Research Grant Proposal for 2018 - State Horticultural Association of Pennsylvania

Title: Evaluating the efficacy of a new postharvest fungicide and developing tools to monitor fungicide resistance in blue mold populations.

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Duration of Project: One year (2018) - new

Justification: Apples are a multibillion-dollar industry and consumers expect a fresh supply of high quality fruit year around. Pennsylvania is the fourth largest apple producer in the United States (U.S. Apple Association). Adams county Pennsylvania grows nearly 75% of the apple fruit for the state as 65% is consumed fresh, and the remaining 35% is used for processing. Postharvest research is vital to the pome fruit industry (growers, packers, and processors) as fungal pathogens not only cause losses during storage which reduces revenue, but also negatively impacts fruit quality, produce mycotoxins (i.e. patulin) and significantly contributes to food waste. Everyone, (i.e. the grower, packer, processor and consumer), succumbs to the negative impacts of postharvest decay.

Blue mold caused by *Penicillium* spp. is one of the most economically important postharvest fruit rot diseases in the US and worldwide (Xiao and Boal, 2009, Rosenberger 1990). There are 4 fungicides (Academy, Mertect, Penbotec, Scholar) labeled for the control of postharvest blue mold decay on pome fruits. However, practical resistance has been detected for Mertect and Penbotec and reduced sensitive isolates for Scholar (Gaskins et al., 2015; Jurick Unpublished results; Yan et al., 2014). In 2016, Academy (containing two active ingredients fludioxonil and difenoconazole) was released by Syngenta to control blue mold on apple fruit. Dr. Xiao in Washington State elucidated baseline sensitivity and determined that a discriminatory dose of 0.5ppm fludioxonil was effective for detecting Scholar resistant *Penicillium* spp. isolates (Li and Xiao, 2008). However, it is **NOT** known: A) what the sensitivity range is for *Penicillium spp.* populations from Pennsylvania to difenoconazole B) what dose(s) of

difenoconazole can be used to detect early shifts in sensitivity to difenoconazole in the fungus, and C) the efficacy of Academy on Pennsylvania populations of blue mold fungi as a curative or preventative postharvest treatment. This work will utilize our collection of previously characterized *Penicillium* spp. from MD and PA storage, obtained from packing and store front facilities, previously funded by SHAP.

Potential Benefits and Products of the study: The results of this project address several research priorities under three different sections which are: Horticulture: Stone and other Pome fruit research, maintaining fruit quality; Plant Pathology: Disease Resistant Management for Key Diseases; Post-Harvest Physiology: control of post-harvest pathogens. The information gained from this study will be of direct benefit to the fruit growers, packers, processors, and the agricultural research community. Data from this study will solve multiple problems including: determining the levels of sensitivity to difenoconazole, one of the active ingredients in the new postharvest fungicide in Academy. From this, a discriminatory dose will be developed for rapid, simple yes/no screening for resistance management. Lastly, data from this study will help indicate the curative and preventative activity of Academy using isolates from Pennsylvania to control blue mold on apple fruit.

Objectives:

1) Determine baseline sensitivity to difenoconazole for *Penicillium* spp.

2) Develop a discriminatory dose for monitoring difenoconazole resistance.

3) Evaluate the efficacy of Academy to control blue mold on apple fruit

Procedures: Determining baseline sensitivity for difenoconazole: The range of sensitivity to difenoconazole in a baseline population of *Penicillium* spp. isolates, will be determined via estimated concentration to inhibit 50% fungal growth (EC_{50}). Technical grade difenoconazole will be dissolved in acetone, adjusted to a concentration of 10mg/ml stock, and added to PDA in in 4.5 cm Petri plates containing concentrations of 0, 0.0001, 0.001, 0.01, 0.1, 1.0, 10, and 100µg/ml difenoconazole. The concentration of acetone in the medium, including the medium without fungicide will not exceed 0.1 ml/L. Each concentration will be represented in triplicate and conidial suspensions of each *Penicillium* spp. isolate will be added to each plate. Conidia will be harvested from 7-day old PDA plates using 2 ml of 0.05% Tween 20-treated water and adjusted to 1×10^5 conidia per ml with the aid of a hemacytometer and a compound microscope. One hundred microliters of conidial suspensions of each isolate will be added to each plate and placed in a temperature controlled incubator at 20°C, for 72 hours. Colony diameters will be measured with a digital micrometer and recorded. EC_{50} values for each isolate will be determined using best-fit regression of the percent inhibition of

growth relative to the unamended control versus the logarithmic transformation for concentrations of difenoconazole using SAS (PROC LOGISTIC). The EC_{50} value for each isolate will be calculated based on the model and frequencies of EC_{50} will be determined. The experiment will be repeated and the average EC_{50} values from several independent experiments will be pooled for data analysis pending post hoc testing showing that the individual experiments were not statistically different ($P>0.05$).

