\*  $p < 0.01$ , \*  $p < 0.05$ , ns, not replicates).

#### Conclusions

MTs are the major site of  $\text{Ca}^{2+}$  regulation in *Ae. aegypti*.

- $\text{Ca}^{2+}$  contents of both whole body and MTs increased significantly in response to the  $\text{Ca}^{2+}$ -containing diets (Fig. A).
- Approximately 40% to 80% of the  $\text{Ca}^{2+}$  increase of whole body can be attributed to MTs (Fig. A).
- The increase of  $\text{Ca}^{2+}$  content in MTs was concentration-dependent, while the increase in whole body was not (Fig. B).

The expression level of PMCA in MTs is up-regulated in response to increasing dietary  $\text{Ca}^{2+}$  loads (Fig. C).

- This result is consistent with the hypothesis that PMCA is responsible for transporting  $\text{Ca}^{2+}$  out of tubular cell for excretion (Fig. 2).

#### Discussions

- To further confirm the function of PMCA in MTs, immunofluorescent localization assay will be conducted.
- Preliminary data on blood-fed *Ae. aegypti* show a dynamic change of  $\text{Ca}^{2+}$  level in MTs after blood meals, which is in accordance with the hypothesized role of MTs in  $\text{Ca}^{2+}$  regulation. The study of how blood meals impact the mRNA abundance of  $\text{Ca}^{2+}$ -ATPases is currently in progress.
- Time permitting, RNAi will be used to knock down the expressions of  $\text{Ca}^{2+}$ -ATPases in *Ae. aegypti* to determine if they may serve as valuable biochemical targets for insecticide development.

#### Acknowledgements

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WOOSTER

Correspondence:  
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# Ph.D. Competition Winners

# 2022 CFAES Poster Competition

## Ph.D. Category, Third Place

CFAES

SCHOOL OF ENVIRONMENT AND NATURAL RESOURCES

### Hormonal Responses to Multiple Stressors and Their Behavioral Consequences

Bethany L. Williams, Lauren M. Pintor, Suzanne M. Gray

**Bethany Williams**  
School of Environment &  
Natural Resources  
Advisors: Dr. Suzanne Gray  
and Dr. Lauren Pintor

#### Introduction

- Many studies focus on how single stressors affect an animal, but animals must manage many stressors simultaneously, increasingly including stressors caused by humans<sup>1,2</sup>
- Reproduction is energetically costly, so dealing with additional stressors may reduce reproductive success<sup>3</sup>.
- In this study we considered how reproductive hormones (i.e., testosterone and estradiol; Figure 1) and behavior in fish are affected by low-oxygen and turbidity (muddy water), two stressors both increasing as consequences of agriculture like runoff and eutrophication<sup>4,5</sup>.
- Low oxygen and turbidity both affect reproductive behavior and/or hormone concentrations individually<sup>5,6,7</sup>, but less is known about their combined effects.
- Understanding how human-induced stressors affect reproductive hormones and behavior is crucial to understand how changing environments will affect a species' ability to survive and reproduce.



Figure 1: The aromatase enzyme converts testosterone to estradiol. Both reproductive hormones are important for regulating courtship and competitive behaviors in male fish<sup>8,9</sup>.

#### The Objectives of This Study Were To:

- Quantify how oxygen and turbidity affect reproductive hormones in fish.
- Determine how changes in reproductive hormones due to aromatase inhibition affect behaviors of males during courtship and competition.

#### We Predicted That:

- Low oxygen and turbidity would increase the ratio of testosterone to estradiol (through aromatase inhibition) as seen in other species of fish in low oxygen sites and based on previously noted behavioral responses to turbidity<sup>5,6</sup>.
- Aromatase inhibition would increase the rate of competitive and courtship behaviors due to its effect on male hormones.

#### Materials and Methods

- Fish (Figure 2) were reared under four combinations of oxygen and turbidity: high-oxygen/clear, high-oxygen/turbid, low-oxygen/clear, and low-oxygen/turbid.
- To test the effects of oxygen and turbidity on hormone production we measured excretion rates of testosterone and estradiol (n=77) in male cichlids using enzyme-linked immunoassays according to manufacturer's instructions (Cayman Chemical).
- In a second experiment, we conducted mate choice (n=20) and male competition (n=20) trials (Figure 3), and we compared the behavior of control males to males given a mild dose of aromatase inhibitor.

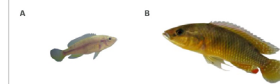


Figure 2: Picture of a female (A) and male (B) cichlid of the species *Pseudocrenilabrus multicolor*. *P. multicolor* is a widespread African cichlid found in sites that vary dramatically in oxygen and turbidity levels.

