ASSESSING POSTHARVEST PATHOGENS IN SUGARBEET STORAGE PILES FROM NORTH DAKOTA AND MINNESOTA

Shyam L. Kandel, Malick Bill and Ela J. Montalvo

USDA-ARS, Northern Crop Science Laboratory, Fargo, ND

In the red river valley of Minnesota and North Dakota, postharvest sugarbeet roots require storage as the high tonnage of the crop exceeds immediate sugar factory processing capabilities. Sugarbeet roots are piled in factory yards, piling grounds, or ventilated sheds to allow the industry flexibility in sugar processing. Maintaining healthy sugarbeet roots in storage is essential to limit storage loss. Root pathogens in the production field, environmental conditions during harvest, varietal differences, and mechanical injuries from harvest and downstream operations all contribute to postharvest losses (Bugbee 1979; Klotz and Finger 2004; Strausbaugh 2018). Postharvest pathogens predominately infect injured sites on the root and can rapidly deteriorate roots depending on environmental conditions in the piles causing elevations in respiration rate and temperature inside the pile (Campbell and Klotz 2006; Mumford and Wyse 1976). These postharvest pathogens not only decrease sugar yield but also increase costs, as severely decayed roots may need to be disposed of without processing. Also, the roots that are processed typically might have higher concentrations of contaminants that can increase sucrose loss to molasses. Genetic resistance to storage diseases may alleviate postharvest losses, however, such resistance in sugarbeet cultivars has not been explored. The lack of knowledge on the predominant pathogens causing postharvest sugarbeet disease in each factory district have slowed the development of host resistance to storage diseases. Multiple fungal and bacterial strains are reported as causal agents for storage-related rots in sugarbeet growing areas in the US. However, limited information is available on the spectrum of postharvest pathogens in sugarbeet piles throughout the storage duration or if the factory districts have unique storage pathogens. Scientific understanding of the identity and abundance of postharvest pathogens will be the first key step to implement management strategies to minimize postharvest losses in sugarbeet storage. This study was conducted to understand the incidence of plant pathogens infecting sugarbeet roots in storage.

Materials and Methods

Symptomatic sugarbeet roots with microbial infestation or suspected roots in the vicinity of symptomatic roots were collected from factory yard and outside non-ventilated piles during the 2022/23 survey. A total of 150 symptomatic roots were collected from five factory districts (Moorhead, Hillsboro, Crookston, East Grand Forks, and Drayton). From each factory district, root samples were collected from three different non-ventilated piles (factory yard, Minnesota outside and North Dakota piles) at three time points i.e., mid-November, mid-December, and mid-December. Two sample bags with five beet roots each were collected from individual non-ventilated pile at each time point. The collected samples were transported to the USDA-ARS facility, Fargo, ND, and stored at 4°C until processing. Root tissues were thoroughly washed with sterile distilled water and incubated on the potato dextrose agar (PDA), de Man Rogosa Sharpe agar (MRS) and nutrient agar (NA) amended with antibiotics using the protocol of Woodhall et al. (2020). Microbial isolates were further grown on the respective media or water agar until a pure culture of single isolates were received. The pure cultures of individual microbes were transferred into either 30% (bacteria and yeast) or 15% (filamentous fungi) glycerol in 2-mL cryovials and stored at -80°C.

The representative pathogen isolates were used to amplify and sequence ITS or 16S rRNA gene for fungi (filamentous and non-filamentous, yeast species) and bacteria, respectively, using sanger sequencing platform (Psomagen Inc., Rockville, MD). The ITS or 16S rRNA gene sequences were submitted for BLASTN search into the National Center for Biotechnology Information nucleotide database to identify the pathogen isolates. To test for pathogenicity of the *Penicillium* spp., healthy sugarbeet root samples were plug-inoculated with 8-mm diameter PDA plug into each of the two 15-mm-deep holes created on the shoulder of the roots (Strausbaugh, 2018). The diameter (measured by a ruler) as well as the weight of the rot was recorded to assess the pathogenicity of the isolates.

