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: Tal-Like Proteins In Endohyphal Symbiosis

PD: Carter, Morgan, E	**Institution:** Cornell University, Ithaca, NY
CO-PD: PD/PI 2 Name (Last, First, MI)	**Institution:**
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CO-PD: PD/PI 5 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 6 Name (Last, First, MI)	**Institution:**
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This Predoctoral Fellowship Application research project addresses the **plant heath and production and plant products priority area** under the mentorship of Dr. Adam Bogdanove, a professor at Cornell University.

The bacterium *Burkholderia rhizoxinica* (Brh) and its fungal host *Rhizopus microsporus* (Rmm) are a model system for studying endohyphal bacteria, an emerging field of interest with ecological and agricultural impact. Transcription Activator-like (TAL)-like type III secretion effector proteins were identified in the Brh genome (Burkholderia TAL-like; BTL), but vary in structure from canonical virulence-enhancing TAL effectors from phytopathogenic *Xanthomonas* spp. While the Brh type III secretion system is essential for symbiosis establishment and maintenance, the role of BTLs is unknown. My preliminary data supports the type III secretion and nuclear localization of BTL proteins in heterologous systems. **I hypothesize that BTL proteins transit the type III secretion system of Brh into Rmm where they localize in the nucleus and directly affect host transcription by binding specific DNA sequences; thus, all Rmm-associated *Burkholderia* strains will have *btl* gene(s).** I will assess secretion and localization during symbiosis through fluorophore fusion and confocal microscopy. RNA-seq and dual luciferase assays will address the effect of BTLs on host transcription and promoter activity. A western blot survey of Rmm isolates containing *Burkholderia* will detect presence/absence of BTLs in related endohyphal *Burkholderia* strains; follow-up sequencing could identify common BTL targets. **Discerning roles of effector proteins in fungal endosymbionts could provide new paths for biocontrol of phytopathogenic fungi or enhancements of symbioses in soil communities and the phytobiome.**

Project Narrative

I. Training/Career Development Plan:

My career goal is to enable our understanding of symbiosis as a research professor and be active in science policy. This research fellowship would enable me to make progress toward that goal by allowing me to focus full-time on a microbial symbiosis with the potential to affect soil health, phytopathology, and ecology. To develop a professional network, I will maintain my current memberships in AAAS and ASM, and participate in annual meetings including the International Society for Plant-Microbe Interactions Meeting in 2019 through fellowship travel funding. I have already completed required and optional graduate teaching assistantships. I will continue to advise undergraduate researchers in summers, as our lab regularly hires many through REUs and grant outreach, and during the academic year when possible.

I was re-elected president of the Plant Pathology GSA for 2017-2018 and will continue advocating for graduate student input in our academic unit (Section). My vice-president and I founded a School of Integrative Plant Science Graduate Student Council to expand communication among the Sections of plant sciences at Cornell and to establish graduate student representation at the school level. Sustained involvement in this council will contribute to my preparation for a faculty position. I will keep engaging in Cornell advocacy at the state and national level and participate in programs like the AAAS Catalyzing Advocacy in Science and Engineering workshop. The application of scientific findings to public policy is crucial for scientific advances to help society; this relationship drives my passion for science and for outreach to legislators, which I plan to do when in Washington, DC for the PD meeting.

II. Mentoring Plan:

Dr. Bogdanove and I meet biweekly to discuss experimental progress, undergraduate mentorship, and professional development opportunities. The Bogdanove lab has weekly lab meetings with a paper discussion and research update, where we exchange ideas and provide critical feedback on each other's projects. We also have a yearly retreat where we evaluate the overall progress and impact of our work and brainstorm new projects or solutions. Dr. Bogdanove sets up meetings for me with visiting lecturers and facilitates my participation in collaborations with labs at other universities that we regularly interact with through conference calls. He continues to recommend me to editors to review papers and has guided me through the reviewer process. I regularly attend the weekly Plant Pathology seminar series, as well as other plant science sections' seminars, and an interdisciplinary symbiosis lecture series that I will present at next year.