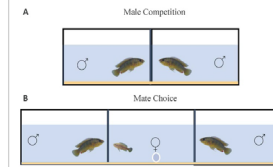


Figure 3: Conceptual diagram of (A) male competition and mate choice (B) trial tanks. During mate choice trials, females could hide from males. Trials were filmed for 30 minutes. To reduce the risk of injury due to aggression, a barrier was placed between males and females. Fish in the behavioral trials were reared under high-oxygen/clear conditions.

#### Results

##### Reproductive Hormones

- Both turbidity and low oxygen increased the ratio of testosterone relevant to estradiol (LMM, Oxygen:  $t = -3.26$ ,  $p = 0.001$ ; Turbidity:  $t = -3.31$ ,  $p = 0.002$ ; Figure 4A).
- Our aromatase inhibition treatment successfully increased the ratio of testosterone to estradiol (LM, Treatment:  $t = 2.49$ ,  $p = 0.020$ ; Figure 4B).
- The rate of competitive behaviors was not affected by a male's individual hormone levels or hormone treatment (LMM; Treatment:  $t = 0.572$ ,  $p = 0.580$ , Hormone Level:  $t = -1.06$ ,  $p = 0.311$ ; Figure 4C).
- The rate of courtship behaviors was positively related to a male's individual hormone level, but aromatase inhibition decreased the rate of courtship behaviors (LMM; Treatment:  $t = -2.46$ ,  $p = 0.026$ , Hormone Level:  $t = 2.64$ ,  $p = 0.017$ ; Figure 4D).

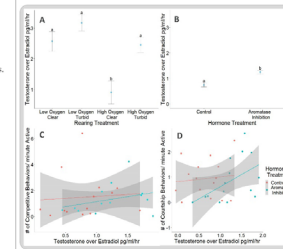


Figure 4: Average ( $\pm$  standard error) excretion rate of testosterone over estradiol of fish in the rearing experiment (A) and fish in the hormone manipulation experiment (B), and the excretion rate of testosterone/estradiol versus the rate of competitive (C) and courtship behaviors (D).

#### Conclusions and Discussion

- Low-oxygen and turbidity increased the ratio of testosterone to estradiol, indicative of aromatase inhibition as previously seen in fish in low-oxygen<sup>7</sup>.
- Mild aromatase inhibition treatment increased the rate of courtship behaviors, though it did not affect male competition.
- Overall, this indicates that changes in hormone levels due to the environment are likely to have behavioral consequences, though they may differ depending on the context and degree of hormone disruption.
- The mechanism of hormonal change (e.g., aromatase inhibition) may also influence behavioral responses as fish in the aromatase inhibition group had lower rates of courtship behaviors at a given hormone level when compared to the control group.
- Because agriculture has been linked to increased turbidity and low oxygen as a result of runoff and increased nutrient loads<sup>10</sup>, it is crucial to understand the potential consequences for aquatic organisms.
- The results of this study will help answer the question of how human-induced stressors affect reproductive success and in turn, population and community dynamics<sup>11</sup>.

#### Acknowledgements and References

We thank the Uganda Commissioner of Fisheries Resources Management and Development, the Uganda National Council for Science and Technology, and Fisheries Laboratory (Bahr el Jebel Station for Fisheries Support and Research) for their support. This research was funded by a NSF Grant to Dr. Lauren M. Pintor and Dr. Suzanne M. Gray. Research support provided by the NSF and the University of Illinois at Urbana-Champaign. We thank the University of Illinois at Urbana-Champaign, and the University of Illinois at Urbana-Champaign for their support. We thank the University of Illinois at Urbana-Champaign for their support. We thank the University of Illinois at Urbana-Champaign for their support.

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# 2022 CFAES Poster Competition

## Ph.D. Category, Second Place

**Caralee Shepard**  
Entomology  
Advisor: Dr. Mary Gardiner

**CFAES**

Department of Entomology

### Urbanization as a filter for the regional bee species pool

Caralee Shepard,<sup>1</sup> Sarah Scott,<sup>1</sup> Kayla Perry,<sup>2</sup> Katherine Turo,<sup>3</sup> Frances Sivakoff,<sup>4</sup> Mary Gardiner<sup>1</sup>

#### Introduction

- Vacant land is common in legacy cities where substantial population declines have occurred.<sup>1</sup>
- Over 20% of Ohio's bee species use vacant land as habitat.<sup>2,3</sup>
- A bee's ability to access and benefit from vacant land is likely-influenced by its functional traits, such as body size and nesting behavior.<sup>4,5</sup>
- Our goal was to determine what functional traits limit a bee species from utilizing urban vacant lots. **This information can inform future management plans to address these limitations posed by the urban environment.**

#### Objective:

Determine how urbanization filters the urban species pool of bees utilizing vacant land.

#### Hypothesis:

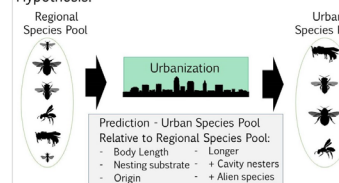


Figure 1. Hypothesis that urbanization will filter the urban species pool relative to the region species pool based on the functional traits of body length, nesting substrate and origin.

