Progress Report and Proposal - Pear Research Review, 2000

TITLE Blue mold epidemiology

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JUSTIFICATION

Blue mold is one of the major causes of loss to decay in the Washington apple industry. *Penicillium expansum* Link and *P. solitum* Westling are the major causal agents for blue mold on pome fruit in the Northwest (Sanderson & Spotts, 1995). Currently, only thiabendazole (TBZ) and captan are registered for postharvest use against this disease. Many fruit packers are hesitant to use captan, because it is not accepted in some important overseas markets. Recently we found that 30% of isolates of *P. expansum* recovered from DPA drenches were insensitive to TBZ. In the Mid-Columbia area, up to 50% of isolates of *P. expansum* recovered from fruit and dump tanks in the mid 1970's were benomyl-tolerant (Bertrand & Saulie-Carter, 1978). Although some biological fungicides are being developed for postharvest use, those currently registered are not very effective. With the new Food Quality Protection Act, new chemical fungicides are unlikely to be registered for postharvest use.

Dose/response relationships have been determined for conidia of *P. expansum* on McIntosh apple (Blanpied & Purnasiri, 1968) and *P. expansum* on Anjou fruit (Spotts, 1986). These relationships were developed using inoculum of each pathogen alone suspended in fresh water. In addition, we are developing curves for naturally occurring pathogens in DPA drenches. The fact that dose/response curves can be determined suggests that if the amount of inoculum on fruit or in postharvest water systems was reduced, the probability of decay developing in fruit would be smaller.

Spores of *Penicillium* spp., especially *P. expansum* are recovered from the air in packing houses. In a study of apple and pear sheds in the Mid-Columbia area, spores of *P. expansum* were recovered from the air in increasing numbers as the packing season progressed (Spotts & Cervantes, 1986). In a study we conducted (unpublished data) in Wenatchee pear sheds, high numbers of spores of *P. expansum* were detected in the air early in the season before sporulating blue mold lesions were observed on fruit. It is not clear where these spores originate.

The purpose of the proposed research is to determine when and where inoculum is produced in the field and how fruit become inoculated. If we can then manipulate the system through cultural practices to reduce the populations of *P. expansum* and other postharvest decay causing organisms we may be able to reduce our risk of loss. Likewise, populations of *Penicillium* spp. are present in the packing house. If we can identify their source and reduce their numbers, the risk of decay will be lessened.

OBJECTIVES

1. Determine environmental, temporal, spacial, and biological parameters that favor inoculum production of blue mold causal agents (especially *P. expansum* and *P. solitum*) in the field, fruit storages and packing houses.

2. Determine environmental, temporal, spacial, and biological parameters that favor dispersal of inoculum of *Penicillium* spp. in the field, fruit storages and packing houses.

PROGRESS

It is empirically evident that inoculum of *Penicillium* spp. is brought into packing and storage facilities with fruit at harvest. Surfaces of bins and fruit may be contaminated in the field and serve to transport inoculum into storage and packing facilities. Surfaces of field bins stacked in bin storage yards were assayed for the presence of decay causing fungi in the Mid-Columbia area several weeks before the start of Bartlett harvest (Sanderson & Spotts, 1995). Although some isolates of *Penicillium* spp. were recovered, especially under the remains of decayed fruit that had been left in the bins from the previous season, they were not common. However, field bins are commonly placed in the orchard two or more weeks before harvest. In the field, spores of these fungi could be dispersed onto bins and subsequently carried into storage and packing facilities. It may also be possible that some of these fungi could begin to grow on the bin surfaces thereby increasing te potential inoculum load on the fruit bins.

Similarly, fruit surfaces may be vehicles for transport of these fungi into storage and packing facilities. This study was done to assess population densities of *P. expansum* and *P. solitum* on bins and on fruit surfaces at harvest.

Procedures

Field bins. Population densities of *Penicillium* spp. on field bins were assessed at harvest in eight orchards. A sampling scheme was developed in which each of two outer sides, one inner side, the inside bottom, outside bottom and one skid were assayed from each of 6 bins in each orchard. Samples were taken from each of eight randomly selected sections (1-cm^2) on each surface. Sections were wetted with sterile water with a cotton swab and scraped with a sterile scalpel. Scrapings from each surface were collected into a tube containing 4 ml sterile water and brought back to the lab to be cultured.