III. Project Plan:

III.A. Introduction and Preliminary Data:

The bacterium *Burkholderia rhizoxinica* (Brh) lives intracellularly in *Rhizopus microsporus* var. *microsporus* (Rmm), a phytopathogenic fungus that causes Rice Seedling Blight and occupies other saprophytic niches [1]. Brh produces rhizoxin, a major toxin responsible for necrosis of the rice plant, which was initially erroneously identified as a mycotoxin [2, 3]. While the fungal and bacterial partners can be cultured independently, both partners have reduced genomes compared to free-living relatives. By plating on antibiotics, fungi can be cured of their Brh symbionts, but are then incapable of sporangia formation and asexual sporulation until reinfected by co-culturing the two partners [4]. Invasion of Rmm by Brh relies on type II secretion of chitinolytic enzymes from the bacterium, suggesting potential for bacterial horizontal transmission [5]. However, Rmm-inhabiting *Burkholderia* strains are hypothesized to descend from a common ancestor and control of host reproduction is thought to maintain the mutualism on behalf of the

bacterium, which suggests that the bacterium is primarily vertically transmitted in spores [6]. The Brh type III secretion (T3S) system is a key component in establishing and maintaining the symbiosis; T3S-deficient mutants struggle to colonize the host and do not restore fungal sporulation, indicating that the T3SS is essential for reproductive control [7].

Putative T3S effector genes in the Brh genome include ones that encode transcription activator-like (TAL)-like proteins referred to as Bats, BurrH, or BTLs (Burkholderia TAL-Like) that have been briefly studied for their use in biotechnology [8-10]. Canonical *Xanthomonas* TAL effectors each have a T3S signal, nuclear localization signal(s) (NLS), DNA-binding central repeat region (CRR), and eukaryote-compatible transcription activation domain (**Fig 1A**); after secretion into the host cell, they localize in the nucleus and bind predictable DNA sequences (effector binding elements; EBEs), specified by the repeat modules of the CRR, to activate gene expression [11]. TAL effectors contribute to bacterial virulence when targeting susceptibility genes in the plant host, such as SWEET genes in cotton and rice [12-14]. BTLs are the first TAL-like proteins identified in a fungal symbiont and in a mutualism [15, 16]. BTLs bind DNA, but vary significantly from the canonical TAL structure, showing more polymorphism among repeats in the CRR and truncated N- and C- termini that lack a strong predicted T3S signal, activation domain, or NLS (**Fig 1B**). BTLs' role host-symbiont interaction is unclear, particularly given the atypical structure compared to canonical TAL effectors. Our lab is uniquely positioned to identify this role, because of our expertise in T3S, TAL effectors, and transcription, and our place in a community of mycology and microbiology experts at Cornell.

My research focuses on Rmm isolate ATCC52813 because the associated Brh has a single *btl* gene, *btl19-13*. By expressing a fusion of BTL19:13 to adenylate cyclase (Cya) in *Pseudomonas syringae* pv. *tomato* DC3000 infiltrated into *Nicotiana benthamiana* leaves, I showed that BTL19-13 transits the T3S system, and that its T3S signal resides in the first 45 amino acids (**Fig 1C**) [17]. Protein stability was confirmed by Western blot (data not shown) with a commercial anti-Cya primary antibody. I also determined that BTL19-13 contains a functional NLS in the C-terminus by expressing a truncated BTL19-13:eGFP fusion in *Saccharomyces cerevisiae*. Alanine substitution of the motif abolished nuclear localization (**Fig 1D**). Additionally, a co-worker in the lab has generated 2 knock-out strains of BTL19-13 (BrhΔbtl). Phenotype tests showed no significant change in host sporulation, pathogenicity, or growth of RMM ATCC52813 under laboratory conditions when infected with BrhΔbtl.

The retention of *btl* genes in the reduced Brh genome and these preliminary findings support the hypothesis that BTLs are secreted via T3S into the host fungus where they translocate to the nucleus and affect transcription. Given the diverse environments Rmm occupies, including opportunistic human infection, food spoilage, and rice seedling blight, the possible targets of BTLs are wide-ranging. To further understand Rmm-Brh symbiosis establishment and maintenance, I aim to verify the subcellular location of BTLs, assess the effect BTLs have on Rmm transcription, and determine the prevalence of BTLs in the Rmm/Brh symbiosis.

