A PROPOSAL TO THE BAKER FUND COMMITTEE

TITLE OF PROJECT: Characterizing the "Magic 8" of Gravitropic Signal Transduction

NAME OF APPLICANT: Sarah E. Wyatt

STATUS: _____ Asst. Prof. _____Assoc. Prof. _X_ Prof. _____ Administrator

DEPARTMENT: Environmental and Plant Biology
CAMPUS ADDRESS: 315 Porter Hall
E-MAIL ADDRESS: wyatts@ohio.edu

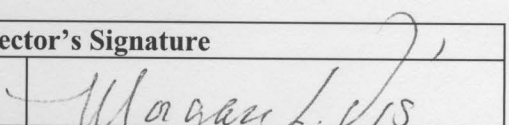
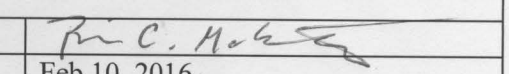
RE-SUBMISSION: ___ YES (Original Submission Date _____)
X NO

BUDGET: Total Request **$11,739.65**
(May not exceed $12,000)

IRB AND IACUC APPROVAL:
To ensure that the University is in compliance with all federal regulations, complete the checklist below. Note: your proposal can be approved prior to IRB or IACUC approval, but funding will be withheld until notification of approval or exemption.

Yes	No	Office of Research Compliance	Policy #
	X	Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
	X	Animal Species: Institutional Animal Care & Use Committee (IACUC) Approval #: Expiration Date:	19.049

SIGNATURES

Applicant's Signature		**Chair/Director's Signature**	
Signature		Signature	
Name	Sarah E. Wyatt	Name	Morgan Vis
Dept/School	PBIO	Unit	PBIO
Date	Feb 9, 2016	Date	Feb 10, 2016
Dean's Signature			
Name	Brian McCarthy	Signature	
College	Arts & Sciences	Date	Feb 10, 2016

✓ **Optional:**
If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises.

Signature:  Date: Feb 10, 2016

2. Baker Fund Proposal Checklist

Applicants **must** complete and sign the checklist. The checklist should be included as the second page of the application (following the cover page).

✓ Cover page	use Baker form
✓ Checklist	use Baker form
✓ Abstract*	1 double-spaced page
☐ Introduction (*for continuations or resubmissions only*)*	1 double-spaced page
✓ Discussion	10 double-spaced pages
☐ Glossary/Definition of Terms* (*not required*)	2 double-spaced pages
✓ Bibliography (*not required*)	3 pages
✓ Biographical Information (*applicant(s) and key personnel*)	3 pages per person
✓ Other Support (*applicant(s) and key personnel*)	1 page per person
✓ Budget and Justification	no limit specified
✓ Appended Materials	10 pages; no more than 10 minutes of footage
✓ Recommended Reviewers	5 required
✓ Electronic copy of proposal	Single Acrobat file, containing entire proposal and required signatures

* These sections should be written in language understandable by an informed layperson to assist the committee in its review.

****Please note: The committee has the right to return without review any proposals that do not conform to these format requirements.****

Applicant signature: 

3. Abstract

Have you ever planted a seed? Did you plant it right side up? Of course you never had to think about it; there is no *right-side-up*. Germinating seedlings know which way is up, because of gravity. Gravity is a fundamental stimulus directing plant growth and development. Yet, even so, we still don't fully understand how plants respond to it. One of the biggest questions remaining is that of signal transduction, how plants convert the physical information provided by gravity into a biochemical response. That is the question we attempt to address, at least in part, in this proposal. In previous work, using a cold effect to isolate the events of signal transduction, we looked for genes that were expressed differently in the model plant Arabidopsis when the plants were turned on their side as compared to those held vertically. Surprisingly, of the 341 genes we identified, only eight were found at the earliest time point (2 minutes) after reorientation. These eight we dubbed the 'Magic 8'. Here, we propose to complete initial characterization of these genes by independently confirming gene expression profiles of each gene using qRT-PCR (Aim1), obtaining plants putatively defective in each gene (Aim 2) and assessing those plants for root and shoot responses to gravity (Aim 3) by measuring curvature in seedlings and mature plants after reorientation with respect to gravity. We have begun collecting data on the Magic 8 and invested considerable effort in developing techniques. So far, we have performed the validation work necessary to assess the gene expression profiles, obtained mutant lines for seven of the genes, bred six of those to be homozygous for the mutation, confirmed the mutation to completely disrupt the gene for four and have curvature data on two. Although we have a firm start of the project, we have considerable work to do. The data obtained will complete the data necessary for a peer reviewed publication and form the foundation for a proposal to the National Science Foundation (NSF) to follow up on the most promising.

5. Discussion

A. Specific Aims. The main objective of the proposed work is to complete initial characterization of eight genes for their potential role in gravitropism (a plant's response to gravity) for publication and to provide preliminary data for a grant proposal to NSF. The genes, dubbed the 'Magic 8', were identified via analysis of gene expression across time after plants were turned on their sides (reoriented with respect to the gravity vector) in the model plant *Arabidopsis thaliana*. The Magic 8 were the only genes expressed at the earliest time point after reorientation and were not expressed at later time points. Our hypothesis is that these genes are components of the earliest events responsible for the gravity response in plants. To test this hypothesis, we will accomplish the following three aims:

Aim 1. Confirm expression profiles of each gene during the initial phases of gravitropism using quantitative real time polymerase chain reaction (qRT-PCR).

