OMB Number: 0524-0039
Expiration Date: 12/31/2021

AFRI PROJECT TYPE

Instructions:

Who completes this form: Each project director (PD) applying to the Agriculture and Food Research Initiative (AFRI) Request for Applications (RFA).

How this template is completed:

* Check one Project Type Box and one Grant Type Box
* For FASE Grants, select an appropriate sub-category. NOTE: New Investigators may also qualify for a strengthening sub-category.

Project Type

- [x] Research
- [ ] Education
- [ ] Extension
- [ ] Integrated

Grant Type

- [ ] Standard Grant
- [ ] Coordinated Agricultural Project (CAP) Grant
- [ ] Conference Grant
- [ ] Other:
- [x] Food and Agriculture Science Enhancement (FASE) Grant
  - [ ] New Investigator
  - [x] Postdoctoral Fellowship Grant
  - [ ] Predoctoral Fellowship Grant
  - [ ] Strengthening
    - [ ] Sabbatical
    - [ ] Equipment
    - [ ] Seed
    - [ ] Strengthening Standard
    - [ ] Strengthening CAP
    - [ ] Conference Grant
    - [ ] Other:

Supplemental Information Form

OMB Number: 0524-0039
Expiration Date: 12/31/2021

Please complete this form in conjunction with the SF-424 Application for Federal Financial Assistance.

1. Funding Opportunity

Funding Opportunity Name

Agriculture and Food Research Initiative - Education and Workforce Development

Funding Opportunity Number

USDA-NIFA-AFRI-007252

2. Program to which you are applying

Program Code Name

Postdoctoral Fellowship

Program Code

A7201

3. Type of Applicant

H: Public/State Controlled Institution of Higher Education

4. Additional Applicant Types

1862 Land-Grant University

5. Supplemental Applicant Types *(Check all that apply)*

- [x] Alaska Native-Serving Institution
- [x] Cooperative Extension Service
- [x] Hispanic-Serving Institution
- [ ] Historically Black College or University (other than 1890)
- [ ] Minority-Serving Institution
- [ ] Native Hawaiian-Serving Institution
- [ ] Public Nonprofit Junior or Community College
- [ ] Public Secondary School
- [ ] School of Forestry
- [x] State Agricultural Experiment Station
- [ ] Tribal College (other than 1994)
- [ ] Veterinary School or College

6. ASAP Recipient Information

Does the legal applicant have an active Automated Standard Application for Payments (ASAP) Recipient Identification Number for NIFA awards?

- [x] Yes
- [ ] No

What is the ASAP Recipient ID (which corresponds with this applications's DUNS and EIN) to be used in the event of an award?

0448408

7. Key Words

Endohyphal bacteria, Fusarium, fungal-bacterial interactions, TnSeq, virulence

8. Conflict of Interest List

ConflictofInterestCombinedx.pd

Add Attachment | Delete Attachment | View Attachment

Project/Performance Site Location(s)

Project/Performance Site Primary Location

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Arizona Board of Regents, University of Arizona

DUNS Number: 8063456170000

* Street1: 1140 E. South Campus Drive

Street2: PO Box 210036, 303 Forbes

* City: Tucson

County:

* State: AZ: Arizona

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 85721-0036

* Project/ Performance Site Congressional District: AZ-003

Project/Performance Site Location 1

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City:

County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code:

* Project/ Performance Site Congressional District:

Additional Location(s)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 8063456170000 **Enter name of Organization:** Arizona Board of Regents, University of Arizona

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 1** **Start Date:** 07/01/2021 **End Date:** 06/30/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Morgan	Elizabeth	Carter	Ph.D.	51,000.00	12.00			51,000.00	8,976.00	59,976.00

Project Role: PD/PI

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 59,976.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
1	Undergraduate Students	0.06			1,560.00	28.00	1,588.00
	Secretarial/Clerical						

1 **Total Number Other Personnel**

Total Other Personnel 1,588.00

Total Salary, Wages and Fringe Benefits (A+B) 61,564.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item **Funds Requested ($)**

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file

Total Equipment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,000.00
2.	Foreign Travel Costs	2,500.00
	Total Travel Cost	5,500.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	11,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	DNA Sequencing	1,000.00
9.	Meeting Registration	500.00
10.	Institutional Allowance	3,000.00
	Total Other Direct Costs	15,500.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	82,564.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

DHHS, Jeanette Lu, 415-437-7820

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	82,564.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	82,564.00

L. Budget Justification

(Only attach one file.) BudgetJustification_CarterAug6.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 8063456170000 **Enter name of Organization:** Arizona Board of Regents, University of Arizona

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 2** **Start Date:** 07/01/2022 **End Date:** 06/30/2023

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Morgan	Elizabeth	Carter	Ph.D.	52,530.00	12.00			52,530.00	9,245.00	61,775.00

Project Role: PD/PI

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 61,775.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
1	Undergraduate Students	0.06			1,607.00	29.00	1,636.00
	Secretarial/Clerical						

1 **Total Number Other Personnel**

Total Other Personnel 1,636.00

Total Salary, Wages and Fringe Benefits (A+B) 63,411.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file

Total Equipment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1,500.00
2.	Foreign Travel Costs	
	Total Travel Cost	1,500.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	12,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	DNA Sequencing	2,000.00
9.	Meeting Registration	500.00
10.	Institutional Allowance	3,000.00
	Total Other Direct Costs	17,500.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	82,411.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

DHHS, Jeanette Lu, 415-437-7820

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	82,411.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	82,411.00

L. Budget Justification

(Only attach one file.) BudgetJustification_CarterAug6.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		121,751.00
Section B, Other Personnel		3,224.00
Total Number Other Personnel	2	
Total Salary, Wages and Fringe Benefits (A+B)		124,975.00
Section C, Equipment		
Section D, Travel		7,000.00
1. Domestic	4,500.00	
2. Foreign	2,500.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		33,000.00
1. Materials and Supplies	23,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	3,000.00	
9. Other 2	1,000.00	
10. Other 3	6,000.00	
Section G, Direct Costs (A thru F)		164,975.00
Section H, Indirect Costs		
Section I, Total Direct and Indirect Costs (G + H)		164,975.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		164,975.00

BUDGET JUSTIFICATION

A. Senior/Key Personnel:

Principal Investigator, Dr. Morgan Carter will devote approximately 1.0 FTE per year towards this project. She will be responsible for overseeing the project, coordinating with her mentor and supervising the undergraduate student. A total of $103,530 is budgeted for Dr. Carter over the 2-year project period.

B. Other Personnel:

Undergraduate Student (To Be Named): Funds are requested to hire one undergraduate student from the Baltrus Lab who will assist in fungal isolations, bacterial extraction/curing, and/or pathogenicity assays, under the mentorship of the PI. The undergraduate student will be paid $13/hour for approximately 120 hours in each calendar year.

A 3% cost of living increase has been included for both salaries starting in year 2 due to inflation.

Total Salaries & Wages: $106,697

Fringe Benefits/Employee Related Expenses (ERE). The University of Arizona defines fringe benefits as direct costs and estimates benefits as a standard percent of salary applied uniformly to all types of sponsored activities, and charges benefits to sponsors in accordance with the Federally-negotiated rates in effect at the time salaries are incurred. The rates used in the proposal budget are based on those in the current Federally-negotiated Rate Agreement. Current DHHS-approved rates for faculty, research staff, and students can be accessed via this link: https://www.fso.arizona.edu/sites/default/files/2020-06/ere2021.pdf, and the applicable types in this proposal are as follows: 17.6% for postdoctoral research associates and 1.8% for undergraduate students. A total of $18,278 is budgeted for fringe benefits/ERE.

Total Personnel & Fringe Benefits: $124,975

C. Equipment: N/A

D. Travel Costs: $7,000

Domestic Travel ($4,500): Funding in the amount of $4,500 is requested for domestic travel expenses over the duration of the project. The PI/PD will attend a Project Director (PD) meeting, as required by the fellowship, estimated at $1,000 in Year 1 only.
She also plans to participate in one workshop, such as the Kansas State University Fusarium Laboratory Workshop, estimated at $2,000 in Year 1 only, to be held in 2021.
She will attend the Annual American Phytopathological Society's Plant Health meeting (location to be determined) in 2022 (Year 2). This meeting is estimated at $1,500. Domestic travel costs will cover airfare, per diem, hotel, and local transportation costs. Costs based on estimates found online at https://www.gsa.gov/.