III.B. Rationale and Significance:

Determining the direction and execution of this research project will be a pivotal learning experience on the way to achieving my goals. My diverse research experiences have inspired my focus on molecular exchange in closely interacting organisms, with a specific interest in direct transcriptional regulation of one organism by another. The Rmm-Brh mutualism is a model for endohyphal bacteria (EHB), including evolution and maintenance of symbiosis, and is especially useful given the prevalence of *Burkholderia*-related bacteria as identified EHB [18-21]. The

proposed fundamental research will contribute to the **plant health and production and plant products priority area**, as EHB continue to be identified within phytopathogenic, mycorrhizal, and endophytic fungi [18, 19, 22-25]. Many culturing techniques cure fungi of endosymbionts, so we only recently began to understand the extent and diversity of EHB, making them new potential tools or targets for more sustainable agricultural practices [23]. Evaluating the role of effector proteins in symbiosis maintenance will be essential for targeting EHB of phytopathogens for biocontrol methods. Alternatively, promotion of endobacteria in soil fungi or endophytes could enhance soil health and microbial communities, as there is increasing evidence of T3S involvement in vibrant bacterial-fungal communities in soil [26]. Knowledge of the interactions in the rhizosphere and phytobiome can only benefit US agriculture, leading to more productive soils and sustainable alternatives to increasing chemical sprays. This project will additionally identify genes important in the symbiosis and/or in interactions of Rmm with its environment through probing direct transcription control by BTLs. An additional potential impact of this work is the establishment of BTLs or other TAL effectors, delivered by Brh, as genetic tools to interrogate genes in the mucorales, which include genetically intractable human and plant pathogens and food spoilage microbes such as *Rhizopus stolonifer*.