Establishing a discriminatory dose for difenoconazole: To identify a discriminatory dose for difenoconazole-resistant *Penicillium* spp. isolates, Minimum Inhibitory Concentration (MIC) phenotyping will be utilized. A discriminatory dose of difenoconazole will be selected based on empirical data obtained from the EC_{50} assays and will be added to 4.5cm PDA plates as above and examined for growth and colony diameters will be measured. Isolates capable of growing on the discriminatory dose of fungicide will be categorized as resistant, those that cannot will be labeled sensitive (Li and Xiao, 2008; Li et al., 2014)

Testing blue mold control on apple fruit: To assess the practical impact of difenoconazole resistance in *Penicillium* spp. in decay failures on the fungicide-treated fruit after harvest, “sensitive” and “resistant” *P. expansum* isolates will be analyzed *in vivo* for their ability to cause decay on Academy® (difenoconazole + fludioxonil) treated apple fruit. Organic ‘Goldrush’ or ‘Crimson Crisp’ apples will be obtained from our collaborator Dr. Kari Peter and will be stored at 1°C until the experiment is conducted. Apples will be surface disinfested for 5 min in 0.6% sodium hypochlorite solution, rinsed three times with sterile water, and air-dried. Each fruit will be wounded with the point of a 3-mm-diameter finishing nail to a 3mm depth. Conidial suspensions (1×10^5 conidia/ml) will be obtained from 7-day-old PDA cultures grown at 20°C and used for inoculations. Curative treatments will be conducted by inoculating apples with 50 µl of the conidial suspension with a micropipette into each wound. Approximately 1 hour after inoculation, fruit will be dipped for 1 min in either sterile water as a positive control or in an Academy fungicide solution per the labeled rate indicated by the manufacturer. Preventative treatments will be conducted in the same manner, except that the fungicide will be applied first, then the fruit will be wounded, inoculated with the pathogen and stored. Fruit will be air-dried for 15 min after dip treatment and then placed on fruit trays and stored in cardboard boxes at 1°C to mimic commercial storage conditions. Fruit will be assessed monthly for decay, and each treatment will have three replicate trays each containing 20 fruit. Lesion diameters will be measured with a digital micrometer (% severity) and fruit with decay (% incidence) will be calculated and recorded (Jurick et al., 2011). Fungicide dip tests will be conducted by Dr. Wayne Jurick II and student hire in Dr. Kari A. Peter’s lab at the Penn State Fruit Research and Education Center in Biglerville, PA for access to cold storage and fungicide materials.

Data Analysis: All experiments will be conducted three times each having three replications. Data from multiple experiments will be included in one analysis for differences between treatments by generalized linear analysis of variance in SAS (GLM ANOVA) and means will be compared by the Fisher's protected LSD at $P \leq 0.05$.

Budget: Dr. Jurick II is requesting a total of $9,643.20 to conduct the research outlined in this proposal and the USDA-ARS will receive all funds for this study.

Supplies: $2,000 – will cover costs for lab supplies including but not limited to: Petri plates, media, technical grade fungicides, pipettes, apple boxes, trays, and other materials as needed during the study.

Student salary: $6,643.20 (GS3 step 1 salary ($13.84/hour – part time summer salary 40hrs/week for 12 weeks). The temporary hire will work in Dr. Jurick's lab in Beltsville, Maryland and will be a USDA-ARS employee that will also collaborate and work with Dr. Peter in her lab at Penn State, FREC as needed to conduct the research project.

Travel: $1,000 – to support the scientists on the project to present data from this SHAP-funded research at scientific and professional stakeholder-based meetings (i.e. American Phytopathological Society & Mid-Atlantic Fruit and Vegetable Conference in Hershey, Pennsylvania)

Other Support: Aside from base funds that support the laboratory in general from ARS, there are no other sources of funding for this project that are currently available or pending.

1. Gaskins, V., Vico, I., Yu, J., and W.M. Jurick II. 2015. First Report of *Penicillium expansum* isolates with Reduced Sensitivity to Fludioxonil from a Commercial Packinghouse in Pennsylvania. Plant Disease. DOI:10.1094/PDIS-11-14-1161-PDN
2. Jurick II, W.M., Janisiewicz, W.J., Saftner, R.A., Vico, I., Gaskins, V.L., Park, E., Forsline, P.L., Fazio, G., Conway, W.S. 2011. Identification of wild apple germplasm (*Malus* spp) accessions with resistance to the postharvest decay pathogens *Penicillium expansum* and *Colletotrichum acutatum.* Plant Breeding. DOI: 10.1111/j.1439-0523.2011.01849.x
3. Li, H.X., and Xiao, C.L. 2008. Characterization of fludioxonil-resistant and pyrimethanil-resistant phenotypes of *Penicillium expansum* from apple. Phytopathology. 98: 427-435.
4. Li, H.X., Ortuno, D., Grabke, A., and Schnabel, G. 2014. Resistance to fludioxonil in *Botrytis cinerea* isolates from blackberry and strawberry. Phytopathology. 104:724-732.
5. Rosenberger, D.A. 1990. Blue mold. p. 54-55. In: Compendium of Apple and Pear Diseases. A.L. Jones, and H.S. Aldwinkle (eds.), APS Press.

6. Xiao, C. L., and Boal, R. J. 2009. Residual activity of fludioxonil and pyrimethanil against *Penicillium expansum* on apple fruit. Plant Disease. 93:1003-1008.

7. Yan,H., Gaskins, V.L., Vico, I., Luo, Y., and W.M. Jurick II. 2014. First Report of *Penicillium expansum* isolates resistant to pyrimethanil from stored apple fruit in Pennsylvania. Plant Disease. 98:7.