Aim 2. Obtain Arabidopsis lines defective in each gene, confirm that each mutant line is a pure breeding line (homozygous for the mutation) and confirm that each mutant does not express the gene (is a complete knock-out for the gene), and finally

Aim 3. Assess the phenotype (visual expression) of each mutant line for shoot and root responses to gravity.

B. Significance: Generally, people don't think much about gravity, and especially not as it relates to plants. However, on Earth, gravity is a constant stimulus governing plant growth and the orientation of plant organs from the germination of seedlings to positioning of flowers for pollination and seed for dispersal. Dogma suggests that the shoots go up and the roots go down, however, a plant's response to gravity is much more complex than that. The effect of gravity on plant growth is never more obvious than when shopping for landscape or household plants. Because they add novelty to our environment and 'fill' unused space, we often select the

weeping trees and weeping or trailing plants for hanging baskets. These plants have a natural defect in their response to gravity, sometime such a strong defect that weeping trees are often grafted onto 'normal' root stock, creating a knot on the stem. In *All I Need to Know I Learned in Kindergarten,* Robert Fulgham states "Remember the seed in the Styrofoam cup: the roots go down and the plant goes up and nobody really knows how or why." And he isn't that far off.

To study gravitropism, researchers typically reorient plants with respect to the gravity vector (turn them on their side). And predictably, the shoots bend up and the roots bend down. They return to their "normal" position with respect to gravity. For the sake of research, the plant's response to gravity has been separated into four phases: perception of the gravity stimulus, transduction of that perception event into a biochemical signal, transmission of the signal to the site of response, and the differential growth response (curvature) of the plant organs. Although we know quite a bit about perception (for review see Kiss 2000), transmission (via the plant hormone auxin, for review see Muday and Rahman 2008) and the response, we still struggle with transduction, how the plant converts the biophysical stimulus into a biochemical signal. Over the last two decades, we have added substantially to our knowledge of potential components involved in the signal transduction pathways, but we still don't have all of the pieces of the puzzle nor do we know how those pieces fit together. (Fig. 1).

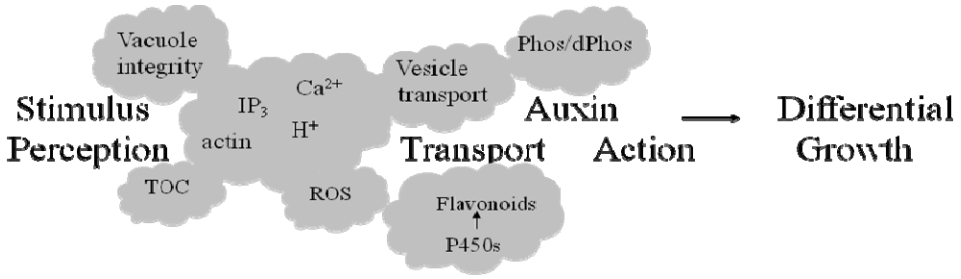


Figure 1. The 'grey cloud' model. A diagrammatic representation of the current understanding of the gravitropic signal transduction pathway. We have moved from a 'black box' to a 'grey cloud'. The position of the "clouds" is suggestive of the possible position in the pathway, but no direct relationship has been experimentally confirmed (Adapted from Wyatt and Kiss 2012).

The work proposed here will add to our understanding of the signaling molecules and may lead to insights into signaling events that would form the foundation of a grant submission to the Integrated Organismal Systems division of NSF.

C. Preliminary Studies of Applicant: To isolate the signal transduction phase of the gravitropic response, we used a novel cold treatment, referred to as the gravity persistent signal (GPS) response (Wyatt et al. 2002). When plants are reoriented with respect to gravity in the cold (4°C), perception of the stimulus occurs, but transmission of the signal (auxin transport) is significantly reduced and thus limits the response; however, when plants are returned to vertical at room temperature (RT), auxin transport is restored and plants bend in response to the reorientation in the cold (Wyatt *et al.*, 2002). Yes, plants 'remember' what happens to them in the cold. This 'memory' indicates that some component of signal transduction is inhibited in the cold. Most recently, we have taken advantage of the GPS response to perform a gene expression study to identify additional molecular components involved in the gravitropic pathway. We assessed gene expression at four time points after plants were reoriented in the cold (Fig 2).

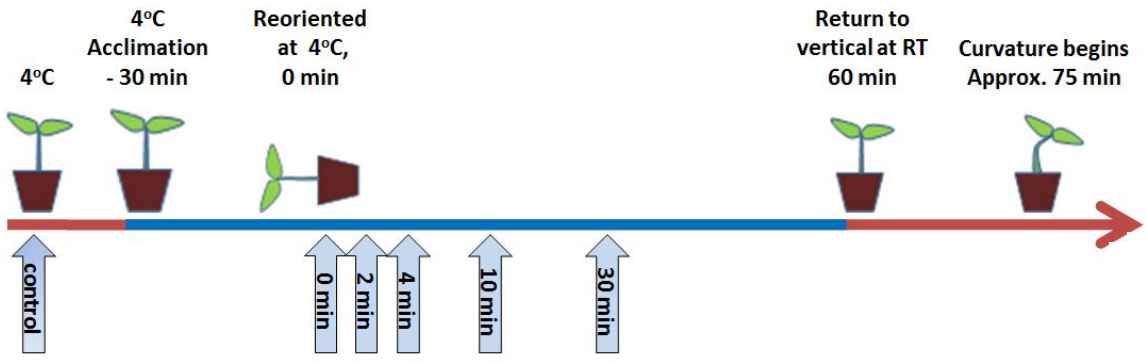


Figure 2. Schematic representation of the GPS response and the time points (vertical arrows) selected for mRNA isolation for the gene expression experiment. The -30 time point allows for an adjustment period to the cold temperature prior to reorientation. The presentation time for Arabidopsis is 3 minutes so the majority of the time points focus around this time frame after the initial reorientation and before return to vertical. Room temperature is indicated by the red line, cold temperature (4°C) is represented by the blue line.

