PennState
College of Agricultural Sciences

PROPOSAL TRANSMITTAL

Date: 1/4/2018 **PSU Ref. No: 199297**

Title: Assessment of resistance to pre- and postharvest site-specific fungicides in populations of Colletotrichum fioriniae (Bitter rot fungus) in Pennsylvania orchards

Submitted to: Patti Keller
Patti@acnursery.com
State Horticultural Association of Pennsylvania
697 Mountain Road
Orrtanna, PA 17353

Submitted by: Kari Peter
717-677-6116
kap22@psu.edu

Proposed Project Period: **04/01/2018 - 03/31/2019** **Total Project Request:** **$11,894**

AUTHORIZED UNIVERSITY OFFICIAL

Kelley Benninghoff DATE 1/4/2018

Kelley Benninghoff
Research Administrator - Pre-award
College of Agricultural Sciences
107 Agricultural Administration Building
University Park, PA 16802-2602
Tel: 814-865-5419
Fax: 814-865-0323
Email: L-AG-contgrts@lists.psu.edu

John W. Hanold DATE 1/4/18

John W. Hanold
Assoc. VP for Resrearch
Office of Sponsored Programs
The Pennsylvania State University
110 Technology Center Building
University Park, PA 16802-2602
Tel: 814-865-1372
Fax: 814-865-3377
Email: osp@psu.edu

EIN: 24-60000376
DUNS No: 00-340-3953

The Pennsylvania State University employs individuals and accepts students and graduate research students from a multitude of national backgrounds. As an entity, the University is subject to, and works diligently to obey, federal regulations regarding the export of controlled technologies and data. Sponsor, as an independent entity, is individually responsible for ascertaining its compliance with federal export laws and procedures. If Sponsor anticipates disclosure or provision of controlled technology or data to University as part of the proposed sponsored project, Sponsor should inform University, in writing, of the existence of, and information concerning the scope and extent of, such anticipated disclosures or provisions.

Please reference PSU Ref. Number in all correspondence.

2018 RESEARCH GRANT PROPOSAL
STATE HORTICULTURAL ASSOCIATION OF PENNSLYVANIA PROGRAM

Title: **Assessment of resistance to pre- and postharvest site-specific fungicides in populations of *Colletotrichum fioriniae* (Bitter rot fungus) in Pennsylvania orchards**

Personnel:
PI: Dr. Kari Peter, Assistant Professor or Tree Fruit Pathology, Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University Fruit Research and Extension Center, Biglerville, Pennsylvania Phone: (717) 677-6116 Ext. 223 Email: kap22@psu.edu

Funding period: April 1, 2018 – March 31, 2019

Amount requested: $11,894

Justification:

Apple fruit rot diseases are prevalent for East Coast apple growers thanks to high temperatures and humid conditions experienced during the growing season. Diseases causing apple fruit decay can have a significant impact on yield and quality, as well as potentially cause loss during storage. A fruit rot that is problematic both before and after harvest is bitter rot. Bitter rot, which was a disease once relegated to the south, has increased its territory into northern apple production areas over the last twenty or more years. Factors that may have increased its prevalence include changes in climate, planting of susceptible cultivars, and fungicide label changes (i.e. the 77 day PHI of mancozeb) (Rosenberger and Cox, 2016).

Bitter rot is caused by fungi in the *Colletotrichum* spp. Up until recently, the culprits linked to causing bitter rot were *C. acutatum*, *C. gloesporioides*, and *Glomerella cingulata* (Gonzales et al., 2006). In the last several years, the playing field has become more complex as there are now at least 17 recognized species of *Colletotrichum* that can cause bitter rot on pome fruit: these are now included in the *C. acutatum* species complex (Damm et al., 2012) and *C. gloesporioides* (Weir et al., 2012). The symptoms caused by the various *Colletotrichum* species are virtually identical; however, the pathogen biology and management strategies could potentially differ.

