2021 RESEARCH GRANT PROPOSAL
STATE HORTICULTURAL ASSOCIATION OF PENNSYLVANIA

Title: **Identification, fungicide resistance profile, and management alternatives of pathogens causing postharvest rots on apples in Pennsylvania**

Personnel:
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Year 2 of 3: Funding period May 1, 2021 – April 30, 2022

Amount requested: $12,500

Justification:

Postharvest rots cause significant economic losses on apple production, especially because these losses include the accumulated costs of field production, transportation, and storage. Between 1% and 15% of apples in the United States are lost each year as a consequence of postharvest rots[14], which translates into approximately 52,000-780,000 tons and up to $2,500 million (estimation based on data from FAOSTAT 2019[8] and the USDA National Report, June 2019[22]). Even though, it is recognized that postharvest rots cause significant losses in the United States, there is a lack of information about the frequency of the most important pathogens causing postharvest rots on apples from Pennsylvania and their fungicide resistance profiles, the main sources of spores, and optimal management options. For 2021, we aim to complete the objectives of our 2020 funded SHAP grant titled "*Identification and fungicide resistance profile of pathogens causing postharvest rots on apples in Pennsylvania*", and include a new objective in order to explore alternatives to synthetic fungicides for management of the most frequent pathogens found causing postharvest rots in commercial packinghouses from Pennsylvania (PA) and Maryland (MD).

Published research indicates that *Penicillium* spp. (blue mold) are the most important postharvest pathogens of apples[7,10], contributing to almost half of postharvest rots[9]. The results of the samplings performed in 2019 and 2020 in multiple packinghouses from PA and MD (objectives 3 and 4 of the 2020 funded SHAP grant), indicate that the air of the packinghouses and cold rooms are significant reservoirs of *Penicillium* spp. spores. On the contrary, field sources, such as picking bags, bins, and freshly harvested fruit did not account for a significant source of spores. Preliminary results of the DNA sequencing of the *Penicillium* spp. isolated, indicate that multiple species (*P. expansum, P. solitum, P. echinulatum, P. glabrum, P. citrinum, P. cainii,* and *P. herquei*) are prevalent in the packinghouses sampled. With the exception of *P. herquei* and *P. cainii*, the identified fungi are reported causing blue mold on apples, with *P. expansum* as the most important species in multiple countries[7,18,19,23].

Botrytis cinerea (gray mold), *Colletotrichum* spp. (bitter rot), and quarantine pathogens, such as *Neofabrea* spp. are also important pathogens causing postharvest apple decay in different countries[5,11,13,20]. The results of our 2019 and 2020 samplings show no evidence of spores of these pathogens on the fruit surface or throughout the postharvest chain of freshly harvested apples. However, latent infections caused by *B. cinerea*, *Colletotrichum* spp., and *Neofabrea* spp. can be present at harvest without evident symptoms and appear after storage[4,5]. During the 2020-2021 season, we expect to corroborate how frequent is blue mold rot in PA packinghouses and if any other latent pathogens are frequently found causing postharvest rots (objectives 1 and 2).

The first objectives of this proposal are intended to provide the foundation for finding alternatives to synthetic fungicides for postharvest apple rot management. Although multiple treatments have been evaluated to control postharvest apple rot, synthetic fungicides applied in pre and postharvest are the main strategy used by PA and MD growers. The main reasons for this are the high efficacy and relatively low

economic cost of this practice. In some of the packinghouses, especially in those with controlled atmosphere (CA) long-term storage, fludioxonil and pyrimethanil are the postharvest fungicides used for apple rot management. Both chemicals are single-site mode of action, and due to this specificity, single-site mutations in the pathogen's DNA can confer resistance[3], as reported in PA for *P. expansum* and *B. cinerea*[24,25]. Therefore, relying on fungicides for apple rot management is not sustainable long-term, and the substitution of fungicides is considered a research priority for apple decay management worldwide[12].