Analysis of gene expression. Arabidopsis var. Columbia seed were sown into soil and grown to maturity in white light on a 16 h light: 8 h dark cycle. When inflorescence stems reached 8-10 cm, plants were the subjected to the GPS treatment as follows. All plants were initially held vertically at 4°C for 30 min as a cold acclimation period. Half the plants were then reoriented 90° (treatment) with respect to the gravity vector, and half the plants were gently moved to simulate the movement of reorientation but maintained vertical (control), all at 4°C. We isolated messenger (m)RNA (the RNA representing only those genes that are expressed in that tissue at that time) from the apical 4 cm (the region of bending) of the inflorescence stems at 2, 4, 10 and 30 minutes after the plants were placed on their side in the cold (Fig. 2). Because the presentation time (the minimum time required for reorientation to cause curvature) for Arabidopsis has been reported to be between 30 sec (Perbal et al. 1997) and 3 minutes (Fukaki et al. 1996a), the majority of our time points focus on this window just after reorientation and before return to vertical at RT, when the plants would be receiving competing signals from the new vertical gravity vector and the downstream events resulting from auxin redistribution and differential growth. Inflorescence stems were flash frozen in liquid nitrogen at each time point, leaves and flowers removed, and the apical 4 cm of the stems pooled and crushed in liquid nitrogen. Four replicates were performed for each time point for both control and experimental samples, labeled with Cy3 and Cy5 using a dye swap protocol to eliminate dye bias, then hybridized to a total of 16 4X44k dual color Arabidopsis genome arrays (Agilent Technologies, Santa Clara, CA) as described in Cook et al. 2015. The probe arrays were scanned by a SureScan Microarray scanner, and the raw optical intensities were captured by the feature collection software. Raw gene expression data were analyzed using the ArrayOU pipeline (Shen et al., 2012). Using a P-value of 0.05 and $\log_2$ fold change >1 or <-1, we identified 348 genes

that were differentially expressed between the GPS treatment and vertical control (8 genes at 2 min; 91, 4 min; 177, 10 min; and 58, 30 min) (Shen et al. 2014). Some of these genes were differentially expressed at multiple time points (Fig. 3), but of particular interest to us are the eight genes expressed only at the 2 minute time point (Fig. 3, blue). We refer to these as the Magic 8 (Table 1). Not only are they expressed at the earliest time point, potentially during the presentation time, but they also do not continue to be expressed at later time points. This 'spike' in expression is what would be expected of signaling specific genes, genes that flip a switch to turn on a signaling pathway.

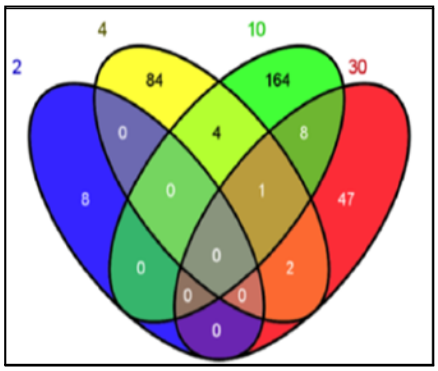


Figure 3. Venn diagram of gene expression after reorientation. Each oval represents the genes expressed at that time point: blue = 2 min, yellow = 4 min, green = 10 min and red = 30 min. Regions of overlap show genes expressed at multiple time points. Of particular note is the lack of overlap of genes expressed at 2 min (blue).

Table 1. The identity and expression of the 'Magic 8' at 2 min after reorientation to gravity.

Gene Name	Arabidopsis Locus ID	Log_2 fold change*	P-value	Known Function**
ARGOS	At3G59900	1.36	0.01	Regulated by auxin; controls lateral organ size
PP1-A13	At3G61060	1.34	0.02	Encodes a F-Box protein w/unknown substrate
NRT1.12	At3G16180	1.29	0.02	Facilitates nitrate transport among leaves
GATL10	At3G28340	1.09	0.04	Protein involved in galacturonosyltransferase
ARL	At2G44080	1.08	0.03	Regulates cell expansion during organ growth
SAUR1	At4G34770	1.06	0.01	Functions in response to auxin
UPR1	At1G64370	-1.04	0.04	Uncharacterized protein
UPR2	At2G25510	-1.16	0.05	Uncharacterized protein

* Positive numbers indicate upregulation of the gene after reorientation as compared to vertical controls; negative numbers indicate down regulation.

**This is a function currently identified for the gene but is only what is known and doesn't represent all possible functions of the gene.