International Travel ($2,500): Funding in the amount of $2,500 is requested in Year 1 only for the PI/PD to attend an international conference, such as the International Symbiosis Society's conference in Lyon, France to be held in 2021. International travel costs will cover airfare, per diem, hotel, and local transportation costs. Costs based on estimates found online at https://www.gsa.gov/.

E. Participant Support Costs: N/A

F. Other Direct Costs: A total of $33,000 is budgeted for Other Direct Costs. A breakdown of each category is provided below.

Materials & Supplies: A total of $23,000 ($11,000 in Year 1 and $12,000 in Year 2) is budgeted for materials & supplies over the 2-year project period. A breakdown of material & supply costs is provided below.

Baltrus Lab: A total of $23,000 ($11,000 in Year 1 and $12,000 in Year 2) is requested for the Baltrus Lab to purchase standard laboratory reagents including petri dishes, agar, enzymes for molecular biology (i.e. DNA polymerases), cryovials, antibiotics for selecting bacterial strains, and pipette tips.

Other: A total of $10,000 over the 2-year project period is budgeted for Other Costs.

(DNA Sequencing) – funds in the amount of $3,000 ($1,000 in Year 1 and $2,000 in Year 2) are budgeted for DNA sequencing costs. This includes Sanger sequencing for 16S rDNA sequencing for isolate identification, Oxford Nanopore Sequencing kits and flow cells for whole genome sequencing, and library preparation and Illumina sequencing for whole genome sequencing and RB-TnSeq.

(Conference Registration) – funds in the amount of $1,000 ($500 per year) are budgeted for conference registration fees.

(Institutional allowance) – per the sponsor guidelines, institutional allowance (in lieu of indirect costs) are budgeted for a total of $6,000 over the 2-year project period ($3,000 per year).

G. Total Direct Costs: $164,975

H. Indirect Costs: $0
Indirect costs are not permitted on Postdoctoral Fellowship Grant awards.

I. Total Direct and Indirect Costs: $164,975

J. Fee: N/A

K. Total Costs and Fee: $164,975

RESEARCH & RELATED Other Project Information

OMB Number: 4040-0001
Expiration Date: 12/31/2022

1. Are Human Subjects Involved? ☐ Yes ☒ No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number:

3. Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. Does this Project Have an Actual or Potential Impact - positive or negative - on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. Project Summary/Abstract Project Summary.pdf | Add Attachment | Delete Attachment | View Attachment

8. Project Narrative MECProjectNarrativeUSDAwithResponse.p | Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited Bibliography.pdf | Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources FacilitiesOtherResources.pdf | Add Attachment | Delete Attachment | View Attachment

11. Equipment Equipment.pdf | Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments ☒

PROJECT SUMMARY

Instructions:
The summary is limited to 250 words. The names and affiliated organizations of all Project Directors/Principal Investigators (PD/PI) should be listed in addition to the title of the project. The summary should be a self-contained, specific description of the activity to be undertaken and should focus on: overall project goal(s) and supporting objectives; plans to accomplish project goal(s); and relevance of the project to the goals of the program. The importance of a concise, informative Project Summary cannot be overemphasized.

Title: Do facultative endohyphal bacteria alter virulence in Fusarium spp. that are critical agricultural pathogens?

PD: Carter, Morgan, E	**Institution:** University of Arizona
CO-PD: PD/PI 2 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 3 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 4 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 5 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 6 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 7 Name (Last, First, MI)	**Institution:**

This postdoctoral project addresses the **plant heath and production and plant products priority area** for two years under the mentorship of Dr. David Baltrus at the University of Arizona. The proposed project will empower the PD for a faculty position in phytopathology focused on the plant health impact of bacterial-fungal interactions. This fellowship will provide extensive opportunities to build on her foundation of molecular biology skills by training in pathogenicity assays and microbial genomics. She will also improve her teaching and mentoring skills and develop systems that will be foundational to her future independent research program.

The presence of endohyphal bacteria (EHB) significantly alters phenotypes across fungal host species. However, there have been no broad screens for the presence or phenotypic consequences of EHB in pathogenic *Fusarium* spp., despite the major diseases caused by this genus across many crops in the United States, from cereals to vegetables. Building on ecological EHB and genomics expertise at the University of Arizona, the PD proposes to survey two pathogenic *Fusarium* spp. for the presence of EHB and determine if EHB contribute to pathogenicity in one pathosystem. Additionally, the PD will identify bacterial genes necessary for fungal infection using RB-TnSeq, a high throughput transposon mutagenesis technique. **A more thorough understanding of the prevalence, impact, and genetic underpinnings of interactions between phytopathogenic fungi and EHB could provide new avenues for effective manipulation to benefit plant health and production, which is of increasing importance given decreasing efficacy of reliable antifungal treatments.**

Response to Previous Review

This proposal is a resubmission of my USDA AFRI EWD Postdoctoral Fellowship proposal "Do facultative endohyphal bacteria alter virulence in *Fusarium* spp. that are critical agricultural pathogens?" (proposal #2019-07244). My previous proposal was ranked non-competitive, although this rating may have been affected by a clerical error that resulted in letters of support not being included in the reviewer packet. While revising this package, I have included updated letters of support from my references and primary mentor, Dr. David Baltrus. I have also included an additional letter from Dr. Gary Bergstrom that details his support and expertise as a collaborator for work focused on *Fusarium graminearum*, analogous to the letter of support from Dr. Barry Pryor for work focused on *Fusarium oxysporum* f.sp. *lactucae*.

Updates to Research Approach: Although each reviewer saw merit in the goals of the original proposal, there were also a variety of ways that the reviewers suggested that my research approach could be strengthened. First is the lack of preliminary evidence for the objectives dealing with endohyphal bacteria (EHB) presence in *Fusarium oxysporum* f.sp. *lactucae*. Given that I officially began my postdoctoral work at the University of Arizona right at the time of this submission, I have not been able to include additional preliminary data. However, I have updated the literature cited to include new discoveries of *Fusarium*-EHB which support my hypothesis that *Fol* will harbor EHB, and I simplified alternative strategies for Objective II. I further clarified parts of the proposal that caused some confusion to the reviewers. For example, one concern was the ability to generate fungi with and without symbionts with the same genetic background to control for the variable virulence across strains. Passage of the fungus on media with and without antibiotics will generate genetically comparable fungal strains without and with EHB, respectively. The largest change is the focus of the third objective to unify the research throughout the proposal. In my previous submission, I proposed to work with an EHB that is actively being studied in the Baltrus lab, but does not come from a *Fusarium* host. I altered this third objective to focus on *Fusarium*-EHB and will be undertaking the previously proposed experiment outside of the scope of this grant, as a proof of concept and point of comparison.

Updates to Training and Mentorship: Within the training and mentoring goals, I was encouraged to seek out more networking, engagement, and learning opportunities. I have updated my budget and proposal to include money for attending a workshop on genomics or *Fusarium* spp. depending on domestic workshop offerings during the fellowship period. I have also emphasized my involvement in professional societies like the American Phytopathological Society and more explicitly detailed how I plan to continue networking and contributing through service. I clarified my timeline and added in the expectation of regular meetings with Dr. Pryor and Dr. Bergstrom for accountability and insight. One comment suggested that I would benefit from additional stakeholder engagement. I connected with the Yuma Center of Excellence for Desert Agriculture (University of Arizona) to develop an Extension Outreach section of my training goals; an additional letter of support from the executive director is also attached. Based on farmer interest, I will be working with the center to contribute a talk on the soil microbiome and will attend field days in the late fall. Finally, I added to my mentorship plan to include training from Dr. Baltrus on grant writing and peer review.

