Northwest Potato Research Consortium

Annual and Final Reports for FY 2017-18 and FY 2016-17, respectively

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Northwest Potato Research Consortium

FY2018 Final Report

Title: **Targeted Herbicide Programs for Weed Control in Potatoes: Educating Potato Growers and Ag Industry about Herbicide Resistance and Weed Shifts.**

Personnel: Pamela J.S. Hutchinson, UI; Joel Felix, OSU; Timothy Miller, Steven Seefeldt and Ian Burke, WSU.

Reporting Period: Final Report FY2018

Summary of accomplishments:

Issues addressed with NPRC funding:

The threat of crippling yield losses due to noncontrolled, metribuzin-resistant weed populations in PNW potato production areas is real. *If PNW growers and others in the potato industry could be better informed about the need for using herbicides with mechanisms of action other than that of metribuzin, then perhaps what has occurred in cropping systems which rely heavily on only a few mechanism of action herbicides*, such as Roundup Ready® corn and soybeans, *will not happen*. In other words, hard-to-control herbicide resistant weeds will not dominate a large number of fields used for growing potatoes.

Herbicides with a novel mechanism of action have not been developed in more than 25+ years. However, there is a mindset about metribuzin as a must for potato herbicide programs to the exclusion of alternative, available herbicides with different mechanisms of action for controlling the same weed species as does metribuzin. Unfortunately, there has been confirmation of metribuzin-resistant common lambsquarters and redroot pigweed weed populations in some PNW potato production fields. *However, similar to what has happened in those previously mentioned Roundup Ready cropping areas, PNW potato growers and potato industry do not always believe there is metribuzin-resistance and/or believe it does not matter because there will be always be new herbicides to replace metribuzin.*

Confounding these serious weed control issues is the fact that what we are calling "the hairy nightshade herbicides:" Matrix, Outlook, Eptam, and Chateau, do not satisfactorily control c. lambsquarters. Therefore, a trend towards these weed species dominating weed populations is more than likely occurring.

Accomplishments and Impacts:

The 2017 study goal was to use multi-location, targeted tank-mix weed control trials to demonstrate, update, and further enable critical extension education efforts for proactive rather than reactive resistance and weed-shift management started in 2016. In both years, NPRC-funded targeted herbicide tank-mix trials were located at a total of four sites in ID, OR, and WA and demonstrated that common lambsquarters and redroot pigweed control by tank mixtures without metribuzin can be equal to or better than when metribuzin is the herbicide included to control these weeds. NOTE: these weed populations were not metribuzin-resistant. Field days/ tours in ID, OR, and WA attended by approximately 150 potato industry growers/representatives each

year featured the trials and were valuable in herbicide resistance and weed shift extension education. In addition, the reasons for the trials and the research results have also been disseminated at many venues, including University of Idaho Potato Conferences Weed Workshops attended by approximately 250 people and Western Society of Weed Science meetings attended by research and extension weed scientists.

Many at the field days/tours, conferences, and meetings had an "aha" moment when the length of time without new herbicide mechanisms of action was mentioned and occurrence of herbicide resistance in other areas despite availability of more than one herbicide mechanism of action.

Experiments and Activities conducted:

The trials conducted, featured, and discussed were located at the U of I Aberdeen R&E Center, OSU Malheur Experiments Station, WSU NW R&E Center (Mt. Vernon, WA) Field Day, and fields near Pasco, WA.

- Herbicide treatments were Eptam, Outlook, or Matrix tank mixed with metribuzin, Linex, or Prowl H2O and applied preemergence (after hilling) then sprinkler incorporated.
- Weed populations at the four locations included common lambsquarters, redroot pigweed, and nightshade sp. Other weeds were also present at Mt Vernon (see below).
- Depending upon location, Russet Burbank, LaSoda, Yukon Gold, and/or Ranger Russet was planted Spring 2017. Trials were harvested Fall 2017 and tuber yield and quality was recorded.

Results:

At Aberdeen, Malheur and Othello in 2017, two-way tank mixtures of Outlook, Eptam, or Matrix w/o metribuzin provided comparable season-long control of common lambsquarters and redroot pigweed to those with metribuzin and control was greater than 95% (Figures 1A, 1B. 1C).

At Mt Vernon, Outlook + metribuzin, or Linex, or metribuztin + Linex which provided 92, 89, or 82% control, respectively, of a mixed weed sp. population (Figure 1D).

- Otherwise, Eptam or Matrix with Linex provided relatively greater control at 68 and 77%, than when these two herbicides were tank mixed with metribuzin or Prowl H2O (less than 60% control). Outlook + Prowl H2O only provided 70% control.
- The mixed population consisted of common lambsquarters, redroot pigweed, hairy nightshade, common chickweed, common groundsel, henbit, and shepherspurse.

Tuber quality and yields reflect season long weed control.

- All treatments generally resulted in greater U.S. No. 1 and Total tuber yields of Russet Burbank at Aberdeen and Ranger Russet at Malheur than those of the nontreated weed control (Figures 2A, 2B).
- Somewhat surprisingly, treatments with Linex as the tank-mix partner did not always result in the relatively greatest LaSota/Yukon Gold total tuber yields at Mt Vernon even though those treatments generally provided better overall weed control than when metribuzin was used (2C).
- At Pasco in 2017, although there were some numerical differences, there were no significant differences between any treatment and the nontreated control for market yield, US 1 & 2s greater than 6 oz tons A^{-1}, and percent counts for US 1s and US 2s greater than 4 ounces,

US 1 & 2s greater than 6 ounces, and culls (Table 1).
  - At Othello, WA in 2016, all herbicide treatments significantly increased market yield, US 1 and 2's greater than 6 oz Tons A^{-1}, and percent number of US 1's greater 4 oz and US 1 and 2's greater than 6 oz compared to the nontreated control (data not shown). There was also an increased in percent number of culls for the nontreated control (62%) compared to all in the herbicide treatments(less than 30%).
- In-season crop injury was less than 10% regardless of location (data not shown).

Conclusions and Usefulness.
The trials and results have increased PNW grower and potato industry understanding that herbicides with a different mechanism of action than metribuzin can control common lambsquarters and redroot pigweed as well as when metribuzin is the tank-mix partner AND there are no yield differences between metrbuzin and non-metribuzin combinations. Control of a mixed species weed population at Mt Vernon with metribuzin tank mixtures translated to total tuber yields less than when

- Therefore, proactive measurements will likely be taken to prevent or delay metribuzin resistance.
- In addition, if the populations had been metribuzin resistant, herbicide combinations including metrbuzin would have resulted in poor or no control. That end result would've shown that it's the metribuzin resistance, not some other reason, such as incorrect rate or improper application techniques affecting control.

The results at Aberdeen, Malheur, and Pasco clearly show that when Prowl H2O or Linex were tank-mixed with the nightshade sp herbicides, Outlook, Eptam, and Matrix, then common lambsquarters and redroot pigweed control was similar to control when metribuzin was the tank-mix partner.

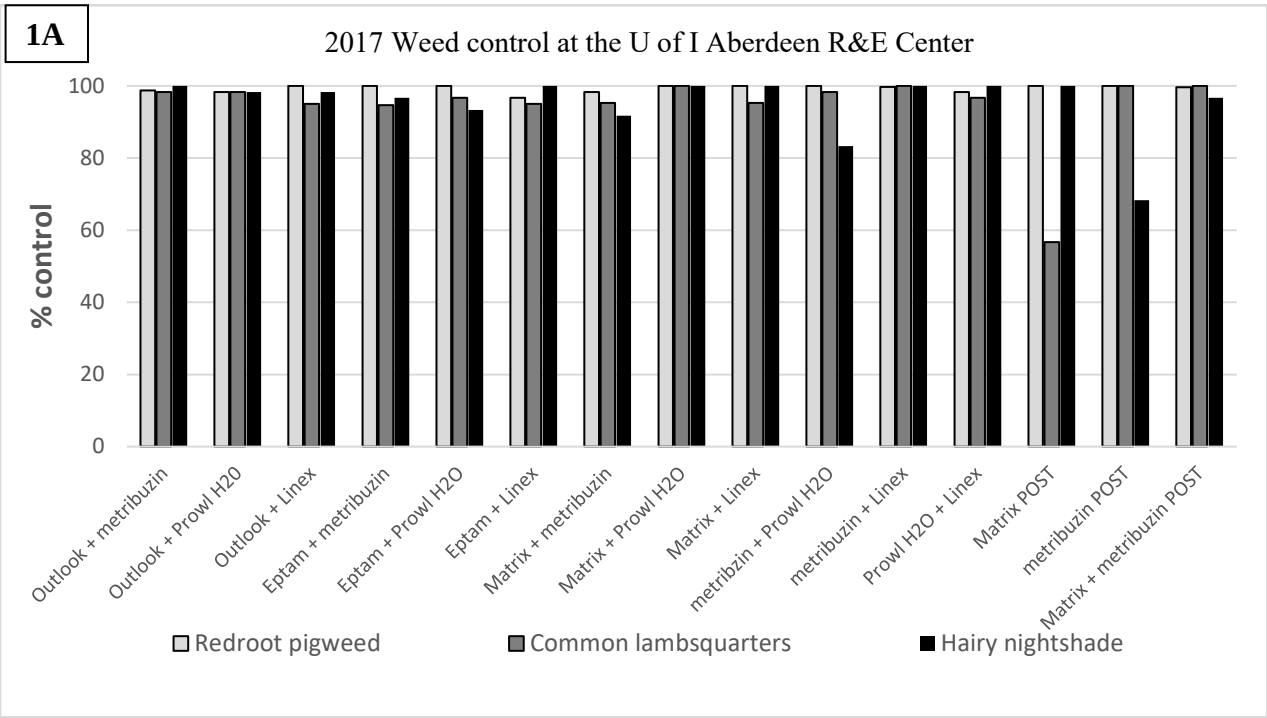
- Tuber quality and total yields reflected season-long weed control Figures 2A, B, and C; Table 1).

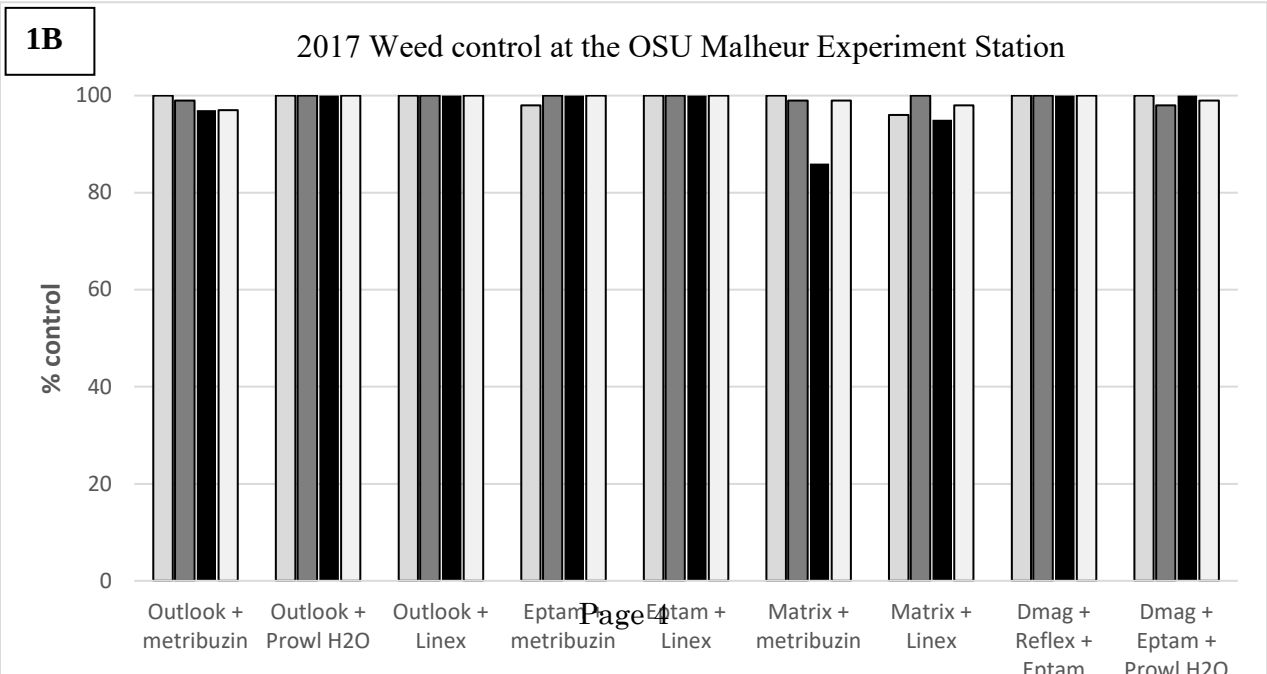
Matrix alone did not control common lambsquarters at the Aberdeen R&E Center (Figure 1A), which is evidence that at least this hairy nightshade herbicide requires tank mixing with a herbicide which can control that weed. Metribuzin alone or with Prowl H2O did not provide satisfactory hairy nightshade control at Aberdeen.

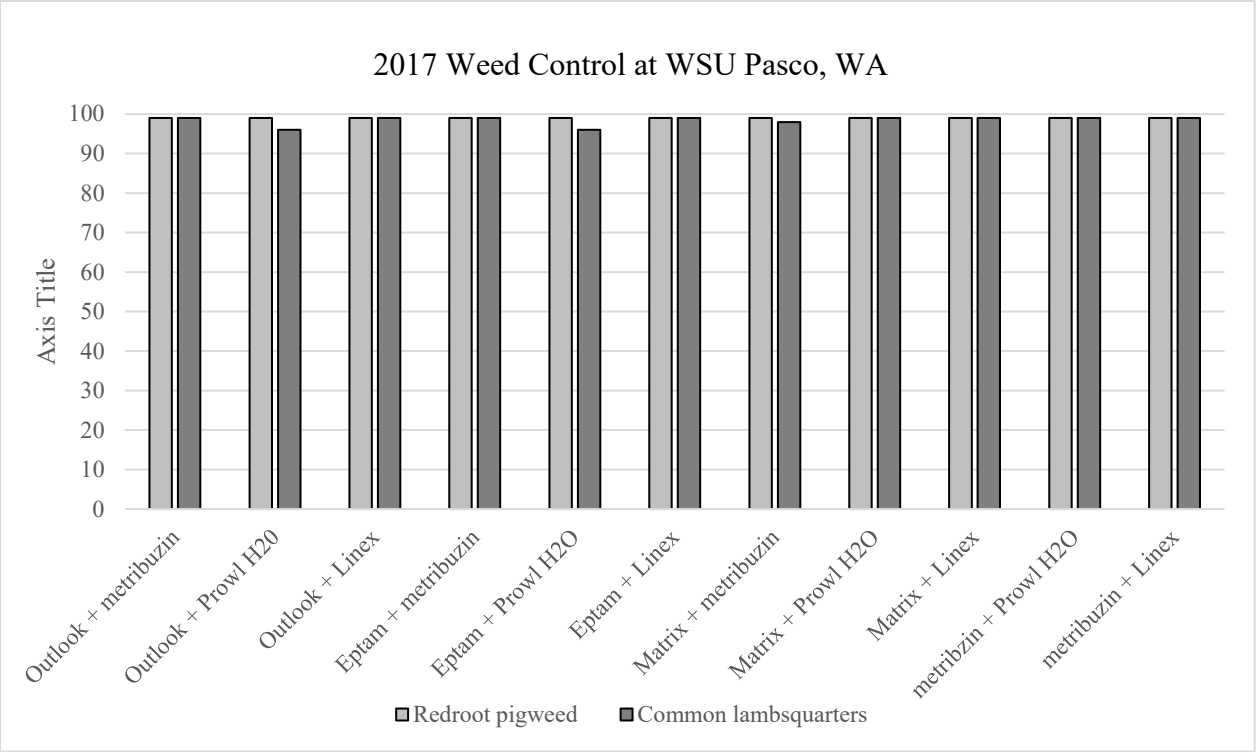
Publications:
None at this time. A PNW extension bulletin is planned, as well as trade magazine articles. Presentation abstracts will be published in meeting proceedings.

Presentations & Reports:
2016 and 2017 U of I Aberdeen R&E Center and OSU Malheur Experiment Station annual pest management field tours.
2016 and 2017 WSU NW R&E Center (Mt Vernon, WA) and Othello, WA field days.
2017 and 2018 U of Idaho Potato Conference presentations (Weed Management Workshops)
2018 Western Society of Weed Science (WSWS) meeting presentation.
NPRC Quarterly and Final reports.
WSWS 2018 Proceedings.

Figure 1A, B, C, D. Weed control by Outlook, Eptam, or Matrix with and without metribuzin in 2017 at the U of I Aberdeen R&E Center, OSU Malheur Experiment Station, WSU Pasco, WA and WSU NW R&E Center (Mt. Vernon, WA).