#### Materials and Methods

- The regional species pool was defined using updated source files shared by Ascher and Pickering.<sup>6</sup>
- Five years of published bee collection data was used in a presence absence matrix for all regional bee species.
- A functional trait database was created using data gathered from online and published resources.
- Statistics: Community-weighted-means (CWM), Null model communities, Standardized effect sizes (SES), Wilcoxon signed rank analysis.

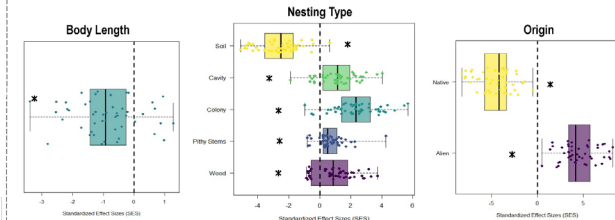


Figure 2. Graphs of results from tuning Standardized Effect Sizes (SES) for each functional category based on Null model communities. The x axis shows the SES and asterisks show significant differences ( $p < .05$ ). Colored points represent the SES of each site, while bar graphs show descriptive statistics for all sites.

#### Results

- Body length of urban species pool was smaller than expected ( $p < .05$ )
- Fewer soil nesting and a greater number of cavity, colony, pithy stem, and wood nesting species than expected ( $p < .05$ )
- A greater number of alien bee species and fewer native species than expected ( $p < .05$ )

#### Conclusion and Implications

- Urban environments lack:
  - Enough bare, stable soil to support soil nesting bees,
  - Large resource habitats to support larger bees with greater nutrient needs
- Conservation projects should provide nesting sites for soil nesting bees on larger patches of greenspace that could provide habitat for larger bee species.

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- Department of Entomology, The Ohio State University, Columbus, OH.
- Department of Biological Sciences, Kent State University, Kent, OH.
- Department of Ecology, Evolution, and Natural Resources, Rutgers University, New Brunswick, NJ.
- Department of Ecology, Evolution, and Organismal Biology, The Ohio State University, Columbus, OH.

# 2022 CFAES Poster Competition

## Ph.D. Category, First Place

**Michelle Pham**  
Entomology & Environmental  
Sciences  
Advisor: Dr. Mary Gardiner

**CFAES**

Environmental Sciences Graduate Program, Department of Entomology

### Reclaiming Vacant Land to Support Urban Bee Habitat

Michelle A. Pham, MaLisa R. Spring, Frances S. Sivakoff, Mary M. Gardiner

#### INTRODUCTION



Figure 1. A green roof at Ohio State University, Columbus campus. (Image from NBBU)

- Green infrastructure (GI) refers to engineered elements that are integrated with natural habitat to support ecosystem services in urban areas. [1]
- Bioswales, green roofs, rain gardens, and private urban gardens are all examples of GI. [1, 2]
- GI can occupy small patches of green space, making it ideal for conservation in cities which lack contiguous habitat. [3]
- Vacant land provides an opportunity to establish low-cost GI that manages stormwater and provides insect habitat. [3]

**Research Question:** Does investment in green infrastructure on vacant land provide dual ecosystem services of stormwater management and bee habitat?

**Objective:** Compare bee abundance across green infrastructure treatments (bioswales, rain gardens) and control habitat (vacant lots).

**Hypothesis:** Investments in low-cost (rain garden) and high-cost (bioswale) green infrastructure can enhance local bee biodiversity.

#### MATERIALS & METHODS

From 2014 to 2015, treatment and control sites were established in the Slavic Village neighborhood (Cleveland, Ohio):



Figure 2. Research sites. (MaLisa Spring)

#### RESULTS

##### Top 5 Bee Genera Collected

1. <i>Lasiosiglossum</i> (n = 1100)	
2. <i>Hylaeus</i> (n = 833)	
3. <i>Colletes</i> (n = 200)	
4. <i>Melissodes</i> (n = 123)	
5. <i>Anthidium</i> (n = 100)	

Figure 5. The majority of bees collected were smaller, solitary species. (MaLisa Spring)



Figure 3. Bee bowls (A) and yellow sticky cards (B). (MaLisa Spring) Bees were sampled by quadrat using bee bowls and yellow sticky cards once a month (Jun-Aug) from 2014-2016. [4] Vegetation variables such as bloom richness, percent cover, and vegetation height were also measured. [4]



Figure 4. A pinned *Agapostemon* (A) and *Megachile* (B) bee under a microscope.

Using light microscopy, specimens were identified to species when possible. [5, 6] Bee, vegetation, and sampling data were analyzed in R. [7] Statistics: generalized linear mixed models, PLS regression [8]

##### Key Findings:

1. The 3 most abundant bee species were all native species to Ohio.
2. Bee abundance was similar within GI treatments and control vacant lots.
3. No habitat variables significantly and consistently predicted bee abundance.