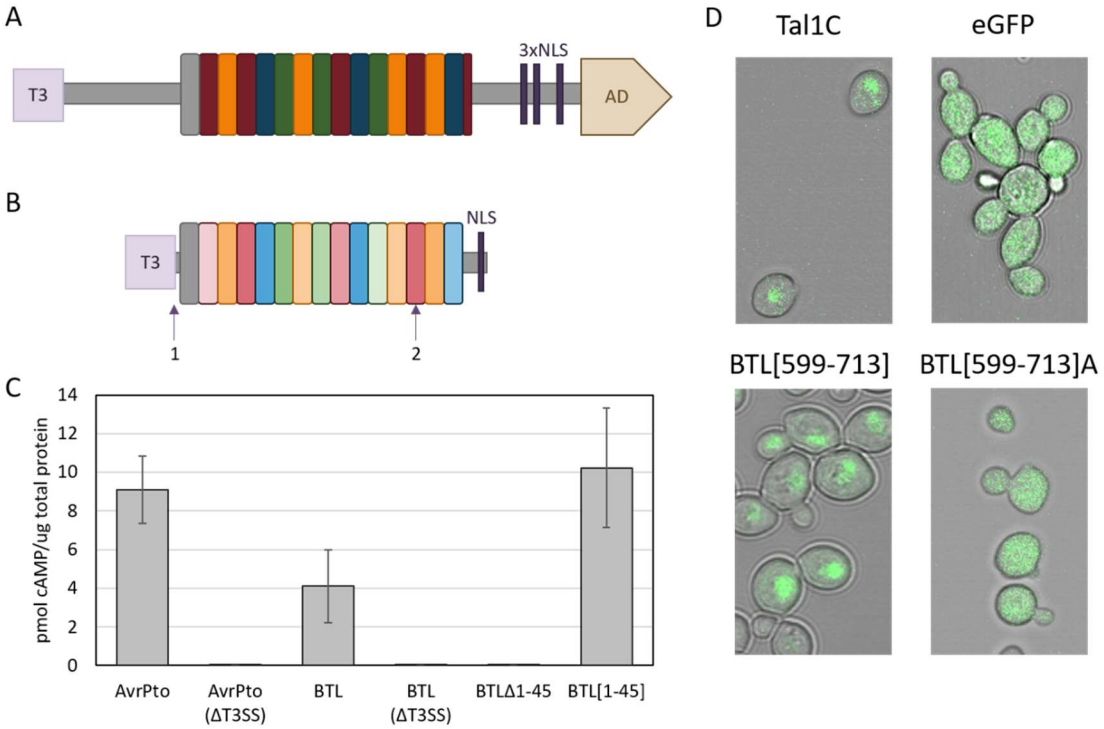

Figure 1. BTL structure and preliminary findings. (A) A *Xanthomonas* TAL effector showing the central repeat region, N-terminal Type III secretion signal (T3), three nuclear localization signals (NLS), and C-terminal eukaryotic activation domain (AD). **(B)** A BTL annotated with arrows where truncations were made to test T3S (1) and nuclear localization (2). **(C)** cAMP in a *N. benthamiana* leaf 6 hpi with Pst DC3000 strains expressing Cya fusions with a DC3000 T3S effector (AvrPto), BTL19-13 (BTL), or truncated BTL proteins; strains with non-functional T3S system denoted as (ΔT3SS) **(D)** Confocal micrographs of yeast expressing eGFP alone, or fused to a *Xanthomonas* TAL effector (Tal1c), a truncated BTL19-13, or a truncated BTL19-13 with a C-terminal alanine substitution of the NLS.

III.C. Approach:

III.C.i Hypothesis and Specific Objectives:

a. Hypothesis: BTL proteins transit the T3S system of Brh into Rmm where they localize in the nucleus and directly affect host transcription by binding specific DNA sequences; thus, all Rmm-associated *Burkholderia* strains will have *btl* gene(s).

b. Objectives:

1. Assess T3S- and NLS-dependent nuclear localization of BTL19-13 during symbiosis
2. Determine the effect of BTL19-13 on host transcription
3. Survey the distribution of BTLs across a collection of Rmm-Brh symbioses

III.C.ii Safety: I have completed Laboratory Safety, Chemical Waste Disposal, Carcinogen Safety, and Fire Safety trainings and participate in regular laboratory safety exercises.

III.C.iii Methods:

Western blot and RT-PCR to check for expression and protein stability

a. Technique: Determining the conditions under which BTL19-13 is expressed is essential for experiments detailed in this proposal. Brh-infected Rmm will be cultured on various types of media and sampled at different developmental stages. Reverse-transcriptase PCR (RT-PCR) will be used to check for expression of a segment of the *btl* mRNA outside of the repetitive region. Samples will also be subjected to western blot with a recently generated BTL-specific antibody (the antibody does not bind *Xanthomonas* TALs, but binds BTL19-13 and a BTL from a different strain, BTL21-17; data not shown). All future experiments will be done under conditions in which BTL19-13 is expressed, unless otherwise noted.

b. Expected Outcomes and Data Interpretation: From previous data on transcription during the infection event, we know that BTL19-13 is expressed during the initial interaction between Brh and Rmm [27]. However, I expect BTL19-13 to be expressed throughout the symbiotic association [28]. RT-PCR and western blot experiments will test this prediction.

c. Pitfalls and Limitations: If expressed constitutively, I will test BrhΔbtl under an identical condition to discount the possibility of a recurring false positive. BTL19-13 may not be expressed under any conditions besides initial infection, making future experiments more difficult because of the tenuous nature of this stage. However, this would provide new hypotheses about the role of functional *btl* genes as initiators of the symbiotic relationship.

Fluorophore fusion and confocal microscopy to confirm localization in vivo

a. Technique: Standard cloning procedures will be used to construct broad host range vectors with BTL19-13 driven by its native promoter, and with a C-terminal mCherry fluorophore, which is compatible with the *Burkholderia* T3S [29]. Additional constructs will be made for an alanine substitution of the NLS and a N-terminal truncation. Constructs will be introduced into BrhΔbtl strains, and confirmed transformants will be used to reinfect cured Rmm. Cultures will be imaged with a Zeiss 710 confocal microscope, with DAPI staining to identify nuclei.

b. Expected Outcomes and Data Interpretation: Interpretation of the localization results will be straightforward. I expect that my previous conclusions from heterologous systems, that BTL19-13 is a T3S effector and localizes to the nucleus, will be confirmed by localization *in vivo* of the BTL19-13:mCherry fusion protein within the DAPI stained fungal nuclei. I further expect that translocation into the fungus will depend on the N-terminal T3S signal and that localization in the nucleus will be abolished in the alanine substitution mutant. Because these experiments will be carried out in the native context, results will **satisfy Objective 1.**

c. Pitfalls and Limitations: If too little protein is produced or secreted into the fungal cell, visualization may not be possible. However, concentration in the nucleus will increase the likelihood of detection [30]. Increasing expression by switching to a strong, constitutive promoter can lead to localization artifacts. Instead, if visualization at native expression levels is not possible, localization will be attempted by subcellular fractionation and western blotting with the BTL antibody. My lab has used this technique before for effector localization *in planta*, and I expect it could be readily adapted for Rmm tissue.