1D

2017 WSU Mt Vernon: Overall weed control - mixed population of C. lambsquarters, R. pigweed, H. nightshade, common chickweed, common groundsel, henbit, and shepherdspurse

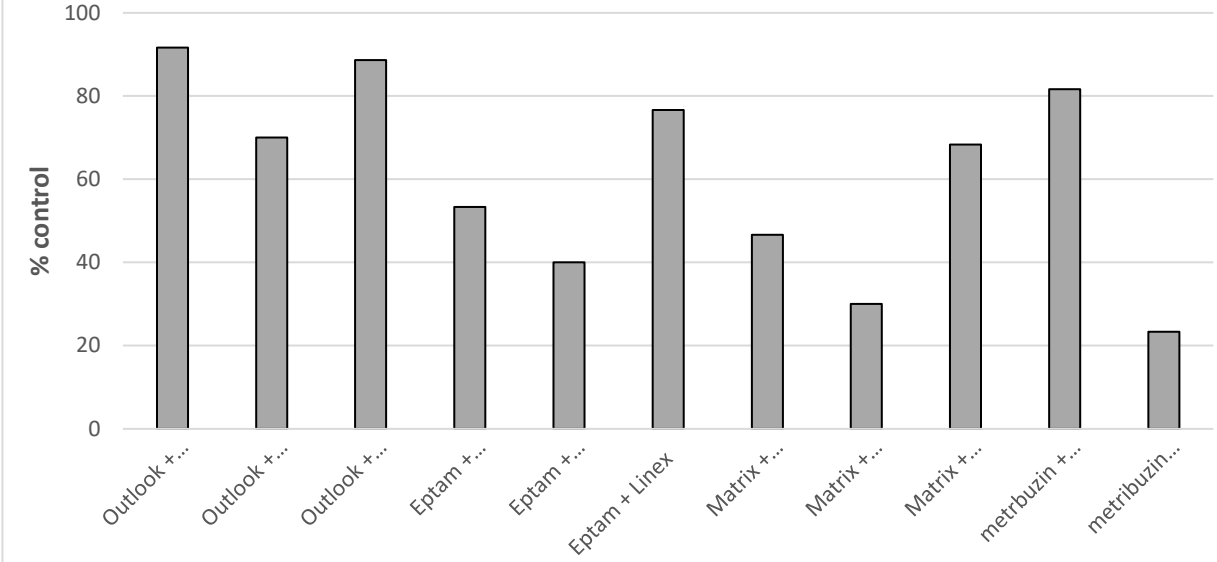
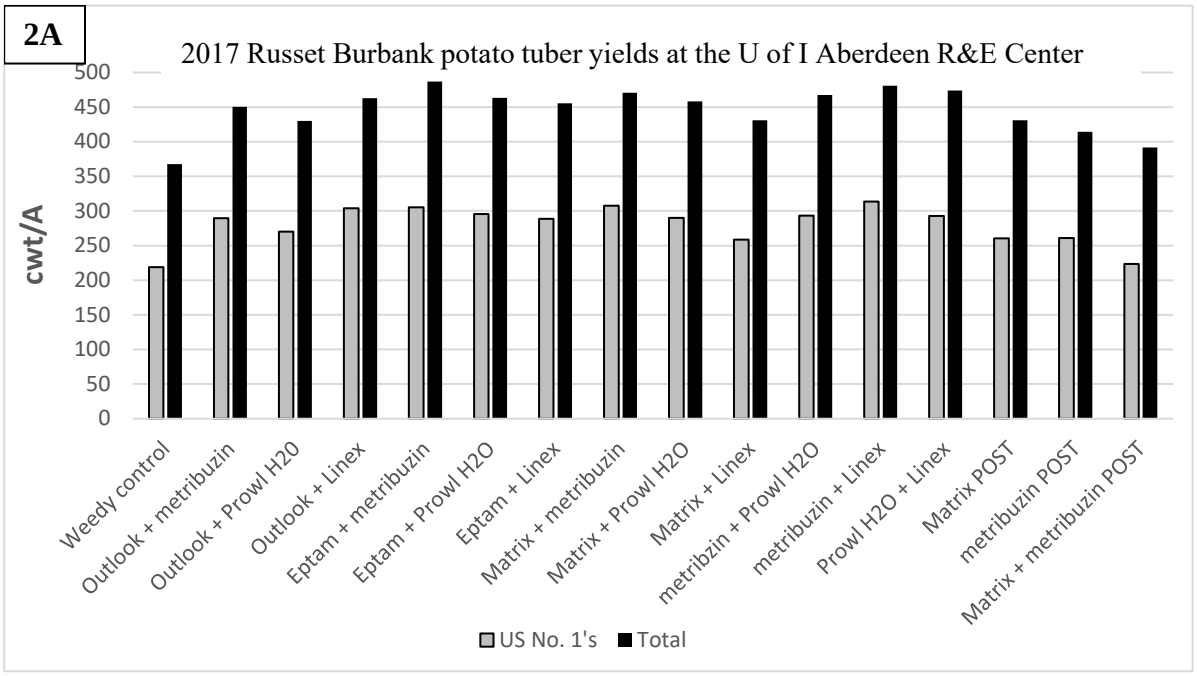
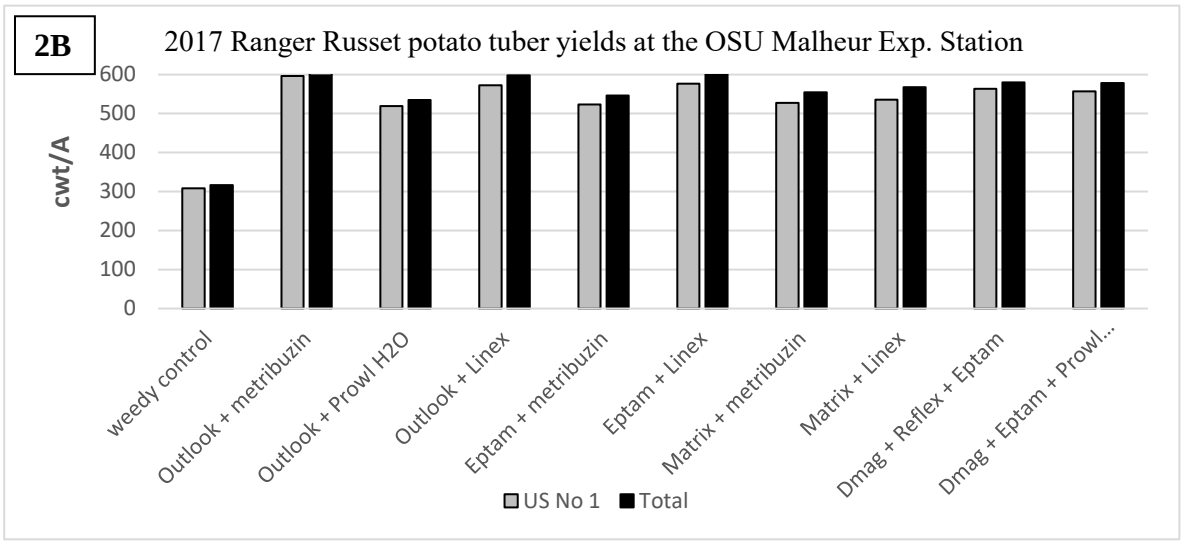


Figure 2A, B, and C. 2017 U.S. No. 1 and total tuber yields at the Aberdeen R&E Center and OSU Maheur Experiment Station and Total tuber yields at the WSU NW R&E Center when Outlook, Eptam, or Matrix was tank-mixed with or without metribuzin. Yields from a nontreated, weedy control are included for comparisons.





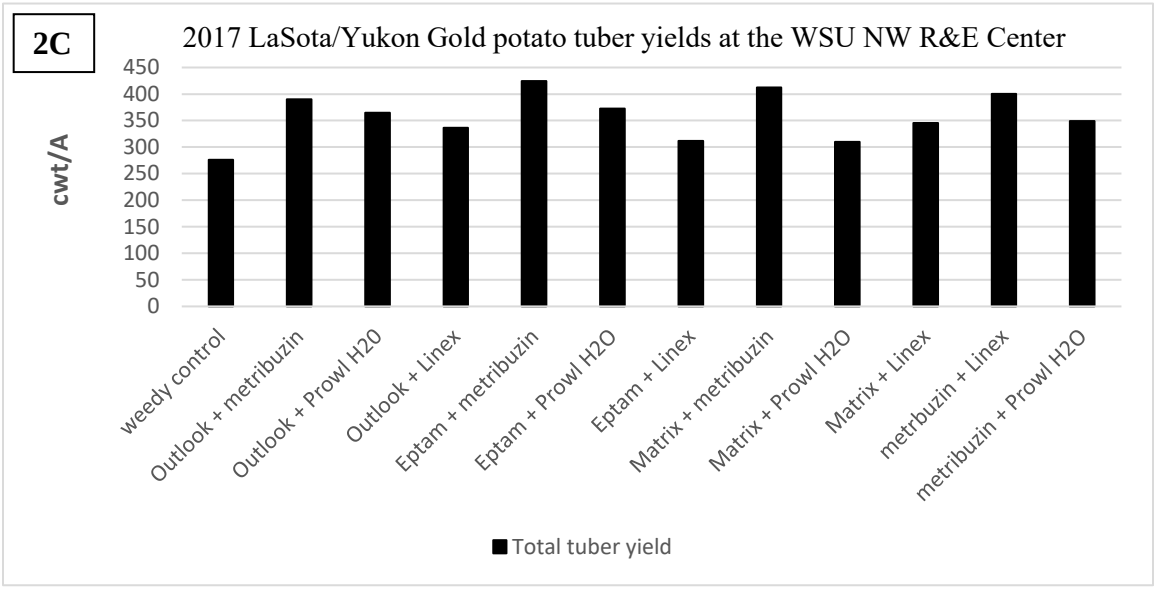


Table 1. Average potato counts by grade size and yield at harvest for 2017 potato study in Pasco, WA using either Outlook, Eptam, and Matrix in combination with other herbicides. Pasco, WA, 2017. DAT = days after treatment. Means followed by the same letter are not statistically significantly different (α = 0.05).

			Counts				Yield	
			September 22, 2017				*September 22, 2017*	
Treatment	**Rate**		**US 1s > 4 oz**	**US 2s > 4 oz**	**US 1 & 2s > 6 oz**	**Culls & < 4 oz**	**US 1 & 2 > 6 oz**	**Market**
	field rate	**lb ai/A**	**%**	**%**	**%**	**%**	**Tons/A**	**Tons/A**
Nontreated			17	0	61	21	15	24
Outlook Sencor	18 fl oz/A 10.7 oz/A	0.84 0.5	28	0	54	18	15	28
Outlook Prowl H2O	18 fl oz/A 2.1 pt/A	0.84 1.0	30	1	45	24	12	24
Outlook Linex	18 fl oz/A 24 fl oz/A	0.84 0.75	46	0	43	23	10	23
Eptam Sencor	6 pt/A 10.7 oz/A	5.25 0.5	20	2	60	17	18	29
Eptam Prowl H2O	6 pt/A 2.1 pt/A	5.25 1.0	19	1	61	18	20	33
Eptam Linex	6 pt/A 24 fl oz/A	5.25 0.75	27	2	46	24	16	30
Matrix Sencor	1.47 oz/A 10.7 oz/A	0.023 0.5	26	2	43	28	10	22
Matrix Prowl H2O	1.47 oz/A 2.1 pt/A	0.023 1.0	41	1	42	15	13	27
Matrix Linex	1.47 oz/A 24 fl oz/A	0.023 0.75	47	1	35	17	10	24
Sencor Linex	10.7 oz/A 24 fl oz/A	0.5 0.75	28	1	51	19	17	33
Prowl H2O Linex	2.1 pt/A 24 fl oz/A	1.0 0.75	19	1	50	28	15	27
		LSD	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

PROGRESS REPORT
Annual Report FY2017-2018

TITLE: Characterizing Fusarium species associated with and refining management of potato dry rot in the Pacific Northwest

PERSONNEL: Kasia Duellman, Phillip Wharton, James Woodhall, Kenneth Frost, Debra Inglis, Donald McMoran

REPORTING PERIOD: Quarterly, February 2018

ACCOMPLISHMENTS/RESULTS:

Objective 1: Collect isolates
Thus far, 372 isolates of *Fusarium*-like species have been obtained from a total of 182 tubers from 46 samples. The samples included the following 19 varieties:

All Blue
Almera
Alturas
Chieftain
Clearwater
Dakota Pearl
Dark Red Norland
Elfe
Pacific Russet
Payette
Premier Russet
Princess
Ranger Russet
Russet Burbank
Russet Norkotah
Teton Russet
Umatilla
Viviana

Additional samples are expected to be collected after symptoms develop in storage after this year's harvest. Expected sampling times will span from February through planting in 2018. Our protocol will also facilitate isolating from non-symptomatic tubers; however, we are specifically targeting tubers that show symptoms of Fusarium dry rot after a period of being stored.

Objective 2: Characterize isolates based on morphology and molecular tools
A portion of these isolates have been partially molecularly characterized by amplifying and sequencing one genomic target: the internal transcribed spacers (ITS) region using the universal primers ITS5 and ITS4 (White et al, 1990, pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds). To date, 156 isolates have been sequenced using the ITS5/4 primers, and

we have tentatively identified the following: 35 of the sequences share high homology with *F. sambucinum*; 9 with *F. equiseti*; 7 with *F. culmorum* (at least two of which also showed homology with *F. cerealis*); 4 with *F. solani*; 5 with *F. acuminatum* or *F. tricinctum*; 5 with *F. graminearum*; and 7 with *F. avanaceaum*. Screening of isolates will continue with ITS5 and ITS4 primers; selected isolates will be further characterized with additional molecular targets to provide a higher degree of confidence in species identification.

Objective 3: Perform Koch's postulates
Not yet started.

Objective 4: Determine fungicide sensitivity profile
Not yet started.

Objective 5: Perform virulence assays
Not yet started.

Objective 6: Perform phylogenetic analyses
Not yet started.

Concerns regarding progress:
Molecular characterization of isolates collected in 2016 and 2017 was expected to be completed by this time, and we expected to initiate Koch's postulates and virulence assays. These delays have in large part stimulated the acquisition of a PhD student, beginning end of June 2018. This project will comprise a significant portion of the PhD student's research.

Collection of 2018 isolates is still on schedule, and we expect to receive more samples from Washington, Oregon, and Idaho. Preparation of duplicate long-term storage sets of isolates collected to date are underway, which will facilitate the collection of data for fungicide sensitivity and phylogenetic analyses.

PUBLICATIONS:
None to date

PRESENTATIONS & REPORTS:
None to date

ANNUAL PROGRESS REPORT

TITLE: Development of Verticillium Wilt-Suppressive Soils and Evaluation of Fungicidal and Biorational Products for Northwest Potato Production

PERSONNEL: Project Leaders: **Dennis A. Johnson**, Plant Pathologist, Washington State University, dajohn@wsu.edu, phone: 509-335-3753; fax: 509-335-9581; **Kenneth Frost**, Extension Plant Pathologist, Oregon State University, kenneth.frost@oregonstate.edu, phone: 541-567-8321 ; **Mike Thornton,** Plant Physiologist, University of Idaho, miket@uidaho.edu, phone: (208) 722-670 (Ext. 211); **Phil Wharton,** Plant Pathologist, University of Idaho, 208-397-7000 (Ext. 108); **James Woodhall,** Plant Pathologist, University of Idaho, jwoodhall@uidaho.edu, phone: (208)-722-6701; **David L. Wheeler**, Graduate Research Assistant, david.wheeler@wsu.edu, phone: 215-880-3024; **Nick Vincent**, Graduate Assistant, Vinc7517@vandals.uidaho.edu, phone: 509-366-7217; **Ransey Portenier**, Research Specialist, ranseyp@uidaho.edu, phone: 208-772-6701; **Daniel H. Farber**, Research Associate, daniel.farber@wsu.edu, phone: 509-335-1998

REPORTING PERIOD: January 2017-January 2018

SUMMARY OF ACCOMPLISHMENTS:

Objective 1. The richness, evenness, and diversity of *V. dahliae* isolates from potato and asymptomatic crops were similar by most metrics and were not genetically diverged. The isolates from asymptomatic crops likely originated from potato and a subset of these isolates retained the ability to cause disease on potato. Inoculum from asymptomatic crops is most likely from potato and does not represent a new population. These results provide evidence that isolates from the asymptomatic crops originated from potato but are likely only contributing marginal amounts of inoculum to potato crops.

Objective 2. Two cycles of serial inoculations of rotation crops were completed and we determined that future cycles can be expedited by inoculating with a soil-drench, using potatoes from tissue-culture, and recovering isolates as soon as 10-15 days after inoculation. Therefore the next three cycles should be completed by late spring. Trials designed to determine if mustard, barley, and potato can select for genotypes of *V. dahliae* that are less aggressive towards potato are complete. The effect of inoculum comprised of mixes of up to three isolates of *V. dahliae* on potato wilt was not synergistic but was limited by the least aggressive isolate. The isolates recovered from each crop will be genotyped to determine if specific crops select for specific isolates of *V. dahliae*.

Objective 3. Potatoes co-inoculated *with Penicillium oxalicum* (*Pox*), a potential biocontrol agent, and *Colletotrichum coccodes* exhibited significantly reduced microsclerotia formation relative to potatoes inoculated with only *Cc* in one of two trials.

Objective 4. Two commercial biological fungicides (Bio-Tam, Serenade ASO) were shown to have limited impact on Verticillium wilt, regardless of application timing or rate. Both Velum Prime and Elatus showed some potential for reducing disease symptoms and improving tuber yield.

ACTIVITIES OR EXPERIMENTS CONDUCTED:

Objective 1. Test the hypothesis that *V. dahliae* isolates from potato and rotation crops are genotypically and phenotypically different: Isolates of *V. dahliae* that were recovered from potato, mustards, grasses, mint, and other symptomatic crops were genotyped with microsatellite markers and the mating-type of each isolate was determine to infer the possibility of sexual reproduction (Table 1). Analyses were performed to determine (i) if the number of loci (i.e. genomic regions) was sufficient to characterize the genetic diversity of our samples, (ii) the diversity of alleles (i.e. DNA variants) per locus, (iii) genotypic richness (i.e. the number of multilocus genotypes), evenness (i.e. the frequency of multilocus genotypes), and diversity (i.e. the number and abundance of multilocus genotypes), (iv) genotypic similarity among isolates from different crops, (v) the co-occurrence of genotypes in isolates from different crops, (vi) the origins of isolates from asymptomatic crops, and (vii) the movement of inoculum between potato and the asymptomatic crops.

Phenotypic characterization of the *V. dahliae* isolates included (i) inoculation of potato, brown mustard ISCI 99, and baronesse barley with a genotypically representative subset of 16 isolates and (ii) determination of the vegetative compatibility group (VCG). The pathogenicity trials are complete but we are still quantifying the amount of *V. dahliae* inoculum from each crop. The mutants required to determine the VCG of each isolate were developed but the mutants still need to be paired with known "tester-strains" to determine the VCG of each isolate. The completion of data analysis for the pathogenicity trials and VCG determination will be finished by spring 2018.

Objective 2. Test the hypothesis that *V. dahliae* isolates lose aggressiveness towards potato after serial infections of rotation crops: Potato, brown mustard ISCI 99, and baronesse barley were inoculated with three isolates of *V. dahlia* (653, 111, and 461) in a greenhouse, evaluated for disease symptoms, and *V. dahliae* was recovered from each plant. Isolates from each plant were then increased in the lab and used to inoculate the same plant (cycle 2). Each trial was repeated once. Ten replicates were used for each treatment to allow destructive sampling of each crop and thereby determine the earliest possible sampling time at which *V. dahliae* can be recovered. Inoculum is currently being quantified from each plant. Subsequent trials are expected to be completed by spring 2018.

To determine if brown mustard ISCI 99, baronesse barley, and potato select for specific strains of *V. dahliae* each crop was grown in potting media infested with one of eight different mixtures of *V. dahlia* isolates. Treatments for each crop included:

1. Mix of three strains: 653 (from potato), 111 (from mint) and 461 (from tomato)
2. Mix of two strains: 653 & 111
3. Mix of two strains: 653 & 461
4. Mix of two strains: 111 & 461
5. One strain: 653 (positive control)
6. One strain: 111
7. One strain: 461
8. Non-inoculated control (negative control)

To avoid confounding each treatment with inoculum density the inoculum density of each pot was adjusted to 20 colony-forming units (CFU) of *V. dahliae* /g of soil. Plants were grown in a greenhouse, evaluated for disease, and biomass or tuber yield were measured at harvest. Inoculum is currently being quantified from each plant. After the inoculum is quantified the strains that infected each crop will be identified by genotyping *V. dahliae* recovered from each plant.

Objective 3. Assess the efficacy of selected fungicides and *Penicillium oxalicum* mixed to a depth of 8 inches in soil bands in controlling Verticillium wilt and black dot of potato: To investigate inhibition of *Verticillium dahliae* (*Vd*) and *Colletotrichum coccodes* (*Cc*) populations, which cause Verticillium wilt and black dot of potato, respectively, by *Penicillium oxalicum* (*Pox*), seedlings approximately 10 cm tall were transplanted into 3.8 L pots with SunGro LC1 soilless potting mix. One week after transplanting, plants were pruned, leaving two stems per pot. *Pox*, isolated from potato stems and increased via solid-state fermentation, at 3.95 g of infested corn kernels per liter of soil was co-inoculated with either 3.95 g *Cc*- or *Vd*-infested sand per liter of potting mix. Positive controls containing *Cc* or *Vd* without *Pox* were included, as well as negative controls without either pathogen, both with and without *Pox*. Plants were harvested after all above-ground material had senesced. Tuber weights were recorded, disease severity was estimated from the percentage of 0.5 cm-thick cross sections of one stem from each pot plated on NPX appearing necrotic, and microsclerotia were counted visually using the dissecting microscope from a ground 10 cm section of the other stem from each pot. ANOVA was used to compare disease severity within stem cross-sections between the differing soil treatments. Paired t-tests were performed to compare tuber weights and microsclerotia counts between treatments with or without *Pox*, and between the positive and negative controls.

Objective 4. Evaluate conventional and biorational pesticides for their ability to suppress Verticillium wilt under field conditions.