#### CONCLUSIONS & DISCUSSION

- In the short term, GI can improve stormwater management without reducing the abundance of bees that use a habitat patch as forage.
- GI may not require high financial investment to yield measurable ecological benefits.

#### SIGNIFICANCE & FUTURE DIRECTIONS



Figure 6. A native wildflower prairie planted in a vacant lot. (Gardiner Lab)

- Future work will validate findings from preliminary analyses and compare bee functional diversity across treatments and species.

• A remaining challenge to urban conservation is managing GI, such as native wildflower plantings (Figure 6), so it is aesthetically pleasing and accepted by local residents. [9, 10]

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I would like to thank the Environmental Sciences Graduate Program for funding through the Fay Fellowship and the undergraduate field assistants who helped collect these data. Funding for this research was provided by the NSF CAREER Grant Program (223787).

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Environmental Sciences Graduate Program  
Department of Entomology  
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# Postdoctoral Competition Winners





# 2022 CFAES Poster Competition

## Post Doc Category, Second Place

CFAES

THE OHIO STATE UNIVERSITY / DEPARTMENT OF ENTOMOLOGY

### The microbiome of *Amblyomma americanum* reflects known westward expansion.

Luis Eduardo Martinez, Paula Lado, Hans Klompen, Risa Pesapane, Sarah M Short.

Luis Martinez  
Entomology  
Advisor: Dr. Sarah Short

#### Introduction

Tick borne diseases (TBDs) e.g. Lyme disease, Anaplasmosis, Ehrlichiosis have almost doubled in the past 12 years<sup>1</sup>. The reasons behind these increases are multifactorial, one being the geographic expansion of their vectors possibly driven by climate change. Among them, *Amblyomma americanum*, has historically been found in the southern USA, rapidly expanding its range westwards and northwards in the past decades. Study of the tripartite vector-pathogen-microbiome relationship is critical to understanding the ecology of TBDs.

#### Significance and overarching goal

Tick microbiomes can show spatial structuring, though the majority of these reports come from *Ixodes*<sup>2</sup> and *Dermacentor*<sup>3</sup>. Thorough understanding of the microbiome's role in tick biology requires systematic studies of more species on a broader spatial scale. Here, we investigated whether the microbiome of *A. americanum* is structured across its historic and expanded range, and specifically if there is a **microbial signature of expansion**.

#### Methods



Figure 1. Map showing the geographic origin of the sampled ticks. The expanded range of *A. americanum* is shown in dark brown, and the historic range in light green. The circles mark sampling locations, and the size of the circle reflects the number of specimens analyzed for each location. The color of the circles indicates different regions: black, historic range (HC); yellow, northeast (NE); purple, midwest (MW); red, west (W). [figure from Lado et al. 2022]

187 *A. americanum* ticks were collected from 4 geographic regions encompassing their historic and expanded range (Figure 1).

Sex typed ticks, subjected to amplicon sequencing (V4) 16S rRNA to characterize their microbiome.

Bioinformatic and microbial ecology downstream analyses.

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#### Results

Females are less diverse than males (Figure 2).

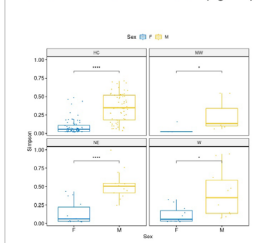


Figure 2. Differences in alpha diversity (i.e. Inverse Simpson Index) between ticks sorted by sex and shown by geographic region. Mean differences estimated with a KW test followed by Dunn test ( $p < 0.05$ ). Historic range (HC); northeast (NE); midwest (MW); west (W).

Community composition of ticks from west region is significantly different from all other sampled regions (Figure 3).

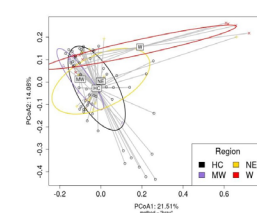


Figure 3. Ordination analysis (PCoA) based on Bray-Curtis dissimilarities among male ticks. Centroids for each geographic region are shown. SD ellipses follow the color code in the legend, with abbreviations representing regions as in Figure 1. PERMANOVA analysis showed tick sex ( $p=1e-5$   $R^2=0.21$ ) and region ( $p=1e-5$   $R^2=0.06$ ) significantly drive beta diversity.

With increasing geographic distance, microbial communities become significantly dissimilar (Figure 4).

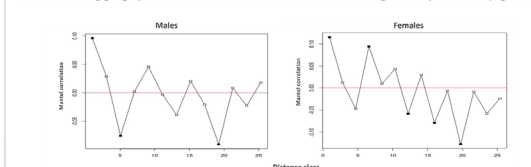


Figure 4. Mantel correlogram showing relationships between the Bray-Curtis dissimilarities and the geographic distances among ticks paired by sex. Distances classes along the x axis are bins following Sturge equation. Dark symbols represent significant Mantel statistics after progressive Bonferroni corrections ( $p < 0.05$ ). Positive and negative correlation values indicate positive and negative relationships between Bray-Curtis values and geographic distances respectively.