Training and Career Development Plan

Previous research outcomes from USDA funding: My work as a USDA Predoctoral Fellow was the first to assign a function to an effector protein from an endofungal bacterial symbiont and opened a new subfield in effector biology focusing on bacterial-fungal interactions. I studied transcription activator-like (TAL) proteins in *Mycetohabitans* spp. (formerly *Burkholderia*). These bacteria live inside of *Rhizopus microsporus,* a fungus used for fermentation and pathogen of both plants and humans, making it relevant to food production and health. Through molecular cloning, microscopy, and sequencing methods, I discovered that *Burkholderia* TAL-like (Btl) proteins transit the bacterial type III secretion system, have a functional nuclear localization signal, are present across *Mycetohabitans* spp., and impact host gene expression and stress tolerance. Btl proteins are likely to provide an opportunity to genetically manipulate mucoromycetes, an understudied branch of fungi affecting food and human safety. Over the period of my 2-year USDA Predoctoral fellowship I presented the work at two conferences (and one after the grant period) and submitted one first-author manuscript for review that was later accepted at PNAS with an additional paper in preparation. Furthermore, I satisfied my policy training goals for the project by attending a policy workshop, advocating for scientific research funding and support for the National Plant Diagnostic Network in Washington, D.C., and serving as an intern with the American Phytopathological Society (APS) Public Policy Board (PPB).

Career goals: My career goal is to become a plant pathology research and teaching faculty member at a land-grant institution to contribute to the community-driven mission of these institutions and do molecular research on questions with applied impact. I am motivated to investigate understudied plant-microbe-microbe interactions to improve the sustainability and resilience of agriculture in new ways. Increasingly fungicide-resistant fungi plague diverse crops causing devastating diseases and wasting resources. Approaching plant health from a phytobiomes perspective that accounts for bacterial-fungal interactions, including endohyphal bacteria (EHB) that live within fungi, will open new avenues of disease control and plant growth promotion. To work in fungal-bacterial-plant relationships, I need a variety of skills and a knowledge of diverse organisms. The researchers at the University of Arizona are at the forefront of molecular research on facultative EHB in endophytes; Dr. Baltrus and Dr. A. Elizabeth Arnold have pioneered recent approaches to study the dynamics and cell-to-cell communication of facultative EHB in diverse fungi. I will apply these methods developed for ecological systems to agricultural systems. Other faculty, including Dr. Barry Pryor, have strong ties to local agriculture in Yuma, the region responsible for producing 90% of the winter leafy vegetables grown in the United States. Emerging soil-borne fungal diseases like *Fusarium* wilt threaten lettuce production, resulting in farmer interest in suppressive soils and microbial controls.

As we learn more about microbe-microbe relationships, especially EHB, plant-associated contexts, we will find ways they can be positively and efficiently manipulated in agricultural systems, which I see as a guiding goal of my future research program. **Through this fellowship I will develop agriculturally relevant EHB systems to build a research program on that will identify new ways to benefit U.S. agriculture through the emerging field of microbe-microbe interactions impacting plant health**. The outcomes from this study would be foundational for future independent research grant applications, such as complementing the bacterial genetics work in Objective III with an investigation of the host genetics affecting EHB recruitment. Understanding if fungal pathogenicity is affected by EHB (Objective II) could be the basis of future work on fungal pathogenicity mechanisms or suppressive soils, uncovering possibilities for pathogen control by targeting EHB. My research program will focus on

interactions in diseases that are difficult to control with current methods, such as soil-borne diseases, and ones that may be amenable to more sustainable biocontrol options.

Professional networking: As an active member of scientific societies, including my primary society APS, I served as an intern for the APS PPB, was interviewed on the International Society of Molecular Plant-Microbe Interactions (IS-MPMI) and APS podcasts, facilitated a workshop and Idea Café at annual meetings, and coordinated virtual outreach on the International Year of Plant Health 2020 Task Force through partnership with Skype A Scientist. I will continue to find opportunities to engage with professional societies including applying to early career editorship positions and attending or facilitating workshops at meetings. **My involvement in APS has grown tremendously, broadening my network and the impact of my work, and I will follow that trajectory.** I will prioritize attendance at society meetings, including APS, to promote my work and develop a network of collaborators and resources. I will continue to engage with other scientists on social media where I have cultivated a community in which I can readily find resources, feedback, guidance, and support.

Training goals: Having worked on fungi, plants, and bacteria, and armed with the following skills, I will be primed to lead research on the impact of fungal-bacterial interactions on plant health, including teaching and mentoring the next generation of plant and microbial scientists.

Research: My undergraduate training in biochemistry and extensive experience with cloning, protein techniques, and microbial manipulation in the lab have solidified my expertise in these areas. I developed this proposal to bring other critical skills to the level of proficiency that I have with molecular biology and genetics. First, I will hone my conventional plant pathology skills of pathogenicity assays, isolation, and characterization through Objectives I and II. Second, I will build on my bioinformatics experience from genomics and gene expression analysis by undertaking bacterial genome sequencing in Objective I and the TnSeq experiment in Objective III. I have included funds in my budget to attend a workshop covering at least one of these skill areas, such as the Fusarium Laboratory Workshop hosted by Kansas State University to improve my isolation and fungal biology skills. The research outcomes and skill development proposed here will be foundational to my future independent research program.

Teaching: I have demonstrated that undergraduate and graduate student progress and career development are a passion of mine and will continue to be a focus of my career. I was a teaching assistant for three courses over five semesters and received positive written feedback from students. Beyond teaching assistantships, I have developed and delivered guest lectures and workshops, and taken an online STEM education class. Going forward, I plan to attend minicourses and workshops provided by the University of Arizona Office of Instruction & Assessment. I will seek to guest lecture in upper-level undergraduate courses at the University of Arizona such as Principles of Plant Microbiology, Plant Biotechnology, and Microbial Genetics, a course taught by Dr. Baltrus, as well as virtually delivering guest lectures at other institutions.

Mentoring: From my own experience as an undergraduate researcher and mentor, I am passionate about undergraduate authorship, skill development, and experimental ownership. I have mentored four highschool or undergraduate women and two of my published thesis chapters have undergraduate authors. Dr. Baltrus regularly engages undergraduates in projects in his lab and publishes with undergraduate authors. I have budgeted for the stipend of at least one undergraduate researcher for the duration of this project as I see many potential undergraduate projects within this proposed work. I will work with the student to design a project based on their interests and background knowledge. As in the past, I will plan experiments with my mentee via

Google Docs and collaborate on problem solving, so that they learn experimental design and troubleshooting skills during their experience. I will also encourage their participation in research symposia and attendance at academic meetings through undergraduate travel awards. In addition to undergraduate mentees, the Baltrus lab has three graduate students who have recently joined that lab that I will be able to informally mentor on fellowship applications, experimental troubleshooting, and degree progress.

Extension Outreach: While I have limited experience with extension outreach, one of the unique benefits of working with land grant institutions is facilitation of direct engagement with the agricultural community and the ability to hear their perspective and concerns. I will work with the Yuma Center of Excellence for Desert Agriculture (University of Arizona) to engage with agricultural stakeholders of the lettuce industry (see letter of support). I will be attending annual field days held each fall and will carry on conversations with farmers through both formal seminars on soil microbial communities and informal meetings.

Mentoring Plan

Dr. Baltrus has expertise in comparative and evolutionary genomics, especially of bacterial symbionts and pathogens. His extensive experience with sequencing and genomics will be critical for Objectives I and III and for improvement of my bioinformatics skills under the guidance of a genomicist. I will meet with Dr. Baltrus at regularly scheduled meetings at least once every other week to discuss research and professional development progress. While in the Baltrus lab, I will present at lab meetings as well as ongoing intergroup meetings with other relevant labs. Dr. Baltrus will help me recruit and mentor undergraduate students. As part of my training to become an independent investigator, Dr. Baltrus will include me in preparing other grant proposals, including attending budget meetings and editing proposals. As a journal editor, he will also facilitate my participation in peer review when possible and connect me to opportunities he becomes aware of within societies and journals.

Furthermore, I will form a network with researchers currently working on plant-*Fusarium* interactions. I will work with Dr. Barry Pryor (see attached letter of support) to learn more about fungal isolation from field samples and pathogenicity assays for the lettuce-related goals in Objectives I and II. Regularly scheduled virtual meetings with Dr. Gary Bergstrom (collaborator at Cornell University; see attached letter of support) and Dr. Pryor will serve as timeline reminders and resources, while keeping me connected to *Fusarium* communities.