RNA-seq to assess impact of BTL19-13 on host transcription

a. Technique: Total RNA will be isolated from Rmm infected with Brh wild-type, BrhΔbtl, and complemented strains using the EZNA Fungal RNA Kit (Omega). RNA libraries will be prepared and sequenced using Illumina technology.

b. Expected Outcomes and Data Interpretation: RNA-seq data will be analyzed as described [31] but aligned to the *R. microsporus* ATCC52813 genome [32]. A modified version of TALE-NT 2.0 Target Finder adapted to BTL binding preferences will be used to identify potential BTL19-13 binding sites/ EBEs in the Rmm genome [33-35]. Differentially expressed RNAs will be cross-referenced to the predicted EBE to identify top candidate targets of BTL19-13, **partially satisfying Objective 2.**

c. Pitfalls and Limitations: RNA-seq will not discriminate between indirect and direct changes in transcription by BTL19-13. A reporter assay for BTL functionality, as outlined below, will distinguish that by supporting or refuting a direct transcriptional regulation role. Based on studies with *Xanthomonas* TAL effectors, I expect to find many potential target genes and will narrow down based on putative gene function. Too many putative gene targets could make identifying true targets challenging. In this case, I will carry out a ChIP-exo experiment to find *bona fide* BTL19-13 *in vivo* EBE to cross-reference with differentially expressed genes [36].

Dual luciferase assay to probe direct effects of BTL19-13 on promoter activity

a. Technique: I will determine if BTL19-13 directly activates or represses upstream or downstream transcription using a head-to-head, dual luciferase reporter construct assembled in our lab, driven by an intervening minimal promoter with a BTL19-13 EBE [37]. As a control for gene activation, a designer TAL effector (dTALE) targeting the known BTL EBE will be assembled. Constructs expressing the dTALE or BTL19-13 will be transformed into *Agrobacterium tumefaciens*, infiltrated into *Nicotiana benthamiana*, and analyzed by Dual-Luciferase Reporter Assay (Promega) for luminescence, with replication [37]. Separately, these experiments will be repeated using a constitutively expressed plant promoter amended with a BTL19-13 EBE to assess possible repression by BTL19-13. After defining the effect(s) of BTL19-13 on transcription in this model, I will test the candidate targets of BTL19-13 by replacing the minimal promoter with the respective promoters.

b. Expected Outcomes and Data Interpretation: Significantly increased luciferase luminescence in the presence of BTL19-13 will be evidence of gene activation by BTL19-13, while a significant decrease will indicate repression. Given the lack of activation domain in BTLs and previous uses of artificially truncated TAL effectors as repressors, I anticipate that BTL19-13 will repress target gene expression [38, 39]. **This will partially satisfy Objective 2.**

c. Pitfalls & Limitations: This assay is only testing for effect on the EBE-incorporated promoter region, but BTL19-13 may act within the gene body. Potentially, an EBE could be incorporated after a transcription start site to test effect on transcription elongation. BTL19-13 and fungal promoters may not be functional in plant cells, in which case I will adapt the experiment to yeast.

Western blot and sequencing to identify BTLs in other isolates

a. Technique: Isolates of Rmm with *Burkholderia* endosymbionts, which can be determined by toxin production, will be obtained from Dr. Teresa Pawlowska's lab and culture collections (e.g., ATCC). Infection by Brh will be confirmed by PCR. BTL repertoire will be assessed by western blot of homogenates, using the BTL antibody. For isolates expressing BTLs, genomic DNA will be extracted, and *btl* genes will be amplified by PCR with primers targeted to the non-repetitive 5' and 3' ends and sequenced.

b. Expected Outcomes and Data Interpretation: Based on our three sequenced isolates, I expect that one to a few *btl* genes will be present in every Brh genome, but that the proteins will diverge in repeat length and predicted binding site. Using the sequencing results and TALE-NT 2.0, we can predict EBEs to see if the BTL19-13 target gene is a common target and if there is potential for complementation of BrhΔbtl with another BTL. **These experiments will satisfy Objective 3.**

c. Pitfalls & Limitations: A western blot only tells if BTLs are currently expressed in the additional isolates. To confirm absence of BTL genes in an isolate, and verify none were missed due to lack of expression under the conditions tested, we will do a follow-up Southern blot using *btl*19-13 as a probe. For the sequencing experiments, if some *btl* genes fail to amplify with the primers designed based on the existing *btl* sequences available, I will use partially degenerate primers to increase SNP tolerance, or I will perform whole genome sequencing of select strains.

