2022 RESEARCH GRANT PROPOSAL
STATE HORTICULTURAL ASSOCIATION OF PENNSYLVANIA

Title: **Problem postharvest rots lurking: Investigating Bull's eye rot and Alternaria rot in PA and MD packinghouses**

Personnel:
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Funding period: May 1, 2022 – April 30, 2023

Amount requested: $14,000

Justification:

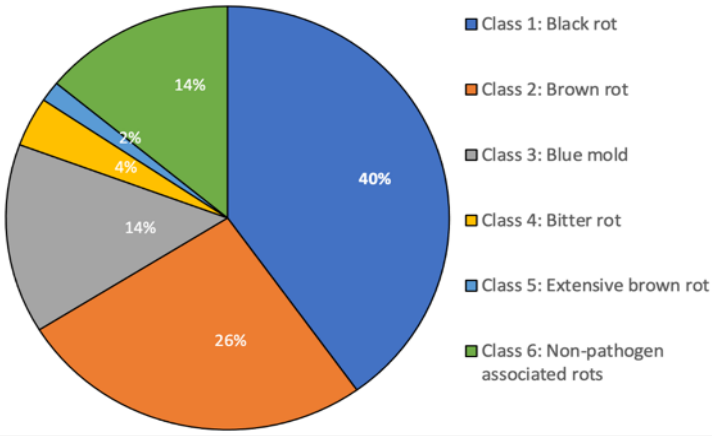

Figure 1. Percentage of rotten apples classified by symptom class. Samplings performed in PA and MD packinghouses (2020-2021 season). N = 450 apples

The proper identification of the causal agents of postharvest apple rots is critical to devise management strategies adapted to the grower's needs. Since 2019, our lab has been studying potential inoculum sources and identifying the most frequent fungi causing postharvest apple rots in Pennsylvania (PA) and Maryland (MD). Results obtained during the 2020-2021 season indicate that the major sources of *Penicillium* spp. (blue mold) are predominantly the packinghouse facilities, cold rooms, and dump tank water. The frequency of blue mold in a random sample of 450 rotten apples from 9 packinghouses in PA and MD was 14% (Figure 1). This contrasts with the importance of this fungus as a postharvest apple pathogen in other regions, such as Washington State, where blue mold accounts for approximately 50% of postharvest rots[5]. The other symptoms observed were classified into additional classes, based on the visual appearance of the lesion, with classes 1 and 2 accounting for 66% of postharvest rots in PA and MD (Figure 1). The fungi causing those symptoms were isolated in the laboratory, and preliminary data based on morphological examination and DNA sequencing indicate that *Alternaria* spp. and *Neofabraea* spp. are the fungi comprising classes 1 and 2, respectively.

Both, *Alternaria* and *Neofabraea* are frequent postharvest pathogens in many apple growing regions worldwide[6,8,10]. *Alternaria* has been associated with core rot and Alternaria rot[16], as well as the risk of mycotoxin contamination of processed apple products[10,11]. *Neofabraea* species cause the disease known as bull's eye rot. The fungus infects apples in the field, any time from

bloom to harvest, and remains latent causing rots after 4-6 months in cold storage. These rots are characterized by circular brown lesions with a lighter brown or tan center, giving the appearance of a "bull's eye"[1,15]. So far, bull's eye rot has not been officially reported in PA and MD. This is a particularly significant disease for growers exporting to Asia and the Middle East because *Neofabraea* spp. are considered quarantine pathogens[1]. Exportation of Washington State apples to China was suspended in 2012 due to the repeated interception of bull's eye rot, speck rot and Sphaeropsis rot, caused by *Neofabraea* spp., *Phacidiopycnis washingtonensis*, and *Sphaeropsis pyriputrescens*, respectively[7,17].

Since our data collected in 2021 suggest that *Alternaria* rot and bull's eye rot are prevalent and highly frequent in orchards and packinghouses in PA and MD, it is necessary to study both diseases in more detail to corroborate symptomatology and identify the associated species. A second season of samplings and DNA sequencing of representative isolates will help to clarify not only the presence, but also the importance of these postharvest diseases in PA and MD. Additional tests, including the inoculation of apples, are also necessary to corroborate symptomatology. This technique is known as Koch's postulates[13] and includes the inoculation of healthy apples with fungi isolated from *Alternaria* or *Neofabraea* infected apples. The original symptoms must be replicated, and the inoculated fungus isolated again from those symptoms. The description of symptoms and the identification of the species associated with Alternaria rot and bull's eye rot is necessary to help growers identify and assess the importance of these diseases in their orchards and packinghouses. Management strategies can also be devised and adopted accordingly.

Objectives:

The objectives of this research proposal address the **2022 SHAP priority under the Post-Harvest Physiology section: Control of post-harvest pathogens.**

Specific research objectives:

1. Identify the *Alternaria* and *Neofabraea* species causing postharvest apple rots in PA and MD.

2. Investigate the symptomatology of *Alternaria* spp. and *Neofabraea* spp. as postharvest pathogens of apples in PA and MD.

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 two packinghouses in MD during the 2022-2023 season.