Results and discussions

The pure cultures of fungal and bacterial isolates were recovered from sugarbeet root tissues displaying the microbial invasion. Fungal and bacterial species were identified by sequencing of internal transcribed spacer regions and 16S rRNA genes in fungi and bacteria, respectively. In total 282 isolates were obtained from 150 root samples received from factory yard and outside during the 2022/23 surveys. Of the seven fungal (non-filamentous) species

obtained, *Penicillim* sp. (31%) and *Mucor circinelloides* (23%) were the most isolated species (Fig. 1A, 2). *Pichia membranifaciens* (16%), *Hansespora valbyensis* (16%), and *Kurtzmaniella quercitrusa* (13%) remained the most isolated yeast species out of the 17 obtained during the 2022 survey (Fig. 1B). Three bacterial species (n = 164) were obtained from rotten roots samples including *Leuconostoc mensenteroides* (88%) and *Gluconobacter cerinus* (11%) (Fig. 3, 4). From the *Penicillium* population from the 2021/22 survey, *Penicillium expansum*, *P. italicum* and *P. firmorum* caused significantly ($P < 0.05$) more rots compared to other *Penicillium* species assessed (Fig. 5).

The study is ongoing to characterize additional isolates and assess pathogenicity tests in sugarbeet cultivars. Furthermore, analysis of more DNA barcoding genes such as beta-tubulin, translation elongation factor 1 alpha gene etc., for fungal isolate characterization will be completed later in 2024.