D. Methods and current status:

qRT- PCR (Aim1). Although microarrays are capable of whole genome profiling, they can also generate numerous false positive signals (Wang *et. al.,* 2006). Thus, qRT-PCR should be performed on RNA collected from wild-type plants to independently validate the expression levels. Two-step qRT-PCR will be performed for each of the Magic 8 with the DyNAmo Color Flash SYBR Green kit (Thermo Fisher Scientific) using the Mx3000P qRT-PCR machine (Stratagene/Agilent Technologies) as described in Cook et al. 2015. RNA will be extracted from the inflorescence stems of wild type Arabidopsis plants at each of the time points after plants were reoriented in the cold during the GPS treatment. To ensure the selection of a stably expressed internal control, the geNorm algorithm (Vandesompele *et al., 2002)* was used to analyze potential reference genes recommended by Czechowski et al. 2005. The gene *PEX4* was selected as the internal control. Assays will be run using template DNA complementary (cDNA) to the mRNA extracted from vertical control and reoriented plants at each of the four time points of the microarray, with each assay in triplicate. Relative quantification of the gene expression levels will be performed using the comparative Cq method.

Progress to date: qRT-PCR primers specific to each of the Magic 8 and *PEX4* have been designed, primer concentrations optimized, and amplification specificity ensured. This work represents all the validation work that must be performed prior to the gene expression profiles being confirmed by qRT-PCR and is no small feat for eight genes.

Selection of Arabidopsis mutant lines (Aim 2). Arabidopsis lines with insertions in the genes of interest were obtained as seed stock from European Arabidopsis Stock Center (NASC) and Arabidopsis Biological Resource Center (ABRC). Seed for two mutant lines for each gene were obtained (Table 2), and we are currently in the process of breeding lines to homozygosity.

Table 2. Mutant lines obtained for each of the Magic 8

Gene Name	Arabidopsis Locus ID	Insertion line 1	Insertion line 2
ARGOS	At3G59900	SALK_105295	SALK_085316
PP1-A13	At3G61060	SALK_152055	SALK_101611
NRT1.12	At3G16180	SALK_013091	SALK_046520
GATL10	At3G28340	SALK_058676	SALK_072808
ARL	At2G44080	GK-079D09	Not Available
SAUR1	At4G34770	SALK_149297	Not Available
UPR1	At1G64370	Not Available*	Not Available*
UPR2	AtG25510	SALK_132861	SALK_13356

*Currently developing mutant lines through CRISPR technology.

Two independent mutant lines for each gene are standard practice to ensure phenotypes seen are truly the result of defects in the gene of interest. For those genes for which mutants are not available, we are currently developing mutant lines using CRISPR technology. For each mutant line, DNA was/will be extracted, and PCR will be performed to confirm homozygosity of the insertion using three primers in each reaction: two specific to the gene of interest and the third complementary to the T-DNA insertion. Once homozygous lines are obtained, each was/will be evaluated by RT-PCR to determine if the insertion results in a complete knock-out of the gene (a null mutation) which is necessary for phenotype analysis. RNA will be collected at 2 min after reorientation in the cold during the GPS treatment, cDNA will be made using the cDNA RT kit by Applied Biosystems and each gene amplified by PCR in both wild-type and each confirmed homozygous line using primers specific for each gene. If the mutant is a complete knock-out, the gene should amplify in the wild-type control but not in the mutant.

Progress to date: see Table 3.

Phenotype Analysis (Aim 3). For each confirmed knock-out line, potential effects on gravitropism will be assessed in both roots and shoots. Seedlings grown on agar in Petri dishes will be evaluated for root skewing and root curvature according to Rutherford and Masson 1996

and Vaughn and Masson 2011. Plants grown in soil will be evaluated for the curvature of inflorescence stems at room temperature and in response to the GPS treatment according to Wyatt et al. 2002. All lines will be also evaluated for overall growth and morphology according to Wyatt et al. 2002 to determine possible pleiotropic effects of the mutations.

Progress to date: see Table 3.

Table 3. Progress to date on the mutant analysis.

Gene Name	Arabidopsis Locus ID	Insertion line 1**	Homozygosity confirmed	Knock-out confirmed	Phenotype analysis completed*			
					RS	RC	SC	GR
ARGOS	At3G59900	SALK_105295	Yes	In progress				
PP1-A13	At3G61060	SALK_152055	Yes	Yes	Yes			
NRT1.12	At3G16180	SALK_013091	Yes	Yes				
GATL10	At3G28340	SALK_058676	Yes	Yes	Yes			Yes
ARL	At2G44080	GK-079D09	Yes	Yes				
SAUR1	At4G34770	SALK_149297	In progress					
UPR1	At1G64370	Not Available*	Lines being developed					
UPR2	AtG25510	SALK_132861	Yes	In progress				

*RS = Root skewing; RC= Root curvature; SC = shoot curvature; GR = GPS response

**The second mutant lines, where available, have been obtained and are currently being bred for homozygosity and, thus, do not appear on the table.