Objective 1

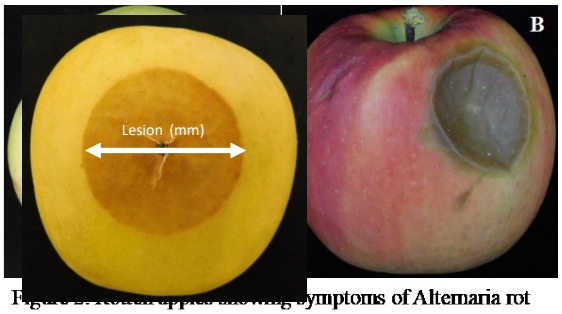

Figure 3. Lesion diameter on rotten apple

In this objective, we aim to study representative isolates of *Alternaria* and *Neofabraea* fungi obtained from rotten apples in PA and MD packinghouses during the 2021-2022 and 2022-2023 seasons. For the 2022-2023 season, samplings will be performed between February-March 2022 to collect apples showing suspected symptoms of Alternaria rot and bull's eye rot (Figure 2). A representative number of isolates (20 for each fungus) will be selected randomly and cultured on Potato dextrose agar (PDA) to describe their characteristics, which include color and texture of the colonies, and any other microscopic features that may help to determine their identity. Since the identification at species level for *Alternaria* and *Neofabraea* frequently requires sequencing DNA from multiple genes[4,9,19], a subsample of 10 isolates per genus will be sequenced including at least three gene regions (GAPDH, β-tubulin, and TEF-1 α), following the protocols recommended in the literature[4,9,19]. Phylogenetic trees, which are graphical representations commonly used to summarize the species and represent the evolutionary relationships between fungal isolates, will be constructed. Pictures of the fungal isolates and the respective symptoms caused on apples will be presented as well, as detailed in Objective 2.

Objective 2

This objective aims to study the symptoms caused by *Alternaria* and *Neofabraea* on apples. This is necessary to help growers and extension personnel identify both diseases, which is a crucial step to make informed management decisions. Surface sterilized 'Golden Delicious' apples harvested from experimental blocks at the Fruit Research and Extension Center, Penn State University will be used. A sterile nail or a cork borer will be used to create two wounds on opposite sides of the fruit equator. A spore suspension or a small plug of the *Alternaria* isolates will be used to inoculate both wounds. After inoculation, the apples will be stored in plastic bins for 7 and 14 days at 23 °C. Preliminary data obtained in 2021 indicate that *Alternaria* requires at least two weeks to develop a lesion of approximately 20-25 mm in diameter; however, evaluation at 7 days is also necessary to compare differences in virulence between isolates. At the end of storage, the lesion diameter will be measured using a digital micrometer, as shown in Figure 3.

The protocols for inoculation and replication of bull's eye rot symptoms differ according to the *Neofabraea* species used [1,3,18]. Therefore, based on the literature and results obtained in objective 1, different methods and culture media will be preliminarily tested to determine the best way to grow the fungus, inoculate apples, and replicate the symptoms observed on stored apples. For instance, spore suspensions obtained from pure cultures grown on specialized media, such as tomato agar, will be used to inoculate wounded apples. For some species, like *Neofabraea vagabunda*, inoculating wounded apples with 20 µl of a suspension containing 10^4 conidia/ml is recommended to replicate bull's eye rot symptoms[2]. For this methodology, fruits will be stored in darkness at 1 °C and incubated for 60, 120, and 180 days, and periodical evaluations will be made to determine the appearance of symptoms[2]. As an alternative methodology, 6-7 mm wounds will be created on opposite sides of the fruit equator and

inoculated with a 6 mm mycelial plug of the fungus growing on PDA. Apples will be stored for 20 days at 20-25 °C and the incidence and severity (lesion diameter) will be determined[12,14].

A total of 10 *Alternaria* and *Neofabraea* isolates, representing the most frequent species identified in Objective 1, will be used for the experiments proposed in objective 2. Results will be presented as a summary of pictures showing the symptomatology on apples, lesion diameter, and morphology on culture media of the fungal species causing Alternaria rot and bull's eye rot in PA and MD.

Once corroborated their presence and identified the *Alternaria* and *Neofabraea* species causing apple rots in PA and MD orchards and packinghouses, management alternatives will be proposed based on information already available for other apple producing regions. Additional research adapted for PA and MD growers will be proposed if necessary.

Budget
Total requested: $14,000

Salaries/Wages – $7,680
Funds are requested to support a seasonal research technician to work in the tree fruit pathology program to assist with this project. The seasonal technician will be paid $12/hr for 40 hr/wk for 16 weeks The seasonal technician is considered Category III Salaries and Wages.

Fringe Benefits - $613
Fringe benefits are computed using the fixed rates of 35.31% applicable to Category I Salaries, 11.26% applicable to Category II Graduate Assistants, 7.98% applicable to Category III Salaries and Wages, 0.35% applicable to Category IV Student Wages, and 24.78% for Category V, Postdoctoral Scholars and Fellows, for fiscal year 2021 (July 1, 2021, through June 30, 2022). 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, 2022, 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 - $5,707
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, of fungal isolates collected. Expenses for materials will be determined from catalog prices using Penn State's Lion Marketplace. 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.

References

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