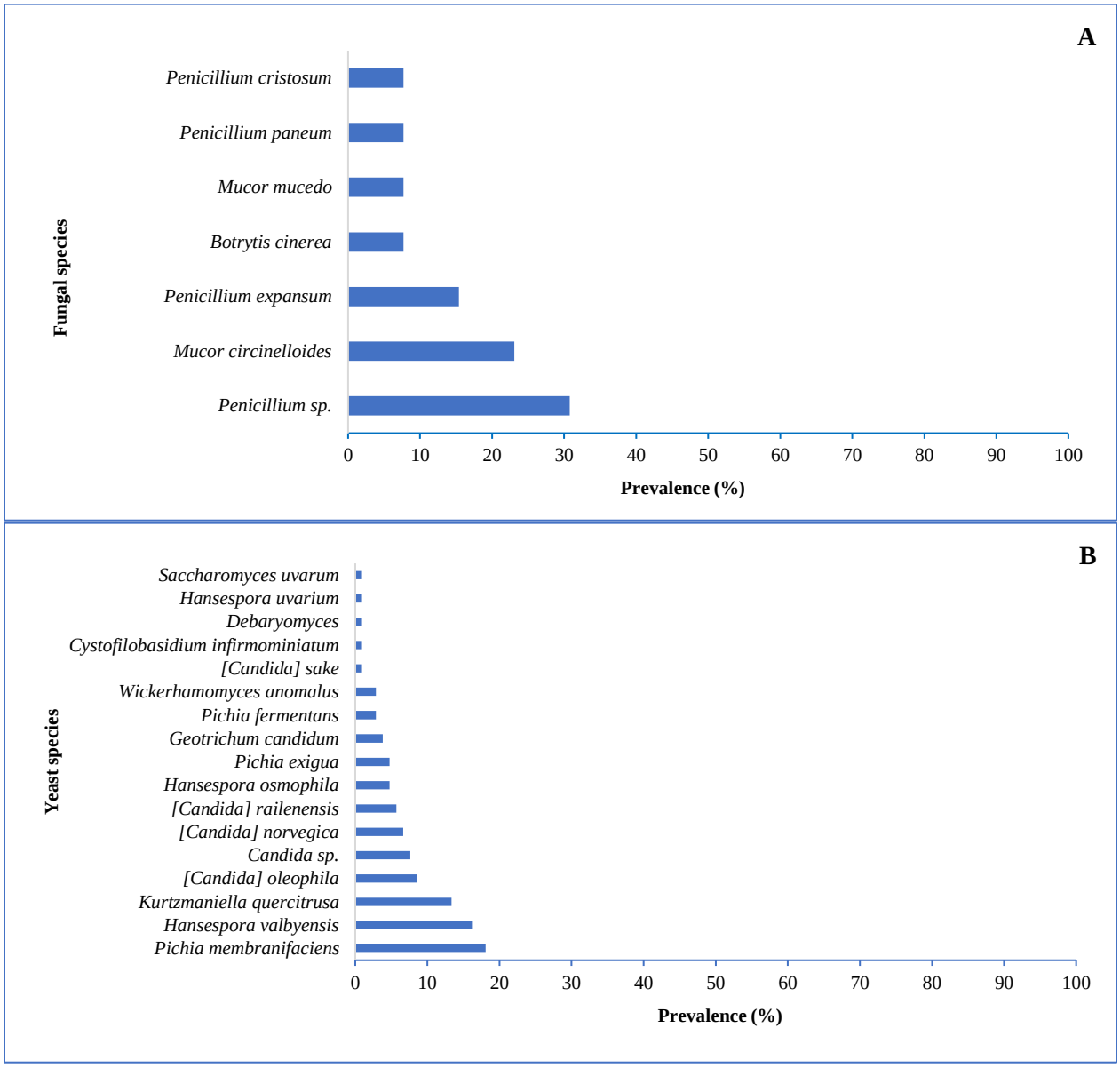

Fig. 1. Prevalence of filamentous fungal (A) and yeast (B) isolates associated with the decaying tissues of sugarbeet roots from storage piles and factory yards during the 2022/23 processing campaign.

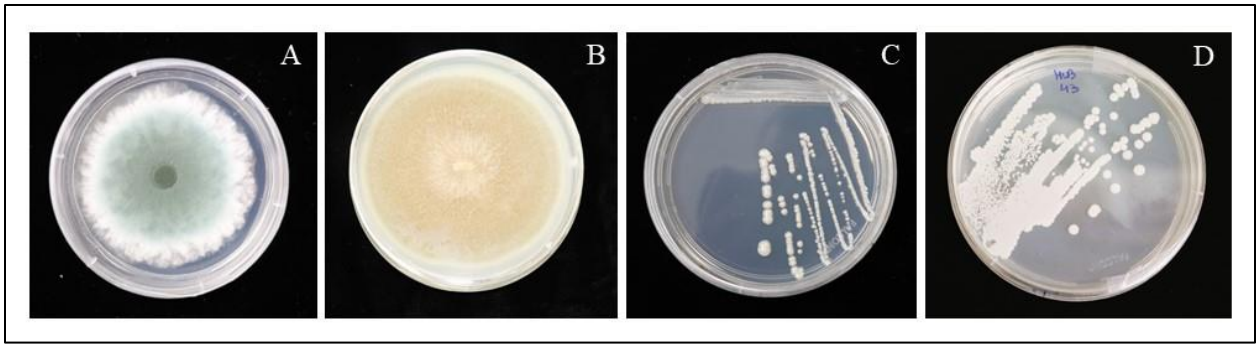

Fig. 2. Potato dextrose agar plates showing the growth of (A) *Penicillium* sp., (B*) Mucor circinelloides*, (C) *Hansespora valbyensis* and (D*) Kurtzmaniella quercitrusa* isolates from the rotten sugarbeet root samples.

.