Samples were diluted and spread onto the surface of CYA+ agar (Czapek's Yeast Agar amended with 200 ppm chloramphenicol and 30 ppm gentamycin) in petri dishes. Colonies of *Penicillium* spp. were enumerated and typed by colony morphology about 5 days later. Representative isolates of each type were transferred to fresh CYA plates for identification of types.

To determine if colonies were developing from mycelial fragments or conidia, samples were spread on ½ strength PDA in petri dishes and incubated for 24 hr at room temperature. Developing colonies were stained with lactophenol acid fuchsin and observed under a light microscope. Ontogeny of at least 30 colonies on each of three plates was assessed.

Fruit. To assess spore loads on fruit surfaces, ten fruit were collected from each of the orchards in which bins were sampled and from trucks at receiving at storage and packing facilities. Fruit were put into new plastic fruit bags, placed in cardboard cartons, and brought back to the lab where they were stored in a walk-in cooler at 33 F until they were processed.

Fruit were individually washed in beakers containing 400 ml sterile distilled water with a drop of Tween 80 added. Fruit were sonicated for 10 min and placed on a rotary shaker for and additional 10 min. Wash water (0.2 ml) was spread onto CYA+ agar in petri dishes (3 dishes/fruit). Population densities of *Penicillium* spp. were assessed as described above.

Results and Discussion

Field bins. The length of time that bins had been in the orchard varied from 1 day to several weeks. Most of the bins sampled had been in the orchard for 1 or 2 weeks before harvest. In general, orchard managers had been careful to place bins on their skids. Most of the bins were several years old and the history of the bins was not known except for those from orchard 4. In that orchard, samples were taken from plastic bins that were new that season and had been used earlier for Bartletts and had not been cleaned after being emptied. These bins had been delivered to the orchard two days before and were stacked at the edge of the orchard. They were the only plastic bins from which samples were taken so any conclusions about the relative level of contamination of plastic bins vs wooden bins would be premature.

Isolates of species of *Penicillium* were recovered from 90% of the wooden bins sampled and half of the plastic bins. A large proportion of isolates of *Penicillium* other than *P. expansum* were *P. solitum*. *Penicillium expansum* was recovered in lower frequencies (42.5% of wooden bins sampled and 20% of plastic bins) and in much lower numbers than were other species of *Penicillium* (Fig. 1 and 2). *Penicillium solitum* was recovered from bins in all orchards from which samples were taken, whereas *P. expansum* was not recovered from one of the orchards.

The highest numbers of isolates of *Penicillium* species other than *P. expansum* were recovered from outer bin surfaces (Fig 2). Considering the relatively small surface area of the skids were heavily contaminated with spores of *Penicillium* spp. (about 25% of the bottom surface area and 40% of the area of the side panels).

Fruit. Fruit are still being processed and data has not yet been compiled. However, spores of several species of *Penicillium* including *P. expansum* are being recovered from fruit surfaces and often in high numbers. Distribution of *Penicillium* spp. on fruit surfaces is highly aggregated, i.e., we are recovering many isolates from some fruit and few from others.

Literature cited

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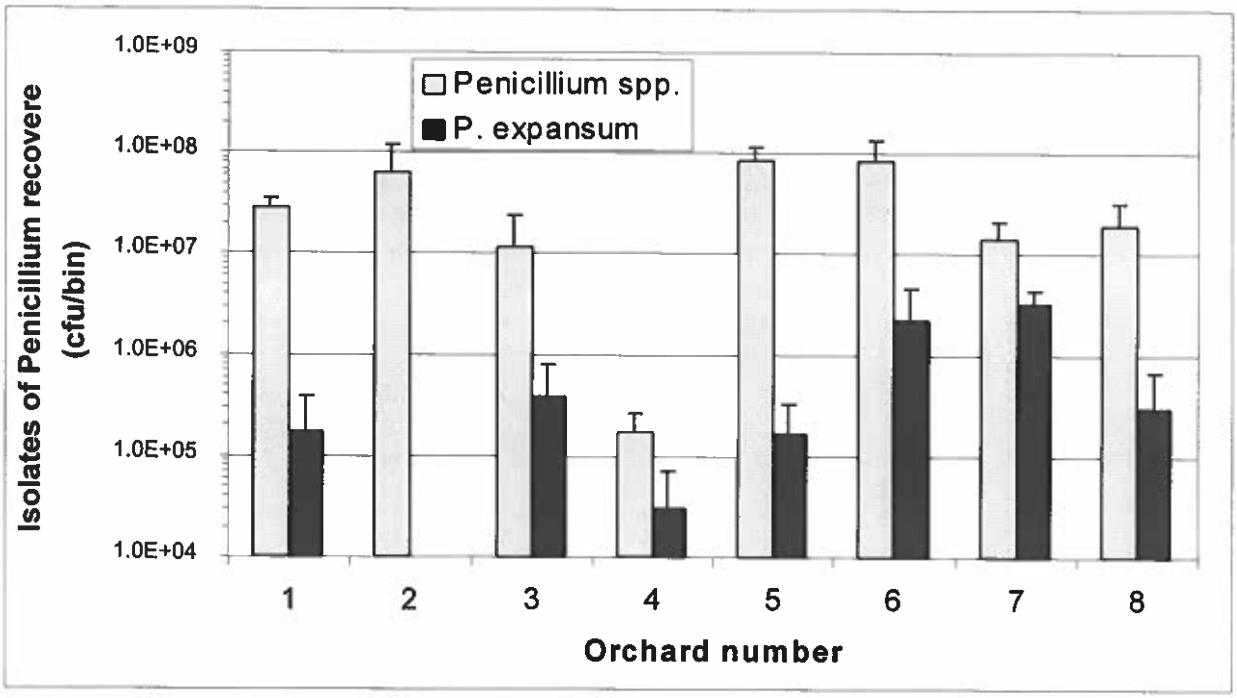