Based on personal observations, as well as conversations with growers, other pathologists, and Extension personnel in the Mid-Atlantic and Northeast, bitter rot incidence is a significant pressing issue for those in the tree fruit industry. Consequently, several labs are studying the bitter rot pathogen. To further research and productivity, we are forming a consortium, which will include Penn State, Cornell University, The University of Kentucky, North Carolina State University, and the USDA-ARS. Issues unique to each region are the infecting species of the *Colletotrichum* pathogen and the status of fungicide resistance. For example, five species of *Colletotrichum* have been found in Kentucky apple orchards (*C. fioriniae*, *C. nymphaeae*, *C. siamense*, *C. theobromicola*, and *C. fructicola*) and the species varied in their sensitivity to several fungicide classes (Munir et al, 2016). As a result of SHAP funding in 2017, we have discovered (to date) the dominant *Colletotrichum* spp. causing bitter rot, at least in Adams County, is *C. fioriniae*. Some of the isolates collected are showing increased tolerance to trifloxystrobin; however, we are in the process of repeating and increasing the number of isolates to better understand how prevalent this finding is. We also attempted to determine the optimal time for controlling bitter rot in the field,

particularly fungicides used during bloom. Preliminary data has suggested bloom to be critical for fruit rot management later in the season. Unfortunately, our results are inconclusive due to low rot incidence overall, especially in our untreated check. We are currently collaborating with Dr. Nicole Gauthier (University of Kentucky) for optimizing a protocol to better understand how critical bloom time is for bitter rot management. **For this proposal, we aim to build on our 2017 data by collecting additional isolates throughout the state to understand the *Colletotrichum* species most prevalent in Pennsylvania orchards and how effective commonly used pre- and postharvest fungicides still manage the disease.** The requested funding from this grant will be supporting the research activities for Mr. Phillip Martin, a Ph.D. student in the Department of Plant Pathology and Environmental Microbiology. The proposed research is to help establish preliminary results to be included in federal grant proposals.

Objectives:

The objectives of this project address the 2018 SHAP priority under the Plant Pathology section: **Disease Resistant Management for Key Diseases:**

1. Collect and characterize bitter rot fungi to species throughout Pennsylvania orchards during the 2018 season.

2. Screen collected bitter rot fungal isolates for sensitivity to the benzimidazole class (FRAC Group 1), the strobilurin class (FRAC Group 11), and the pyrrole class (FRAC Group 13) of fungicides.

Objective 1 Procedures: Apples exhibiting bitter rot symptoms (orange-colored spores on the surface of the fruit arranged in a concentric pattern) will be collected from PA orchards from August until harvest. The search for infected fruit in the coming season will be announced during the 2018 winter meetings, as well as in Fruit Times. In addition, help will be solicited for identifying sites from Extension personnel and consultants. Fungal isolates will be obtained from the apple fruit collected using standard laboratory procedures. We will be confirming the identity using conventional molecular methods: genomic DNA isolation and amplification of conserved genes (β-tubulin, GAPDH, and calmodulin) used to differentiate species of pathogens. Gene products will be sequenced and the resulting sequence data will be analyzed using publically available BLAST software (NCBI) to determine sequence identity. In addition, fungal isolates will be further characterized using standard morphological (culture coloration, spore size, etc.) methods. Using Golden Delicious apples, Koch's postulates will be conducted to verify pathogenicity of bitter rot isolates on the observed host.

Objective 2 Procedures: To evaluate fungicide sensitivity of collected isolates, we will use mycelial growth assays by using the formulations of the following commonly used fungicides during pre- and postharvest: trifloxystrobin (Flint; found in Luna Sensation) and pyraclostrobin (Cabrio; found in Merivon and Pristine); thiophanate methyl (Topsin M); and fludioxonil (Scholar - postharvest). Briefly, 60 mm-diameter petri plates of ½ strength potato dextrose agar (PDA) will be amended with the active ingredient concentrations of 0.01, 0.1, 1, 10, and 100 µg/ml for each of the fungicides listed above. Bitter rot fungi will be screened for sensitivity by transferring from the master plate of a single-spored isolate a mycelial plug from the leading edge of growth and placed in the center of the fungicide amended and unamended plate with three replications per rate for each isolate. The plates will be incubated for 10 days