In Idaho, two field experiments were performed at the Parma Research and Extension Center on Greenleaf silt loam soil. In both experiments, the previous crop was wheat and soil test showed a pH of 8.6 and contained 5 ppm NO_3-N and 6 ppm NH_4-N. The field was fertilized in the fall with 17N-80P-100K. There was an additional topdress application made at hilling, which consisted of 150N as ESN. The relatively low level of nitrogen application was an attempt to increase symptoms of Verticillium wilt.

Both trials were planted using cut certified Russet Norkotah Strain TX278 seed on May 4, 2017. The seed was treated with Emesto Silver fungicide immediately after it was commercially cut. The seed pieces were planted in 36" rows with 10" in-row spacing. The individual plots consisted of 6 rows wide (18 feet) by 40 feet long. The treatments were arranged in a randomized complete block design with 4 replications. Pesticide applications for weeds and insects followed the University of Idaho guidelines. The fields were irrigated to maintain 65-70% soil moisture using a solid-set sprinkler system.

To increase *Verticillium dahliae* populations, the field was inoculated prior to planting and again prior to hilling. The isolate for the first inoculation was obtained from an infected potato plant in Idaho. The second inoculation used an isolate obtained from a mint plant in Idaho. On both dates, the inoculum mixture was spread onto each plot using a hand-held fertilizer spreader and mechanically incorporated.

Biological Fungicide Trial

Two different commercial biological fungicides (Serenade ASO and Bio-Tam) were applied at 4 different rates/timings. The applications consisted of in-furrow and chemigation treatments as follows:

The treatments were as follows:

1- Non-treated check
2- Fumigated check (Vapam)
3- Bio-Tam in-furrow
4- Bio-Tam chemigated (low rate)
5- Bio-Tam in-furrow + chemigated (low rate)
6- Bio-Tam chemigated (high rate)
7- Serenade in-furrow
8- Serenade chemigated (low rate)
9- Serenade in-furrow + chemigated (low rate)
10- Serenade chemigated (high rate)

Fumigation with 40 gal/ac Vapam was carried out in the fall prior to planting on November 11, 2016. In-furrow applications were applied at planting using a CO_2 sprayer with two TeeJet 80015 nozzles operated at 25 psi to treat two rows at a time. Chemigation treatments were applied using a gasoline powered sprayer attached to a boom with eight flood jet nozzles spaced 24 apart operated at 25 psi. This method is designed to stimulate a center pivot irrigation with 0.1 in/acre. The Bio-Tam rates were 4.8oz/ac for in-furrow, 2.5lbs/ac for low rate chemigation, and 5lbs/ac for high rate chemigation. Serenade ASO rates consisted of 2qt/ac for in-furrow, 2qt for low rate chemigation, and 4qt for high rate chemigation. These rates were consistent with the label recommendation. The application dates were June 23, July 7, July 21 and August 4.

Soil and stem samples were taken prior to the first chemigation application and again after all of the applications were made. The samples were analyzed using a real-time polymerase chain reaction (qPCR) method to measure the amount of *Verticillium dahliae* and *Colletotrichum coccodes* DNA present in the soil and stem tissue.

Visual symptoms of Verticillium wilt were rated throughout June, July, and August. The visual ratings were based on a 0-5 scale that increased with symptoms of wilting and chlorosis in the plants. Disease severity over the season was measured by calculating the relative area under the disease progress curve (RAUDPC). A rating of three or higher was considered diseased and was used to calculate incidence of symptomatic plants. The plots were vine-killed with Reglone on August 28 and were harvested on September 12. For each plot the middle 2 rows X 20 feet were mechanically harvested for yield and grade.

Data were analyzed by ANOVA using the SAS statistical program. When the F-test was significant the means were separated using a LSD at the 5% level. Single degree of freedom contrasts were used to conduct planned comparisons as follows: biological fungicides vs check, chemigation vs in-furrow, and Bio-Tam vs Serenade ASO.

Traditional Fungicide Trial

Two commercial fungicides (Velum Prime and Elatus) were applied at different timings; and in combinations with Serenade ASO as follows:

1- Non-treated check

2- Serenade + Velum Prime in-furrow

3- Serenade + Velum Prime in-furrow fb Velum Prime chemigated

4- Elatus in-furrow

5- Serenade + Elatus in-furrow

In-furrow and chemigation applications were made using the same equipment as outlined above. Serenade ASO rate was 2qt/ac, Velum Prime 6.5 oz/ac, and Elatus was applied at 7.26 oz/ac. The Velum Prime chemigation treatment was applied once, near row closure on July 5.

Pathogen population sampling, visual disease ratings, harvest and grading procedures were the same as outlined above. Data were analyzed by ANOVA using the SAS statistical program. When the F-test was significant the means were separated using a LSD at the 5% level. Single degree of freedom contrasts were used to conduct planned comparisons as follows: fungicides vs check and Velum Prime vs Elatus.

In Hermiston, a field experiment was conducted at the Hermiston Agricultural Research and Extension Center (HAREC) south of Hermiston, Oregon. Fall tillage practices included discing twice and roller-harrowing. Wheat was used as a winter cover crop and was killed down with an herbicide application prior to spring tillage and planting. Prior to planting, the field was disced and roller-harrowed. *Verticllium dahliae* inoculum was used to infest potato seed, variety 'Russet Burbank', prior to planting and seed for each treatment (Table 11) was cut and pre-weighed prior to infestation.

To prepare inoculum for field trials, a *V. dahliae* isolate (VCG4A) originally isolated from potato, was cultured on rye seed. Briefly, rye seed was soaked in water for 16 hours and was autoclaved two times in Sun bags (Sigma-Aldrich, Ronkonkoma, NY) containing approximately 0.9 kg of rye seed. The seed was allowed to cool 24 hours between autoclaving. The autoclaved rye seed was allowed to cool to room temperature and was inoculated with PDA plugs containing actively growing cultures of *V. dahliae*. Inoculated rye seed was allowed to incubate at room temperature for approximately 6 weeks and bags was turned periodically to reduce bacterial contamination. After the incubation period, rye seed was dried on trays and homogenized in a coffee grinder. The amount of inoculum (CFU/g dry weight) was quantified by culture plating.

Furrows were opened with a commercial potato planter with the closing discs removed. Seed pieces of all experimental, standard, and control treatments were hand-planted into the open furrows. For plots that were infested with *V. dalhaie*, approximately 8 oz per of ground inoculum was sprinkled into the center two rows of each plot prior to row closure. The experiment included four replicates and was arranged as a randomized complete block design in the field. Seed pieces were spaced 12 inches apart within rows and potatoes were planted on 19 May. Rows were 34 inches apart. Four- row plots were 11.3 ft. wide by 20 ft. long, for a total of 0.0052 acres per plot. Six foot alleys separated replications.

In-furrow biorational chemical treatments were applied at planting with a CO_2 pressurized backpack sprayer operating at 30 psi with a 2 nozzle boom with Tee Jet 8001 flat fan nozzles delivering 12 gpa. In-furrow insecticides were applied in a 4-6" band over seed pieces in the 2 center rows.

Immediately after the in-furrow treatments were applied and all seed piece treatments were placed in the open furrows, all seed was covered by hilling. After planting, plots were maintained according to typical commercial practices (i.e. fertility, weeds, insects) conducted by HAREC staff. Chemigation treatments were applied using a chemigation simulator applying approximately 0.1 inch of water. Chemigation occurred on 7/6, 7/25, 8/3, and 8/18.

Data was collected from the two center rows of each plot. Plant senescence, as a measure of Verticillium wilt, was visually assessed in July and August on a Horsfall-Barratt scale (i.e., 1-12; 1 no senescence, 12 fully senescenced). *C. coccodes* and *V. dahliae* infection was assessed by culturing 5 cross sections from each main stem of the destructively sampled plants on Sorenson's medium. Cross sections were taken from the first 30 cm of stem above the soil line. Stem cross sections were assessed after 14 days by counting the number of cross sections with visible *C. coccodes* scelrotia and *V. dalhiae* microsclerotia. Destructive sampling of plants occurred on 11 July and disease assessment and culture plating occurred the following 2 days.

Potatoes were vine-killed with Reglone on 9 September and harvested on 29 September. Yield and grade of the different size classes was determined for the two center rows of each experimental replication (34' of row after destructive sampling). The trial was graded in the sorting shed in October.

Data were analyzed in R statistical environment and treatment means were separated using ANOVA with a Fisher's Protected Least Squared Difference (LSD) mean separation test (P=0.05). Data resulting from destructively sampled plants (i.e., data from subplot sampling) were aggregated to the plot level prior to conducting an ANOVA and mean separation.

RESULTS:

Objective 1:

Genotypic richness (i.e. the number of multilocus genotypes) and diversity (i.e. the number and abundance of multilocus genotypes, see *H'*, *G,* and corrected λ) were similar for *V. dahliae* populations from potato and asymptomatic crops (Table 2). Genotypic evenness (i.e. the frequency of genotypes, see E_5) was greater for the *V. dahliae* population from asymptomatic crops than for the population from potato (Table 2). All isolates from asymptomatic rotation crops were one mating-type and therefor the possibility of sexual recombination is low (Table 1). The *V. dahliae* population from asymptomatic crops was more similar to *V. dahliae* from potato than to populations from other crops (Figure 1). Additionally, the populations from potato and asymptomatic crops were not significantly differentiated (Table 3). *V. dahliae* genotypes from potato also co-occurred within the genotypes from asymptomatic crops (Figure 2). The origin of isolates from asymptomatic crops is most likely from potato crops grown in the same field soils and, consequentially, the isolates from potato did not arise from the isolates from asymptomatic crops (Figure 3). This claim is supported by our observation that isolates of *V. dahliae* retained the ability to cause disease on potato (Figures 4 & 5) but did not affect potato yield in a greenhouse (Figure 6). Additionally, the one-way movement of inoculum from potato to asymptomatic crops was more likely than the inverse and as likely as two-way inoculum movement between potato and the asymptomatic crops (Figure 7).

Together these results provide evidence that support the hypotheses that (i) *V. dahliae* from asymptomatic crops do not represent a new population or pathogen strain that may jeopardize potato production but rather (ii) evolved from the preexisting potato population and (iii) do not appear to be contributing significant inoculum to potato crops grown in subsequent soil. The marginal amounts of inoculum that the asymptomatic rotation crops may be contributing to potato crops may not increase disease pressure if that inoculum is composed of *V. dahliae* strains that are

less aggressive towards potato than most strains in potato production regions. This question will be resolved upon completion of the objective 2.

Objective 2.

Two cycles of serial inoculations of brown mustard ISCI 99, baronesse barley, and potato were completed and we determined that we could recover *V. dahliae* from infected plants 10-15 days after inoculation. Several issues stifled our progress for this objective but we are confident that we will complete the three remaining inoculations in the coming months (Figure 8) because of the availability of potato plantlets grown with tissue-culture techniques at University of Idaho, undergraduate laboratory assistance, minimal time needed for soil-drench inoculations, and our ability to recover *V. dahliae* from infected plants 10-15 days after inoculation.

When mustard, barley, and potato were grown in soil infested with multiple isolates of *V. dahliae* yield of potato but not mustard or barley was affected (Figure 9). Disease of potato plants grown in soils with a mix of three isolates was marginally greater in soil containing all three isolates, isolates 653 with 111, or isolate 653 alone than the other treatments, especially treatments with isolate 461 (Figure 10). This provides evidence that, under field conditions where inoculum is composed of potentially diverse populations of *V. dahliae*, inoculum is only as aggressive as the least aggressive isolate, unless present with two other aggressive isolates. Potential synergistic effects of multiple isolates on wilt are therefore negligible or too small to detect from this experiment. If this phenomenon is operating in commercial potato fields then disease is expected to be lower in fields with mixes of weak and highly aggressive isolates than in fields with only aggressive isolates. Finally, if the rotation crops select for and increase the least aggressive isolates then using rotation crops could reduce disease pressure in fields with heavy inoculum loads comprised of aggressive isolates by shifting the composition of *V. dahliae* populations from highly aggressive to weakly aggressive. We will determine if rotation crops select specific isolates of *V. dahliae* by spring of 2018.

Objective 3.

Inoculations with the potato pathogens were successful in eliciting high levels of disease severity in plants inoculated with each pathogen. Potatoes inoculated with *Cc* had significantly greater black dot severity than potatoes inoculated with *Vd* or the control (Figure 11), and potatoes inoculated with *Vd* had significantly greater Verticillium wilt severity than potatoes inoculated *Cc* or the control (Figure 12). Potatoes co-inoculated with *Cc* and *Pox* produced greatly differing microsclerotia counts from trial 1 to trial 2. In trial 1, there were no significant differences (Figure 13a), while in trial 2, there were significantly fewer microsclerotia than with *Cc* alone, with means of 1110.4/g and 3109.2/g ground potato stem tissue, respectively (P = 0.018, Figure 13b). However, no significant differences were found in *Vd* microsclerotia counts (Figure 14) between pots containing *Pox* and the control. No significant differences found in tuber weights of potatoes co-inoculated with *Pox* and *Cc* nor *Pox* and *Vd* (Table 4) compared with the positive controls. Disease severity within potato stem cross-sections between plants co-inoculated with *Pox* and *Cc* or *Pox* and *Vd* were not significantly different from the positive controls (Table 5).

In summary, *Pox* may be a valuable biocontrol agent to control black dot of potato. Potatoes co-inoculated with *Cc* and *Pox* exhibited significantly reduced *Cc* microsclerotia counts in one of two trials. Potatoes co-inoculated with *Cc* and *Pox* or with *Vd* and *Pox* exhibited slightly reduced disease severities, but these differences were not statistically significant. Further investigations into the efficacy of *Pox* as a biocontrol agent given higher inoculum loads and with larger pot sizes are underway in the greenhouse, and investigations in field applications are necessary.

Objective 4.

Biological Fungicide Trial

Pathogen populations: In Idaho, initial levels of *Verticillium dahliae* DNA in soil samples were very low and quite variable, but increased dramatically by the time of the post treatment sample near vine kill (Table 6). None of the biological fungicide treatments significantly reduced pathogen levels in soil compared to the non-treated check, regardless of application rate or timing.

V. dahliae levels in stem tissue were also relatively low and highly variable at the first sampling prior to the chemigation treatments, but increased 20,000 to almost 200,000 fold by the end of the season (Table 6). There was no evidence that any of the biological fungicide treatments significantly reduced pathogen levels in stem tissue compared to the non-treated check.

Soil and stem tissue levels of *Colletotrichum coccodes*, the causal agent of black dot disease, were also low and highly variable at the pre-treatment sampling, but increased greatly by the end of the season (Table 7). Similar to the results for *Verticillium*, there was no evidence that any biological fungicide treatment reduced pathogen populations of *Colletotrichum* in soil or stem tissue.

In the field trial at the HAREC, incidence and severity of stem colonization by *V. dalhiae* and *C. coccodes* did not differ among biorational treatments, the non-treated control or the non-infested/non-treated control (Table 13).

Visual symptoms: The plants started to show visual symptoms of wilting ad chlorosis typical of Verticillium wilt by the 3rd week of July, and had almost completely senesced by the 3rd week of August. RAUDPC values ranged from 17 to 26, indicating moderate disease pressure (Figure 15). None of the biological fungicide treatments significantly reduced disease progression compared to the non-treated check. However, it should be pointed out that fumigation with Vapam (grower standard treatment) also did not reduce visual symptoms of Verticillium wilt, indicating that other pathogens may have been responsible for the disease symptoms in this trial. In Hermiston, disease symptoms, measured as plant senescence, did not differ among biorational treatments on any assessment date or when aggregated over the season (Table 12).

Yield and grade: Tuber yields ranged from 26 to 27.7 t/ac, which is typical for Russet Norkotah in this region of ID (Table 8). None of the biological fungicide treatments significantly influenced total tuber yield, regardless of application rate or timing. Likewise, tuber size distribution and specific gravity were not influenced by biological fungicide treatments (Table 8). In Hermiston, yields averaged 30.8 tons per acre. Biorational pesticide applications did not significantly affect total potato yields (Table 14). There were no treatment differences in yields within the < 4 oz., 4-8 oz., 8-12 oz., and > 12 oz. size categories (Table 15) although there were differences among treatment yields within the cull category of potato.

Traditional Fungicide Trial

Pathogen populations: Levels of both *Verticillium dahliae* and *Colletotrichum coccodes* DNA in stem samples were initially low, but increased dramatically by the end of the season (Table 9). While almost all of the traditional fungicide treatments had numerically lower pathogen levels at the end of the season compared to the non-treated check, there were no significant differences due to the large amount of variability among samples.

Visual symptoms: RAUDPC values ranged from 12 to 19, indicating moderate disease pressure (Figure 16). The single degree of freedom contrast indicated that the fungicide treatments significantly (P = 0.0421) reduce disease progression compared to the non-treated check. However, there was no significant differences between the Velum Prime and Elatus treatments.

Yield and grade: Tuber yields ranged from 26.5 to 29.2 t/ac (Table 10). While all of the traditional fungicide treatments had numerically higher total tuber yield compared to the non-treated check (averaging 1.3 t/ac), there were no significant differences.

The fungicide treatments also tended to increase > 6 oz tuber size distribution and specific gravity compared to the non-treated check, but these differences were also not significant.

PROJECTIONS:

In two years of trials during 2016 and 2017 we have not seen any significant reduction in pathogen levels, visual disease symptoms or impact on tuber yield and size from applications of Bio-Tam and Serenade. This indicates that this approach to control of Verticillium wilt does not work consistently under the disease pressure and environmental conditions present in southwest Idaho or the South Columbia Basin. In contrast, Velum Prime and Elatus showed some potential for reducing disease symptoms in Idaho, and tended to improve yield and tuber size under moderate disease pressure. These products should be evaluated again and in multiple locations, both alone and in combination with Serenade or other biological fungicides to confirm these results.

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- Frederick, Z. Cummings, T. and Johnson, D. 2017. Weeds can pose additional threat to potato production. Potato Country – July/August. 3 pp.
- Johnson, D.A. 2017. Managing late blight during late season and into storage. Potato Progress XVII, No 13, 2pp.
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- Frederick, Z.A., and Johnson, D.A. 2017. Specific weed increase Verticillium inoculum in potato fields. Potato Country.
- Frederick, Z.A., Cummings, T.F., and Johnson, D.A. 2017. Susceptibility of weeds from potato production systems to potato aggressive isolates of *Verticillium dahliae*. Potato Progress XVII No.4, 10pp.
- DeLisle, Michelle, and Johnson, D.A. 2017. Gaining an insight into a widespread potato disease. Potatoes Australia. Feb-Mar, pg 26.
- Objectives 1 & 2 will be submitted for publication as 2-3 manuscripts in *Molecular Ecology* or *Phytopathology*

PRESENTATIONS & REPORTS:

- Alternatives to fumigation. Idaho Potato Conference, Pocatello, ID, January 19, 2017.

- Drip irrigation and biofungicides for potato production, Parma Research and Extension Center Field Day, Parma, ID, June 21, 2017.

- The influence of biopesticides on Verticillium wilt of potatoes. Potato Association of American Annual Meeting, Fargo, ND, July 24, 2017.

- Management of soil borne diseases in onions and potatoes. University of Idaho Pesticide License Recertification Course, Caldwell, ID, December 13, 2017.

- Objectives 1 & 2 will be presented at the WA OR Potato conference, the International Congress of Plant Pathology, and the Potato Association of America meeting or the Pacific Division of the American Phytopathological Society meeting.

Table 1. Isolates of *Verticillium dahliae* from different crops, the number of samples (N), the number of multilocus genotypes (MLGs), the mating-type, and vegetative compatibility group (VCG).