Introduction

Background: Fungi and bacteria interact across the phytobiome, in the underground rhizosphere and surrounding soil as well as endophytically and epiphytically in the foliage and stems of the phyllosphere. Studies on microbial interactions within the phytobiome are often limited to one-to-one interactions, but are interesting for their insights into microbial warfare and partnerships (*1-3*). Bacterial-fungal relationships can alter disease severity, such as the interaction between *Burkholderia glumae* and *Fusarium graminearum* in rice plants resulting in increased toxin and spore production (*4*). Conversely, the natural ectosymbiotic bacterial consortium of *Fusarium oxysporum* MSA 35 inhibits pathogenicity on lettuce, converting the fungus to a plant growth-promoting endophyte and antagonist to plant pathogenic *F. oxysporum* isolates (*5-7*).

In some cases, fungal-bacterial interactions are so close that bacteria live intracellularly within fungi, as was first found in arbuscular mycorrhizal fungi in the 1970s (*8*). For decades, only a handful of EHB were known, largely in obligate relationships involving mycorrhizal fungi (*9*). In 2005, the first case of a plant pathogen relying on an EHB to cause a plant disease was

discovered; *Rhizopus microsporus* requires a toxin produced by facultative endosymbiotic *Mycetohabitans* spp. to cause rice seedling blight (*10*). *Mycetohabitans* and other EHB that were initially discovered were characterized as Burkholderiaceae, which are commonly found as symbionts across the tree of life and occupy a varied set of niches (*11, 12*).

Within just the past decade, screens of plant-associated fungi have found that EHB are much more diverse than was previously appreciated and that these relationships range from facultative to obligate (*13, 14*). Various EHB isolated from endophytic fungi of tropical and coniferous trees do not have reduced genomes and seem to be horizontally transmitted, suggesting they have undergone less coevolution with their fungal hosts, compared to *Mycetohabitans* and EHB from mycorrhizal fungi. (*14, 15*). These larger screens and isolate-specific interaction studies have revealed that EHB can affect their fungal hosts in a variety of ways including altering carbon use, encouraging growth of germinating spores, altering plant cell wall degrading enzyme activity, and controlling sporulation (*16-19*). EHB also appear to vary in how they establish infections within fungal hosts. For example, *M. rhizoxinica* requires a Type III secretion system to infect *R. microsporus*, but many of the facultative EHB sampled thus far lack such structures (*15, 20*).

Developing new systems to probe EHB interactions will clarify the full repertoire of ways that these partnerships impact plant health. **The diversity of functions discovered so far within plant-associated EHB suggests potentially broad impact of these relationships on agriculture, plant health, and pathogen virulence.** EHB can affect the plant host negatively through toxin production or positively through production of plant hormones like indole-3-acetic acid (IAA) (*21-23*). The pathogen *Rhizoctonia solani* harbors an *Enterobacter* sp. that is required for increased toxin production by the fungus and full virulence on creeping bentgrass (*24*). Still, some EHB play unclear roles in their fungal hosts lifestyle; most EHB in seed-associated fungi are not important for seed colonization by the fungus (*25*). Two strains of the plant pathogen *Ustilago maydis* have an intracellular *Bacillus* sp. capable of nitrogen fixation, though it is unknown whether this relationship affects pathogenicity of *U. maydis* on corn (*26*). There are major questions surrounding the role and specificity of EHB: (1) can closely related species and strains form the same partnerships, (2) what genetic capabilities are required for the bacteria to associate with fungi, and (3) do these interactions impact pathogenicity of fungal hosts.

Fusarium spp. are documented pathogens and endophytes of an extensive array of plants, with complicated phylogenetic relationships (*27*). Pathogenic *Fusarium* spp. cause disease in many major crops leading to yield loss and toxin contamination. In larger screens of endophytic fungi, isolates from tropical trees that group with *Fusarium solani* and *Fusarium oxysporum* have been shown to contain diverse endohyphal bacteria (*14, 28*). Based on the known interactions and larger screens, we hypothesize that many fungi form facultative relationships with bacteria in a plant-associated context, including phytopathogenic *Fusarium* spp. (*10, 13, 14, 24, 26*). **Here I propose to do a survey of EHB in two distinct phytopathogenic *Fusarium* species complexes that affect US agricultural products:** *Fusarium graminearum* (*Fg*), causal agent of Fusarium head blight in wheat and barley and Giberella ear and stalk rots in maize, and *Fusarium oxysporum* f.sp. *lactucae* (*Fol*), causal agent of Fusarium wilt of lettuce.

Fg causes episodic outbreaks in grains across the US based on regional weather conditions, resulting in yield losses of tens of millions of dollars in years like 2008 (*29*). While most *Fusarium* spp. are soilborne, spores of *Fg* are aerially dispersed and infect flowering cereals and wild grasses. Disease management relies mostly on targeted fungicide sprays of moderately resistant crops (*30, 31*). Haphazard weather associated with changing climate has the

potential to increase the number of outbreaks seen if rain corresponds to flowering times and affects spray timing. *Fg* is particularly dangerous because of its production of highly regulated mycotoxins that affect humans and animals through exposure to contaminated grains. Many screens for biocontrol agents have been conducted, though these have not caught on commercially due to the relative success of fungicides for disease control (*32*). However, with the rising popular interest in fungicide resistance and the impact on human health outcomes, alternative options to chemical control should be considered (*33*).

In contrast, *Fol* is soilborne and can persist in the soil for many years, making treatment difficult once the pathogen is there (*34*). *Fol* was first found in Arizona in 2001, after being found in California previously (*35*). As more fields become infested with the fungus, there is an increase in incidence and losses, making Fusarium wilt one of the emerging diseases in lettuce in the US. Current methods for controlling Fusarium wilt are limited mostly to cultural methods like planting when temperatures are lower and using pathogen-free seed (*36*). Both nonpathogenic isolates and pathogenic forma specialis of *Fusarium oxysporum* broadly colonize plants and are interrelated; pathogenic isolates typically cause disease in only one plant host. It is possible that the pathogenicity of some *F. oxysporum* isolates is mediated by bacterial ecto- or endosymbionts and not merely fungal genetics, as is the case for previously mentioned *F. oxysporum* MSA 35 (*6*). Because control of *Fol* is so difficult, biocontrols like suppressive soils could be a viable option with increased knowledge of microbial interactions (*37-39*).

In 2012, a ranking of the most important plant pathogens according to fungal pathologists placed *F. graminearum* and *F. oxysporum* as fourth and fifth (*40*). Mycotoxin contamination and yield loss continue to be issues across crop species infected by *Fusarium* fungi, despite current control measures. We know that endophytic *Fusarium* spp. can harbor EHB and that phytopathogenic *Fusarium* spp. have documented interactions with bacteria that alter disease development. A single study has found that *Enterobacter*-containing *Fusarium fujikuroi* produces consistently higher levels of the FDA-regulated toxin, fumonisin (*41*). Yet no one has investigated EHB in plant pathogenic *Fusarium* spp. on a broad scale, especially between two *Fusarium* spp. with such different disease cycles and lifestyles**. The work proposed here will be the jumping off point for a research program that dives into the molecular mechanism of *Fusarium*-bacterial interactions, how they impact plant health in important crops, and applying this knowledge for better and more sustainable disease control.**

Preliminary Data: Based on the findings that EHB partner with *Fusarium* spp. (*14, 17, 41*) and my previous experience working with barley, I screened *Fusarium* spp. isolates from grasses in New York State (*42*) from Dr. Gary Bergstrom's collection, while I was conducting my predoctoral work. I screened for bacteria by PCR amplification from fungal DNA preparations using the universal 16S bacterial primers 10F and 1507R. Of the 16 *Fusarium graminearum* (Figure 1A) and 20 related *Fusarium* spp. isolates screened, all had a PCR product of ~1.5 kb, the expected size for bacterial 16S rRNA, while the negative no template control did not. I chose 10 for Sanger sequencing of the PCR amplicon and found that there were two groups of related bacteria based on a BLAST search: *Burkholderia* spp. within the *Burkholderia cepacia* species complex in *F. graminearum* isolates and *Pseudomonas geniculata/Stenotrophomonas maltophilia* in other grass-associated *Fusarium*. Both of these bacterial groups have members that are known to associate with plants. Some *S. maltophilia* and *P. geniculata* strains have been characterized for fungal biocontrol, including *Fusarium* spp., while others are known to produce

IAA or fix nitrogen and generally be plant growth promoting (*43-45*). I have further been able to successfully isolate a *P. geniculata/S. maltophilia* strain from one of the fungal isolates.

