PennState
College of Agricultural Sciences

PROPOSAL TRANSMITTAL

Date: 12/19/2018 **PSU Ref. No: 206050**

Title: Completing the Picture: Characterizing Bitter Rot Fungal Isolates and Verification of Lab Results with Field Trials

Submitted to: Patti Keller

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Proposed Project **05/01/2019 - 04/30/2020** **Total Project Request: $11,000**

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2019 RESEARCH GRANT PROPOSAL

STATE HORTICULTURAL ASSOCIATION OF PENNSLYVANIA

Title: **Completing the Picture: Characterizing Bitter Rot Fungal Isolates and Verification of Lab Results with Field Trials**

Personnel:
PI: Dr. Kari Peter, Assistant Professor of Tree Fruit Pathology, Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University Fruit Research and Extension Center, P.O. Box 330, 290 University Dr., Biglerville, PA Phone: (717) 677-6116 Ext. 223
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Funding period: May 1, 2019 – April 30, 2020

Amount requested: $11,000

Justification:

Bitter rot of apple was an exceptionally severe problem in 2018, in part due to the wet conditions throughout the growing season. As challenging as this disease is, progress is being made in understanding its life cycle and determining the most effective management tools. SHAP funded studies in 2017 found that *Colletotrichum fioriniae* was the most prevalent causal fungal species in the orchards sampled, followed by *C. fructicola* (Martin et al. 2018). In 2018, the sampling of bitter rot apples was expanded to include growers from around Pennsylvania. With 2018 being such a bad bitter rot year we had a better than expected response, sampling over 500 apples from more than 30 orchards. Preliminary results show that the causal fungal species distribution is similar to that found in 2017, with the addition of *C. siamense* and *C. henanense*, the latter being a species that has not previously been identified as a cause of bitter rot. Due to the abundance of samples received, the diversity of fungal species, and the identification of a new bitter rot species, the fungal isolates collected need additional characterization to complete the picture of the fungi causing bitter rot in Pennsylvania.

Preliminary studies showed both *C. fioriniae* and *C. fructicola* mycelial growth was only weakly inhibited by the FRAC Group 11 fungicide, trifloxystrobin (active ingredient in Flint and Luna Sensation) (Martin et al. 2018). An expanded fungicide sensitivity test found that the most common fungal species, *C. fioriniae*, has reduced sensitivity to the popular summer cover spray thiophanate methyl (FRAC Group 1, active ingredient in Topsin M), as compared to the species *C. fructicola* (unpublished results). Field fungicide trials show Captan (FRAC Group M4) has decent effectiveness if it doesn't wash off. Merivon (FRAC Groups 7 & 11) has also shown decent effectiveness, but growers are limited to 4 sprays per season. The evidence points to potential weakness in the ability of the cover sprays Captan and Topsin M to limit bitter rot in years with ample rainfall. Completing the fungicide sensitivity picture requires a two-pronged approach. First, mutations in the β-tubulin and cytochrome b genes have been identified as sources for insensitivity to FRAC Groups 1 and 11, respectively (Hu et al. 2015; Forcelini et al. 2016). Pennsylvania isolates need to be checked for the presence of those mutations to inform resistance management. Second, lab results need to be confirmed with field trials, especially field trials of alternative cover spray products such as Omega (active ingredient fluazinam, FRAC Group 29) and biological plant defense inducers such as Regalia (FRAC Group P5). Regalia has been described to

increase lignification, which strengthen cell walls. This may be beneficial for Honeycrisp, which is extremely susceptible to bitter rot.

With regards to the life cycle of *C. fioriniae*, we have discovered that spores are being dispersed in orchards from bloom to harvest, and that infections can become established by late May (Proceedings of 2018 Cumberland Shenandoah Fruit Workers Conference, in press). Much of the molecular work to complete the life cycle picture is being funded by Northeast SARE. However, a key missing element is field trials to narrow down the period of apple susceptibility to infection. In Japan, trials indicated that their key infection period is in the first 60 days after full bloom (Nekoduka et al. 2018). We need to conduct field trials to try and narrow down the period of apple susceptibility to infection in Pennsylvania.

Objectives:

The objectives of this project address the 2019 SHAP priority under the Plant Pathology section: **Disease Resistant Management for Key Diseases:**

1. **Complete the characterization of fungal species causing bitter rot in Pennsylvania**
2. **Look for mutations that could be conferring resistance to commonly used fungicides**
3. **Test alternative cover-spray fungicides and plant-defense inducing biologicals in the field**
4. **Find the period of greatest apple susceptibility to bitter rot with field trials**

Objective 1 Procedures

Fungal isolates have been collected from apples with bitter rot from across Pennsylvania. These isolates have been broadly grouped by morphology and many have had genes sequenced to definitively identify them to species. The remainder of the isolates also needs to be definitively identified to species with gene sequencing and, for a sample of each species, several additional genes need to be sequenced to identify the species genotype. This is especially important for the species *C. fructicola* and *C. siamense*, which have not been previously identified as causing bitter rot in Pennsylvania, and for *C. henanense*, which, to the best of our knowledge, has not previously been identified as causing bitter rot anywhere in the world. Koch's postulates will be completed on *C. fructicola, C. siamense*, and *C. henanense* to confirm pathogenicity. DNA has been or will be extracted with a NucleoSpin Microbial kit (Macherey Nagal, Bethlehem PA), and the genes GAPDH, β-tubulin, calmodulin, glutamine synthetase, APn2/MAT1-2 and possibly histone 3 will be amplified with PCR, sequenced with Eurofins Genomics (Louisville KY), aligned with MEGA software, and compared to existing fungal isolate databases using publically available BLAST software (NCBI).