#### Results

Stochasticity coupled with dispersal limitation are the main drivers of microbiota dissimilarities among individuals within sexes (Figure 5).

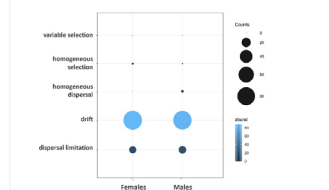


Figure 5. Relative contribution of community assembly processes. Dot size and color gradient are a visual proxy for the percentage assigned to each type of process.

#### Discussion and conclusions

→ Lower diversity in females may be a function of their *Rickettsiales* burden.

→ Microbiota is an additional layer of information to understand and potentially detect range expansion. Westward populations seem a plausible example of **beta diversity turnover** due to geographic distance.

→ Dispersal limitation combined with drift drive spatial turnover in community composition for both sexes. This is potentially consistent with geographic isolation and warrants further investigation. Factors such as elevation, vegetation, and season, concurrently with the time scale of the expansion have to be considered when contextualizing this results.

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# 2022 CFAES Poster Competition

## Post Doc Category, First Place

Gonzalo-  
Miyagusuku  
Cruzado

Food Science & Technology  
Advisor: Dr. Monica Giusti

Department of Food Science and Technology

## Viable production of novel colorants for the food industry: More efficient pyranoanthocyanin formation using 4-vinylphenol

Gonzalo Miyagusuku-Cruzado, Danielle M. Voss, Yesen Cheng, and M. Mónica Giusti.

CFAES

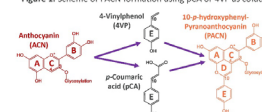
### Introduction

Consumer demand for clean labels and healthier foods is driving the food industry to look for replacements for artificial colorants. Anthocyanins (ACN) are naturally sourced pigments that can be potential replacements, but their limited stability difficult their application by the food industry. Pyranoanthocyanins (PACN) are ACN-derived pigments with superior stability and resistance to thermal, pH-, and bleacher-induced degradation<sup>1,2</sup>, making them ideal replacements for artificial colorants in most food applications. However, PACN limited availability in nature hinders their potential for industrial application.

Our previous study showed that PACN can be formed by reacting ACN with hydroxybenzoic acids under heated incubation in as short as 72 hr<sup>3</sup>. This was a significant improvement considering that PACN formation usually takes anywhere from a few weeks to several years<sup>4</sup>.

A secondary, potentially more efficient pathway of PACN formation involves 4-vinylphenols, decarboxylated hydroxybenzoic acids, as cofactors. 4-Vinylphenols can be produced by microorganisms as a self-defense mechanism against the antimicrobial activity of hydroxybenzoic acids<sup>5</sup>. Our previous study showed that lactic acid bacteria efficiently biotransformed p-coumaric acid (pCA) into 4-vinylphenol (4VP), enabling its use as a cofactor for PACN formation at an industrial scale. Although promising, a systematic study on the efficiency of 4VP as a cofactor for PACN production has yet to be conducted. We hypothesized that 4-vinylphenols could be more efficient cofactors for PACN production, showing higher yields in shorter times.

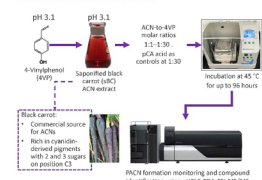
Figure 1. Scheme of PACN formation using pCA or 4VP as cofactors



### Objective

To evaluate 4VP (decarboxylated pCA) as cofactors for PACN production, model their formation kinetics, and provide insight on their formation mechanisms.

### Materials and Methods



### Results

Figure 2. Incubation for 96 hr of sBC ACN with 4VP or pCA resulted in the formation of new pigment peaks (3 and 4). Peak sizes suggested that this formation was dependent on the ACN-to-cofactor molar ratio

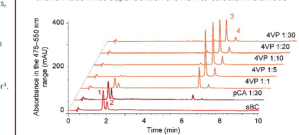


Figure 3. New peak formation was time-dependent as evidenced by the increasing peak sizes over time until a plateau was reached

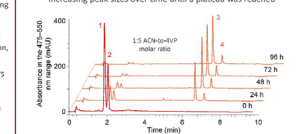


Table 1. Pigment identification using uHPLC-PDA-ESI-MS/MS. Peaks 1 and 2 correspond to the initial anthocyanins in sBC. Peaks 3 and 4 were consistent with their 10-p-hydroxyphenyl-pyranoanthocyanin derivatives

Peak	$\lambda_{max}$ (nm)	[M] <sup>+</sup> m/z	Fragment [M-1] <sup>+</sup> m/z	Identity
1	516	743	287	Cyanidin-3-O-glucosyl galactoside (C3G-Gal)
2	517	581	287	Cyanidin-3-O-glucosyl galactoside (C3G-Gal)
3	503	859	403	10-p-hydroxyphenyl-pyranoanthocyanin (10-PH-PACN)
4	501	897	403	10-p-hydroxyphenyl-pyranoanthocyanin (10-PH-PACN)

Figure 4. Additional peaks detected in the uHPLC-PDA-ESI-MS/MS (peaks 5' and 6') were consistent with direct condensation products between 4VP and ACN, intermediate compounds in PACN formation.

