PennState
College of Agricultural Sciences

PROPOSAL TRANSMITTAL

Date: 12/19/2018 **PSU Ref. No: 206057**

Title: Investigating and Understanding Different Sources For Fruit Rot Fungi in the Packhouse and Field to Better Control Postharvest Decay of Stored Apple Fruit

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Proposed Project **05/01/2019 - 04/30/2020** **Total Project Request: $16,500**

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2019 RESEARCH GRANT PROPOSAL
STATE HORTICULTURAL ASSOCIATION OF PENNSYLVANIA

Title: **Investigating and Understanding Different Sources For Fruit Rot Fungi in the Packhouse and Field to Better Control Postharvest Decay of Stored Apple Fruit**

Personnel:

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Funding period: May 1, 2019 – April 30, 2020

Amount requested: $16,500

Justification:

The importance of postharvest rots has been demonstrated to reduce quality in apples (Janisiewicz and Jurick II 2017, Naets *et al*. 2018). In Pennsylvania, different fungal species have been identified as postharvest pathogens, especially *Penicillium expansum*, *Botrytis cinerea,* and *Colletotrichum* spp. (Kou *et al*. 2014, Yan *et al*. 2014 (I), Yan *et al*. 2014 (II)). Fungi can infect apples either in the field or during postharvest handling/storage, and once apples are harvested, there are multiple sources of inoculum (e.g spores, mycelium). Field bins, and air from packinghouses and cold storages have been identified amongst the most important sources of fungi causing apple rots (Amiri and Bompeix 2005, Hansen *et al*. 2010).

Multiple strategies to reduce fungal spores and postharvest rots in apples during storage have been recommended; however, these have currently not been adapted. For bin sanitation, hot water at 176 °F (Hansen *et al*. 2010) or chlorine-based sanitizers, peroxides and quaternary ammonium (Sholberg 2004) have been proven effective. Also, the use of postharvest fungicides such as Mertect (thiabendazole), Penbotec (pyrimethanil), Scholar (fludioxonil), and Academy (difenoconazole + fludioxonil), have been the main strategy to control apple rots (Lima *et al*. 2011, Jurick II *et al*. 2018), with the associated challenges of fungicide resistance frequently reported for pathogens such as *P. expansum* and *B. cinerea,* which has hampered decay management (Yan *et al*. 2014 (I), Yan *et al*. 2014 (II), Amiri *et al*. 2017, Ali and Amiri 2018).

Postharvest rot impacting apple fruit quality during and after storage remains a significant problem for packinghouse operators and the negative effects have been mentioned in different sources related to academia and industry (Penn State Extension 2017, Washington State University 2018, Good Fruit Grower 2018), which justifies our study to continue with research focused on gaining a better understanding of the relationship between sources of inoculum and the occurrence and management of postharvest rots.

This study aims to achieve a better understanding of what are the main sources of apple postharvest rots in Pennsylvania, and what are the fungicide resistance profiles as they relate to the source of inoculum. Also, we feel this is timely topic considering fruit rots in the field are increasing in incidence, especially during 2018, and it is uncertain what this impact will have in the postharvest environment. Previous research performed in other states is very specific in terms of the inoculum source reported, and even less information is available in Pennsylvania, which increases the importance of this

study to provide an up to date profile of postharvest rots. This will help apple growers to improve or incorporate new cost-effective management practices to better control postharvest rots, focusing on specific sources of inoculum and fungicide resistant spores, such as field equipment, packinghouse facilities and/or cold storages. It will also help producers determine which are the most effective materials to apply for optimal decay control.

Objectives:

The objectives of this research proposal address the 2019 SHAP priority under the post-harvest physiology section: **Control of post-harvest pathogens,** and the Plant Pathology section: **Disease resistant management for key diseases**.