in the dark at 25°C and mycelial growth of the cultures monitored by recording the diameter with a slide caliper. Mycelial colony growth will be measured in two perpendicular directions from the edge of each colony. The mean diameter will be used to calculate percent inhibition relative to the unamended medium. In vitro sensitivity data will be subjected to a separate analysis of variance to determine any trends in population shifts. Pending our results of the individual evaluations of each fungicide class, we will take a subset of isolates with increased tolerance and analyze for cross-resistance to multiple fungicides.

Budget:

Wages for part-time seasonal employee ($9.25/hr, 20 h/wk, 12 weeks):	$2,200
Wage fringe benefits (7.9%):	$174
Laboratory supplies:	$6,000
Purchased services:	$2,000
Travel:	$1,500
Total requested:	**$11,894**

Budget Justification:

Salaries/Wages - $2,220

Funds are requested for a seasonal wage employee will be hired for the summer season and will contribute 50% of their time during their 12 week summer employment ($9.25hr/20 h/wk for 12 weeks) to assist with the collection and processing of samples.

Fringe Benefits - $174

Fringe benefits are computed using the fixed rates of 41.60% applicable to Category I Salaries, 15.40% applicable to Category II Graduate Assistants, 7.90% applicable to Category III Salaries and Wages, 0.10% applicable to Category IV Student Wages, and 26.30% for Category V, Postdoctoral Scholars and Fellows, for fiscal year 2018 (July 1, 2017, through June 30, 2018). If this proposal is funded, the rates quoted above shall, at the time of funding, be subject to adjustment for any period subsequent to June 30, 2018, if superseding Government approved rates have been established. Fringe benefit rates are negotiated and approved by the Office of Naval Research, Penn State's cognizant federal agency.

Materials and Supplies - $6,000

Funds are requested for microbiological media, plates, laboratory disposables, genomic DNA extraction kits, and reagents for molecular evaluations (primers, PCR master mixes) to support the growth, analysis, and evaluation of fungicide resistance of fungal isolates collected. Expenses for materials will be determined from catalog prices using Penn State's eBuy.

Purchased services - $2,000

Funds are requested for sequencing of DNA samples of collected *Colletotrichum* isolates to identify to species. Sequencing will be done by Penn State Genomics Core Facility or Eurofins Scientifc.

Travel - $1,500

Funds are requested to support PPEM graduate student, Phillip Martin, to attend the Annual American Phytopathological Society meeting in Boston, MA July 29 – August 3, 2018. He will be presenting a

poster about the bitter rot research he has conducted from 2017 – 2018 resulting from SHAP support. Funds will be used to supplement additional funds that the PPEM Department will be providing students and ultimately all financial support will cover transportation, hotel, and meal costs for the trip.

References:

Damm, U., Cannon, P.F., Woudenberg, J. H. C., and Crous, P. W. 2012. The *Colletotrichum acutatum* species complex. Studies in Mycology 73: 37-113.

Gonzalez, E., Sutton, T. B., and Correll, J. C. 2006. Clarification of the etiology of Glomerella leaf spot and bitter rot of apple caused by Colletotrichum spp. based on morphology and genetic, molecular, and pathogenicity tests. Phytopathology 96: 982-992.

Munir, M., Amsden, B., Dixon, E., Vaillancourt, L., and Gauthier, N. A. W. 2016. Characterization of *Colletotrichum* species causing bitter rot of apple in Kentucky orchards. Plant Dis. 100:2194-2203.

Rosenberger, D. A. and Cox, K. 2016. Preventing bitter rot in apples. Scaffolds Fruit Journal 25 (22) 1-4. Online: http://www.scaffolds.entomology.cornell.edu/2016/SCAFFOLDS-8-29-16.pdf

Weir, B., Johnston, P. R., and Damm, U. 2012. The *Colletotrichum gloeosporioides* species complex. Studies in Mycology 73: 115 – 180.