Conclusion. The Magic 8 project originated as an unanticipated result from a NSF funded project to perform network analysis on gene expression across the GPS response. To date, all work on the Magic 8 has been performed by talented undergraduate researchers, including the original discovery, funded through PACE, my research incentive, course credit and as volunteers. Significant preliminary work has been done toward the qRT-PCR (Aim1). Mutant lines have been identified for all the genes or are currently being developed, several have been bred to homozygosity with more in progress, a couple have been confirmed to be true knock-outs (all in Aim 2) and phenotype analysis of the mutants has been started for a few (Aim 3). To

publish the work, we need to complete characterization of all 8 genes. Two of the undergraduate working on the project will graduate this spring, and the other two may not be available this summer. And regardless, I do not have funding to support them. To complete this project in a reasonable time frame, especially in time for submission of an external, federal proposal, I need a more dedicated effort. Alexander Meyers, a PBIO PhD student, transferred to the lab in February 2016. He has previous molecular and Arabidopsis phenotype experience and would be perfect to complete this project if given the time to commit to it. If this proposal is funded, it would not only allow me to complete this initial characterization, but would allow him to gain experience in the lab and develop his own project, possibly building on one of the Magic 8.

Timeline and Management Plan. The project should be completed in the timeline of the Baker funding period (1 year) with the personnel requested. I will supervise the overall project, with the graduate student responsible for completing all experiments with the help of the undergraduate. Three undergraduates are currently working on the project (spring quarter), but have limited time with their course load. Realistically, qRT-PCR may be completed for a few of the genes, and the students may be able to isolate additional homozygous lines. The graduate student (Alexander Meyers) will work with them to get acquainted with the project and, with the support provided by this proposal, should be able to complete Aims 1 & 2 and make substantial progress toward the completion of the phenotype analysis (Aim 3) during the summer with the help of the undergraduate researcher requested. Any remaining work can be completed during the fall semester. Data should be available for the NSF IOS preproposal deadline in January with the manuscript submitted during spring semester 2017.

E. Collaborations: N/A

F. Confidentiality: N/A

6. Glossary or Definition of Terms: N/A

7. Bibliography (Reference Cited)

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8. Biographical Information – Dr. Sarah E. Wyatt

Department of Environmental and Plant Biology
508A Porter Hall
Ohio University
Athens, OH 45701
Web page: www.wyattlab.wordpress.com

Telephone: (740) 593-1133
FAX: (740) 593-1130
e-mail: wyatts@ohio.edu

Higher Academic Degree: Ph.D., Plant Physiology, Purdue University, 1995

Experience:

Jan- July 2016	Visiting Scientist (FFL), GeneLab, NASA Ames Research Center Mountain View, CA
2013- present	Professor, Department of Environmental and Plant Biology Ohio University, Athens, Ohio
2006-2013	Associate Professor, Department of Environmental and Plant Biology Ohio University, Athens, Ohio
2000-2006	Assistant Professor, Department of Environmental and Plant Biology Ohio University, Athens, Ohio
1996-2000	Research Associate, NASA Specialized Center of Research and Training Department of Botany, North Carolina State University, Raleigh, NC

Publications (graduate students in bold, undergraduates in italics) (last 5 years):

Kruse C, Shen K, Wyatt, SE (in preparation) Network analysis of gene expression data provides insights in pathway prediction for gravitropic signaling. *BMC Plant Biology*

Cook CA, Tucker A, **Shen K** and Wyatt SE. (2015) Microarray identifies transcription factors potentially involved in gravitropic signal transduction. *Gravitational and Space Research* 3:18-27.

Basu, P., Luesse, D.R., and Wyatt, S.E. (2015) "Proteomic approaches and their application to plant gravitropism" IN: *Methods in Molecular Biology* Elison Blancaflor (Ed.) Springer – Humana Press.

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Selected Papers Presented and Published Abstracts (45 total, last 5 years)

Basu, P., Luesse, D. R., Wyatt, S. E. "BRIC20: Identification of Differentially Expressed Proteins in Seedlings Grown on the International Space Station" American Society of Gravitational and Space Biology, (Alexandria, VA; November 2015)

Wyatt, S. E., **Basu, P.**, **Kruse, C.P.S.**, Luesse, D. R. "BRIC20: Proteomics Analysis of Arabidopsis Seedlings in Microgravity" American Society of Gravitational and Space Biology, (Alexandria, VA; November 2015).

Benson, C., Held, J., Pugh, J., Cook, C. A., Wyatt, S. E. "Gene expression analysis identifies 8 genes involved in early gravitropic signaling in Arabidopsis" American Society of Gravitational and Space Biology, (Alexandria, VA; November 2015)

Wyatt, S. E., *Cook, C. A.* "Transcriptions Factors AtAIB and WRKY 18 may play a Role in Gravitropic Signaling" American Society of Gravitational and Space Biology, (Alexandria, VA; November 2015)

Kruse, C. P. S., Luesse, D. R., Wyatt, S. E. "Transcriptomic Analysis of Seedlings Grown in Microgravity" American Society of Gravitational and Space Biology, (Alexandria, VA; November 2015)

Wyatt, S. E., and Luesse, D. R. "BRIC-20: Proteomics analysis of Arabidopsis seedlings in microgravity" GeneLab Workshop, American Society of Gravitational and Space Biology, (Alexandria, VA; November 2015)

Wyatt, S. E., **Kruse, C. P.S.** "Plant Gravitropic Signal Transduction: A Network Analysis Leads to Gene Discovery" Plant Biology 2015 (Minneapolis, MN; July 2015)

Basu, P., Luesse, D. R., Wyatt, S. E. (Author Only), "A Discovery-based, Proteomic Analysis of Arabidopsis Seedlings Grown in Microgravity" Plant Biology 2015 (Minneapolis, MN; July 2015)