Both the PD and Mentor have previously worked on EHB, including developing EHB infection and extraction protocols and carrying out genetic manipulations of the bacteria. These protocols will be used in the work proposed here as the starting point for these new systems (*46, 47*). Dr. Baltrus and colleagues developed techniques for curing and reassociation of the previously isolated endophytic fungus *Pestalotiopsis* sp. strain 9143 and its bacterial symbiont *Luteibacter* sp. stain 9143, as well as the closely related *Luteibacter* sp. strain 9145 and its fungal host *Microdiplodia* sp. strain 9145 (*13, 46, 47*). *Luteibacter* sp. strain 9143 is amenable to transformation and shown to be endocellular via a fluorophore gene insertion (Figure 1B). Complete genome sequences of these bacterial strains and many other EHB have been assembled by Dr. Baltrus and colleagues.

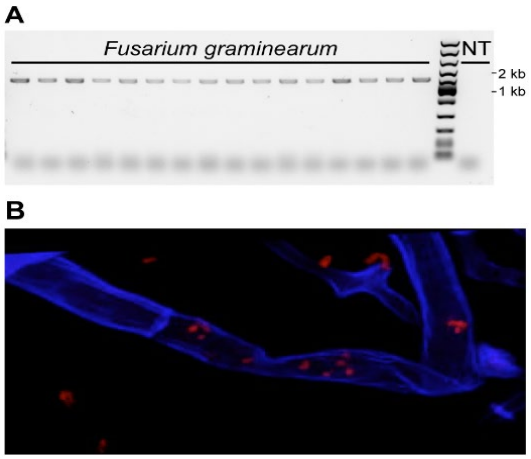


Figure 1. A) Agarose gel electrophoresis of bacterial 16S rRNA PCR products amplified from *F. graminearum* DNA preparations. NT – No template. **B)** *Luteibacter* sp. 9143 fluorescing red, from Tn7 insertion of mCherry, within fungal hyphae stained blue with Calcofluor white.

Rationale and Significance

This project addresses the AFRI area of Plant Health and Production and Plant Products, because of the significance of microbial interactions within the phytobiome to plant health. Such interactions have been the basis for biocontrols of plant pathogens, like the use of *Bacillus* spp. to reduce infection by fungi including *Fusarium* spp. (*37, 48*). Unfortunately, many biocontrols have less impact in the field than expected (*49*). It is crucial to better understand how microbes interact within the phytobiome and modulate each other's behavior and abilities to develop more effective biologicals-based amendments. Indigo Ag has licensed a patent owned by Dr. Baltrus and Dr. Arnold for fungal-bacterial reassociation techniques, indicating a clear interest from commercial stakeholders in determining the role of EHB on plant health. Making agriculture more sustainable and resilient relies on improved tool development to overcome mounting disease pressure, changing climate, increasing public opposition to chemical usage, and pathogen resistance to synthetic chemicals.

Mechanistic and functional studies have only scratched the surface of known EHB, and few phytopathogens have been assessed for EHB, especially on a scale broader than single isolates (*10, 24, 26, 41, 50*). My proposed project will not only assess the presence and variety of EHB in phytopathogenic *Fusarium* spp. but will also begin to investigate how these associations are established and whether they impact pathogenicity. One could imagine selecting bacterial inoculant strains not only for plant growth promotion abilities, but their ability to associate with pathogenic fungi and reduce fungal virulence. This has been demonstrated in other situations like the release of *Wolbachia*-infected mosquitoes to reduce human virus transmissibility (*51*). EHB that contribute to a reduction in virulence or toxin production have the potential to be targeted or developed into a post-harvest fungal control, which is an issue especially in *F. graminearum*-contaminated grains (*41*). Furthermore, much in the way that studying how microbes control the plant immune system has led to discoveries that inform resistance breeding and engineering,

studying bacteria that alter fungal pathogenicity could offer new insight into fungal pathogenicity mechanisms, informing control both chemically and biologically.

Approach

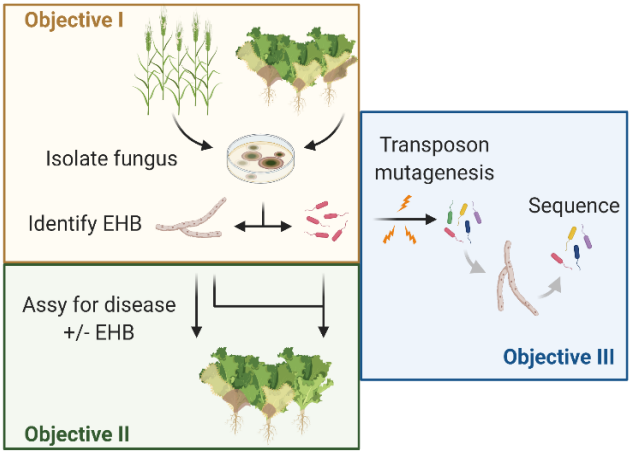
Hypothesis: Agriculturally relevant *Fusarium* fungi form facultative associations with genetically capable endohyphal bacteria that alter fungal virulence or pathogenicity.

Objectives:

I. Survey and identify EHB from two agriculturally relevant *Fusarium* spp. that are from key pathogen groups
   a. *Fusarium graminearum* (Fusarium head blight) in small grains from New York
   b. *Fusarium oxysporum* f. sp. *lactucae* (Fusarium wilt) in lettuce from Arizona

II. Determine if pathogenicity and virulence of selected *F. oxysporum* isolates depend on EHB presence

III. Investigate bacterial genes required for association of EHB with fungal hosts



Methods:

Objective I: Survey and identify EHB from two agriculturally relevant Fusarium spp. that are from key pathogen groups

Understanding the presence and diversity of EHB in two phytopathogenic *Fusarium* spp. will help determine how widespread these relationships are, in fungi with contrasting lifestyles. For my survey of *Fusarium graminearum* (Fg), I will continue to screen isolates provided by Dr. Bergstrom (see Letter of Support). By using an existing collection and new isolates of Fg from regular surveying, I can efficiently use my time and resources for EHB screening. The Bergstrom lab collects isolates from various agricultural sites and natural preserves distributed across New York State. Collected plant tissues are surface sterilized and plated on artificial growth media containing 0.2 g/L streptomycin and 0.24 g/L neomicyin. *Fusarium* spp. that grow from the plant tissue are transferred to potato dextrose agar with no antibiotic, identified morphologically, and glycerol stocked. For my survey of *Fusarium oxysporum* f.sp. *lactucae* (Fol) from lettuce in Arizona, I will work with Dr. Pryor to obtain *Fol* isolates from diseased lettuce from local fields, some of which will be collected during an ongoing survey that his research program is conducting. Like *Fg* samples, *Fol* will be identified morphologically and genomic DNA will be extracted for verification and EHB screening.

As done in the preliminary screen, I will screen isolates by PCR using the 16S rDNA 'universal' primers 10F/1507R and 27F/1492R following previous studies (*13, 52*). Amplicons from positive samples will be sent for Sanger sequencing. I will then stain fungal mycelia with a Live/Dead fluorescent stain and image them to visually inspect for bacteria by microscopy (*10, 13*). I will attempt to extract the bacteria from fungal isolates by using a variety of previously established techniques, including incubation at high temperature and mechanical tissue lysis followed by filtration (*47, 53*). Bacteria that are culturable will be assessed for transformation amenability by Tn7 insertion of a fluorophore. For the fungal partner, I will try methods of

removing bacterial symbionts by curing through serial single spore propagation or serial/extended plating on antibiotics (*10, 18, 47*). Basic fungal morphology on plates will be assessed to determine any phenotype between fungi with and without EHB. I will then experiment with reforming the relationships with bacterial partners and their hosts and try to swap host/symbiont partners to assess partner specificity. Reassociation of fluorophore-tagged bacteria with their fungal hosts will confirm endocellular localization.