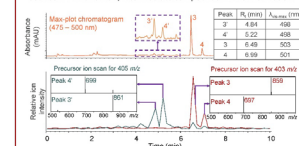


Figure 5. Kinetic modeling of PACN yields showed that 4VP was a more efficient cofactor than pCA

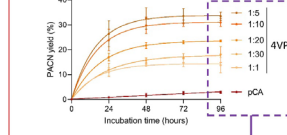


Figure 6. All PACN yields with 4VP were significantly higher than those with pCA after incubation for 96 hr

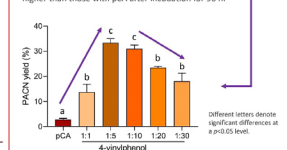
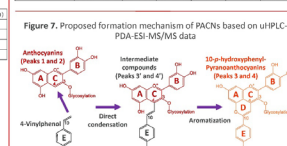


Table 2. PACN formation with 4VP followed pseudo-first order kinetics reaching higher yields (plateau %) with faster formation rates (k) than with pCA.

	p-Coumaric acid	4-Vinylphenol
ACN-to-cofactor molar ratio	1:30	1:1
Pseudo-first order	Zero-order	Pseudo-first order
R <sup>2</sup>	0.85	0.93
Plateau (%)	14.38	33.75
k	0.031 h <sup>-1</sup>	0.062 h <sup>-1</sup>

Figure 7. Proposed formation mechanism of PACNs based on uHPLC-PDA-ESI-MS data



### Discussion

- Incubation of ACN with 4VP resulted in the formation of new compounds (peaks 3 and 4 in Fig. 2 and 3). The UV-Vis spectral characteristics and the mass-per-charge ratios of these newly formed compounds were consistent with 10-p-hydroxyphenyl-PACN (Table 1).
- PACN yields were time-, cofactor-type-, and cofactor-concentration-dependent (Fig. 2, 3, and 5).
- Yields with 4VP were up to +12 times greater than those observed with pCA (Fig. 5 and 6). Yields with 4VP were up to 70% greater than those previously reported for more reactive hydroxybenzoic acids under similar incubation conditions<sup>3</sup>.
- ACN-to-cofactor molar ratios of 1:1 and 1:1.10 had significantly higher yields (p<0.05) than those observed for ratios of 1:20, 1:30, and 1:1 (Fig. 6).
- PACN formation with 4VP as a cofactor followed a pseudo-first-order kinetic model whereas formation with pCA followed a zero-order model (Fig. 5 and Table 2). This may be one of the first reports on the formation kinetics of PACN.
- Additional compounds detected support a direct condensation and aromatization mechanism of formation (Fig. 3 and 7). A similar mechanism has been reported for PACN from malvidin-derived ACN with 4-vinylguaiacol (decarboxylated ferulic acid)<sup>6</sup>.

### Conclusions

- 4VP was a more efficient cofactor than its precursor, pCA, for PACN production showing greater yields in shorter times.
- Forming PACN at a faster rate with higher yields will enable their viable use by the industry. This study will aid the food industry in its transition towards naturally sourced pigments.

### Future work

- Future experiments will focus on the bioreactor scale-up of the production of PACN using pigment-rich food waste and lactic acid bacteria capable of biotransforming hydroxybenzoic acids into 4-vinylphenols. This project is to be funded by the CFAES IGP Intellectual Accelerator Program.

### Acknowledgements

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The Giusti Phytochemicals Laboratory





# Research Staff Competition Winners

# 2022 CFAES Poster Competition Research Staff Category, Second Place

CFAES

College of Food Agricultural and Environmental Sciences/ Entomology

## Residual effect of Cyclaniliprole and Flonicamid on *Hippodamia convergens* and *Aphidius colemani*

Nuris Acosta<sup>1</sup>; Luis A. Canas<sup>1</sup>; Arnol Gomez<sup>1</sup>; Carlos E. Bográn<sup>2</sup>

**Nuris Acosta**  
Entomology  
Supervisor: Dr. Andy Michel  
and Dr. Luis Canas

### INTRODUCTION

Insecticides are a commonly used pest management tool to control aphids in ornamental plants grown in controlled environments. The increasing use of natural enemies in this production system makes it vital to study the impact of insecticides on these organisms. The objective of this study was to determine the residual effect of two active ingredients cyclaniliprole, flonicamid and the combination of both on green peach aphid (*Myzus persicae*) (Sulzer) and two of its natural enemies: the parasitoid, *Aphidius colemani* Viereck and the predator *Hippodamia convergens* Quénin-Ménéville.