Hosts	Crops	N	MLG	Mating Type	VCG
Symptomatic					
	Potato	174	42	*MAT1-2*	4A,4B,4A/B,2A/B
	Mint	117	18	*MAT1-2*	2B.2A/B
	Other	58	29	*MAT1-2 & MAT1-1*	1,3,2A,2B,2A/B,4A,4B
	Total	349	89		
Asymptomatic					
	Mustards	107	27	*MAT1-2*	TBD
	Grasses	15	8	*MAT1-2*	TBD
	Total	122	35		
All	Total	471	107		

Table 2. Genetic richness, diversity, and evenness of *Verticillium dahliae* recovered from symptomatic and asymptomatic crops. Summary statistics include the number of samples (N), the number of multilocus genotypes (MLG) (i.e.richness), the expected number of MLGs from the smallest rarefied sample ≥ 10 (eMLG), Nei's unbiased gene diversity (H_{exp}), Shannon-Wiener index of MLG diversity (*H'*), Stoddart-Taylor index of MLG diversity (*G*), the corrected Simpson index (corrected λ), and evenness (E_5). Clone-corrected data (where each genotype is represented only once) is represented in parentheses.

Crops	N	MLG	eMLG	H_{exp}	*H'*	*G*	Corrected λ	E_5
Potato	174	42	8 ± 2	0.44	2.45	4.71	0.78	0.35
			(10 ± 0.0)	(0.59)	(3.74)	(42)	(0.97)	
Mint	117	18	4 ± 1	0.18	1.09	1.61	0.38	0.31
			(10 ± 0.0)	(0.66)	(2.89)	(18)	(0.94)	
Other	58	29	10 ± 2	0.74	2.88	10.51	0.91	0.56
			(10 ± 0.0)	(0.76)	(3.37)	(29)	(0.96)	
Mustards	107	27	8 ± 2	0.28	2.50	6.99	0.85	0.53
			(10 ± 0.0)	(0.33)	(3.30)	(27)	(0.96)	
Grasses	15	8	8 ± 1	0.31	1.93	6.08	0.83	0.85
			(8 ± 0.0)	(0.37)	(2.08)	(8)	(0.87)	
Total	471	107	10 ± 2	0.69	3.26	10.02	0.90	0.36
			(9.8 ± 0.0)	(0.68)	(4.62)	(93.8)	(0.98)	

Table 3. Analysis of molecular variance (AMOVA) of populations of *Verticillium dahliae* recovered from potato and asymptomatic crops including mustards and grasses. Raw and clone-corrected data, where each genotype is used only once, were used. This table is interpreted like an analysis of variance (ANOVA). For example, there is very weak evidence ($P = 0.332$) that *V. dahliae* from potato and asymptomatic crops are different because most genetic variability is within crops and samples ($P = 0.0001$).

Source of variation	df	Sum of squares	Variance	Variance explained (%)	*P*-value
Among crops [a]	1	181.53	0.43	13.24	0.332
Among samples within crops	1	36.13	0.92	28.33	0.0001
Within samples	293	556.62	1.89	58.42	0.0001
Total	295	764.30	3.25	100	.
Among crops [b]	1	15.17	0.01	0.52	0.332
Among samples within crops	1	7.06	0.39	15.42	0.0004
Within samples	74	160.12	2.16	84.04	0.0001
Total	76	182.35	2.57	100	.

[a] Raw data and [b] clone-corrected data

Table 4. Mean tuber weights per potato plant. P-values are from paired t-tests comparing each pathogen treatment co-inoculated with *Penicillium oxalicum* (*Pox*) (Y) or without *Pox* (N).

Pox	Pathogen	Tuber Weight	P-value
N	*Cc*	287.17	0.68
Y	*Cc*	272.10	0.68
N	Ctrl	269.66	0.97
Y	Ctrl	268.87	0.97
N	*Vd*	291.61	0.67
Y	*Vd*	270.57	0.67

Table 5. Mean severities of black dot and Verticillium wilt. P-values are from paired t-tests comparing each pathogen treatment co-inoculated with *Penicillium oxalicum* (*Pox*) (Y) or without *Pox* (N).

Pox	Pathogen	Black Dot	P-value	Verticillium wilt	P-value
N	*Cc*	0.58	0.41	0.00	0.34
Y	*Cc*	0.42	0.41	0.01	0.34
N	Ctrl	0.05	0.06	0.03	0.78
Y	Ctrl	0.37	0.06	0.02	0.78
N	*Vd*	0.07	0.88	0.44	0.46
Y	*Vd*	0.08	0.88	0.32	0.46

Table 6. Influence of biological fungicide applications on *Verticillium dahliae* levels in soil and stem samples taken pre and post application as determined by qPCR. The values are means of 4 reps.

Treatment		*V. dahliae* soil pre-trt (pg/g)	*V. dahliae* soil post-trt (pg/g)	*V. dahliae* stem pre-trt (pg/g)	*V. dahliae* stem post-trt (pg/g)
Non-treated	1	0	25	1	1012648
Fumigated check	2	2	609	1	1674898
Bio-Tam in-furrow (IF)	3	0	101	0	105813
Bio-Tam chemigated low (CL)	4	1	773	8	313335
Bio-Tam IF + CL	5	0	8	35	728965
Bio-Tam chemigated high (HL)	6	0	800	0	316183
Serenade in-furrow (IF)	7	1	11	1	822023
Serenade chemigated low (CL)	8	0	588	0	3917565
Serenade IF + CL	9	2	0	23	1547560
Serenade chemigated high (HL)	10	1	525	3	660380
		ns^	ns	ns	ns
Contrasts					
Non-treated		0 ^{ns}	25 ^{ns}	1 ^{ns}	1012648 ^{ns}
Biological fungicides		1	351	9	1051478
In-furrow		1 ^{ns}	30 ^{ns}	15 ^{ns}	801090 ^{ns}
Chemigated		1	671	3	1301866
Bio-Tam		0 ^{ns}	420 ^{ns}	11 ^{ns}	366074 ^{ns}
Serenade ASO		1	281	6	1736882

^NS = main effect or single degree contrast not significant at the P=0.05 level.

Table 7. Influence of biological fungicide applications on *Colletotrichum coccodes* levels in soil and stem samples taken pre and post application as determined by qPCR. The values are means of 4 reps.

Treatment		*C. coccodes* soil pre-trt (pg/g)	*C. coccodes* soil post-trt (pg/g)	*C. coccodes* stem pre-trt (pg/g)	*C. coccodes* stem post-trt (pg/g)
Non-treated	1	380	1218	106	37905
Fumigated check	2	628	1305	198	28743
Bio-Tam in-furrow (IF)	3	173	4388	23	35865
Bio-Tam chemigated low (CL)	4	863	1455	360	24425
Bio-Tam IF + CL	5	83	1960	1051	36970
Bio-Tam chemigated high (HL)	6	166	3938	318	28793
Serenade in-furrow (IF)	7	150	2423	62	27080
Serenade chemigated low (CL)	8	588	4373	4268	29523
Serenade IF + CL	9	65	5970	43	35150
Serenade chemigated high (HL)	10	358	2948	166	13533
		ns^	ns	ns	ns
Contrasts					
Non-treated		380 ns	1218 ns	106 ns	37905 ns
Biological fungicides		305	3432	786	28917
In-furrow		117 ns	3685 ns	295 ns	33766 ns
Chemigated		493	3179	1278	24069
Bio-Tam		321 ns	2935 ns	438 ns	31513 ns
Serenade ASO		290	3929	1135	26322

^NS = main effect or single degree contrast not significant at the P=0.05 level.

Table 8. The influence of biological fungicide applications on yield, size distribution and specific gravity of Russet Norkotah Strain 278 potatoes grown at Parma, ID in 2017. The values are means of 4 reps.

Treatment		Total yield (t/ac)	Over 6 oz (%)	Over 10 oz (%)	Specific gravity
Non-treated	1	26.5	67	34	1.075
Fumigated check	2	27.7	66	25	1.079
Bio-Tam in-furrow (IF)	3	26.6	74	38	1.079
Bio-Tam chemigated low (CL)	4	26.3	69	30	1.077
Bio-Tam IF + CL	5	26.3	69	29	1.076
Bio-Tam chemigated high (HL)	6	26.1	71	37	1.075
Serenade in-furrow (IF)	7	27.6	68	30	1.079
Serenade chemigated low (CL)	8	26.6	71	33	1.078
Serenade IF + CL	9	26.0	68	32	1.077
Serenade chemigated high (HL)	10	25.8	69	33	1.076
		ns^	ns	ns	ns
Contrasts					
Non-treated		26.5 ns	67 ns	34 ns	1.075 ns
Biological fungicides		26.4	70	33	1.077
In-furrow		26.6 ns	69 ns	32 ns	1.078 ns
Chemigated		26.2	70	33	1.077
Bio-Tam		26.4 ns	70 ns	33 ns	1.077 ns
Serenade ASO		26.5	69	32	1.078

^NS = main effect or single degree contrast not significant at the P=0.05 level.

Table 9. Influence of Velum Prime, Elatus and Serenade ASO fungicide applications on levels of *Verticillium dahliae* and *Colletotrichum coccodes* in stem samples taken pre and post application as determined by qPCR. The values are means of 4 reps.

Treatment		*V. dahliae* stem pre-trt (pg/g)	*V. dahliae* stem post-trt (pg/g)	*C. coccodes* stem pre-trt (pg/g)	*C. coccodes* stem post-trt (pg/g)
Non-treated	1	1	1012648	106	37905
Serenade + Velum Prime in-furrow	2	0	164698	830	28623
Serenade + Velum Prime in-furrow fb Velum Prime chem	3	14	983963	78	18945
Elatus in-furrow	4	1	225720	63	12138
Serenade + Elatus in-furrow	5	1	581515	1285	18165
		ns	ns	ns	ns
Contrasts					
Non-treated		1 ns	1012648 ns	106 ns	37905 ns
Fungicides		4	488974	564	19468
Velum Prime		7 ns	574331 ns	454 ns	23784 ns
Elatus		1	403618	674	15152

^NS = main effect or single degree contrast not significant at the P=0.05 level.

Table 10. The influence of Velum Prime, Elatus and Serenade ASO fungicide applications on yield, size distribution and specific gravity of Russet Norkotah Strain 278 potatoes grown at Parma, ID in 2017. The values are means of 4 reps.

Treatment		Total yield (t/ac)	Over 6 oz (%)	Over 10 oz (%)	Specific gravity
Non-treated	1	26.5	67	34	1.075
Serenade + Velum Prime in-furrow	2	27.4	74	40	1.076
Serenade + Velum Prime in-furrow fb Velum Prime chem	3	29.2	70	35	1.078
Elatus in-furrow	4	27.9	69	36	1.079
Serenade + Elatus in-furrow	5	26.8	66	31	1.076
		ns^	ns	ns	ns
Contrasts					
Non-treated		26.5 ns	67 ns	34 ns	1.075 ns
Fungicides		27.8	70	35	1.077
Velum Prime		28.3 ns	72 ns	37 ns	1.077 ns
Elatus		27.3	67	33	1.078

^NS = main effect or single degree contrast not significant at the P=0.05 level.

Table 11. List of treatment, products, and application rates and timings.

Treatment	Infested	Rate	Application[a]
1	N	NA	NA
2	Y	NA	NA
3	Y	BioTam 4.8oz	IF
4	Y	BioTam 2.5lbs	CG
5	Y	BioTam 4.8oz (IF); 2.5lbs (CG)	IF, CG
6	Y	BioTam 5.0lbs	CG
7	Y	Sernade 2qt	IF
8	Y	Sernade 2qt	CG
9	Y	Sernade 2qt (IF); 2qt (CG)	IF, CG
10	Y	Sernade 4qt	CG
11	Y	SoilGard 2lbs	IF
12	Y	SoilGard 2lbs	CG
13	Y	SoilGard 2lb (IF); 2lbs (CG)	IF, CG
14	Y	SoilGard 4lbs	CG
15	Y	RhizoPro 43 Gal (1); 20 Gal (2-4)	CG
16	Y	RhizoPro 22 Gal (1); 10 Gal (2-4)	CG

[a] IF = In-furrow at planting; CG = Chemigation. Chemigation treatment were applied on 7/6, 7/25, 8/3, and 8/18.

Table 12. Effect of biorational chemical treatment on visual disease rating.

		Disease						
Infest.	**Rate**	**7-Jul**	**17-Jul**	**3-Aug**	**18-Aug**	**6-Sept**	**Average**	**AUSPC**
N	NA	0.0	0.3	0.8	2.8	9.0	2.6	148
Y	NA	0.0	0.0	0.8	2.3	9.5	2.5	141
Y	BioTam IF	0.0	0.0	1.5	2.8	8.3	2.5	158
Y	BioTam LO	0.0	0.3	1.8	5.0	9.3	3.3	204
Y	BioTam IF LO	0.0	0.5	0.3	3.0	9.8	2.7	154
Y	BioTam HI	0.0	0.3	0.8	3.3	9.0	2.7	156
Y	Sernade IF	0.0	0.0	2.0	4.5	10.5	3.4	208
Y	Sernade LO	0.0	0.0	1.5	4.8	10.0	3.3	200
Y	Sernade IF LO	0.0	0.3	0.3	2.8	8.0	2.3	130
Y	Sernade HI	0.0	0.0	0.8	3.5	9.8	2.8	164
Y	SoilGard IF	0.0	0.0	0.3	2.8	8.0	2.2	127
Y	SoilGard LO	0.0	0.3	1.0	3.5	9.3	2.8	167
Y	SoilGard IF LO	0.0	0.3	0.3	3.3	9.5	2.7	153
Y	SoilGard HI	0.0	0.3	0.8	3.8	9.5	2.9	169
Y	RhizoPro HI	0.0	0.3	0.5	2.8	9.8	2.7	151
Y	RhizoPro LO	0.0	0.0	1.5	4.0	10.5	3.2	192
	LSD	NS	NS	NS	NS	NS	NS	NS
	F (P-value)	0.00	0.84	0.29	0.38	0.52	0.27	0.23

Table 13. Effect of chemical treatment on the incidence and severity of potato stem colonization by V. dalhiae and C. coccodes

Infest.	Rate	*V. dahlaie* Stem Incidence	*V. dahlaie* Stem Severity	*C.coccodes* Stem Incidence	*C.coccodes* Stem Severity
N	NA	0.3	0.1	0.9	0.4
Y	NA	0.4	0.2	0.8	0.5
Y	BioTam IF	0.1	0.0	0.5	0.2
Y	BioTam LO	0.3	0.1	0.8	0.5
Y	BioTam IF LO	0.6	0.3	0.6	0.3
Y	BioTam HI	0.4	0.2	0.7	0.4
Y	Sernade IF	0.4	0.1	0.8	0.5
Y	Sernade LO	0.4	0.1	0.8	0.4
Y	Sernade IF LO	0.5	0.2	0.8	0.5
Y	Sernade HI	0.4	0.3	0.7	0.5
Y	SoilGard IF	0.4	0.3	0.8	0.5
Y	SoilGard LO	0.3	0.2	1.0	0.6
Y	SoilGard IF LO	0.5	0.2	0.8	0.5
Y	SoilGard HI	0.5	0.4	0.8	0.5
Y	RhizoPro HI	0.4	0.3	0.8	0.5
Y	RhizoPro LO	0.3	0.2	0.6	0.4
	LSD	NS	NS	NS	NS
	F (P-value)	0.87	0.83	0.70	0.96

Table 14. Effect of biorational chemical treatment on the total potato yield.

		Yield (Tons per Acre)	
Infest.	Rate	**Total**	**Total- No Culls**
N	NA	34.3	32.0
Y	NA	31.4	28.3
Y	BioTam IF	29.6	28.3
Y	BioTam LO	27.9	26.5
Y	BioTam IF LO	28.3	27.1
Y	BioTam HI	30.5	29.2
Y	Sernade IF	26.7	24.7
Y	Sernade LO	30.3	28.9
Y	Sernade IF LO	33.4	31.3
Y	Sernade HI	29.7	27.5
Y	SoilGard IF	33.4	31.7
Y	SoilGard LO	27.5	26.7
Y	SoilGard IF LO	31.9	30.4
Y	SoilGard HI	35.7	34.4
Y	RhizoPro HI	33.1	31.0
Y	RhizoPro LO	29.7	28.7
	LSD	NS	NS
	F (P-value)	0.83	0.86

Table 15. Effect of biorational chemical treatments on the yields of different size categories of potato.

		Yields (Tons per Acre)					
Infest.	Rate	**Culls**		**< 4 oz.**	**4-8 oz.**	**8-12 oz.**	**> 12 oz.**
N	NA	2.3	ab	4.4	13.6	6.7	7.3
Y	NA	3.0	a	4.1	12.2	7.5	7.2
Y	BioTam IF	1.3	bcd	3.8	9.2	8.1	7.1
Y	BioTam LO	1.4	bcd	3.7	10.4	7.2	7.0
Y	BioTam IF LO	1.3	bcd	2.9	11.5	6.2	6.8
Y	BioTam HI	1.3	bcd	4.9	12.0	7.7	6.7
Y	Sernade IF	2.0	abc	3.3	10.6	5.7	6.7
Y	Sernade LO	1.4	bcd	4.0	13.2	8.2	6.5
Y	Sernade IF LO	2.1	abc	3.8	10.9	9.4	6.1
Y	Sernade HI	2.2	ab	3.8	9.4	7.6	6.0
Y	SoilGard IF	1.8	bcd	4.2	12.4	8.4	5.3
Y	SoilGard LO	0.8	d	3.1	9.6	7.2	5.1
Y	SoilGard IF LO	1.6	bcd	4.4	11.8	7.2	4.6
Y	SoilGard HI	1.3	bcd	4.5	14.8	8.9	4.5
Y	RhizoPro HI	2.1	abc	3.5	12.8	8.8	3.6
Y	RhizoPro LO	1.0	cd	4.6	13.8	7.2	3.2
	LSD	1.12		NS	NS	NS	NS
	F (P-value)	0.029		0.11	0.19	0.76	0.3

Figure 1. The genetic similarity of *Verticillium dahliae* from asymptomatic mustards and grasses, potato and other crops is displayed. Each group of isolates is represented by a color and circumscribed by an ellipse. The closer the points the more genetically similar the isolates.

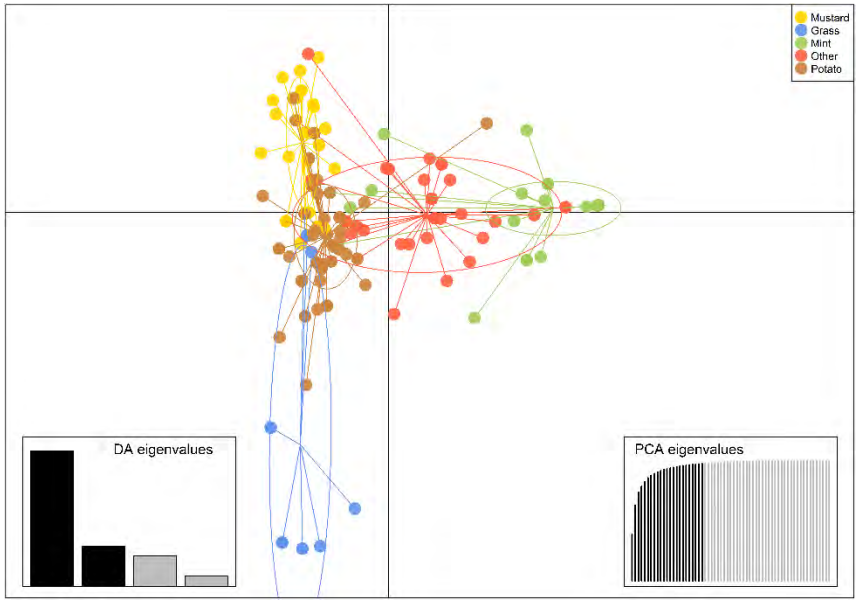


Figure 2. The relatedness of *Verticillium dahliae* recovered from asymptomatic mustards and grasses, potato and other crops. Each circle represents a group of multilocus genotypes (MLGs) with a size proportional to each sample size. The co-occurrence of two or more crop colors within one circle means that isolates recovered from those crops share the same MLG. For example, isolates from potato share several MLGs with mustards and grasses.