Methods that work to isolate or cure an EHB strain may not work for all, so I will focus on isolates that vary in 16S rDNA sequence first to hone methodology and then expand to related bacteria. Based on the diversity of bacteria identified, I plan to sequence the genomes of select isolates to compare them to previously sequenced facultative EHB from endophytic fungi. I will use a combination of Oxford Nanopore sequencing and Illumina sequencing that is commonly being employed in the Baltrus lab, which will contribute to my training goal of increased proficiency with bacterial genomics (*54*). Determining the size of bacterial genomes and diversity of EHB will show whether phytopathogenic *Fusarium* form transient relationships with many partners or more co-evolved, stable relationships. **The *Fusarium*-EHB partnerships characterized in this Objective will be systems that I can investigate further in my own independent research program to fully characterize their role;** teasing apart the details of partnership establishment and maintenance will be critical for assessing biocontrol potential and impact on plant health. The isolation and sequencing techniques that I plan to employ in this objective are skills that I outlined as important for my career development in my training plan.

Expected Results and Pitfalls*:* The methods and techniques in this section have been used across endohyphal bacterial systems and should work with reasonable modifications. If visual methods determine that associated bacteria are not actually within the cell, I can continue to investigate the extracellular interactions in similar ways. Based on previous surveys of fungi related to *Fusarium* spp., I expect to find a diverse set of EHB inhabiting *Fol* and *Fg* (*14*). However, preliminary results from screening grass-associated *Fusarium* spp. have resulted in identification of only two groups of bacteria after sequencing 10 isolates, so it may be more limited. The greatest pitfall is the possibility that the initial step of culturing *Fusarium* isolates on antibiotics for isolation will have cured the fungi of any EHB. However, I expect this does not cure all EHB from fungi, given the brief period of culturing on antibiotics and the preliminary evidence of associated bacteria I have found so far. Most curing protocols for EHB require extended or sequential plating on antibiotics for all bacteria to be removed (*6, 16*). If this ultimately does pose an issue, I will attempt isolating without antibiotics, though this presents its own challenges.

Objective II: Determine if pathogenicity and virulence of selected *F. oxysporum* isolates depends on EHB presence

One of the potential roles of EHB is virulence suppression or enhancement of the host fungus, especially given known cases where EHB or extracellular bacteria contribute to toxin production or virulence (*4, 6, 24, 41*). For this objective, I will focus on the *Fol*-lettuce system to capitalize on the resources at the University of Arizona. I will cure EHB-harboring *Fol* isolates identified in Objective I and test the impact on pathogenicity. Fungi will be passaged through media with or without antibiotics to create a cured strain and strain that has been cultured for an equivalent length of time as the "wildtype". I will aim to reassociate the cured isolate with extracted EHB to then test for complementation of any phenotypes resulting from curing.

Inoculations will be carried out as described in other studies (*55, 56*). Briefly, fungi with and without EHB will be grown on potato dextrose agar until they sporulate. Spores will be

scraped from the plate in 0.5% potassium chloride and filtered. Susceptible lettuce seedling roots by will be rinsed and dipped in spore suspension or a mock inoculation of 0.5% potassium. Inoculated seedling will be placed in a greenhouse in a randomized block design and monitored for three weeks for symptom development which will be rated on a 0 to 3 scale: 0 = no symptoms, 1 = mild stunting, 2 = severe stunting and some chlorosis or necrosis, and 3 = dead plant (*56*). Statistical analysis of the disease ratings will determine if the virulence of the fungi was affected by the presence of EHB.

***Expected Results and Pitfalls*:** I expect to find *Fol* virulence will be affected by at least some EHB, based on previous findings with ectosymbiotic bacteria (*6*). This may be just a decrease due to nutritional sharing with a symbiont, or a larger effect, which would be investigated in the future. This objective does rely on finding endohyphal bacteria in *Fol* or alternatively establishing a relationship with a previously isolated EHB strain from endophytic *F. oxysporum*. Based on known *F. oxysporum* association with endo- and ectohyphal bacteria, I expect that *Fol* will be able to harbor EHB (*6, 22*). However, if it does not, I will switch pathogenicity experiments to the *Fg*-wheat system where I have already identified putative endosymbionts. Though not as relevant to Arizona and more difficult to carry out there, I have experience with pathogenicity assays in small grains and this is an experiment that is equally of broad interest.

Objective III: Investigate bacterial genes required for association of EHB with their hosts

I hypothesize that fungal and bacterial genes are involved in determining which EHB partnerships can form. This hypothesis is based on preliminary, unpublished results from the Baltrus lab that a lab strain of *Pseudomonas stutzeri* cannot infect the endophytic fungus *Pestalotiopsis* sp. 9143 that naturally harbors *Luteibacter* sp. 9143. As part of my current work in the Baltrus lab, I will be developing a Random Bar code Transposon-site Sequencing (RB-TnSeq) library for *Luteibacter* sp. 9143 to identify genes of interest that play a role in colonization of fungal hosts (*57*). Dr. Baltrus and colleagues have already shown that transposon mutagenesis is possible in *Luteibacter* sp. 9143 by inserting a fluorophore tag with a transposon insertion plasmid (Figure 1B). Additionally, quantitative reassociation assays have been developed for this specific system (*46*). **Creating an RB-TnSeq library of a *Fusarium*-associated EHB to compare and contrast with the in-progress *Luteibacter* sp. 9143 library will be done to satisfy Objective III and my training objective to learn large scale genomic techniques.** To find a bacterial symbiont amenable to transformation and transposon mutagenesis, I will start with the *P. geniculata/S. maltophilia* strain that I have been able to culture from a NY *Fusarium* sp., but may switch to other EHB once identified.

For RB-TnSeq experiments, a transposon mutagenesis library of the bacterium of interest is made and sequenced to associate bar codes with insertion events. Then cured fungus is reassociated with the library, selecting for mutants that are still able to form the relationship. Bacteria will be re-isolated by adapting a published protocol for bacterial isolation from leaves that involves disrupting the host cells, filtering the lysate, and centrifuging (*58*). The resulting bacterial cell pellet will be the template for DNA purification, PCR amplification, DNA library preparation, and sequencing of the bar codes. The amplification of relevant DNA sequences from the insertion sites reduces efficiency effects from excess bacterial or fungal genomic DNA. Sequence analysis will be done following the publicly available computation pipeline for RB-TnSeq (*57*). Fitness values will be calculated by dividing the relative abundance of a barcode after associating the library with a host by the relative abundance of a barcode before association.

By creating an RB-TnSeq library for both *Luteibacter* sp. 9143 and a *Fusarium*-

associated symbiont, I will develop valuable resources for the experiment proposed here as well as future high throughput experiments, such as assessing the capability of these bacteria to live endophytically or infect other fungal hosts. I will expose both libraries to both their respective fungal hosts if they can host swap; we know that *Luteibacter* sp. 9143 can infect both *Pestalotiopsis* sp. 9143, the host from which it was isolated, as well as *Microdiplodia* sp. 9145. By exposing bacterial libraries two or more hosts, I will identify genes that are unique to the infection of a specific host, and shared genes needed to establish an endohyphal infection.

Expected Results and Pitfalls: I expect that secretion systems may be important for relationship establishment, given examples such as the critical nature of the type II and III secretion systems for *Mycetohabitans rhizoxinica* invasion of *Rhizopus microsporus*, though *Luteibacter* sp. 9143 does not have a type III secretion system (*15, 20, 59*). Transporters and metabolic pathways are also likely to be important for adaptation to an intracellular lifestyle, though we do not know which and these may vary by fungal host. Motility is a large component of occupying different niches, so these genes may be affected as well (*60*). **Genes identified provide new hypotheses for individual characterization to reveal potential targets for EHB control and engineering as part of a future research program**. This experiment provides a technical foundation to build upon for other EHB for a more global view of relationship establishment.

Given the widespread use of TnSeq, my own experience with diverse gram-negative bacteria, and the ability to transform non-obligate EHB so far, this experiment is feasible in EHB isolated from *Fol* or *Fg.* Identifying a strain to conduct this experiment will be an early priority while satisfying Objective I. If I cannot find an amenable strain within a reasonable timeframe, I can do comparative genomics of EHB strains and *Luteibacter* sp. 9143, drawing on the results of that RB-TnSeq experiment, yielding information about whether core genes are shared across EHB. However, it is possible for the association of EHB and a fungal host to be determined more by the fungal genetics, i.e. host recruitment, than by bacterial genetics. If I find that very few genes appear to be important for endohyphal fitness, I have even more reason to investigate how the fungal host is selecting symbionts in a future study.