Some alternatives, such as thyme oil, chitosan, and natamycin have shown promising results for postharvest disease management under experimental conditions[12,16,21]. For instance, on inoculated apples, chitosan and thyme oil reduced apple rot up to 87%, and natamycin inhibited spore germination of *P. expansum* and *B. cinerea* in vitro[12]. We propose to evaluate these three alternatives to manage postharvest apple decay. These alternative fungicides are considered GRAS (Generally recognized as safe) fungicides and will be evaluated as single treatments or as mixtures with synthetic fungicides. Considering that fungicide resistance is already present in PA apple packinghouses[24,25], mixing synthetic and GRAS fungicides may also help to eradicate fungicide resistant spores in packinghouses where synthetic fungicides are considered essential for long-term storage. On the contrary, for packinghouses with short-term storage (less than six months), applying GRAS fungicides in pre or post-harvest can be an option to avoid using synthetic chemicals to control postharvest rots. Using mixtures can also help to obtain additional benefits, such as the induction of fruit defense, already reported for chitosan and thyme oil[1,15]. This is important for latent pathogens, such as *B. cinerea*, which are not easily controlled by synthetic fungicides sprayed on the fruit surface during postharvest. Chitosan and essential oils have also the advantage that can be applied either in pre or postharvest[2,17], which opens more possibilities for growers without equipment and technology for postharvest application of fungicides.

Objectives:

The objectives of this research proposal address the **2021 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:

<u>2020 Funded Grant (continued during 2021)</u>

1. Identify the most frequent fungi causing postharvest rots on apples in Pennsylvania packinghouses located in different apple growing regions.
2. Study the fungicide resistance profile of the most frequent fungi causing postharvest rots on apples in Pennsylvania packinghouses in different apple growing regions.
3. Determine the location of apple rot spores with respect to field equipment, picking bins, packing facility and storage environments.
4. Investigate the fungicide resistance profiles of apple rot isolates obtained from fruit, field equipment, packinghouse facilities, and storage environments.

<u>2021 additional objective</u>

5. Evaluate the efficacy of thyme oil, chitosan, and natamycin to control postharvest apple rot when used as stand-alone treatments or in mixtures with synthetic fungicides.

Procedures:

The research will be performed at the Penn State Fruit Research and Extension Center (FREC), located in Biglerville, PA and in eight commercial packinghouses from southeastern and southwestern PA, and three packinghouses in MD during the 2020-2021 and 2021-2022 seasons.

Objectives 1 through 4

These objectives will be completed during the 2020-2021 season. So far, 11 packinghouses from PA and MD have been sampled and the most frequent fungi colonizing non-symptomatic apples, bins, picking bags, dump tank water, and the air of the packinghouse facilities and cold rooms have been isolated. The next steps include the identification at species level of fungicide resistant and non-resistant isolates using morphological characteristics and DNA sequencing (objectives 3 and 4). Our preliminary results indicate that there is a higher diversity of blue mold species in postharvest environments than we originally expected; therefore, the number of isolates to be sequenced will increase in order to separate the species associated with blue mold from those that are not pathogenic on apples. Each packinghouse will be sampled again in 2021 to isolate the fungi causing postharvest rots during storage (up to 11 months) and the species identity and their fungicide resistance profile (isolates growing on thiabendazole, fludioxonil, and pyrimethanil) will be determined (objectives 1 and 2).

Objective 5

In this objective, we aim to evaluate the efficacy of GRAS fungicides (thyme oil, chitosan, and natamycin) to control postharvest rots on artificially inoculated apples and on non-inoculated apples kept under simulated commercial storage. The apples for the experiments will be harvested from 'Golden Delicious', 'Aztec Fuji', and Honeycrisp experimental blocks at FREC, Penn State University.

5.1 Fungicides: Aqueous solutions of GRAS and synthetic fungicides will be used in all the experiments and the active ingredient concentrations in the solution are specified in Table 5.1.

Table 5.1. Description of the fungicides and active ingredients concentration to be applied on apples.

Commercial product	Origin	Active ingredient concentration
Scholar SC	Synthetic	255 mg L^{-1}
Penbotec 400 SC	Synthetic	1000 mg L^{-1}
Thyme Guard	Essential oil from *Thymus vulgaris*	1%
Chitosan Acetate 1%	Crustacean shells	1%
BioSpectra 100 SC	Actinomycete (*Streptomyces natalensis*)	470 mg L^{-1}

*Active ingredient concentration selected according to the commercial product label (highest commercial dose of fludioxonil and pyrimethanil) and literature (thyme oil, chitosan, natamycin)[1,16].