Sternberger, A. L., Ballard, Jr., H. E., Wyatt, S. E., "Genome Assembly and Investigation into the Chasmogamous / Cleistogamous Mixed Breeding System of *Viola pubescens* (Violaceae)" Plant Biology 2015 (Minneapolis, MN; July 2015)

Wyatt, S.E., Shen, K., Hayden, M. "Plant gravitropic signal transduction: A network analysis leads to gene discovery" American Society for Gravitational and Space Research (Pasadena, CA; October 2014)

Cook, C.A., Tucker, A., Wyatt, S.E. "Microarray of GPS Treatment Reveals Novel Genes Involved in Signal Transduction." American Society for Gravitational and Space Research (Pasadena, CA; October 2014)

Hall, Z., **Hayden, M.**, *Osika, M.*, Wyatt, S.E. *"Identification of the gene defective in gravity persistence signal (gps) 6 of Arabidopsis thaliana."* American Society for Gravitational and Space Research (Pasadena, CA; October 2014)

Basu, P., Wyatt, S.E. "A proteomic study of *Arabidopsis* seedlings grown in microgravity on the International Space Station" American Society for Gravitational and Space Research (Pasadena, CA; October 2014)

Wyatt, S.E., **Shen, K.,** *Hayden, M.*, Fenstemaker, S., Tucker, A., Moore, M., Brita, A.D., "Plant gravitropic signal transduction: A network analysis leads to gene discovery" COSPAR 2014 (Moscow, Russia; Aug 2014)

Tucker, A., *Cook, C.A., Osika, M., Pugh, J.*, Wyatt, S. E. "Microarray analysis of the GPS treatment reveals novel transcription factors involved in gravitropic signal transduction". Plant Biology 2014 (Portland, OR; July 2014)

Wyatt, S.E., Luesse, D.R. "Proteomics Analysis of Arabidopsis Seedlings in Microgravity" International Space Station (ISS) Joint CSA/ESA./JAXA/NASA Science Symposium (WebEx; June 2014)

Tucker, A., Abrams Z., **Shen, K.**, Wyatt, S.E. "Transcriptome analysis to identify novel factors of the gravitropic signal transduction pathway" Plant Biology 2013 (Providence, RI: July 2013)

Craig Schenck, Vijayanand Nadella, *Jessica Lindner*, S.E. Wyatt "Proteomics profile of gravitropic signaling" Plant Biology 2012 (Austin, TX: July 2012)

Kaiyu Shen, Razvan Bunesco, SE Wyatt "The application of machine learning to gene discovery from genome-wide analyses" Plant Biology 2012 (Austin, TX: July 2012)

Tim Williams, Gar Rothwell, Sarah Wyatt. "Rescuing a fossil morphology with exogenous auxin treatments of *Isoetes*". Botany 2012 (Columbus, OH; July 2012)

Abrams, Z., Armbruster, M., Clay, S., Fenstemaker, S., Garcia, K., Hayden, M., Johnson, T., Lyall, K., Presley, W., Thompson, O., Williams, D., Williams, T., Wyatt, S.E. "Mining and Annotation of Gene Lists: A comparative study" Great Lakes Bioinformatics (Ann Arbor, MI: May 2011)

Abrams, Z., Nadella, V., Wyatt, S.E. "A database for adding in miRNA analysis" Great Lakes Bioinformatics (Ann Arbor, MI: May 2012)

Tim Williams, Gar Rothwell, Sarah Wyatt. "Predicting Polar Auxin Transport Regulation in *Isoetes* through Conserved Protein Functions in Disparate Evolutionary Lineages". Great Lakes Bioinformatics 2012 (Ann Arbor, MI; May 2012)

Kaiyu Shen, Razvan Bunesco, SE Wyatt "An interactome approach to analyze genomic data". Great Lakes Bioinformatics 2012 (Ann Arbor, MI; May 2012)

Sarah Wyatt, **Kaiyu Shen**. Building an interactome to identify signaling components. Rocky Mountain Bioinformatics 2011 (Snowmass, CO: December 2011)

Kaiyu Shen, Vijay Nadella, Sarah Wyatt. A Gravitropic Signaling Interactome. American Society of Gravitational and Space Biology Annual Meeting (San Diego, CA: November 2011)

8.b Biographical information – Alexander Meyers

Department of Environmental and Plant Biology
315 Porter Hall
Ohio University
Athens, OH 45701

Telephone: (315) 228-8604

e-mail: am920114@ohio.edu

Academic Background

Ph.D. Molecular and Cellular Biology, anticipated 2019
Ohio University, Athens, Ohio 45701

Bachelor of Science (Cum laude), Biochemistry, 2013
State University of New York at Cortland, Cortland, NY 45701

Academic Positions & Related Employment

2014-present	Graduate researcher, Ohio University, Department of Env. & Plant Biology Athens, Ohio 45701
2013-2014	Microbiology lab technician, Morrisville State University of New York Morrisville, NY 13408
2012-2013	Undergraduate research assistant, State University of New York Cortland, NY 13045
2012	Undergraduate research assistant, University of Exeter Exeter, UK EX4 4SB

Honors and Awards

Top Graduating Senior in Chemistry, Cortland State, 2013
Cortland Undergraduate Research Fellowship, $2000, 2012
Phi Beta Delta Honor Society for International Scholars, inducted 2012
Spiegel Wilcox Cortland College Orchestra Scholarship, 2010

Teaching

Guest lectures: Foundations of Plant Biology
- Fall 2014- Introduction to Plant Genetics
- Spring 2015- What is Science: Combatting the Bad by Defining the Good
- Fall 2015- Cellular Life
- Fall 2015- Biotechnology

TA Positions:
- Foundations of Plant Biology Labs. Fall 2014-present. Primary lab instructor.