**Hypothesis:** We hypothesize that these insecticides will negatively impact the survival rate of the natural enemies.

### MATERIAL AND METHODS

#### Greenhouse:

- Plants:** Zinnias plants (*Zinnia elegans*-Fig. 2A) were grown in a greenhouse at 24°C/16:10 (L:D) light. They were watered and fertilized using drip irrigation system.
- Treatments (5):** Untreated; Cyclaniliprole, 16.4 Fl oz/100 Gal; Flonicamid, 4.28 Fl oz/100 Gal; Cyclaniliprole + Flonicamid at 15.5 Fl oz/100 Gal and Mainspring, 8 Fl oz/100 Gal as chemical control (Figure 1).
- Application:** Treatments were applied as a drench to D0.
- Variables:** The residual effect of the treatments at 24 and 72 h; survival of green peach aphid and their natural enemies was evaluated over time: D0, D7, D14, D21, and D28.

#### Laboratory:

- Leaf collection:** At each evaluation time, zinnia leaves were collected, cut and placed inside a petri dish (100 X 15 mm) with wet plaster (Fig. 2-B).
- Evaluation:** for aphids, 20-2<sup>nd</sup> instar nymph were placed inside each petri dish, and for the natural enemies (Arbico Organics) 10 adults of each (*Aphidius* or *Hippodamia*), as well as 10-2<sup>nd</sup> instar aphid used as prey were used. In all petri dishes, a sugary cotton wick inserted in a 1.5 ml vial was used as food (Fig. 2-C).
- Analysis:** Each treatment was repeated five times and was set up in a laboratory bench (24°C-Fig. 2-C) in a completely randomized block design. SAS Ver 9.4 software was used to analyze the data.

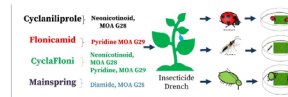


Figure 1: Diagram of methodology.

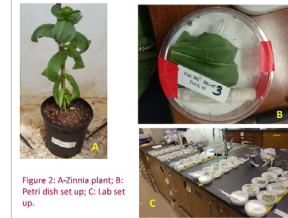


Figure 2: A-Zinnia plant; B: Petri dish set up; C: Lab set up.

### RESULTS

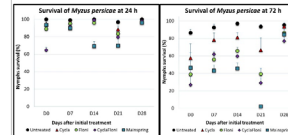


Figure 3: Insecticides affected the survival of aphid nymphs 24h (left) evaluation time in D0, D14 and up to D21 ( $P < 0.05$ ), especially the treatment Mainspring. After 72h, Mainspring and the combination Cyclaniliprole + Flonicamid followed by Flonicamid alone caused significant decrease in survival, up to D28 ( $P < 0.05$ ).

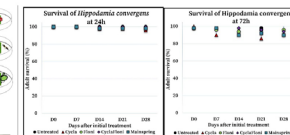
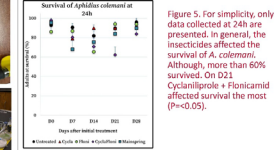


Figure 4: None of the insecticides had impact on the survival of *H. convergens* over time either at 24 or 72h.



### CONCLUSION

- In the present study we evaluated the indirect effect of Cyclaniliprole, Flonicamid, Cyclaniliprole + Flonicamid and Mainspring (industry standard) as drench over time on the survival of green peach aphid and two of its natural enemies.
- Myzus persicae:** Our results show that the highest mortality was observed at 72h and that residual of Mainspring, Flonicamid and Cyclaniliprole + Flonicamid was still present up until day 21 after treatments were applied.
- Hippodamia convergens:** None of the treatments significantly reduced the survival of the ladybug *H. convergens* at 24 or 72h of evaluation. *H. convergens* was not affected even when products such as Cyclaniliprole were applied directly (Long and Godfrey 2015).
- Aphidius colemani:** Flonicamid showed residual effects until day 21 reducing the wasp survival at 24 h evaluation. More striking was the effect observed when products were sprayed (Payton 2018). When combined with Cyclaniliprole, the residual effect of these insecticides on the wasp lasted three weeks (D21).
- We accept the hypothesis that some insecticides do affect survival of some natural enemies.

### DISCUSSION

- Insecticidal residue sometimes may be more toxic to natural enemies than it is to the pest but even contact residual activity of insecticides is variable among natural enemy species (Roubos et al. 2014).
- Flonicamid and cyclaniliprole in combination can be used after day 21 as part of IPM tactics for the control of green peach aphids.
- The impact of insecticides on biological control agents varies depending on the active ingredient and the type of biological control agent. Therefore, studies need to evaluate each of these interactions.
- Toxicological evaluations that include probit analysis make help establishing the impact of active ingredients on beneficial organisms.