Timeline and Evaluation Plan

Timeline: My timeline for completion involves finishing Objective I within the first year so that I will have strains that may be incorporated into Objectives II and III. I will also focus on creating the RB-TnSeq library for Objective III in the first year as soon as I identify appropriate strains, with the goal of completing this experiment by the middle of year two. Pathogenicity assays in Objective II will also be done in year two. Manuscripts will be prepared throughout the grant period as data is collected and analyzed (see Management Plan for timetable).

Milestones: **(1)** I will make at least one presentation at a scientific conference each year, such as ISS Congress 2021 and APS Plant Health 2022. **(2)** I will have at least two teaching experiences per year: a guest lecture and a workshop/minicourse. I will request feedback from undergraduate mentees and students about my mentoring and teaching skills as **indicators** of teaching progress and to identify areas to improve on. **(3)** I will aim to publish three papers, one on each of the objectives proposed here, and to have those submitted by the end of the grant period. These studies will be published in relevant journals of interest to a diversity of plant and microbial-related fields, to promote my work and to distribute my findings as broadly as possible.
(4) During the fellowship, I will be actively applying to new grants and faculty jobs. Successful applications will serve as an **indicator** of my training through this grant.

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Facilities & Other Resources

Laboratory: The Mentor (Dr. David Baltrus) has a modern research laboratory on the University of Arizona's campus with multiple workbenches, one of which will be dedicated to the Project Director (Morgan Carter) with another available to undergraduate researchers working on this project. This laboratory is maintained at a BSL-2 status and has appropriate workspaces and equipment to safely carry out microbial and genetics experiments such as those proposed here.

Office: The laboratory contains office space for Baltrus, as well as desk space for 8 graduate students/postdoctoral fellows, one of which will be dedicated for Carter.

Computers: We have access to shared research computing clusters at the University of Arizona accounts through the UA which enable calculations to be performed on approximately 3,000 cores singly or in parallel. We additionally have access to, and close ties with, open access computing clusters associated with Cyverse, which is located on the UA campus (http://www.cyverse.org/)

Greenhouse: The Baltrus lab has growth chambers for small plant assays. Dr. Barry Pryor's (collaborator) lab has greenhouse space for lettuce growth and pathogenicity assays.

Other: Sequencing and Equipment. The UA Genomics Core (UAGC) is a centralized sequencing facility within walking distance of the Baltrus laboratory. The UAGC facility provides competitively priced, high quality, and fast-turnaround sequencing services on an ABI3730xI, as well as massively parallel next-generation sequencing through an Illumina HiSeq and NovaSeq. More information can be found at http://uagc.arl.arizona.edu/. The Baltrus lab has access to a shared media facility within the Bio5 Institute, which is used to prepare a variety of reagents. more information can be found at http://www.bio5.org/facilites/research-services/cores/media-facilities.

Equipment

The Baltrus lab has the required equipment to complete the objectives proposed in the project narrative. The lab currently has a New Brunswick refrigerated shaking incubator, a non-refrigerated New Brunswick shaking incubator, and two stationary incubators for propagating bacterial and fungal cultures. There are currently two -80 freezers for long term culture storage. Equipment needed for DNA extraction and sequencing is available, including thermocyclers, microcentrifuges, and gel electrophoresis equipment. An autoclave is available for common use on the same floor of the building.

Key Personnel

The PD, Morgan Carter, conducted the preliminary experiments in grass-associated *Fusarium*. Carter developed the research goals proposed here, with the help of the Mentor, Dr. David Baltrus, to refine them and establish collaboration with Dr. Barry Pryor for his knowledge of lettuce-associated *Fusarium* isolation and pathogenicity assays. Pryor and Dr. Gary Bergstrom will serve as advisors to Carter on research objectives related to their expertise and will meet with Carter regularly for updates on research plans and outcomes. They will also provide samples or facilitate sample collection as necessary. Carter will oversee at least one undergraduate researcher over the duration of the project period. She will be responsible for experimental design and execution, as well as data analysis, with consultation with the Baltrus. He will advise the PD through regular meetings and help evaluate the progress of Carter. Attendance at scientific meetings and workshops proposed in the training plan will be done by Carter. Baltrus and Carter will seek out and create teaching opportunities for Carter. Publications will be co-written by the Carter and Baltrus

Situation: Postdoctoral training in techniques and knowledge needed for developing an independent research program studying uncharacterized symbiotic bacterial-fungal interactions

Inputs

- Mentoring:
  - Time
  - Mentor
- Professional Development:
  - Data
  - Travel funding
  - Institutional support
  - Undergraduate Student
- Project:
  - Knowledge
  - Materials/Funding
  - Laboratory space
  - Sequencing
  - Growth space
  - Fungal Isolates

Activities

- Mentoring:
  - Lab meetings
  - 1-on-1 meetings
  - Network building
  - Teaching opportunities
- Professional Development:
  - Conference presentations
  - Manuscript preparation
  - Undergraduate mentoring
- Research:
  - Fungal isolation
  - Pathogenicity assays
  - Sequencing and analysis
- Extension Outreach
  - Farmer engagement

Outputs

- Identification of bacterial-fungal interactions involving phytopathogenic *Fusarium*
- New systems of organisms to work in for future experiments
- 3 scientific publications
- Larger network of colleagues
- Increased technique proficiency
- Scientific training of an undergraduate researcher
- Informational lecture or video for farmers

Outcomes

Personal

- Increased capacity for independent investigation
- Improved scientific communication skills, including teaching
- Improved leadership, mentoring, and teamwork abilities

Public

- Increased knowledge of bacteria-fungi interactions
- New avenue for sustainable agriculture research/biocontrols
- A trained agricultural scientist advancing in academia

Assumptions

- Bacteria and fungi are closely associated in plant systems and will be isolated together
- Microbes influence each other's behaviors and abilities

External Factors

- Disease incidence in the field
- Scientific conferences and teaching opportunities occurring during time frame of project

Management Plan

The PD (Dr. Morgan Carter) will oversee at least one undergraduate researcher over the duration of the project period. The Mentor (Dr. David Baltrus) and Carter will seek out and create teaching opportunities for Carter as possible, with the goal of two teaching experiences per year. Carter will be responsible for experimental design and execution, as well as data analysis, with consultation with Dr. Baltrus through biweekly meetings. These meetings will help evaluate the progress of the project and Carter's training. When necessary or helpful, Carter will reach out to others at the University of Arizona, such as Dr. Betsy Arnold (fungal ecology), and abroad to request training or resources and establish collaborations if beneficial to the project outcomes. These additional contacts may also be requested to review prepared manuscripts for critical feedback or act in an advisory capacity as needed. Carter will work with the Yuma Center of Excellence for Desert Agriculture to engage with farmers and industry stakeholders through workshops, videos, and/or field days. Dr. Pryor and Dr. Gary Bergstrom will have regular meetings with Carter to assess progress and offer insights.

Attendance at scientific meetings and workshops proposed in the training plan will be done by Carter annually. This will allow for input on the proposed work from the scientific community at-large. Publications will be co-written by Carter and Baltrus and will be published in peer-reviewed journals to assess their quality and impact. The research project and techniques used shall be recorded in an electronic laboratory notebook with copies kept by both Carter and Baltrus, while new strains and data will be stored in the Baltrus lab with accurate documentation. Carter plans to continue working on these systems and questions after this project period ends and will then move duplicates of isolates to her new location as necessary.

Expected Timeline:	**Su21**	**F21**	**W21**	**Sp21**	**Su22**	**F22**	**W22**	**Sp22**
Research Objectives								
I. Identify EHB								
II. Pathogenicity								
III. Mutagenesis								
Training Objectives								
Undergraduate Mentorship								
Teaching Experiences		Lecture	Work-shop			Lecture	Work-shop	
Communication								
Conference presentations								
Manuscript preparation					Sub-mission		Sub-mission	Sub-mission
Extension/ outreach		Field Day				Field Day	Seminar /Video	

Data Management Plan:

Sharing of results and protocols
Results, data analyses, and detailed experimental protocols will be published in a timely fashion, with preference given to open access journals. Sequencing reads and assemblies will be included as supplemental files in publications through Figshare.