Outreach

Dept. of Environmental and Plant Biology Graduate Student Association
Charter Member, 2015

9. Other Support, Grants Submitted to Outside Agencies:
I have no funds available for the completion of the work. The preliminary work for this proposal was funded by a 2 year NSF award that was continued as a no cost extension. Those funds ended May 2014. We have completed manuscripts using the data completed during the grant period: one recently published (Cook et al. 2015) with the other one close to submission (Kruse et al.). All data for that manuscript is complete. The support requested here is to complete data for a 3rd manuscript based on preliminary data from the award.

A. Previous University Funding: none within the last 3 years. My last ended in 2011. I have previously been very successful at turning OURC and Baker funding into external awards and publications.

B. External Funding:
   2015 Collaborative: A re-iterative systems approach to gravitropic signaling, National Science Foundation, Molecular and Cell Biology division Wyatt, S. E., Luesse, D. R. (Co-PI), Ercal, G. (Co-PI), $664,169.00, (Currently Under Review)
   2015 Preproposal: Collaborative Research: Plant gravitropic signaling: a multi-faceted approach to understanding the regulatory network. National Science Foundation, Integrative Organismal Systems. Wyatt, S. E. (PI), Luesse, D. R. (co-PI), Not Invited.
   2015 NIAC step A - Nanoplants for Advanced Life Support, NASA, Wyatt, S. E., $0.00, Letter of Intent. Not Invited.
   2014 NASA, GeneLab supplement to BRIC 20. "Proteomics Analysis of Arabidopsis seedlings in microgravity. $63,000 (1/2015-12/2015)
   2014 Collaborative Research: Molecular Characterization of Gravity Persistent Signal (GPS) Mutants in *Arabidopsis thaliana*, National Science Foundation, Molecular & Cellular Biology division, Federal, ($450,000) Wyatt, S. E. (PI), Luesse, D. R.(co-PI), Not Funded.
   2012 NASA, Research Opportunities in Space Flight. "BRIC 20: Proteomics Analysis of Arabidopsis seedlings in microgravity. $383,052 (9/2013-8/2015)
   2012 National Science Foundation, Organism and Environment Cluster, IOS. "Gravitropic Signal Transduction: A network approach to identifying the regulatory mechanisms," $250,000 (Funded, 6/2012-5/2014).
   2011 Plants and gravity: a multi-faceted approach to understanding the regulatory network, National Science Foundation, Wyatt, S. E., $250,000.00, Funded. June 2012 - May 2015.

Sustainability - Possible Continued Funding for this Research. Data from Kruse et al. (in preparation) served as preliminary data for a NSF MCB proposal submitted Nov 2015 that is currently under review. If the work proposed here is completed, it should not only provide the necessary data for a manuscript in a peer reviewed journal but also provide preliminary data to support a submission to NSF's Integrated Organismal Systems program. This is the program that funded the original research that led to the discovery.

10. Budget and Justification

A. Consumable Supplies: $2456.50

Materials needed for plant growth and phenotype analysis both of seedlings on growth media in petri plates and mature plants grown in soil in pots. For statistical power, a minimum of 30 individual plants per mutant and wild-type are necessary. For 8 mutant lines and wild-type with 2 independent mutant lines each, 30 plants per line for 2 root assays and 30 plants per line for each of 2 assays for mature plants, we will needed a minimum of 90 plants/line for seedlings totally 1080 seedlings and 1080 adult plants. Supplies are also requested for the molecular assays including DNA extraction and PCR to confirm homozygosity of the insertions, RNA extraction and RT-PCR to confirm that the insertion results in a knock out of the gene. These costs are detailed in the following table.

Item	**Unit of purchase**	**Company**	**Cost per unit**	**Amount needed**	**Total request**
Plant Growth					
Pots, soil, fertilizer etc	30 plants/ 16 mutant lines + WT	per H. Blazier	N/A	Minimum 1080 plants	$ 270
Culture plates, agar, media, etc	30 plants/ 16 mutant lines + WT	Fischer Scientific	N/A	Minimum 1080 plants	$ 432
Molecular assays (PCR, RT-PCR, qRT-PCR)					
Taq polymerase & buffer	500 units	Omega Bio-tek	$95	1 vial	$ 95
Primers	Per primer	IDT Inc.	$6	2/line, 33	$ 198
Nucleotides	1.5 ml vial of NTP for 100 reactions	Lucigen	$57	1 vial	$ 57
cDNA RT kit	100 rxn/vial	Applied Biosystems	$270	1 vial	$ 270
DyNAmo Color Flash SYBR Green kit for qRT-PCR	100 rxn/kit	Thermo Fisher Scientific	$ 86	1 kit	$ 86