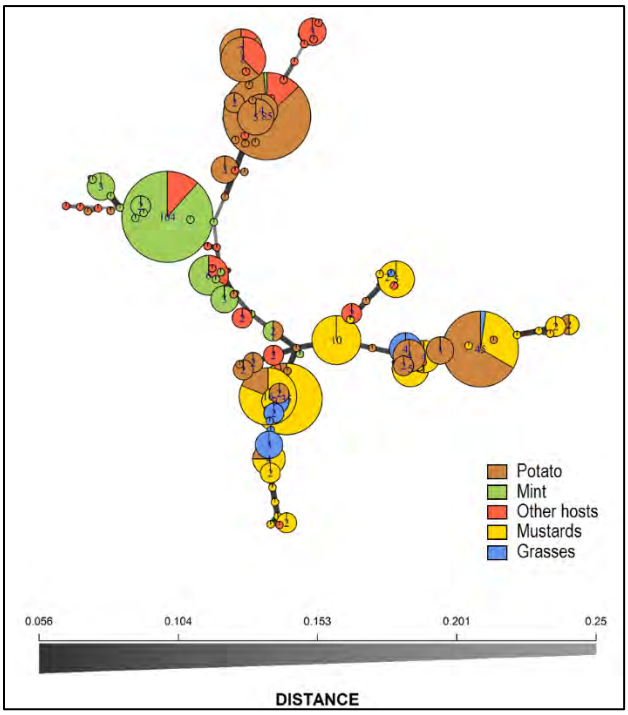


Figure 3. The two possible evolutionary scenarios that lead to the emergence of asymptomatic infections of rotation crops were tested. Scenario A describes the isolates from asymptomatic crops (in black) evolving from the isolates from potato (in grey) when the two groups diverged at some historical time (t) before being sampled at present time (t0). Scenario B describes the isolates from potato evolving from the isolates from the asymptomatic crops. The posterior-probability (PP) of the scenario, credibility intervals (C.I), and type 1 (false-positives) and type 2 error rates (false-negatives) are presented. Scenario A is more likely than scenario B, given the higher PP and the C.I that does not envelope the C.I of scenario B

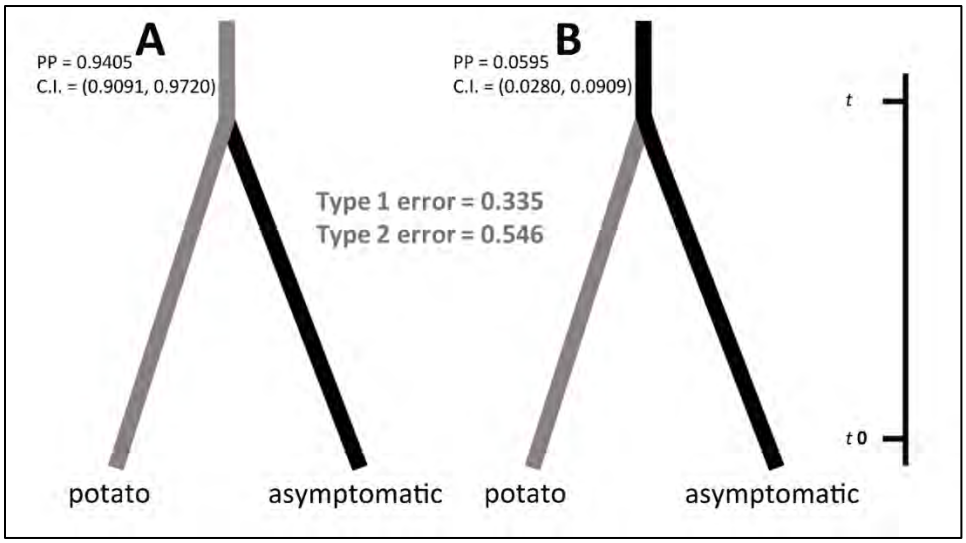


Figure 4. A representative subset of n = 16 isolates of *Verticillium dahliae* recovered from asymptomatic mustards and grasses caused disease on potato (left) and produced microsclerotia (black specks) on mustards (right) without causing observable symptoms.

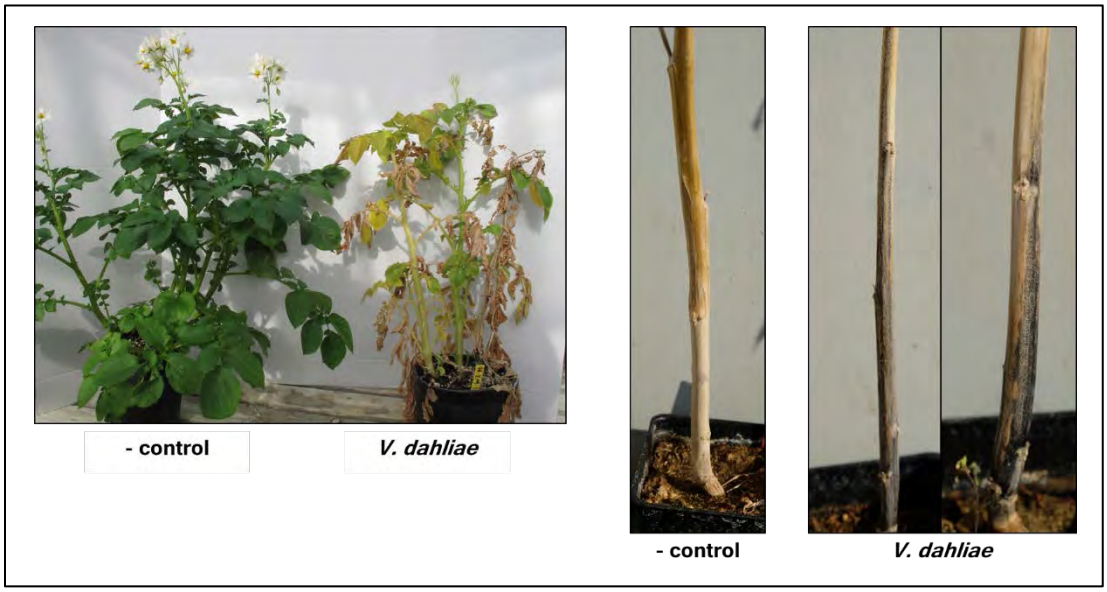


Figure 5. Yields of potato, mustard, and barley crops inoculated by sixteen isolates of *Verticillium dahliae* recovered from potato (isolate 653) and asymptomatic grasses (isolate ZmVd-1) and mustards (all other isolates). Yield was not significantly affected by any isolate.

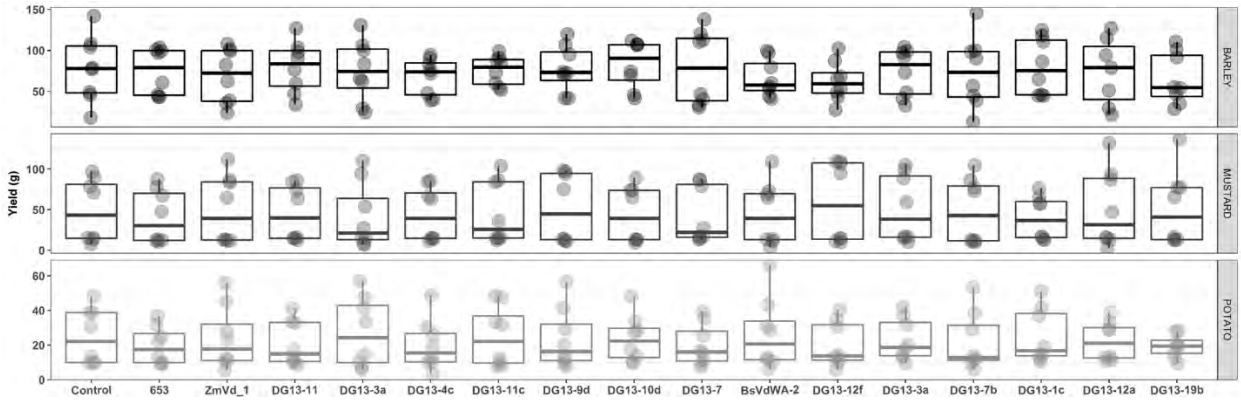


Figure 6. Disease, as reported by the area under the senescence progress curve (AUSPC), is presented for potato plants inoculated with a known pathogenic isolate (653) and sixteen isolates recovered from asymptomatic mustards and grasses caused disease on potato. The letters above each boxplot signify the ranking of means from greatest to lowest, from a to f. Treatments that share a letter(s) are not statistically significant.

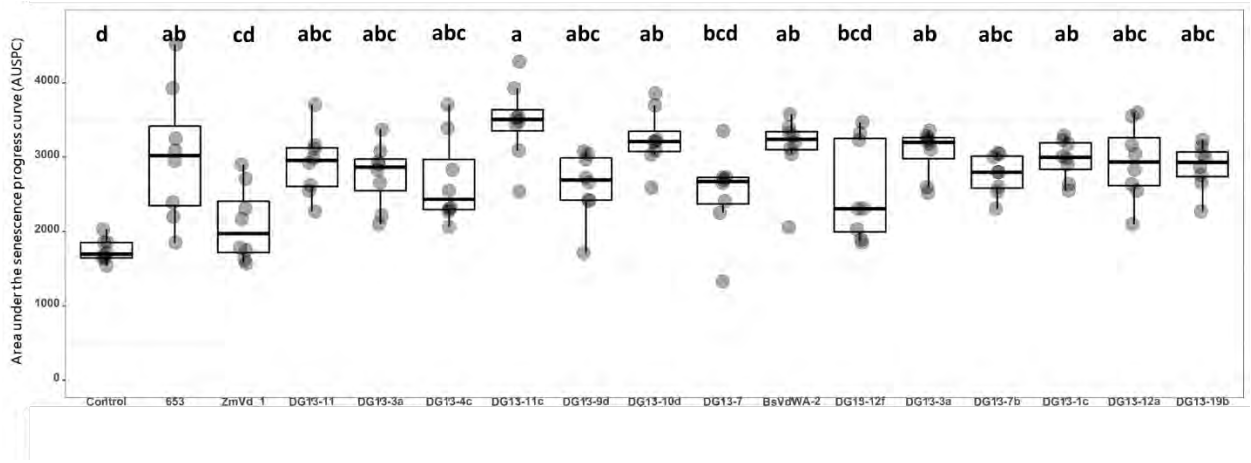


Figure 7. Three possible scenarios of inoculum movement were evaluated. Each scenario is displayed on the x-axis of the bottom-most boxplot image. From left to right, the first scenario describes the two-way movement of *Verticillium dahliae* inoculum to and from potato (large grey circle) and the asymptomatic crops (smaller black circle), the second scenario describes the onew-way movement of inoculum from potato to the asymptomatic crops, and the third scenario describes the one-way movement of inoculum from the asymptomatic crops to potato. The bottom plot displays the estimated size of the population (θ), the middle plot displays the migration rate or inoculum movement (M), and the top plot displays the log-likelihood (LnL) of each scenario where larger likelihoods are more likely scenarios. Each dot represents a different replicate of the analysis, completed 5x/scenario. The first two scenarios are the most likely therefor it is unlikely that asymptomatic rotation crops are contributing significant amounts of inoculum to subsequent potato crops.

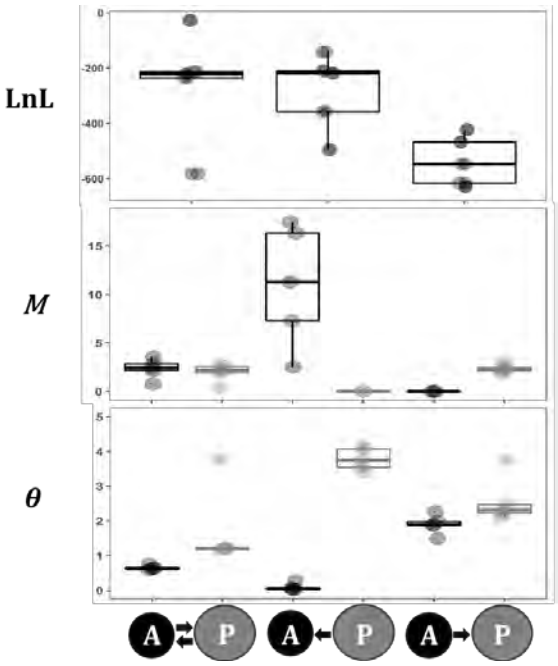


Figure 8. The design of the serial inoculations experiment where potato, mustard, and barley are all inoculated with three isolates (only one show) of *Verticillium dahliae*. After each cycle (T_n) isolates are recovered from each crop and used to inoculate the same crop for a total of five cycles. After the fifth cycle all isolates will be used to inoculate potato. We predict that isolates recovered from mustard and barley after 3-5 cycles will cause less disease on potato than isolates recovered from potato.

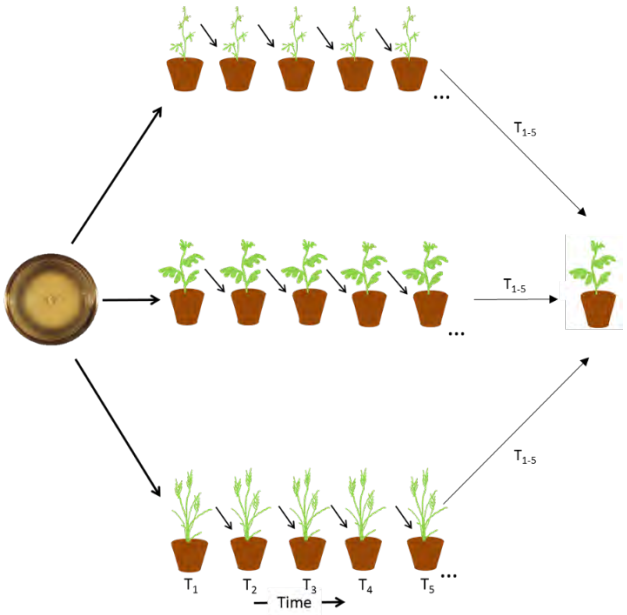


Figure 9. Yields of potato, mustard, and barley grown in soil infested with mixes of three, two, or one isolate of *Verticillium dahliae.* Yield was only affected in potato plants where non-inoculated control plants had significantly greater yields than all inoculated treatments.

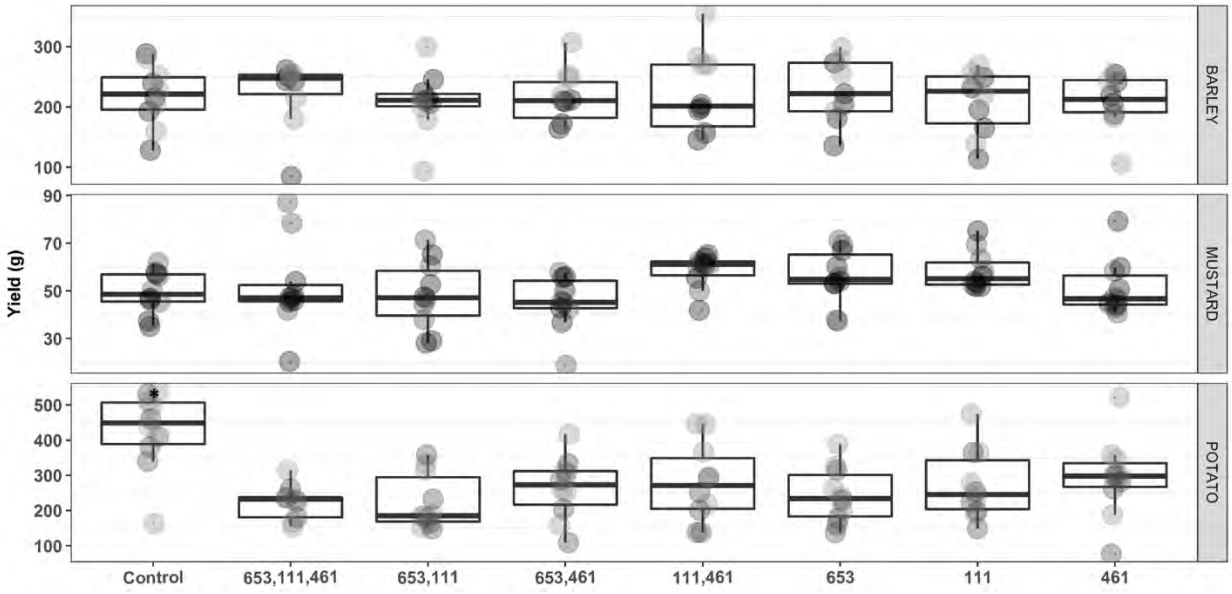


Figure 10. Disease, as reported by the area under the senescence progress curve (AUSPC), varied among potato plants grown in soil infested with mixes of three, two, or one isolate of *Verticillium dahliae*. The letters above each boxplot signify the ranking of means from greatest to lowest, from a to c. Treatments that share a letter(s) are not statistically significant.

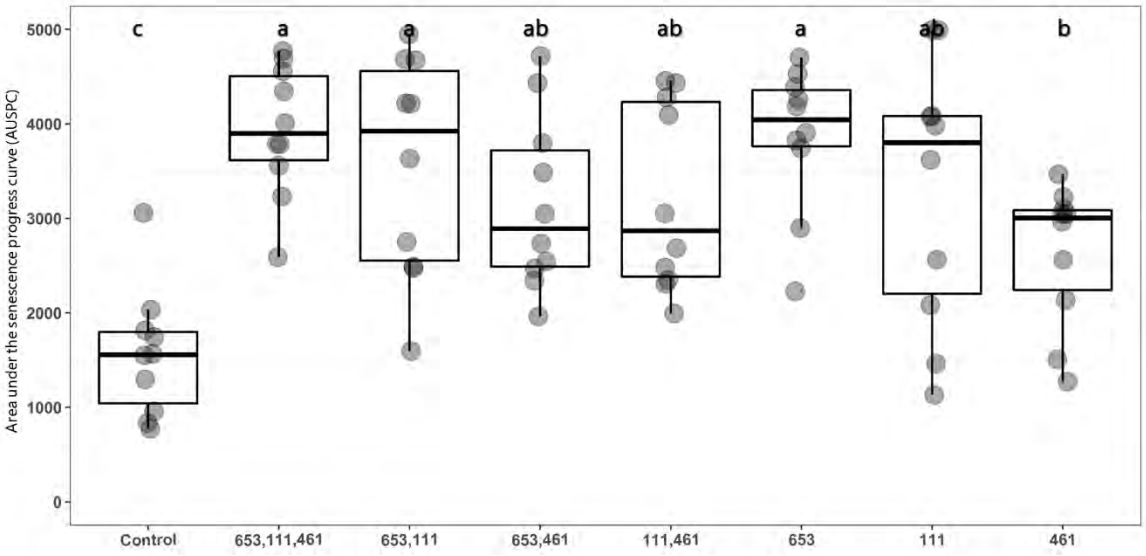


Figure 11. Mean black dot of potato severity, given inoculation with *Colletotrichum coccodes* (Cc), *Verticillium dahliae* (VD), or neither (Ctrl).