Figure 1. Total population density of Penicillium species recovered from field bins at harvest. Error bars indicate ½ of the standard deviation of the mean.

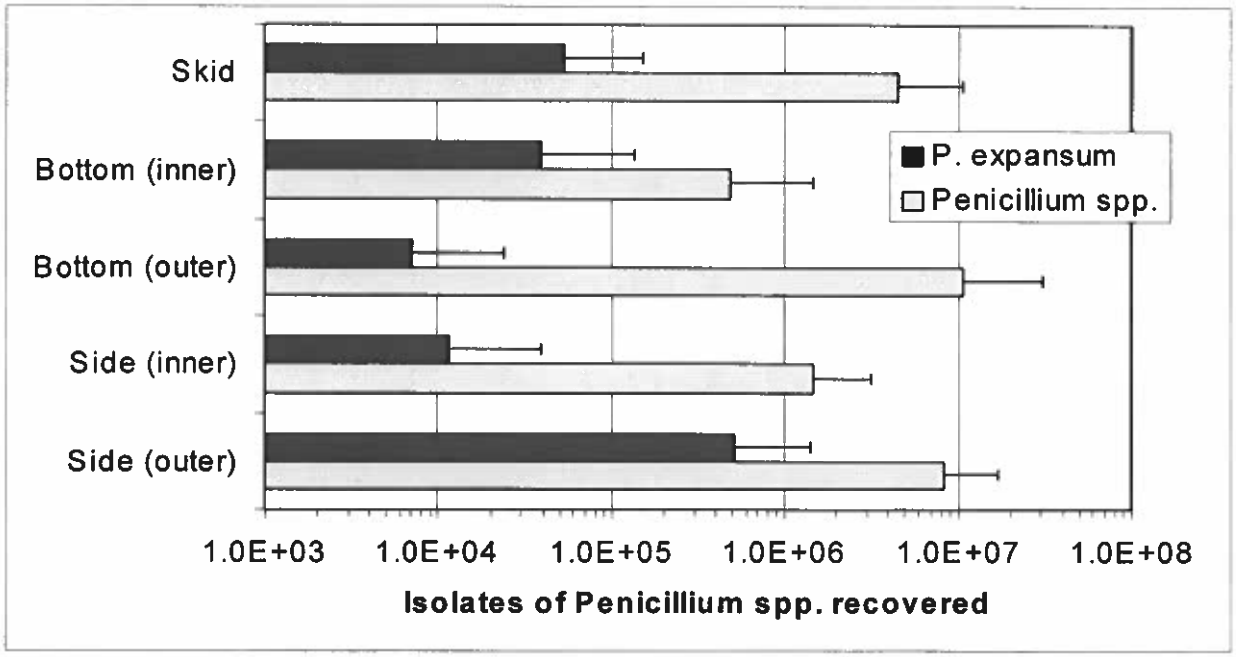

Figure 2. Population densities of Penicillium species recovered from different locations on field bins at harvest. Error bars indicate ½ of the standard deviation of the mean.