Reagents for RNA extractions					
RNeasy mini kit	25 rxn/vial	Qiagen	$146.50	2 vials	$ 293
DNA extractions					
	100 ml	Acros organics	$67.83	1	$ 67.83
	500 ml	Fisher	$58.37	1	$ 58.37
	500 ml	Sigma-Aldrich	$71	1	$ 71
	100 g	Sigma-Aldrich	$50.20	1	$ 50.20
	100 g	Promega	$29	1	$ 29
	500 ml	Fisher	$95	1	$ 95
	50 g	Sigma-Aldrich	$26.10	1	$ 26.10
Disposables					
1.5 ml Eppendorf tubes	500 /pk	Fisher	$63	4	$ 152
0.2 ml PCR tubes	1000/pk	Thermo Scientific	$103	2	$ 206
Supplies sub total					**$2456.50**

B. Travel: None requested.

C. Student Wages: Summer stipend is being requested for one PhD student (Alexander Meyers) to work full time for the 15 weeks of summer semester. A tuition waiver will be provided by the College of Arts and Sciences should the proposal be funded. Mr. Meyers will be responsible for completing all remaining assays with help from one undergraduate researcher and the writing of the final manuscript. The phenotype analyses (5 in total) are time intensive, plants must be grown and maintained, and capturing phenotypes is time sensitive. To complete the data needed for a high quality journal within the time allotted will require dedicated time as requested. I will supervise the project and work closely with Mr. Meyers and the undergraduate to complete all aspects of the project. All salary numbers were provided by Ms. Karen Keesey, PBIO Accounting Support Specialist.

Personnel	Salary	Amount of effort	Total requested
Summer Stipend: Alexander Meyers, PhD student	$6,120.00*	100% effort	$6,120.00
Workers Comp.	(.00739)		45.23
Undergraduate Researcher (TBA)	$10/hr**	20 hr/week/15 weeks (300 h) + benefits	$3,000.00
associated benefits for UG			117.92
Personnel sub total			**$9,283.15**

*The stipend rate is standard for a PBIO PhD student. A tuition waiver will be provided by the College of Arts and Sciences if the proposal is funded (See Appendix A).

**An hourly rate of $10/h is being requested for the undergraduate due to the technical nature of the work. That rate is standard for an undergraduate researcher in my laboratory.

D. Equipment: None requested

E. Faculty Stipend: None requested.

F. Other: None requested.

G. Total requested: $11,739.65

11. Appended Materials *(10 page maximum; no spacing, margin, or font size requirements)*

A. Email from Dr. Brian McCarthy, Assoc. Dean of Arts & Sciences confirming a tuition waiver if the grant is funded.

From: McCarthy, Brian
Sent: Wednesday, February 10, 2016 8:57 AM
To: Wyatt, Sarah <wyatts@ohio.edu>
Cc: Ritchie, April <ritchiea@ohio.edu>
Subject: Graduate Waiver Request

Dear Sarah,

By way of this email, CAS is willing to provide a one semester (summer) tuition waiver, should your Baker Fund proposal be successful in generating the matching graduate student stipend.

If and when the time comes, please have Connie submit a CAS Footprints ticket on this issue and include this email as an attachment.

Good luck with your proposal!

Best wishes,
Brian

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Brian C. McCarthy, Ph.D.
Assoc Dean and Prof of Forest Ecology
College of Arts & Sciences
207 Wilson Hall Admin
Ohio University
Athens, OH 45701-2979 USA
T: 740-593-2979
M: 740-707-9017
E: mccarthy@ohio.edu
Skype: BCMcCarthyOHIO

12. Recommended Reviewers (listed alphabetically). I know these potential reviewers professionally, but have no personal relationship. None were my mentors nor were they my students.

Dr. Elison Blancaflor
e-mail: eblancaflor@noble.org
Phone: 580- 224-6687
Address: Plant Biology Division
The Samuel Roberts Noble Foundation Inc.
2510 Sam Noble Parkway
Ardmore, OK 73401
Web site: http://www.noble.org/staff/blancaflor-elison/
Expertise: plant cell biology/cytoskeleton, root hair development, gene expression, Arabidopsis, space flight

Dr. Amy Clore
e-mail: clore@ncf.edu
Address: New College of Florida
Division of Natural Sciences
5800 Bay Shore Rd.
Sarasota, FL 34243-2109
Web site: http://www.ncf.edu/clore
Expertise: plant growth and development, cell biology

Dr. Simon Gilroy
e-mail: sgilroy@wisc.edu
Phone: 608-262-4009
Address: Department of Botany
University of Wisconsin
Birge Hall, 430 Lincoln Drive
Madison, WI 5370
Web site: http://www.botany.wisc.edu/gilroy/
Expertise: molecular components of touch and gravity regulation of plant growth and development, Arabidopsis gene expression, signaling, microscopy

Dr. John Z. Kiss, Professor, Dean of the Graduate College
e-mail: jzkiss@olemiss.edu
Address: Department of Biology
100 Graduate House
University of Mississippi
University, MS 38677-1848
Phone: 662- 915-7474
Web site: http://biology.olemiss.edu/people/faculty/john-kiss/
Expertise: gravitropism/light interactions, space flight, microarray gene expression analysis, plant cell biology

Dr. Stan Roux, Professor
e-mail: sroux@uts.cc.utexas.edu
Address: The University of Texas at Austin –MCDB
1 University Station A6700
205 W. 24th St.; BIO 16
Austin, TX 78712-0183
Web site: http://www.sbs.utexas.edu/roux/
Expertise: molecular components of light and gravity regulation of plant growth and development, Ceratopteris (fern) and Arabidopsis as model systems