MPB 2017 Research Report:
Impact of Phosphorous Acid Treatment on the Incidence of Soft Rot Disease (yr 1/2)

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The primary objective of this study was to quantify differences in soft rot disease prevalence after several months in storage for tubers the were treated post-harvest with phosphorous acid versus those not treated. Secondary objectives were to determine if phosphorous acid has a negative impact on soft rot bacterial culture growth, determine the amount of drying time for phosphorous acid treated tubers, and quantify differences in bacterial and fungal community composition existing on diseased and non-diseased tuber surfaces in bins that were treated post-harvest with phosphorous acid versus those that were not treated. The first three objectives have been completed and the final objective is being worked on as DNA extractions from tuber surfaces will be sequenced to generate millions of bacterial and fungal sequences per sample.

In Summer 2017, our lab isolated bacteria that were indicative of soft rot bacteria by producing pitting on CVP plates and PCR tested positive for *P. parmentieri*. Bacterial cultures from these isolates were used in this study to inoculate tubers deployed in storage in order to test incidence of disease distribution under various treatments.

On September 22, 2017 two storage bins at the Aroostook Farm were filled with approximately 300cwt. Tuber temperatures for both bins averaged 62°F at bin filling. Tuber cultivar was Dark Red Norland. Bin 2 was filled without post-harvest tuber treatment while bin 1 was filled with tubers treated with a post-harvest treatment of Phosphorous Acid (57% mono and di-potassium phosphite), at a product rate of 12.8oz per ton, with a total spray volume of 0.5gal/ton. Treatment was applied on the bin piler with an overhead spray bar with 4 nozzles. Tuber piles within each bin measured approximately 9 feet in height.

At the completion of bin-filling, ventilation (approx. 1.5 CFM/cwt) was initiated. Humidity and temperature were maintained at levels of 98% RH and 55F respectively for a three week curing period. After the curing period, bin temperatures were lowered by 0.5F/day, to a holding temperature of 45F, and humidity maintained at 98% RH.

Each bin contained three experimental treatments consisting of 50 tubers per mesh onion bag. Treatments consisted of 2%, 4%, or 0% (negative control) disease incidence that was accomplished with diseased tubers that were inoculated in the lab from the cultures obtained during the summer. Each treatment was replicated 5 times, so a total of 15 bags were deployed in each bin. Tubers were deployed in bin 2 (no post-harvest phosphorous acid treatment) on 10/21/2017 and in bin 1 (post-harvest (phosphorous acid treatment) on 10/28/2017.

Storage bins and treatment bags were assessed on 12/18/2017 and 1/18/2018 for soft rot infections. The data was analyzed with ANOVA, and mean comparisons were conducted using Tukey's HSD, P=0.05. During the first assessment date (12/18/2018) the tuber surfaces in the untreated bin were dry but in the phosphorous acid bin, they were wet. The difference was striking. In the untreated bin, 12 of the 15 source

inoculant tubers exhibited no signs of active disease and the tissue damaged area had dried out. But in the bin treated with phosphorous acid, all source tubers were still active and the disease had spread to additional tubers in some instances. At this time, there was a significant difference in both the 2% and 4% treatments (shown below). The 2% Phos. Acid treatment had an 8% infection rate compared to a 1.2% rate in the 2% unsprayed treatment. The 4% Phos. Acid treatment had a 10.4% infection rate compared to a 6.4% rate in the unsprayed treatment. ANOVA showed a significant difference for each of these treatments, and for all treatments combined (6.1 % for Phos. Acid treatment versus 2.5% for unsprayed).

Mean Percent Active Infections (% of 50 Tubers)

	Inoculated Tubers (% of 50 Tubers)			
Treatment	0	2	4	
Phos. Acid (52 DAI)	0c[x]	8a	10.4a	**6.1A[y]**
None (58 DAI)	0c	1.2bc	6.4ab	**2.5B**
	0*[z]	**4.6****	**8.4*****	
				Overall=4.3

x. Means followed by the same lower-case letter
are not significantly different, Tukey's HSD, P=0.05.
y. Means followed by the same upper-case letter
are not significantly different, Tukey's HSD, P=0.05.
z. Means followed by the same number of (*)
are not significantly different, Tukey's HSD, P=0.05.

However, during the second assessment date (1/18/2018) the tuber surfaces in the phosphorous acid treated bin had dried out similarly to what was evident in the non-treated bin and 5 of the original 15 source inoculant tubers were no longer active. Overall infection rates were much lower (chart below) and there was no significant difference between Phos. Acid treated tubers and unsprayed tubers.

Mean Percent Active Infections (% of 50 Tubers)

	Inoculated Tubers (% of 50 Tubers)			
Treatment	0	2	4	
Phos. Acid (82 DAI)	0b[x]	0.8b	4.0a	**1.6A[y]**
None (88 DAI)	0b	0.8b	2.0ab	**0.9A**
	0*[z]	**0.8***	**3.0****	
				Overall=1.3

x. Means followed by the same lower-case letter
are not significantly different, Tukey's HSD, P=0.05.
y. Means followed by the same upper-case letter
are not significantly different, Tukey's HSD, P=0.05.
z. Means followed by the same number of (*)
are not significantly different, Tukey's HSD, P=0.05.

Lab disk diffusion assays indicated that phosphorous acid has no negative impact on the growth of pathogenic bacteria. Disks were infused with the same post-harvest phosphorous acid concentrations and also at twice that concentration. The results indicate that soft rot bacteria are not inhibited by phosphorous acid and that phosphorous acid may prolong the amount of time it takes for tuber surfaces to dry out. We will be conducting lab assays in a fume hood to determine relative drying time of tuber surfaces sprayed with water versus phosphorous acid.

Zones of inhibition for bacterial culture growth in association with disk diffusion assay

Disk infusion	Zone (mm)
Phosphorous acid 1x concentration	0.00
Phosphorous acid 2x concentration	0.00
Distilled water (negative control)	0.00
Chlorhexidine (positive control)	9.50

* Zones are mean measurements from 10 replicated plates. There were no zones of inhibited growth on any plates for either of the phosphorous acid treatments or for the negative control.

Tuber piles in our bins was 9 feet, but in typical storage can range from 15 - 18 feet. On 12/18/2018, the phosphorous acid tubers had active disease and the pile was still wet. There were less active diseased tubers in 1/18/2018, but by then the pile had dried. So it took approximately 3 months for a 9 foot high pile to fully dry - if the pile was twice that height, it could take up to twice as long, giving pockets of disease more time to establish and spread. Our results support the findings of a study where the prevalence of soft rot disease was increased in association with phosphorous acid application (Dupuis et al. 2008).

Dupuis, B., Garcia, N., & Boels, G. (2008). Efficacy of potato seeds disinfection products to control Erwinia spp. *Communications in agricultural and applied biological sciences*, *73*(2), 343-348.