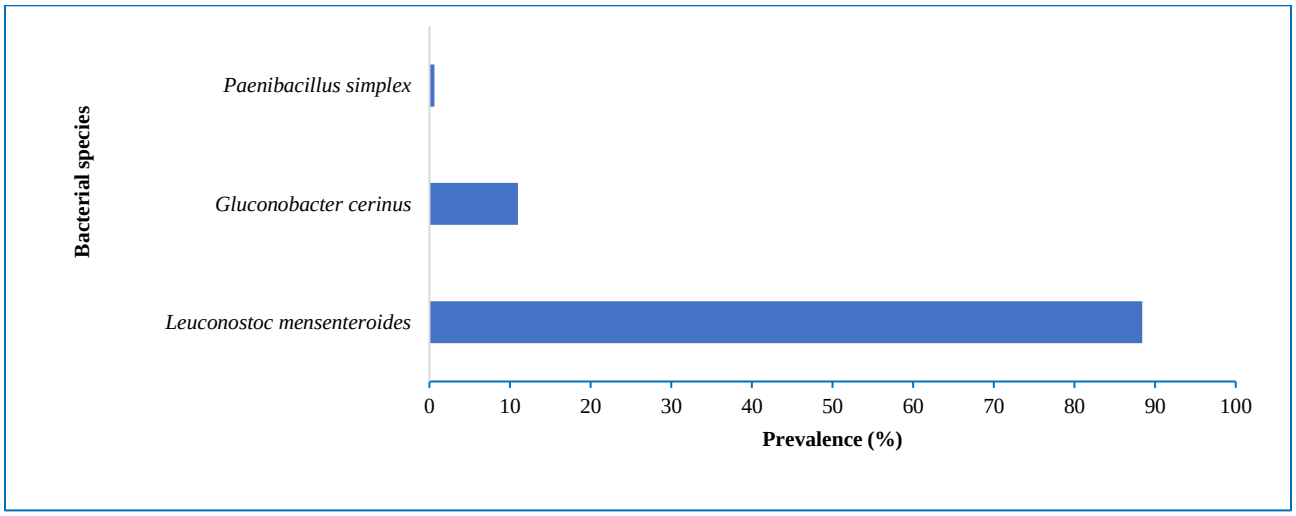

Fig. 3. Prevalence of bacterial isolates associated with the decaying tissues of sugarbeet roots from storage piles and factory yards during the 2022/23 processing campaign.

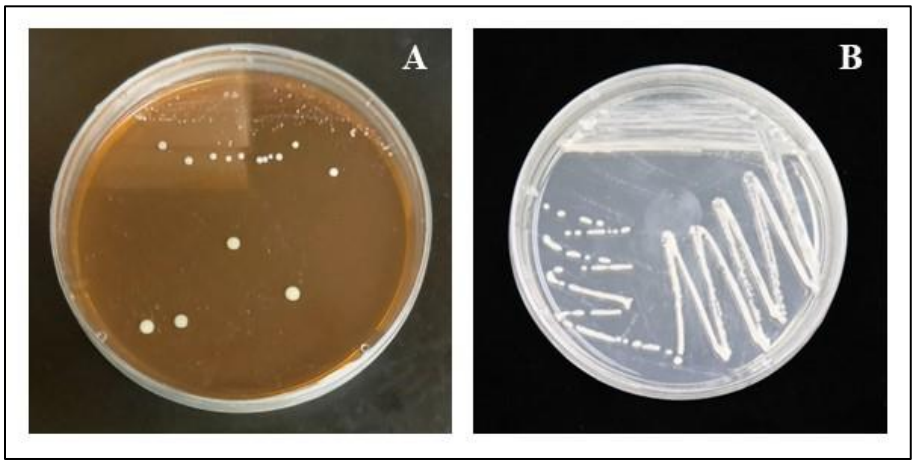

Fig. 4. Agar plates showing the growth of (A) *Leuconostoc mensenteroides* (MRS) and (B) *Gluconobacter cerinus* (PDA) isolated from the rotten sugarbeet root samples.