Specific research objectives:

1. Determine the location of apple rot spores with respect to field equipment, picking bins, packing facility and storage environments.
2. Examine the amount and type of fungal rots that are present on sampled areas.
3. Investigate the fungicide resistance profiles of apple rot isolates obtained from fruit, field equipment, packinghouse facilities, and storage environments.

Procedures:

The research will be performed at the Fruit Research and Extension Center (FREC) located in Biglerville, PA. The samplings will be performed in at least three commercial packinghouses in southcentral Pennsylvania.

Objectives 1 and 2: The location and amount of apple rot spores will be determined in apple fruits selected randomly and in different points during postharvest handling. First, fruits will be selected over time during postharvest: immediately after harvest, after fungicide treatment, mid storage, end of storage. The end of storage and mid storage time points will be defined according to commercial specifications for each variety. Fungi colonizing the fruit surface will be isolated by washing apples in sterilized water, performing serial dilutions, and a sample of the final dilution will be distributed on acidified potato dextrose agar (PDA). After three days at 20 °C, the total colony forming units (CFU) of fungi will be counted. Additionally, apples showing decay symptoms will be sampled to isolate and identify the causal agents. The second approach will be the isolation of fungi from picking bags, field bins, and the air of packinghouses and cold storages. Fungi colonizing picking bags and bins surfaces will be isolated using sterilized cotton Q-tips, washing in sterilized water and plating on acidified PDA, as performed by Hansen *et al*. (2010). Fungal spores in the air of packinghouses and cooling rooms will be collected on PDA media, using an air sampler to obtain the number of CFU m^{-3}, as performed by Amiri and Bompeix (2005). Fifteen replicates per source of inoculum will be used, and each replicate will be a Petri plate containing PDA or PDA/Richards medium amended with fungicide. The morphological characteristics of the three most frequent fungal colonies will be used to define their identity at the genus level, and the species will be confirmed using conventional molecular methods: genomic DNA extraction, PCR amplification, and sequencing the ITS region to determine species identity.

Objective 3: To investigate the fungicide resistance profiles, apple rot fungal isolates will be obtained from fruit, field equipment, packinghouse, and storage facilities, using the same procedures previously

outlined for objective 1. For this purpose, the culture media will be amended with fungicide according to the following specifications:

Culture medium	Fungicide active ingredient	Concentration (ppm)*
PDA	Thiabendazole	5
PDA	Fludioxonil	0.5
PDA	Difenoconazole	5
Richards defined medium	Pyrimethanil	1

*The concentration of active ingredients in postharvest fungicides and culture media to identify resistant isolates was defined according to literature (Yan *et al*. 2014 (I), Yan *et al*. 2014 (II), Amiri *et al.* 2017; Jurick II *et al*. 2018), using *P. expansum* as a model.

As indicated for objective 1, the identity of the most frequent fungicide resistant isolates will be confirmed using morphological and molecular methods, which include colony morphology, DNA extraction, PCR amplification, and sequencing ITS region to determine species identity.

Budget:

2019 Summer stipend for a graduate student (1/2 time, grade 12)	$6,730
Wages for part-time seasonal employee ($9.25/hr, 40 h/wk, 12 weeks):	$4,400
Wage fringe benefits (7.81%):	$868
Materials and Supplies	$3,252
Travel:	$1,250
Total requested:	**$16,500**

Budget Justification:

Salaries/Wages – $11,130

Funds are requested to provide the summer (PPEM) graduate student stipend and a summer seasonal assistant. Both will be working on this project to collect and process the samples.

Fringe Benefits - $868

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 - $3,252

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. Funds are requested to purchase prepaid plates

from Eurofins Scientific for sample cleaning and subsequent sequencing of DNA samples of collected fungal isolates to identify to species.

Travel - $1,250

Funds are requested to support personnel working on this project to attend meetings, including the annual Cumberland Shenandoah Fruit Workers meeting in Winchester, VA, November 29 – 30, 2019, and other regional meetings. They will be presenting a talk or poster about this postharvest fruit rot project supported by SHAP. Funds will cover travel, 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.

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