### FUTURE DIRECTIONS

- Evaluate the effect of the direct impact of new chemical molecules on biological control agents commonly used to control greenhouse pests of ornamental plants.
- Evaluate the residual effect of these new active ingredients on more natural enemies on different cropping systems in control environments.
- Establish guidelines for the compatibility of active ingredients with biological control agents.

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# 2022 CFAES Poster Competition

## Research Staff Category, First Place

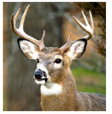
**Pat Boley**  
Animal Sciences  
Supervisor: Dr. Scott Kenney

**CFAES**

OARDC CENTER FOR FOOD ANIMAL HEALTH

### Surveillance for SARS-CoV-2 in White-Tailed Deer Populations of Northeastern Ohio

Patricia A. Boley, Patricia M. Dennis, Vanessa Hale, Linda J. Saif, Scott P. Kenney



#### INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus similar to SARS-CoV and to other betacoronaviruses that have been detected in other species, has spread across the world with dire effects on healthcare systems and economies. SARS-CoV-2 can infect several domestic animal species (e.g., dogs, cats, ferrets) and other animals under human care (e.g., mink, big cats, gorillas) but has not been found circulating in free-ranging animal species other than a few isolated cases [1]. Recent studies have demonstrated that white-tailed deer (*Odocoileus virginianus*) are susceptible to infection [2,3]. We developed two enzyme-linked immunosorbent assays (ELISAs) for detecting antibodies to SARS-CoV-2 nucleocapsid (N) or spike (S) that are both sensitive and specific. We validated the assays for cross-reactivity with other coronaviruses. We then sampled 472 free-ranging white-tailed deer from 10 locations in Northeastern (NE) Ohio (Fig. 1) from November 2020 to March 2021. We tested the sera with the S-based ELISA.

#### AIMS

Optimize and validate ELISA for SARS-CoV-2 that is both sensitive and specific. Establish the seroprevalence of SARS-CoV-2 antibodies in deer serum samples collected.

Figure 1. Sampling Sites

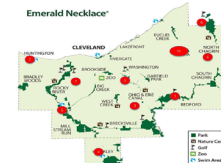


Figure 1. Map of sampling sites in the greater Cleveland area. Red dots with numbers are the reservation sites where deer samples were collected.

Figure 2. The Organization of SARS-CoV-2 genome by protein structure.

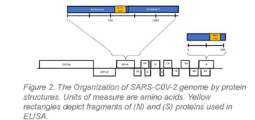


Figure 2. The Organization of SARS-CoV-2 genome by protein structure. Units of measure are amino acids. Yellow rectangles depict fragments of (N) and (S) proteins used in ELISA.

#### METHODS

##### Sample Collection

- Deer harvests occurred at locations that were baited for up to two weeks.
- Once deer were culled, they were uniquely tagged for future sample identification such as collection location. Serum and swabs were collected by an experienced veterinarian who wore PPE. Gloves were changed between each sample. Serum was stored at minus 80°C until transported and tested.

##### ELISA Methods

- A SARS-CoV-2 ELISA was developed for both N and S. Small segments [4] of each were cloned into pRSETA plasmid. Plasmid was transformed into bacteria, harvested, and purified. Purified protein was used as antigen coating in 96 well plates at 2 µg/ml.
- Serum samples were aliquoted and heat-inactivated at 56°C for 30 min. before testing. Samples were tested in triplicate and titrated beginning at 1:10 dilution. S antigen in carbonate buffer (50 µl) was added to plates and incubated overnight at 4°C. Plates were blocked with 5% nonfat dry milk for two hours. Serum samples were added and incubated for 1 hour. Secondary antibody was then incubated for 1 hour. TMB substrate was added for 10 min. and then stopped with 0.3M sulfuric acid.
- Plates were washed 5X with PBS-T between each step.
- Sample absorbances were read at 450 nm.
- Appropriate controls were used on each plate.
- A negative cut off was established for each concentration using pre-covid deer samples from the same area.

Table 1. Reagents Tested for Cross-Reactivity

Accession	Gene	Protein	Result	
Human Coronavirus	U04710	CoV	N	0.00
Human Coronavirus	U04710	CoV	S	0.00
Human Coronavirus	U04710	CoV	E	0.00
Human Coronavirus	U04710	CoV	M	0.00
Human Coronavirus	U04710	CoV	N	0.00
Human Coronavirus	U04710	CoV	S	0.00
Human Coronavirus	U04710	CoV	E	0.00
Human Coronavirus	U04710	CoV	M	0.00
Human Coronavirus	U04710	CoV	N	0.00
Human Coronavirus	U04710	CoV	S	0.00
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Human Coronavirus	U04710	CoV	N	0.00
Human Coronavirus	U04710	CoV	S	0.00

# **Congratulations to all competition winners!**