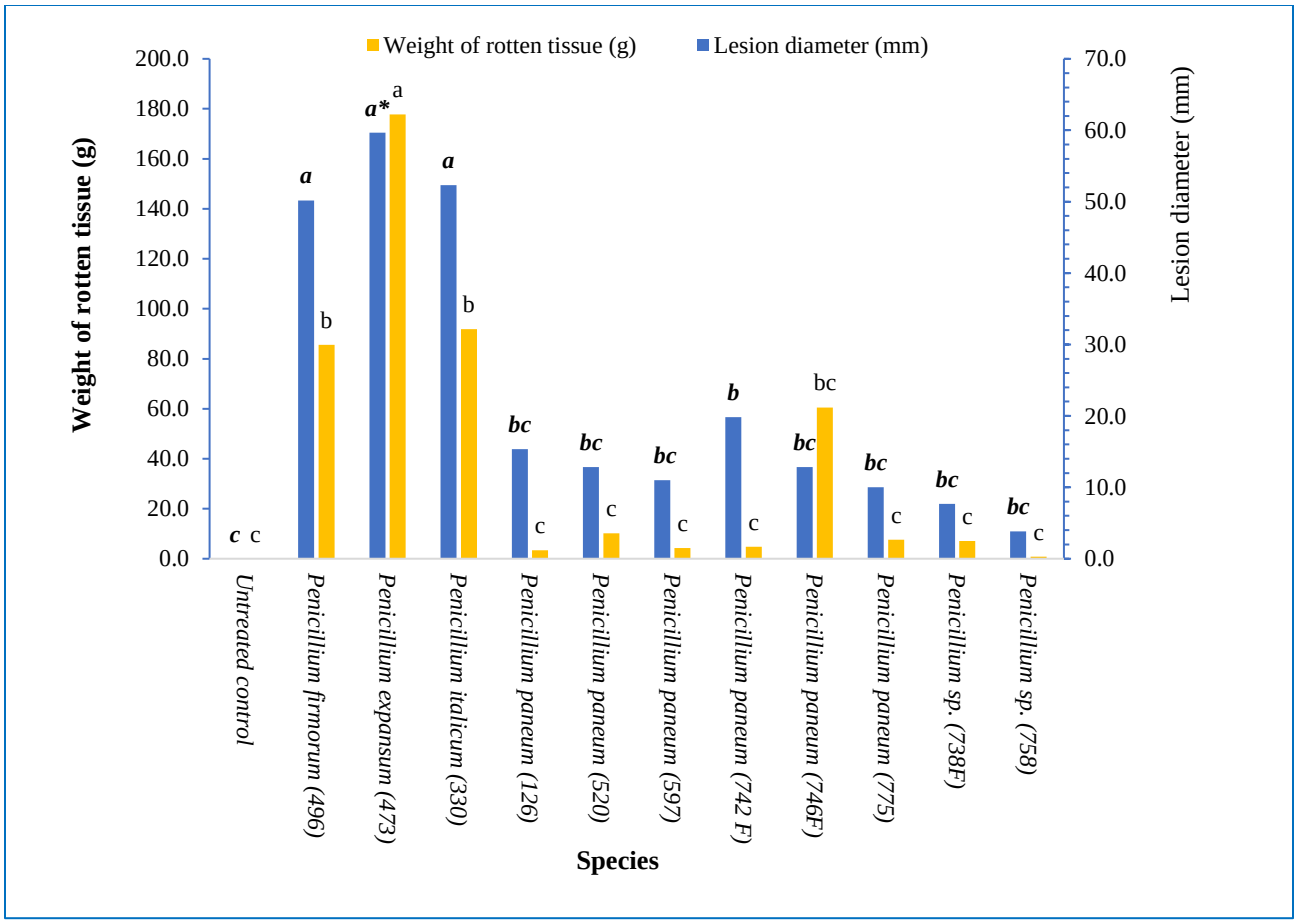

Fig 5. Pathogenicity of *Penicillium* isolates on sugarbeet roots. The asterisk, * indicates significant differences (P < 0.05) that are represented by different letters, which are also in order by highest to lowest.

ACKNOWLEDGEMENTS

We are thankful to the Sugarbeet Research and Education Board of Minnesota and North Dakota for the funding to perform this research. We appreciate help from American Crystal Sugar Company, Southern Minnesota Beet Sugar Cooperative, and Minn-Dak Farmers' Cooperative for collecting and getting sugarbeet root samples.

References:

1. Bugbee, W.M. 1979. The effect of plant age, storage, moisture, and genotype on storage rot evaluation of sugarbeet. Phytopathology 69:414-416.
2. Campbell, L.G. and Klotz, K.L., 2006. Postharvest storage losses associated with Aphanomyces root rot in sugarbeet. J. Sugar Beet Res. 43:113-127.
3. Klotz, K.L., and Finger, F.L. 2004. Impact of temperature, length of storage and postharvest disease on sucrose catabolism in sugarbeet. Postharvest Biol. Technol. 34:1-9.
4. Mumford, D.L. and Wyse, R.E. 1976. Effect of fungus infection on respiration and reducing sugar accumulation of sugarbeet roots and use of fungicides to reduce infection. J. Am. Soc. Sugar Beet Technol. 19:157-62.
5. Strausbaugh, C.A. 2018. Incidence, distribution, and pathogenicity of fungi causing root rot in Idaho long-term sugar beet storage piles. Plant Dis. 102:2296-2307.