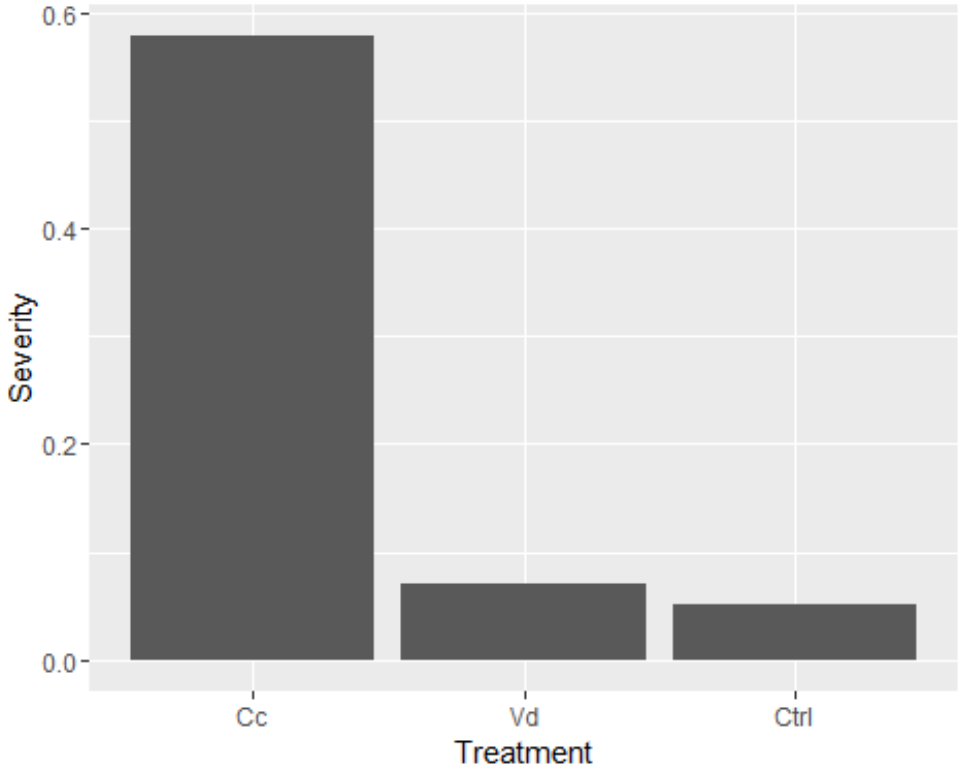


Figure 12. Mean Verticillium wilt of potato severity, given inoculation with *Colletotrichum coccodes* (Cc), *Verticillium dahliae* (VD), or neither (Ctrl).

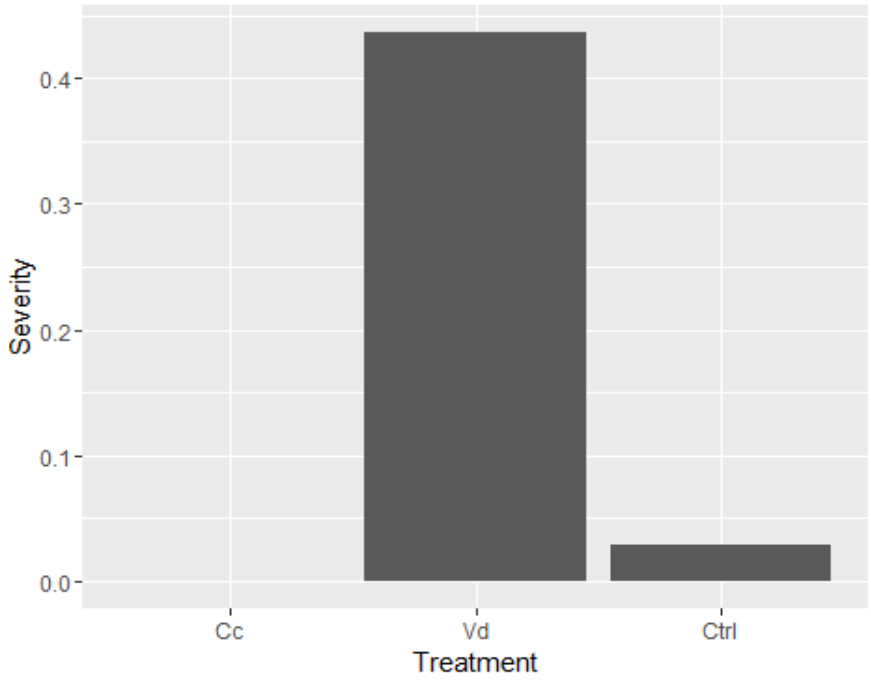


Figure 13. *Colletotrichum coccodes* microsclerotia per 0.4 g ground potato stem tissue plated on NPX, from pots co-inoculated with *Penicillium oxalicum* (P ox) compared with the control (Ctrl), trials 1 (a) and 2 (b).

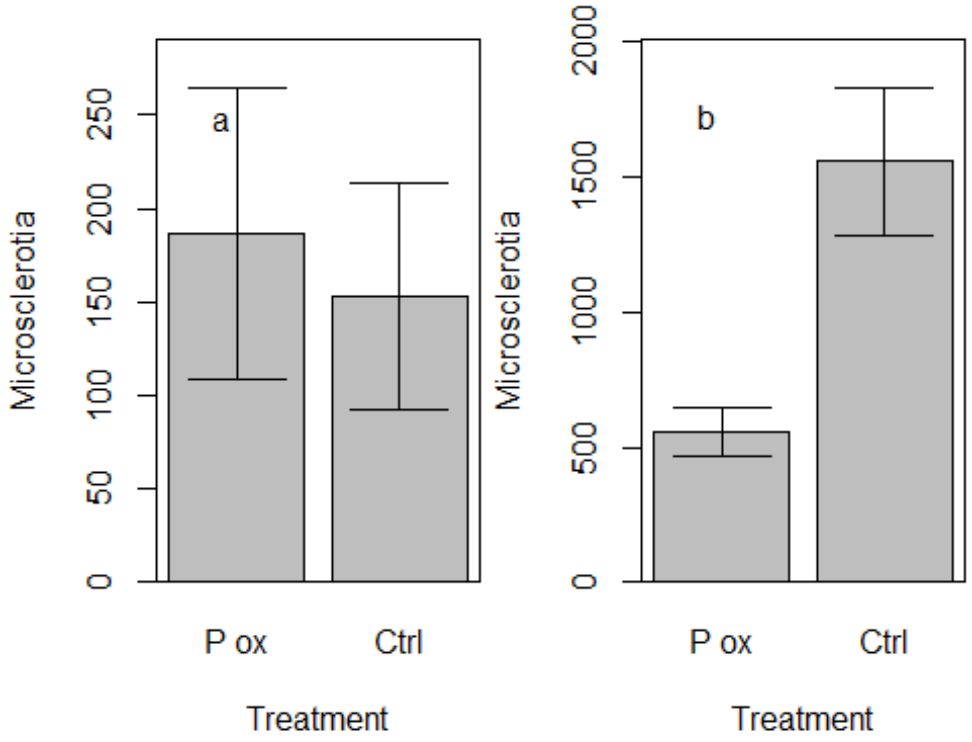


Figure 14. Mean *Verticillium dahliae* microsclerotia per 0.4 g ground potato stem tissue plated on NPX, form pots co-inoculated with *Penicillium oxalicum* (P ox) compared with the control (Ctrl).

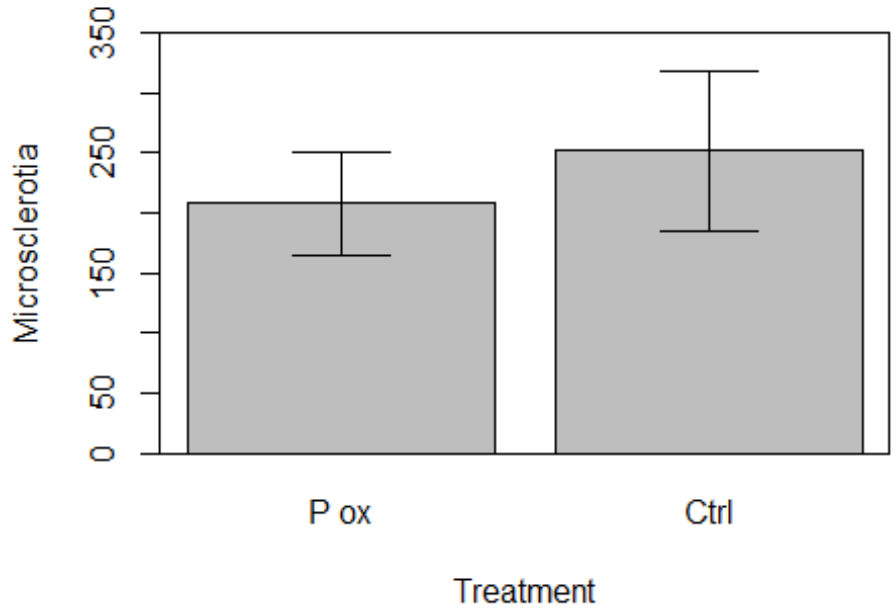


Figure 15. The influence of biological fungicide applications on relative area under the disease progress curve for Russet Norkotah Strain 278 potatoes grown at Parma, ID in 2017. The values are means of 4 reps.

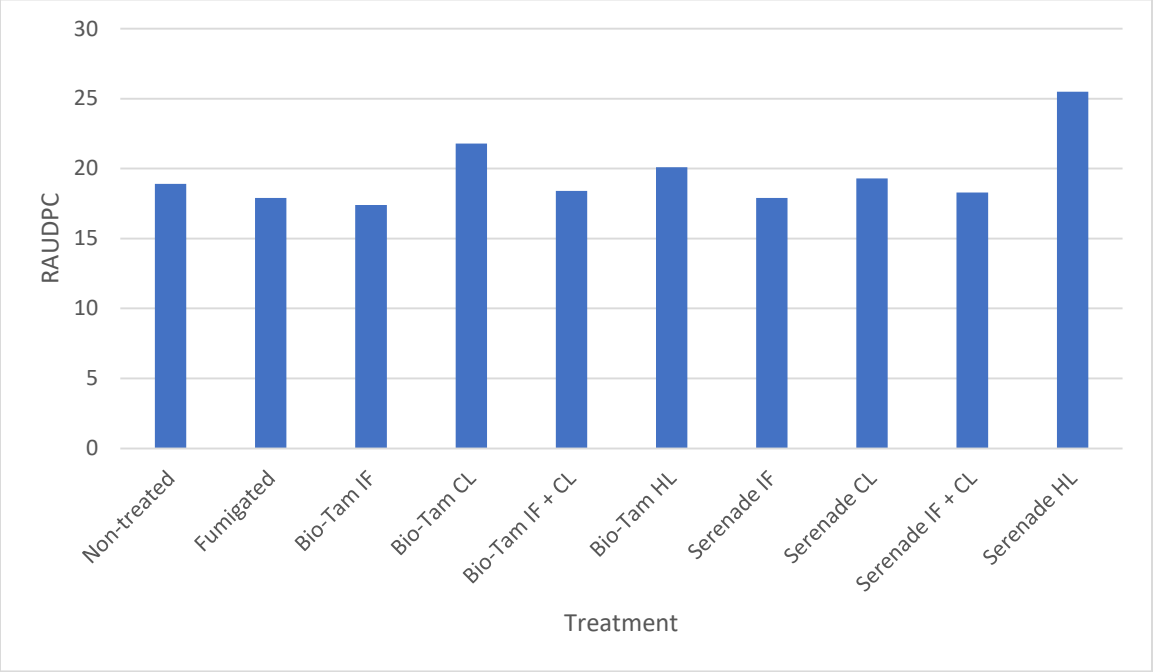
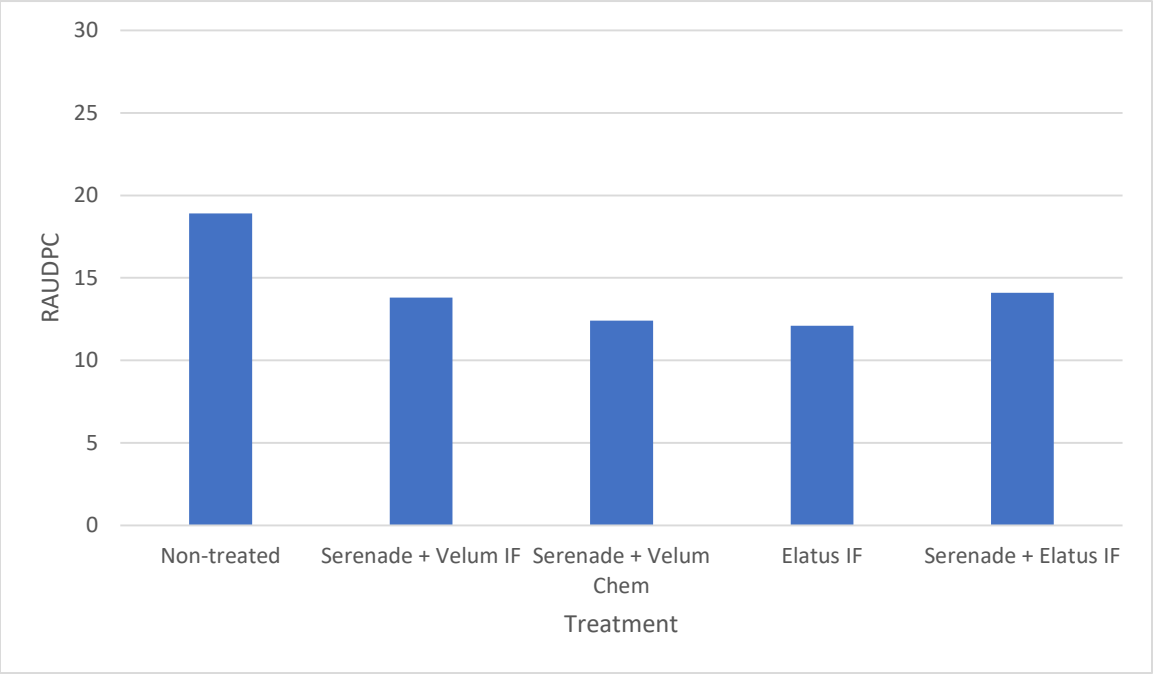


Figure 16. The influence of Velum Prime, Elatus and Serenade ASO fungicide applications on relative area under the disease progress curve for Russet Norkotah Strain 278 potatoes grown at Parma, ID in 2017. The values are means of 4 reps.



Northwest Potato Research Consortium

A Cooperative Effort of the Potato Commissions of ID, OR, & WA

February Quarterly Report FY 2017-18

TITLE:
Assessing Efficacy of Disinfection of Fresh Pack Water on Bacterial Load and Tuber Decay

PERSONNEL:

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COOPERATION:
Mart Produce, Wada Farms, Walker Produce

REPORTING PERIOD: February 2018

ACCOMPLISHMENTS:

We have completed evaluations for the 2016-2017 funding year and included the results in the following report. More data on bruising and decay potential will be added in the final report.

The work for the 2017-2018 year are currently underway, so this is not the final report. The first sampling was scheduled for October 9 in cooperation with Mart Produce. However, this did not occur due to scheduling conflicts. The first sample was collected February 9 and tubers are currently under evaluation. An instrumented sphere was used to measure bruising at different points in the packing operation by Nora Olsen's research team. Additional samples will be collected in the coming weeks.

RESULTS:

See accompanying report. The main take-home message is that applying a disinfectant with a Mafex applicator or spray bar in fresh pack operations does not reduce the potential for tubers to develop soft rot under wet conditions.

PUBLICATIONS:

None to date.

PRESENTATIONS & REPORTS:

Miller Research Potato Pest Management Seminar. January 30, 2018.

2017 Miller Research Potato Pest Management Field Day. August 16, 2017.

Miller J. Potato disease management. Invited presentation given at the 2017 Simplot Grower Solutions NW Regional Training at Idaho Falls, ID on February 7, 2017.

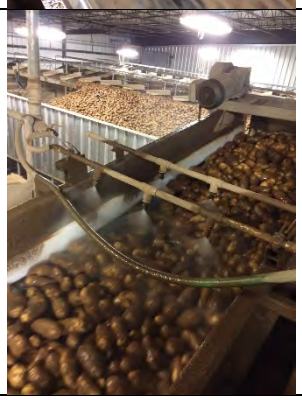
Miller, J. Managing tuber/soil borne diseases of potato. Invited presentation given at the 49th Annual Idaho Potato Conference at Pocatello, ID on January 18, 2017.

Miller, J. Should I Spend the Money? Making Smart Decisions in Disease Management Programs. Invited presentation given at the 2016 Montana Seed Seminar in Missoula, MT on November 3, 2016.

Miller, J. It's Significant to Me! The Purpose of Statistics in Agriculture. *Potato Grower*: June 2016, pp.20-22.

Fresh Pack Operation #1

Potato tubers were sampled from the first fresh pack operation on January 31 and February 22, 2017. Sampling locations were as outlined in Figure 1.

	Location	
1.	Immediately off the truck	
2.	After passing through dirt eliminator	
3.	After wash flume-rock trap/before storage bins	

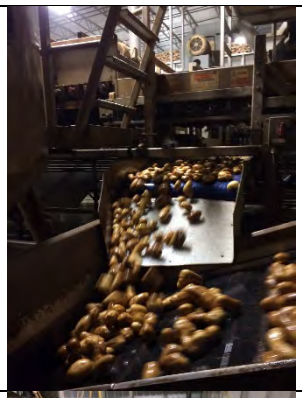
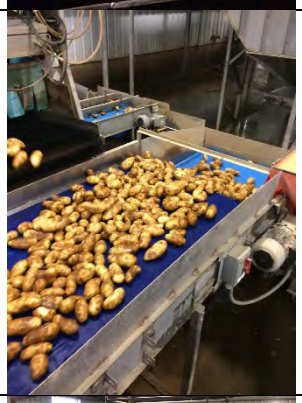
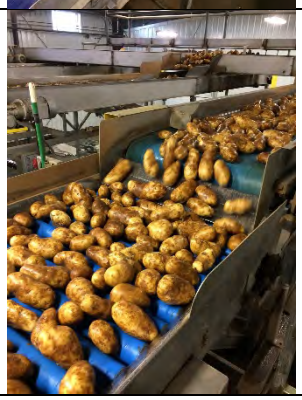
4.	After first spray bar (water only)	
5.	After sizing drop	
6.	After Mafex applicator Treated with Sanidate (80-100 ppm) and SproutNip (3 ppm).	
7.	After sorter/before boxing	(Photo not available)
8.	After sorter/before bagging	

Figure 1. Sampling location at fresh pack operation #1.

At each location, three separate 20-tuber samples were collected (60 tubers total). Two samples were placed in commercial 20-lb capacity poly bags (soft rot evaluation) and one sample

was placed in a 50-lb capacity mesh bag (bruising evaluation). Samples for soft rot were taken to the Miller Research facility near Acequia and the sample for bruising was taken to the University of Idaho Kimberly Research and Extension Center.

Tubers for the soft rot samples were placed in plastic web-bottomed greenhouse trays, and the trays were placed in a humidification tent. The air was humidified for 15 minutes each hour using a commercial storage humidifier. The temperature ranged between 56-66°F. Tubers were evaluated daily for symptoms of soft rot development. When soft rot was observed, the infected tuber was removed from the tray. The number of tubers with soft rot was recorded each day and a running total of the total soft rot incidence was determined for each sample. The ratings over time were used to calculate the AUDPC (area under the disease progress curve):

$$AUDPC = \sum_{i=1}^{N_t-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$$

In the AUDPC equation:

i = the observation number (e.g. first observation = 1, second observation = 2)

y = disease severity

t = time of observation (in days in this case)

The AUDPC incorporates disease ratings over time into a single value.