III.C.iv Communication Plans

I will present findings of this work at professional conferences. I plan to publish two papers, with the first focusing on molecular activity from my preliminary data, confirmation *in vivo*, and the reporter assay results. The second paper will address the role of BTLs in Rmm-Brh symbiosis with the results from the RNA-seq, target validation, and isolate survey.

IV. Timeline and Evaluation Plan:

Evaluation of progress at meetings with Dr. Bogdanove and my dissertation committee will focus on alignment with the timetable below. Publication plans may be revised depending on results. Additional conferences, workshops, and professional development activities may be added or substituted considering special sessions and themes. I will take advantage of opportunities to present my findings orally when possible. I will update my LinkedIn profile monthly with new skills, connections, and accomplishments.

Objective	**Summer 2017**	**Fall 2017**	**Spring 2018**	**Summer 2018**	**Fall 2018**	**Spring 2019**	**Summer 2019**
I. Assessment in vivo	Cloning	Imaging					
II. Role in Transcription	RT-PCR	RNA-seq	Luciferase Assay	Promoter Tests			
III. Survey BTL distribution			Obtain samples	Western Blots	Sequence *btl*s/Brh	Target prediction	
Communication & Development							
Conference Presentations			AAAS Annual	ASM Microbe			IS-MPMI
Manuscript Submissions				1st paper			2nd paper
Other	Undergrad mentor	Symbiosis workshop	CASE workshop	Undergrad mentor			Thesis writing

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Biographical Sketch

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Education:

Cornell University Ithaca, NY	*Ph.D. Candidate* GPA 4.0	Plant Pathology and Plant-Microbe Biology	2014-Present
North Carolina State University Raleigh, NC	*B.S.* GPA 4.0	Biochemistry; Minors in Genetics and Biotechnology	2011-2014
Davidson College, Davidson, NC	 GPA 4.0	Undecided	Fall 2010

Research Experience:

2015-Present	Graduate Thesis Research	Dr. Adam Bogdanove	Cornell University, Ithaca, NY
2014-15	Graduate Research Rotations	Dr. John Lis, Dr. Adam Bogdanove, Dr. Bryan Swingle	Cornell University, Ithaca, NY
2011-14	Plant Biology Research Assistant	Dr. Margaret Daub	North Carolina State University, Raleigh, NC
2013	Summer Undergraduate Research Program in Molecular Biology	Dr. Coleen Murphy	Princeton University, Princeton, NJ
2011-12	Undergraduate Researcher in Biotechnology	Dr. Eric Miller and Dr. Susan Carson	North Carolina State University, Raleigh, NC

Teaching Experience:

Spring 2017	Molecular Biology (BIOG3320)	Cornell University
Fall 2015	Foundations of Biology (BIO1140)	Cornell University
Fall 2012 - Fall 2013	Introduction to Biochemistry (BCH451)	North Carolina State University

Presentations:

NCSU Spring Undergraduate Research Symposium – Poster/Published Abstract

2011 "Potential effect of homing endonucleases on genome heterogeneity in mycobacteriophage Mutaforma13"

2012 "Drivers of genome evolution: homing endonuclease genes in mycobacteriophages"; "Mercury: Fact and Fiction"

2014 "The Role of Polyketide Synthases in the Banana Pathogen *Mycosphaerella fijiensis*" *(Winning Poster)*

State of North Carolina Undergraduate Research and Creativity Symposium

2013 "Expression analysis in *Mycosphaerella fijiensis,* the causal agent of black Sigatoka disease of banana"

NCSU Summer Undergraduate Research Symposium

2011 "Toxin biosynthesis in the banana pathogen *Mycosphaerella fjiensis*"

Graduate Record Examinations (GRE) Scores:

09/21/13 Verbal Reasoning: 164 / 94th Percentile
Analytical Writing: 5.5 / 98th Percentile
Quantitative Reasoning: 167 / 93rd Percentile

Awards and Honors:

2016	NSF Graduate Research Fellowship Honorable Mention
2014	Cornell University Presidential Life Sciences Fellowship
2014	H. Robert Horton Award – Outstanding Undergraduate in Biochemistry
2014	NCSU University, College of Agriculture and Life Sciences, and Biochemistry Honors Programs Graduate
2013	NC Biotechnology Center Undergraduate Biotechnology Research Fellowship
2013	Phi Beta Kappa Honor Society
2013	Phi Kappa Phi Honor Society
2013	Goldwater Scholar
2011-2014	Stephanie Christine Anderson Memorial Scholarship Recipient

Service and Leadership:

Fall 2010	Davidson Students Volunteer for Science Volunteer
Summer 2012	SciWorks Volunteer
Fall 2012	Daniels Center for Math and Science Student Volunteer
2011-2012	Quad Area Council Representative to NCSU Inter-Residence Council *Advocated for programming and policy changes to benefit campus residents and increase community outreach and engagement*
2012-2014	University Honors Program Ambassador
March 2013	North Carolina Science and Engineering Fair Volunteer
2012-2014	College of Agriculture and Life Sciences Senator Academics Committee Chair – *Oversaw legislation related to academic issues; initiated legislation and coordinated feedback about burdensome course requirements and extended add/drop dates* CALS Delegation Leader – *Spoke on behalf of the College of Agricultural and Life Sciences; held meetings to brainstorm legislation*
March 2015	Expanding Your Horizons Workshop Assistant
2015-Present	Plant Pathology Graduate Student Association Executive Council Treasurer/Fundraising Committee Chair *5/15-5/16 – Designed and sold calendars to raise money for the association; handled payments between departments and students; prepared budgets* President *5/16-present – Improved student-faculty communication to facilitate feedback on faculty hires, curriculum changes, and a stream-lined Teaching Assistant hiring process; presided over meetings of the GSA and advised committee chairs*
April 2017	Cornell Advocacy Day – Graduate Students Lobby Congress for Federal Funding

Professional Memberships:

American Association for the Advancement of Science
American Society for Microbiology

Management Plan

The PD will have regular one-on-one meetings with the Mentor to discuss progress and decide on next steps. When necessary or helpful, the PD will reach out to others at Cornell University and abroad to request training or resources and establish collaborations if beneficial to the project outcomes. The PD will hold yearly meetings with her Thesis Committee (Dr. Adam Bogdanove, Dr. John Lis, and Dr. Teresa Pawlowska) for assessment and evaluation of research and career training. Guidance by committee members, chosen for their relevant expertise, will be integrated over the period of the project. The research project and techniques used shall be recorded in the Bogdanove lab collective electronic laboratory notebook, while new strains and data will be stored in the Bogdanove lab with accurate documentation so that other researchers can continue the work after the PD has completed her degree. Regular research updates at the Bogdanove lab meetings by the PD will ensure scrutiny of results and prime other laboratory members for involvement in the project.

For timeline of career development events and research progress, see Project Narrative.

Situation: Graduate training in understudied symbiotic interactions for Morgan Carter, a Plant Pathology Ph.D. candidate with academic career goals and interest in policy outreach

Inputs

- Mentoring:
  - Time
  - Mentor
- Professional Development:
  - Data
  - Travel funding
  - Institutional support
- Project:
  - Knowledge
  - Materials/Funding
  - Laboratory space
  - *Rhizopus* isolates
  - Microscope
  - Sequencing facility

Activities

- Mentoring:
  - Lab meetings
  - 1-on-1 meetings
  - Seminars
- Professional Development:
  - Conference presentations
  - Cornell advocacy
  - Manuscript preparation
  - GSA meetings
  - Undergraduate Mentoring
- Research:
  - Western blotting
  - RNA-seq
  - Microscopy
  - Cloning

Outputs

- Graduate degree
- New technique for manipulating mucorales
- Two scientific publications
- Multiple scientific posters
- Larger network of colleagues
- Policy recommendations to state and national government
- Increased technique proficiency

Outcomes

Personal

- Increased capacity for independent investigation
- Improved scientific communication skills
- Improved leadership and teamwork abilities
- Better problem-solving

Public

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

Assumptions
• Knowledge of specific bacteria-fungi interactions will be translational to agricultural productivity and sustainability

External Factors
• Professional Development opportunities offered • Scientific conferences being held during time frame