5.2 **<u>Wounded inoculated apples</u>**: a sterile nail will be used to cause 3 mm deep wounds in two opposite sides of the fruit equator. Afterwards, the apples will be immersed for 2 min in fungicide solutions at the concentrations specified in Table 5.1 and left to air dry. After one hour, each wound will be inoculated with 5 µl of a spore suspension of *P. expansum* or *B. cinerea* (10^5 spores ml^{-1}) isolated from rotten apples during the 2021 samplings. These are the most frequent postharvest apple pathogens reported in the literature; however, new species can be used in the experiments according to our findings during the 2020-2021 season. After inoculation, the apples will be stored in plastic boxes for six days at 23 °C. At the end of storage, the lesion diameter on both sides of the apple equator will be measured using a caliper, as shown in Figure 1.

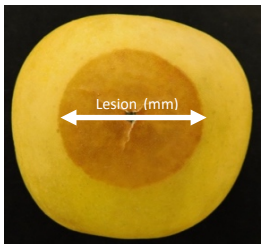

Figure 1. Lesion diameter on apple inoculated with *Penicillium* sp.

The synthetic fungicides Scholar (a.i fludioxonil) and Penbotec (a.i pyrimethanil) will be used for comparison, and sterilized water will be used as control. A total of 20 apples per treatment will be used.

<u>5.3 Non-wounded apples</u>:

The efficacy of thyme oil, chitosan, and natamycin to control postharvest rots will be evaluated on apples stored under simulated commercial storage, at 1 °C and 95% relative humidity, and 5 or 11-months storage. The fungicide solutions will be prepared to obtain the same active ingredient concentration specified in section 5.1. The apples will be immersed for 2 min in the respective fungicide solutions and tap water will be used as control.

Two groups of 40 apples per treatment will be used. One group will be stored for five or eleven months at 1 °C and 95% relative humidity, and the other will be stored under the same temperature and relative humidity in controlled atmosphere (CA) rooms (1% O_2 + 2% CO_2), at Rice Fruit Company. One of the limitations reported in the literature for GRAS fungicides is the low residual activity, which is the time in storage the fungicide is able to control postharvest diseases. For this reason, the apples will be evaluated at five and eleven months to determine possible differences in residual activity. At the end of storage, the number of rotten apples will be counted to determine the disease incidence (# of rotten apples/40 *100). Observations about toxicity or unusual flavor or unwanted smells of the apples at the end of storage, especially for thyme oil will be also made.

5.4 Preharvest applications

The efficacy of a preharvest application of chitosan or thyme oil on postharvest apple rot control will be evaluated. Natamycin is considered a food preservative and commercial formulations are intended only for postharvest use, especially because the active ingredient is sensitive to degradation by UV-light[6].

A total of five apple trees will be randomly selected from 'Golden Delicious', 'Aztec Fuji', and 'Honeycrisp' orchard blocks at FREC and sprayed 7 days before harvest with aqueous solutions of chitosan or thyme oil, both at 1% active ingredient. Five trees will be sprayed with tap water as the control. A total of 80 apples per tree will be harvested and stored for six months at 1 °C and 95% relative humidity. At the end of storage, the number of rotten apples will be counted to determine the disease incidence (# of rotten apples/40 *100). A representative sample of at least five rotten apples per treatment will be used to isolate and identify the fungal species causing rots. This will allow to identify pathogens that are not efficiently controlled by the GRAS fungicides tested.

Budget
Total requested: $12,500

Salaries/Wages – $6,930
Funds are requested ($6,930) to provide the summer (PPEM) graduate student stipend. The summer graduate support is considered Category III Salaries and Wages.

Fringe Benefits - $549
Fringe benefits are computed using the fixed rates of 34.88% applicable to Category I Salaries, 12.35% applicable to Category II Graduate Assistants, 7.94% applicable to Category III Salaries and Wages, 0.31% applicable to Category IV Student Wages, and 23.88% for Category V, Postdoctoral Scholars and Fellows, for fiscal year 2020 (July 1, 2020, through June 30, 2021). 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, 2021, 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 - $5,021
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.

Sponsor does not allow for indirect costs.

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