The number of pectolytic bacteria in clean water (not introduced into the flume/rock trap) and dirty water (in the flume/rock trap) was estimated by plating. A 0.1-ml aliquot was spread on three 10 × 100 mm plastic Petri plates filled with crystal violet pectate agar (CVP). (Water from the flume/rock trap had been diluted 1000 times prior to plating due to higher expected bacterial counts.) Plates were incubated at room temperature (~70°F) for two days, and then examined at 100× magnification. The number of pectolytic bacteria (estimated to be *Pectobacterium* – formerly *Erwinia* – species) was counted. Pectolytic bacteria release enzymes that degrade the upper pectin layer of the agar, causing small pits to form for each pectolytic colony. The total number of soft-rotting bacteria.

Soft rot incidence and AUDPC data were analyzed by analysis of variance (ANOVA) using Agricultural Research Manager (ARM) 2017. When the treatment effect was significant (P<0.10; see "Treatment Prob (F)" at the bottom of each data column in the tables), mean separation was performed using Fisher's protected LSD. Means followed by the same lowercase letter are not statistically different when compared to each other. If the treatment variances were not homogeneous as determined by Bartlett's test for homogeneity, means were transformed prior to analysis. Back-transformed data are listed in the results and specific transformations are listed in the table footnotes.

Results from the first two samples showed that soft rot potential generally increased as tubers progressed through the wash plant (Tables 1 and 2). Some observations from the first soft rot evaluation:

1. Soft rot potential is lowest just off the truck.
2. Traveling through the dirt eliminator does not substantially increase rot potential.
3. Rot potential increases after coming out of the flume.
4. Disinfectant applied through the Mafex applicator did not reduce rot potential.

Table 1. Effect of sampling location on tuber soft rot development for the January 31 sample.

Description	Soft Rot Incidence (%)						AUDPC
Rating Date	Feb-8	Feb-9	Feb-10	Feb-13	Feb-14	Feb-15	Feb-15
Trt Treatment							
1 Off truck	0 d	0 b	3 b	5 d	5 d	10 c	26 d
2 After dirt eliminator	0 d	0 b	0 b	0 d	0 d	3 c	1 d
3 After flume/before bins	1 cd	8 b	10 b	45 c	55 c	65 b	247 c
4 After spray bar (water)	11 bc	30 ab	30 ab	55 bc	63 bc	63 b	362 bc
5 After sizing drop	23 ab	48 a	48 a	78 a	80 ab	83 ab	531 ab
6 After Mafex (Sanidate)	9 bcd	40 a	45 a	68 ab	70 abc	78 ab	441 ab
7 Before boxing	15 abc	45 a	50 a	83 a	85 a	88 a	527 ab
8 Before bagging	41 a	53 a	53 a	88 a	88 a	93 a	593 a
LSD P=.10	16.4 - 27.6	32.2	32.4	22.0	20.5	20.4	29.5 - 189.1
Standard Deviation	1.8t	21.9	22.0	15.0	14.0	13.9	2.9t
CV	57.1t	78.62	74.2	28.5	25.23	23.24	17.43t
Grand Mean	3.2t	27.8	29.7	52.5	55.3	59.7	16.6t
Treatment Prob(F)	**0.0018**	**0.0057**	**0.0054**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

Means followed by same letter do not differ significantly (P=0.05, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.05). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the square root (x+0.5) transformation. Back transformed means are given in the table.

Table 2. Effect of sampling location on tuber soft rot development for the February 22 sample.

Description	Soft Rot Incidence (%)						AUDPC
Rating Unit	Feb-27	Feb-28	Mar-1	Mar-2	Mar-3	Mar-6	Mar-6
Trt Treatment							
1 Off truck	1 f	9 c	7 c	10 c	10 c	13 b	69 d
2 After dirt eliminator	5 f	13 c	13 c	16 b	18 b	18 b	112 c
3 After flume/before bins	85 d	98 ab	99 ab	99 a	99 a	100 a	678 ab
4 After spray bar (water)	66 e	96 b	98 b	98 a	98 a	99 a	659 b
5 After sizing drop	90 cd	99 ab	99 ab	99 a	99 a	99 a	679 ab
6 After Mafex (Sanidate)	94 bc	100 a	100 a	100 a	100 a	100 a	696 ab
7 Before boxing	100 a	100 a	100 a	100 a	100 a	100 a	700 a
8 Before bagging	99 ab	100 a	100 a	100 a	100 a	100 a	699 a
LSD P=.10	2.03 - 5.13	2.33 - 10.38	1.61 - 8.38	5.84	5.27	1.38 - 8.91	38.170 (8%)
Standard Deviation	6.73t	7.21t	5.98t	4.80	4.33	5.55t	31.371
CV	11.64t	10.4t	8.58t	6.22	5.59	7.79t	5.85
Grand Mean	57.87t	69.35t	69.71t	77.19	77.50	71.23t	536.484
Treatment Prob(F)	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

Means followed by same letter do not differ significantly (P=0.05, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.05). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the arcsine square root % transformation. Back transformed means are given in the table.

Bacterial Load of Water

Water samples were collected during the February 22 sampling. Samples of clean water and water from the flume/rock trap were collected both before and after a changing of water in the flume/rock trap.

The number of pectolytic bacteria (able to cause bacteria soft rot) was similar in the clean water. The numbers in the flume/rock trap dropped substantially after changing the water. After the water change, the water was still discolored with soil and debris. But the bacterial counts were 83% lower.

	# Pectolytic Bacteria/ml Water[1]	
	Before water change (~2:15 pm)	After water change (~3:15 pm)
Clean water	18,033	20,400
Flume/rock trap	8,971,111	1,564,444

[1] Pectolytic bacteria are those which are able to degrade pectin. Most of these are assumed to be able to cause soft of potato tubers.

Bruising

Incidence of blackspot bruise was similar for most of the sampling locations (*Figure 2*). Blackspot bruise was significantly higher for tubers going into the bag compared to those placed in boxes. In this particular situation, these tubers go through an extra set of belts, drops, and bins prior to reaching the bagging station.

Shatter bruise increased significantly after leaving the wash flume and dropping through the holding bins. Shatter bruise appears to decrease after that point. This could be due to two possibilities:

1. Tubers were being sorted out after the Bin sample, so fewer damaged tubers would be present in the downstream flow.
2. The tubers we sampled from the Truck, Sand Trap (dirt eliminator), and Flume may not have been from the same lot as those in later sampling locations.

On future collection dates care will need to be taken to ensure that the tubers leaving the truck are the same as those which are flowing through the bins prior to the spray bar.

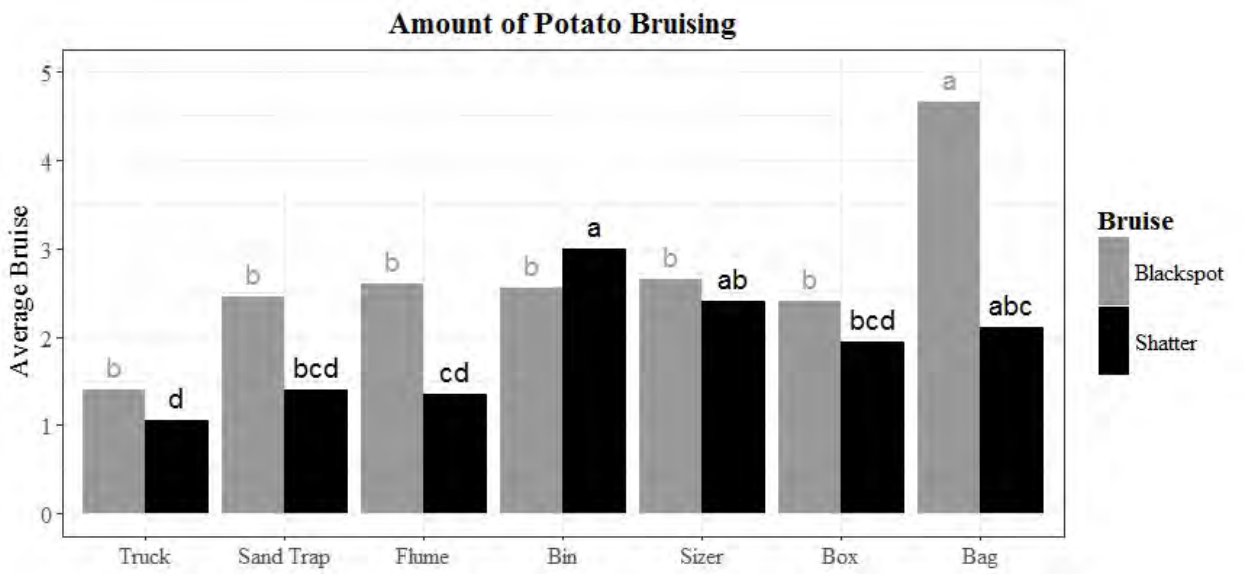


Figure 2. Effect of sample location on tuber bruising.

Evaluation of Tubers for Soft Rot Potential – Second Fresh Pack Operation

Samples of potato tubers (cv. Russet Burbank) were collected at a second fresh pack operation on March 15, 2017. Samples were collected at the following locations in the wash plant:

1. Truck – immediately after being off-loaded from the truck
2. Before bins – right before tubers were deposited in large even-flow bins
3. After bins 1 – taken from the bottom of the bins (line was not running)
4. After bins 2 – taken after tubers had left the bins
5. After spray bar – after tubers had passed under both spray bars, including disinfectant
6. After Odenberg – after tubers went through Odenberg and were dropped
7. Box – taken from tubers which had already been boxed
8. Bag – taken from tubers which had already been bagged

During the sampling procedure on March 15, the line was stopped. As a result, we were not able to collect tubers for sample numbers 4-6. These samples were collected for us by Wada personnel the following day (March 16) and delivered to Miller Research the same day.

Tubers were brought back to Miller Research for evaluation. Tubers were placed in plastic web-bottom greenhouse trays, and the trays were placed in a humidification tent. The air was humidified for 15 minutes each hour using a commercial storage humidifier. The temperature ranged between 56-66°F. Tubers were evaluated daily for symptoms of soft rot development. When soft rot was observed on a tuber, it was removed from the tray. The number of tubers with soft rot was recorded each day and a running total of the total soft rot incidence was determined for each sample. Examples of soft rot symptoms are shown in Figure 1.

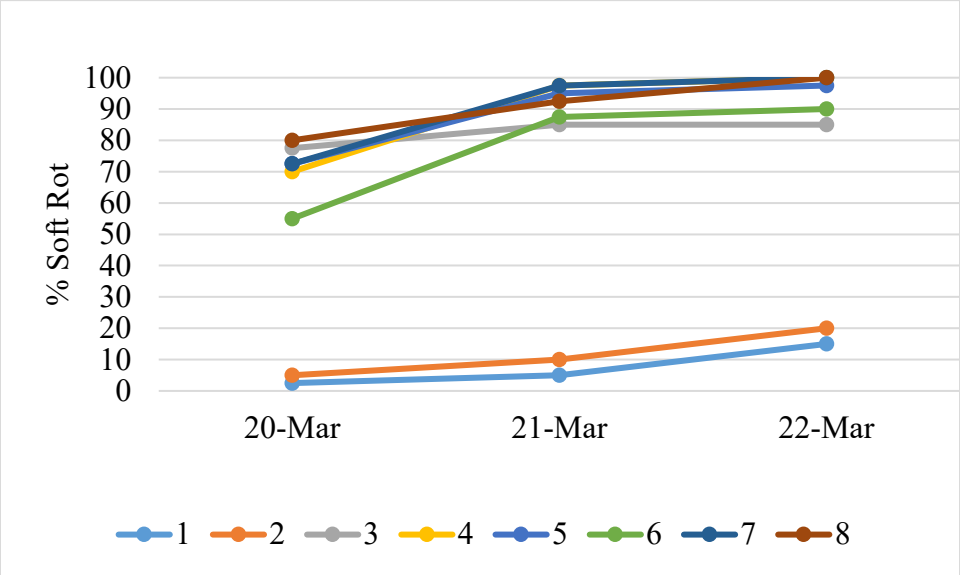
Tubers from treatments 1 and 2 were not washed. These treatments showed very little soft rot development (Table 1). This is consistent with observations we have made at two other wash plants. Tubers from treatment 3 were not washed, but did get wet as they were pulled from the bottom of the even-flow bins. These showed a significantly greater potential for soft rot than tubers from treatments 1 and 2. The tubers which were collected the next day had passed under a spray bar located at the bottom of the even-flow bins. These tubers showed a significantly greater potential for decay on March 21 and 22 than those that which were pulled out the day before. When the tubers from treatment 4 were delivered to Miller Research, we noted that they appeared wetter than those which we collected for treatment 3 the day prior. After leaving the bins (treatment 4), the soft rot potential was similar for all remaining treatments. Passage under a spray bar applying disinfectant did not reduce the rot potential. These observations are also consistent with observations made at other wash plants. Once tubers are washed or exposed to water, the soft rot potential increases substantially, regardless of what is done in the plant.

Table 1. Effect of sample location on bacterial soft rot potential.

Description	% Tubers w/Soft Rot – Cumulative		
Rating Date	Mar-20	Mar-21	Mar-22
Trt Treatment			
1 Truck	2 b	5 c	5 c
2 Before bins	5 b	10 c	10 c
3 After bins - 1	78 a	85 b	85 b
4 After bins - 2	70 a	98 a	98 a
5 After spray bar	72 a	95 ab	95 ab
6 After Odenberg	55 a	88 ab	88 ab
7 Pre-box	72 a	98 a	98 a
8 Pre-bag	80 a	92 ab	92 ab
LSD P=0.05	25.18	10.72	10.72
Standard Deviation	10.65	4.53	4.53
CV	19.58	6.36	6.36
Grand Mean	54.38	71.25	71.25
Treatment Prob(F)	**0.0005**	**0.0001**	**0.0001**

Samples for treatments 1-3 and 7-8 were collected on March 15 and samples for treatments 4-6 (shaded) were collected on March 16. Values followed by the same letter are not significantly different (P<0.05).

Figure 3. Soft rot potential for tubers collected at various locations from a wash plant.



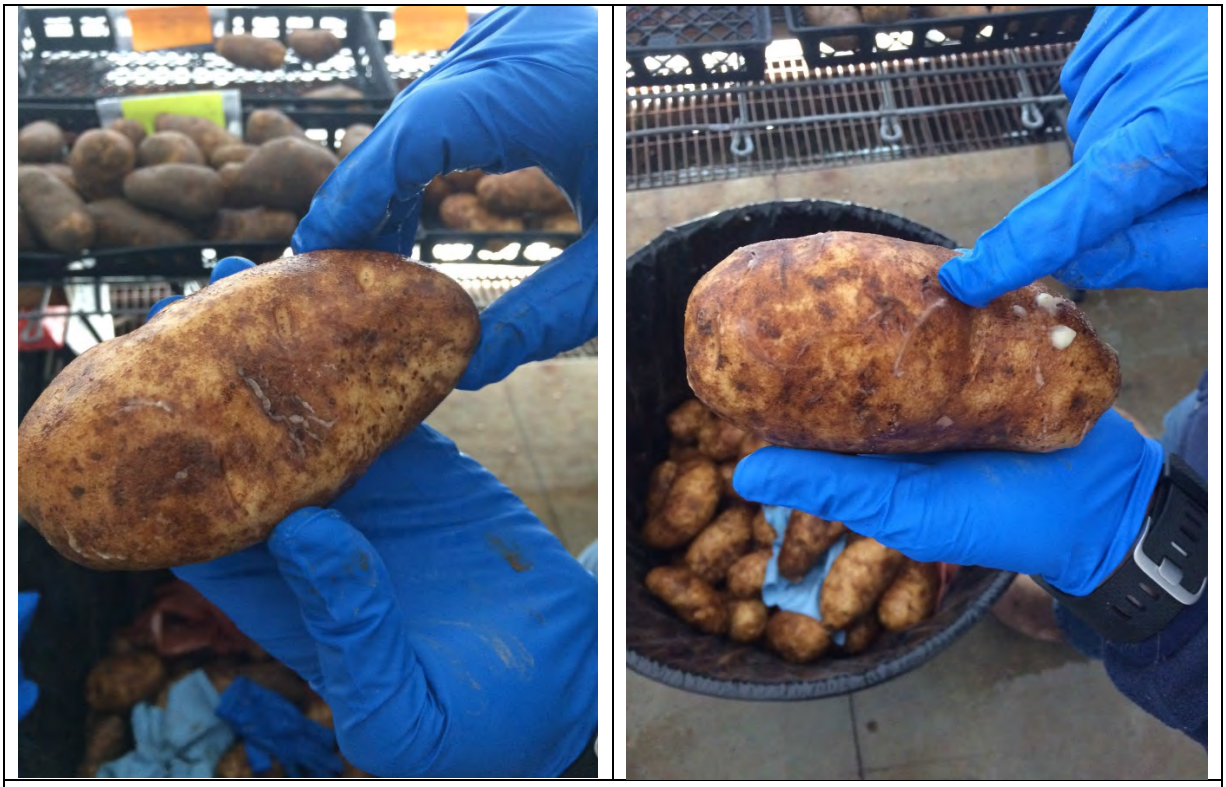


Figure 2. Examples of soft rot development in areas where tubers had been damaged.

Northwest Potato Research Consortium
A Cooperative Effort of the Potato Commissions of ID, OR, & WA
February Quarterly Report FY 2017-18

TITLE:
Comparison of Metam Sodium Fumigation Methods and Alternatives to Metam Sodium Fumigation for Potato Pest Management

PERSONNEL:
Miller, J., T. Taysom, C. Clayton, S. Hansen, T. Miller, and S. Anderson

REPORTING PERIOD:
February 2018

ACCOMPLISHMENTS:
This project was last funded during FY 2016-2017. However, the trial did not work out as well as expected. The trial was repeated this year at no cost to the Consortium. The trial is nearly complete and a summary of accomplishments to date are listed in the following report.

Additional work is still underway. We are currently doing tests to quantify the amount of *Verticillium dahliae* and *Colletotrichum coccodes* in potato stems. The results will be included in an updated final report.

RESULTS:
A summary list of the main results is found in the following page. This report was written with the Results and Discussion first, followed by the Materials and Methods. While this is not standard, it allows the readers to focus first on what we learned. The reader can then view the Methods if they are interested.

PUBLICATIONS:
None to date.

PRESENTATIONS & REPORTS:
1. Miller, J. Potato disease management. Invited presentation given at the 2018 Simplot Grower Solutions NW Regional Training at Idaho Falls, ID on February 13, 2018.
2. Miller, J., Taysom, T. and Hansen, S. Miller Research Potato Pest Management Seminar. Held at Rupert, ID on January 30, 2018.
3. Hansen, S. 2018. Comparison of fumigation methods in potato. Presentation given at the Idaho Association of Plant Protection at Twin Falls, ID on November 1, 2017.
4. Miller, J. Potato disease management. Invited presentation given at the 2017 Simplot Grower Solutions NW Regional Training at Idaho Falls, ID on February 7, 2017.
5. Miller, J. Vydate as an alternative to metam sodium for potato plant health. Invited presentation given at the 49th Annual Idaho Potato Conference at Pocatello, ID on January 18, 2017.
6. Miller, J. Managing tuber/soil borne diseases of potato. Invited presentation given at the 49th Annual Idaho Potato Conference at Pocatello, ID on January 18, 2017.