Objective 2 Procedures

A sample of the fungal isolates that were (or will be) lab tested for fungicide sensitivity will have DNA extracted as described above. Genes known to impart resistance to fungicides such as β-tubulin and cytochrome b will be amplified, sequenced and compared to known resistant and susceptible fungal isolates as described in objective 1.

Objective 3 Procedures

An apple research block comprised of Honeycrisp/M7 at the Fruit Research and Extension Center that experienced heavy bitter rot disease pressure in 2018 will be used to test alternative cover spray products (Omega) and biological plant defense inducers (Regalia), in combination and as standalone. Treatments will be arranged in a randomized complete block with four replications/treatment using single tree treatments. Immature apples will be harvested throughout the season and tested for dormant infections using the surface disinfestation-freezing-incubation method. Fruit will be collected at harvest and rated for infection. Non-infected fruit will be put into cold storage and rated again during the winter for post-harvest rots. Infection rates from the different treatments will be statistically compared using the software SAS (SAS Institute Inc., Cary NC) or R (R Core Team, Vienna Austria).

Objective 4 Procedures

An apple research block comprised of Honeycrisp/B9 at the Fruit Research and Extension Center that experienced minimal bitter rot disease pressure in 2018 will be used to find the period of greatest apple susceptibility to bitter rot. A heavy fungicide program will be used to limit infection outside of select 2-week periods throughout the season. The orchard block will be divided into approximately 10 treatment blocks, in a randomized complete block with four replications/treatment using two trees/treatment. Starting at bloom, select treatment blocks will have a 2-week gap in the fungicide program during which infected apples will be hung in the tree canopy to induce heavy infection pressure during that time. For trees not being treated with fungicides during the 2-week gap, they will be temporarily covered with large tarps while the fungicides are being applied to ensure fungicide drift will not interfere with the trial. The no-fungicide/ heavy infection periods will be applied to successive treatment blocks from bloom to harvest. Immature apples will be harvested during the season and tested for dormant infections using the surface disinfestation-freezing-incubation method. Fruit will be collected at harvest and rated for infection. Non-infected fruit will be put into cold storage and rated again during the winter for post-harvest rots. Infection rates from the different timed infection periods will be statistically compared using the software SAS (SAS Institute Inc., Cary NC) or R (R Core Team, Vienna Austria).

Budget:

Wages for part-time seasonal employee ($9.23/hr, 20 h/wk, 13 weeks):	$2,400
Wage fringe benefits (7.81%):	$189
Laboratory supplies:	$7,411
Travel:	$1,000
Total requested:	**$11,000**

Budget Justification:

Salaries/Wages – $2,400

Funds are requested for a seasonal wage employee to be hired for the summer season. They will assist with the collection and processing of samples.

Fringe Benefits - $189

Fringe benefits are computed using the fixed rates of 38.97% applicable to Category I Salaries, 14.74% applicable to Category II Graduate Assistants, 7.81% applicable to Category III Salaries and Wages, 0.18% applicable to Category IV Student Wages, and 25.34% for Category V, Postdoctoral Scholars and Fellows, for fiscal year 2018 (July 1, 2018, through June 30, 2019). 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, 2019, 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 - $7,411

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 fungal isolates collected, as well as evaluation of fungicide resistance of fungal isolates collected. Expenses for materials will be determined from catalog prices using Penn State's eBuy. Fuds are requested to purchase supplies for field experiments (tarps, flagging) and will be purchased locally. Funds are requested to purchase prepaid plates from Eurofins Scientific for sample cleaning and subsequent sequencing of DNA samples of collected *Colletotrichum* isolates to identify to species.

Travel - $1,000

Funds are requested to support travel for personnel working on this project to attend the annual Cumberland Shenandoah Fruit Workers meeting in Winchester, VA, November 29 – 30, 2019 and another regional fruit workers meeting, such as those in New England or Michigan, to report this research. Funds will cover hotel, meals, and cost of meeting registration. All travel will be in accordance with University travel regulations and mileage will be charged at the current rate on the date of travel.

References

Forcelini, B. B., Seijo, T. E., Amiri, A., and Peres, N. A. 2016. Resistance in strawberry isolates of *Colletotrichum acutatum* from Florida to Quinone-outside inhibitors fungicides. Plant Diseae. 53:1689–1699.

Hu, M.-J., Grabke, A., and Schnabel, G. 2015. Investigation of the *Colletotrichum gloeosporioides* species complex causing peach anthracnose in South Carolina. Plant Dis. 99:797–805.

Martin, P., Krawczyk, T., Lehman, B., and Peter, K. A. 2018. Getting the Upper Hand on Bitter Rot of Apples: Understanding the Fungal Culprits, Epidemiology, and Fungicide Resistance. Pennsylvania Fruit News. 98:26–27.

Nekoduka, S., Tanaka, K., and Sano, T. 2018. Epidemiology of apple bitter rot caused by Colletotrichum acutatum sensu lato. J. Gen. Plant Pathol. 84:262–271.