Large datasets
Sequencing data will be archived on the Baltrus lab server, which is continually backed up by the University of Arizona, and distributed without restriction upon publication. All data will additionally be stored on redundant backup servers at the University of Arizona. Sequencing data not included as available supplementary material on the internet will be distributed upon request.

Access to strains
Strains will be maintained at the University of Arizona and can be distributed upon request after publication, with the expectation that Baltrus and/or Carter will be notified if the strains are further shared. Transport of strains will follow regulations and may necessitate that the recipient is permitted.

Management of intellectual property
Any intellectual property that results from the proposed research in the Baltrus lab will be governed by the University of Arizona policies.

Morgan Elizabeth Carter
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https://orcid.org/0000-0001-5639-2013; https://morganecarter.wordpress.com

Education:

Cornell University Ithaca, NY	*Ph.D.* GPA 4.0	Plant Pathology and Plant-Microbe Biology	2014-2020
North Carolina State University Raleigh, NC	*B.S.* GPA 4.0	Biochemistry; Minors in Genetics and Biotechnology	2011-2014

Selected Research Experience:

2020-	Postdoctoral Researcher	Dr. David Baltrus	University of Arizona,
2014-2020	Graduate Thesis Research	Dr. Adam Bogdanove	Cornell University
	"Translating lessons from effector biology to fungal effector screens, plant resistance mechanisms, and bacterial-fungal interactions" – PhD Conferred May 24, 2020		
2011-2014	Plant Biology Research Assistant	Dr. Margaret Daub	North Carolina State University

Presentations:

2020 - American Phytopathological Society Potomac Division Meeting – **Invited Talk**
"Finding My Voice: Communicating Plant Pathology as a Graduate Student"
2019 - University of Arizona School of Plant Sciences – **Seminar**
"Not just for plant pathogens: a fungal endosymbiont uses TAL effectors to impact host"
2019 - Plant Health (APS Annual Meeting) - I. E. Melhus Graduate Student Symposium **Talk**
"Arabidopsis & barley convergently evolved an engineerable protease detection mechanism"
2019 - International Congress of Molecular Plant-Microbe Interactions - **Poster**
"Thinking outside the plant: TAL effector-like proteins in a bacterial-fungal symbiosis"
2019 - American Association for the Advancement of Science – **ePoster**
"Engineerable pathogen detection mechanism convergently evolved in barley & Arabidopsis"
2018 - International Congress of Plant Pathology – **Poster**
"Convergent evolution of effector protease recognition by Arabidopsis & barley"
2018 - International Symbiosis Society Congress – **Poster**
"TAL effector-like proteins in *Burkholderia-Rhizopus* microsporus symbiosis"
2017 - American Phytopathological Society Northeastern Division Meeting – **Talk**
"A programmable Arabidopsis resistance response conserved in barley?"
2014 - NCSU Undergraduate Research Symposium – **Poster** (*Session Winner*)
"The Role of Polyketide Synthases in the Banana Pathogen *Mycosphaerella fijiensis"*

Professional Society Memberships:
American Association for the Advancement of Science; American Phytopathological Society; International Society for Molecular Plant-Microbe Interactions; International Symbiosis Society

Teaching Experience and Development:

March 2020	Guest Lecture in Intro Virology *"Biotechnology for Virus Resistance"*	Clemson University *Delivered online*
February 2018	Gene Editing in Agriculture: Science, Policy, Story *Training Team Faculty Member*	Cornell Alliance for Science in India
Spring 2017	Teaching Assistant: Molecular Biology (BIOG3320)	Cornell University
Fall 2015	Teaching Assistant: Foundations of Biology (BIO1140)	Cornell University
Fall 2015	An Introduction to Evidence-Based Undergraduate STEM Teaching *Completed MOOC*	Center for the Integration of Research, Teaching, and Learning
Fall 2012 – Fall 2013	Teaching Assistant: Introduction to Biochemistry (BCH451) 3 semesters	North Carolina State University

Selected Awards and Honors:

2019	Barbara McClintock Graduate Student Award – Cornell SIPS ($830)
2019	I.E. Melhus Graduate Student Symposium Award – APS ($500)
2018	USDA AFRI Predoctoral Fellowship ($95,000)
2018	Plant Pathology GSA Outstanding Service Award – Inaugural Recipient
2016	NSF Graduate Research Fellowship Honorable Mention
2014	Cornell University Presidential Life Sciences Fellowship ($32,874+tuition,etc)
2014	H. Robert Horton Award – Outstanding Undergraduate in Biochemistry
2013	NC Biotechnology Center Undergraduate Research Fellowship ($4000)
2013	Goldwater Scholar – Barry Goldwater Scholarship and Excellence in Education Foundation ($7500)

Selected Service, Outreach, and Leadership:

2018-current	Skype A Scientist; 13 sessions with K12 students
2020-current	International Year of Plant Heath Task Force – Virtual Outreach
2018-2020	APS Public Policy Board Early Career Intern
2018-2019	International Symbiosis Society Student Committee Member
March 2018	AAAS Catalyzing Advocacy in Science and Engineering Workshop
2017-2018	Plant-Microbe Biology Faculty Search Committee
April 2017	Cornell Advocacy Day – Graduate Students Lobby Congress
2015-2018	Plant Pathology Graduate Student Association Leadership Roles
2012-2014	College of Agriculture and Life Sciences Senator – NCSU
Fall 2012	Daniels Center for Math and Science Student Volunteer

Mentoring:

3 Undergraduates - Emily Wan, 1.5 year; Ana Maria Restrepo Sierra, 6 months; Autumn Hurd, 2 months --- 1 High school student - Megan Feely, 8 months

Publications:

Carter M. E., et al. (2020). “A TAL effector-like protein of an endofungal bacterium increases the stress tolerance and alters the transcriptome of the host.” Proceedings of the National Academy of Sciences. Jul 2020: 202003857. 10.1073/pnas.2003857117. DOI:

Noar, R., Thomas, E., Xie, D., **Carter M. E.**, Ma, D., and M.E. Daub. (2020). “A Polyketide Synthase Gene Cluster Associated with the Sexual Reproductive Cycle of the Banana Pathogen, *Pseudocercospora fijiensis*.” PLOS One. 14(7): e0220319. DOI: 10.1371/journal.pone.0220319

Carter, M. E. and Helm, M., et al. (2018). "Convergent evolution of effector protease recognition by Arabidopsis and barley." Molecular Plant-Microbe Interactions. 32(5): 550-565. DOI: 10.1094/mpmi-07-18-0202-fi

Carter, M. E., et al. (2018). "A confounding effect of bacterial titer in a type III delivery-based assay of eukaryotic effector function." Molecular Plant-Microbe Interactions. 31(11): 1115-1116. DOI: 10.1094/mpmi-05-18-0128-le

CURRENT & PENDING SUPPORT

Name: Morgan Carter

Instructions:
Who completes this template: Each project director/principal investigator (PD/PI) and other senior personnel that the Request for Applications (RFA) specifies
How this template is completed:

- Record information for active and pending projects, including this proposal.
- All current efforts to which PD/PI(s) and other senior personnel have committed a portion of their time must be listed, whether or not salary for the person involved is included in the budgets of the various projects.
- Provide analogous information for all proposed work which is being considered by, or which will be submitted in the near future to, other possible sponsors, including other USDA programs.
- For concurrent projects, the percent of time committed must not exceed 100%.

Note: Concurrent submission of a proposal to other organizations will not prejudice its review by NIFA.

NAME (List/PD #1 first)	SUPPORTING AGENCY AND AGENCY ACTIVE AWARD/PENDING PROPOSAL NUMBER	TOTAL $ AMOUNT	EFFECTIVE AND EXPIRATION DATES	% OF TIME COMMITTED	TITLE OF PROJECT
Carter, Morgan (PD) Baltrus, David (Mentor)	Pending: USDA AFRI Postdoctoral Fellowship (this proposal)	$164,975	1-July-2021 to 30-June-2023	100%	Do facultative endohyphal bacteria alter virulence in *Fusarium* spp. that are critical agricultural pathogens?