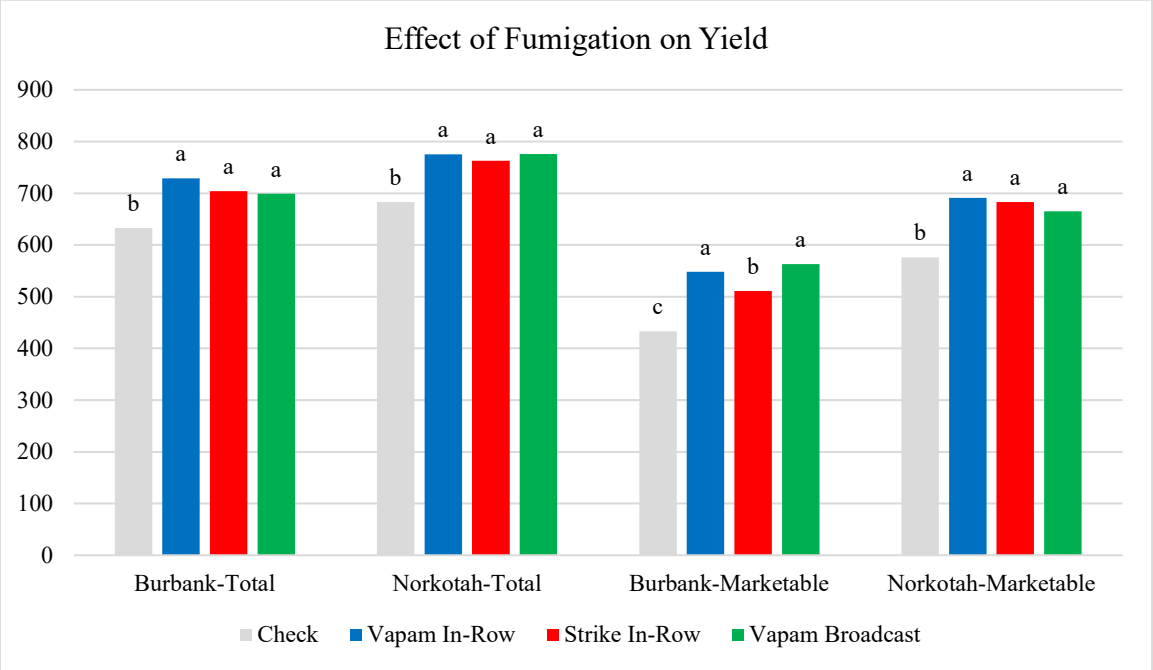
7. Miller, J. Improving Efficiency of Product Usage in Potato. Invited presentation given at the 2016 Syngenta Potato Partners program in San Diego, CA on November 16, 2016.
8. Thornton, M., and Miller, J. 2016. Evaluation of Vydate and Vertisan programs for reducing the reliance on fumigation in potato production. *Am. J. Potato Res*. 93:144.

Effect of Fumigation Methods on Potato Yield and Quality

Miller Research Trial ID: 17P-IPC-01

Summary

1. Nematode pressure was very light and data were not obtained on efficacy against those pathogenic to potato.
2. Broadcast Vapam increased Burbank vigor in late August.
3. All fumigation methods increased Norkotah 296 vigor in late August.
4. Burbank yield and grade was highest with Vapam (both methods).
5. All fumigation treatments improved Norkotah yield.
6. Broadcast Vapam produced larger tubers than other methods.
7. Tuber size was not affected by fumigation for Norkotah.
8. Broadcast Vapam had the highest economic return for Burbank.
9. All three fumigation treatments were similar for Norkotah economic return.



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INTRODUCTION

Treatments

1. Check
2. Vapam (metam sodium), in-row at 30 gal/acre, fall fumigation
3. Strike 80 CP (chloropicrin), in-row at 8.5 gal/acre, fall fumigation
4. Vapam (metam sodium), broadcast at 40 gal/acre, fall fumigation

Trial Map

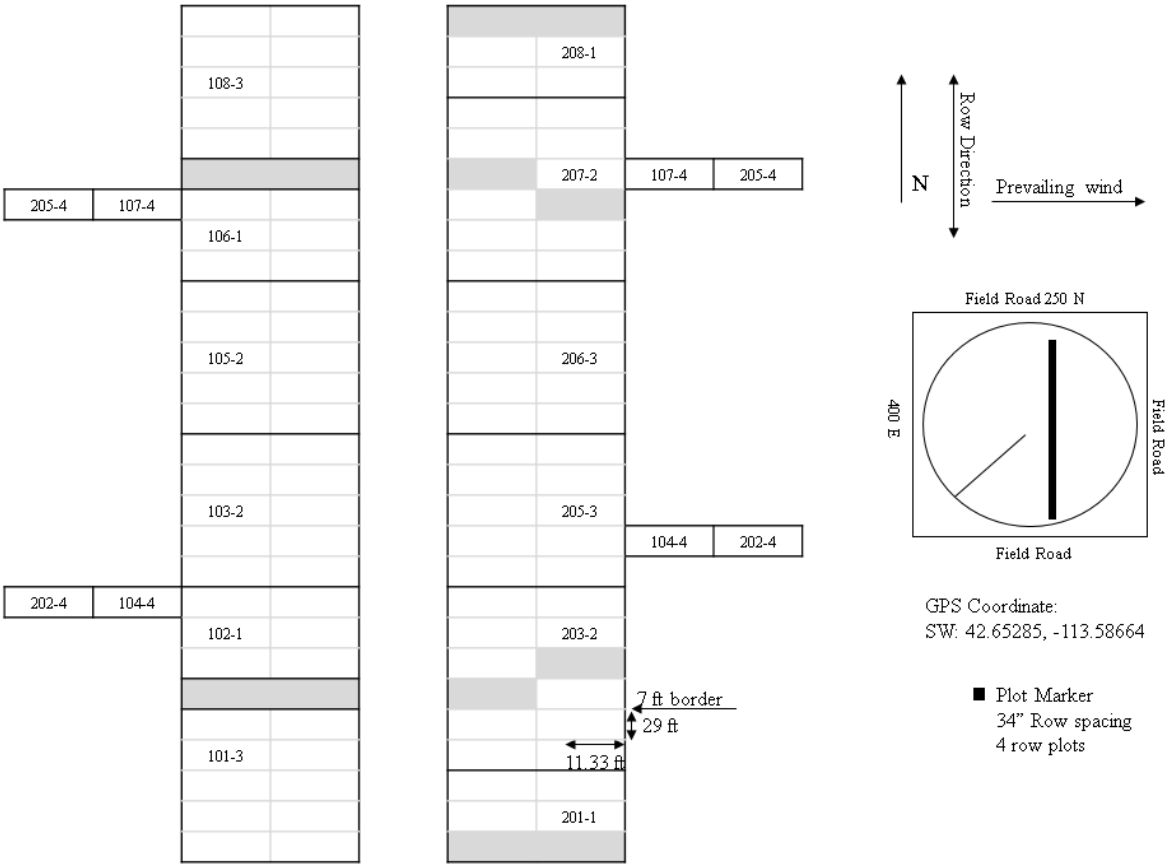

Figure 1. Plot map for the IPC fumigation method trial conducted at Miller Research in Acequia, ID in 2017. The number on the left in each block is the plot number and the number on the right in each block is the treatment number. The plots for treatment 4 (broadcast fumigation) had to be placed adjacent to this trial due to logistics of application.

RESULTS AND DISCUSSION

Crop Vigor

All fumigation methods resulted in similar crop vigor for Russet Burbank on August 10 (Table 1). Very little senescence was observed in this variety at this time. By August 22, however, the broadcast Vapam (treatment 4) had the highest vigor. The other two fumigation treatments were statistically similar, but were also similar to the non-fumigated check.

As expected, Russet Norkotah 296 showed greater senescence and decreased vigor on August 10 compared to Russet Burbank (Table 2). Strike had the highest vigor. Broadcast Vapam was similar. On August 22, all three fumigation methods were equal and showed greater vigor than the non-fumigated check.

Table 1. Crop vigor (cv. Russet Burbank).

Description				% Vigor	
Rating Date				Aug-10	Aug-22
Trt	Treatment	Rate	Unit Code*		
1	Non-fumigated			95 a	79 b
2	Vapam HL	30	gal/a IR	96 a	86 ab
3	Strike 80 CP	8.5	gal/a IR	96 a	86 ab
4	Vapam HL	40	gal/a BC	98 a	92 a
LSD P=.10				3.03	8.10
Standard Deviation				2.33	6.25
CV				2.42	7.27
Grand Mean				96.31	85.94
Treatment Prob(F)				0.3168	**0.0745**

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

Table 2. Crop vigor (cv. Russet Norkotah 296).

Description				% Vigor	
Rating Date				Aug-10	Aug-22
Trt	Treatment	Rate	Unit Code*		
1	Non-fumigated			72 c	46 b
2	Vapam HL	30	gal/a IR	76 bc	66 a
3	Strike 80 CP	8.5	gal/a IR	86 a	78 a
4	Vapam HL	40	gal/a BC	85 ab	64 a
LSD P=.10				8.78	16.18
Standard Deviation				6.77	12.48
CV				8.46	19.67
Grand Mean				80.00	63.44
Treatment Prob(F)				**0.0486**	**0.0387**

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

Nematode Sampling

Unfortunately the trial site did not have a large number of nematodes which are typically pathogenic on potato (i.e. Columbia root knot and root lesion). Some Northern root knot nematodes were present (Table 3). Statistical differences were not observed among treatments, but the non-fumigated check had the highest number of Northern root knot nematodes. All treatments were similar for stubby root nematode counts. This was not surprising since these nematodes are migratory in nature. Pin nematodes (which are not pathogenic) were also reduced by all fumigation methods. Broadcast did provide a significant reduction compared to Strike.

No symptoms of tobacco rattle virus (TRV, also called corky ringspot) were observed in the trial area. TRV is vectored by the stubby root nematode.

Table 3. Nematode sample analysis for August 22.

Description Rating Unit					Northern RK #/500 cc	Stubby Root Number	Pin Number
Trt	Treatment	Rate	Unit	Code*			
1	Untreated				26 a	11 a	140.3 a
2	Vapam HL	30	gal/a	IR	0 a	10 a	0.8 bc
3	Strike 80 CP	8.5	gal/a	IR	3 a	6 a	5.3 b
4	Vapam HL	40	gal/a	BC	2 a	9 a	0 c
LSD P=.10					25.36 - 54.18	10.34 - 46.65	9.52 - 118.56
Standard Deviation					0.97t	0.68t	0.61t
CV					152.88t	68.24t	76.35t
Grand Mean					0.63t	0.99t	0.80t
Treatment Prob(F)					0.2819	0.9643	**0.0034**

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the automatic log transformation of x+1 (all columns). Back transformed means are given in the table.

Yield and Grade

All fumigation methods increased yield for both Russet Burbank and Russet Norkotah 296 (Tables 4 and 5). With respect to Russet Burbank, Vapam (either application method) was more effective than Strike for marketable yield (Table 4). Broadcast Vapam produced the most US#1 tubers (Vapam in-row was similar) and the most tubers over 10 oz.

For Norkotah, marketable yield was similar for all fumigation treatments (Table 5). Differences were not observed for US#1, US#2, or tubers over 10 oz.

Table 4. Yield and grade (cv. Russet Burbank).

Description					Yield (cwt/acre)				
Rating Unit					Total	Marketable	% US#1	% US#2	% >10oz
Trt	Treatment	Rate	Unit	Code*					
1	Untreated				633 b	433 c	48 c	21 a	16 b
2	Vapam HL	30	gal/a	IR	729 a	548 a	60 ab	16 a	18 b
3	Strike 80 CP	8.5	gal/a	IR	704 a	511 b	58 b	15 a	20 b
4	Vapam HL	40	gal/a	BC	699 a	563 a	65 a	16 a	33 a
LSD P=.10					35.91	35.640	7.099	6.234	10.74
Standard Deviation					27.70	27.495	5.476	4.809	8.29
CV					4.01	5.35	9.54	28.85	38.33
Grand Mean					691.26	513.681	57.400	16.669	21.63
Treatment Prob(F)					**0.0051**	**0.0004**	**0.0116**	0.3715	**0.0640**

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

Table 5. Yield and grade (cv. Russet Norkotah 296).

Description					Yield (cwt/acre)				
Rating Unit					Total	Marketable	% US#1	% US#2	% >10oz
Trt	Treatment	Rate	Unit	Code*					
1	Untreated				683 b	576 b	73 a	12 a	40 a
2	Vapam HL	30	gal/a	IR	775 a	691 a	84 a	5 a	46 a
3	Strike 80 CP	8.5	gal/a	IR	763 a	683 a	83 a	6 a	48 a
4	Vapam HL	40	gal/a	BC	776 a	665 a	79 a	6 a	43 a
LSD P=.10					40.84	60.723	9.292	5.877	7.91
Standard Deviation					31.51	46.846	7.169	4.534	6.10
CV					4.21	7.16	8.98	61.58	13.87
Grand Mean					749.21	653.906	79.819	7.363	44.00
Treatment Prob(F)					**0.0065**	**0.0249**	0.1844	0.2624	0.3597

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

Tuber Size

The percentage of undersize (< 4 oz) was similar for all Burbank treatments (Table 6) and for all Norkotah 296 treatments (Table 7). Additionally, the percentage of tubers in the individual size categories for US#1 tubers was similar for all treatments.

In aggregate, the small differences for Burbank translated to a significant difference for total US#1 tubers (Table 4), and this was likely due to the higher percentage values in the 10-14 oz and > 14 oz classes (Table 6). No such trend was observed for Norkotah (Table 7).

Table 6. Tuber size distribution (cv. Russet Burbank).

Description					% US#1			
					%<4oz	4-6oz	6-10oz	10-14oz	>14oz
Trt	Treatment	Rate	Unit	Code*					
1	Untreated				20 a	18 a	22 a	6 a	1 a
2	Vapam HL	30	gal/a	IR	21 a	24 a	23 a	11 a	2 a
3	Strike 80 CP	8.5	gal/a	IR	20 a	19 a	25 a	9 a	4 a
4	Vapam HL	40	gal/a	BC	14 a	15 a	25 a	16 a	6 a
LSD P=.10					5.232	6.120	5.774	8.557	2.983 - 4.147
Standard Deviation					4.037	4.721	4.455	6.601	0.304t
CV					21.61	24.73	18.95	62.2	53.53t
Grand Mean					18.681	19.094	23.513	10.613	0.567t
Treatment Prob(F)					0.1523	0.1290	0.7110	0.2397	0.1097

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the automatic log transformation of x+1 (>14oz). Back transformed means are given in the table.

Table 7. Tuber size distribution (cv. Russet Norkotah 296)

Description					% US#1				
					%<4oz	4-6oz	6-10oz	10-14oz	>14oz
Trt	Treatment	Rate	Unit	Code*					
1	Untreated				11 a	17 a	23 a	18 a	14 a
2	Vapam HL	30	gal/a	IR	8 a	11 a	30 a	22 a	20 a
3	Strike 80 CP	8.5	gal/a	IR	8 a	12 a	28 a	22 a	21 a
4	Vapam HL	40	gal/a	BC	11 a	14 a	26 a	19 a	20 a
LSD P=.10					2.682	4.098 - 4.375	4.352 - 4.697	5.029	7.862
Standard Deviation					2.069	0.096t	0.056t	3.880	6.065
CV					21.64	8.29t	3.87t	19.25	32.06
Grand Mean					9.563	1.157t	1.441t	20.156	18.919
Treatment Prob(F)					0.1916	0.1828	0.1071	0.3575	0.4270

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the automatic log transformation of x+1 (4-6oz, 6-10oz). Back transformed means are given in the table.

Other Tuber Grades

US#2 tubers were similar for all treatments for both varieties (Tables 8 and 9). Both Vapam fumigation methods resulted in the lowest percentage of culls for Burbank (Table 8). Strike in-row was similar to broadcast Vapam. Broadcast Vapam resulted in a significantly higher average tuber weight than all other treatments. Culls and average tuber weight were similar for all treatments with Norkotah.

Table 8. US#2, culls, and average tuber weight (cv. Russet Burbank).

Description				% US#2			Avg. Tuber
				4-10 oz	> 10 oz	% Culls	Weight (oz)
Trt	Treatment	Rate	Unit Code*				
1	Untreated			12 a	8 a	11 a	5.6 b
2	Vapam HL	30	gal/a IR	11 a	5 a	4 c	5.2 b
3	Strike 80 CP	8.5	gal/a IR	9 a	6 a	7 ab	5.5 b
4	Vapam HL	40	gal/a BC	8 a	8 a	5 bc	6.5 a
LSD P=.10				6.240	4.049	2.701 - 4.277	0.728
Standard Deviation				4.814	3.124	0.145t	0.562
CV				48.66	46.07	16.63t	9.88
Grand Mean				9.894	6.781	0.875t	5.688
Treatment Prob(F)				0.5686	0.3776	**0.0226**	**0.0609**

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the automatic log transformation of x+1 (Cull). Back transformed means are given in the table.

Table 9. US#2, culls, and average tuber weight (cv. Russet Norkotah 296).

Description				% US#2			Avg Tuber
Rating Unit				4-10oz	>10oz	% Culls	Weight (oz)
Trt	Treatment	Rate	Unit Code*				
1	Untreated			3 a	8 a	4 a	7.0 a
2	Vapam HL	30	gal/a IR	2 a	4 a	2 a	7.8 a
3	Strike 80 CP	8.5	gal/a IR	1 a	5 a	2 a	7.8 a
4	Vapam HL	40	gal/a BC	2 a	4 a	3 a	7.3 a
LSD P=.10				1.804 - 2.171	4.183	2.630 - 3.489	0.634
Standard Deviation				0.225t	3.227	0.281t	0.489
CV				48.82t	65.36	49.41t	6.53
Grand Mean				0.462t	4.938	0.569t	7.488
Treatment Prob(F)				0.1879	0.3222	0.6353	0.1123

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the automatic log transformation of x+1 (4-10oz, Cull). Back transformed means are given in the table.

Economic Return

Combining yield and grade together for Burbank resulted in broadcast Vapam returning the highest dollar value on both per cwt and per acre bases (Table 10). This was true for both processing and fresh pack. In-row Vapam was similar to broadcast Vapam for processing dollar per acre, but all other treatments were significantly lower for dollar return. Vapam in-row and Strike in-row provided a significantly higher dollar per acre return than the non-fumigated check based on both processing and fresh pack.

For Norkotah 296, in-row Vapam and Strike produced a higher dollar per cwt than the check (Table 11). Broadcast Vapam was intermediate and did not separate from the check. All fumigation treatments did increase the dollar per acre return over the check, and all treatments were similar to each other.

Table 10. Gross dollar return (cv. Russet Burbank).

Description				Processing		Fresh Pack	
Rating Unit				$/cwt	$/acre	$/cwt	$/acre
Trt	Treatment	Rate	Unit Code*				
1	Untreated			7.91 b	3435 c	10.70 c	4660 c
2	Vapam HL	30	gal/a IR	8.06 b	4420 ab	11.49 bc	6343 b
3	Strike 80 CP	8.5	gal/a IR	8.08 b	4131 b	11.67 b	5987 b
4	Vapam HL	40	gal/a BC	8.38 a	4712 a	13.51 a	7615 a
LSD P=.10				0.248	381.5	0.850	803.8
Standard Deviation				0.191	294.3	0.655	620.1
CV				2.36	7.05	5.54	10.08
Grand Mean				8.106	4174.3	11.841	6150.9
Treatment Prob(F)				**0.0426**	**0.0010**	**0.0012**	**0.0007**

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

Table 11. Gross dollar return (cv. Russet Norkotah 296).

Description				Fresh Pack	
Rating Unit				$/cwt	$/acre
Trt	Treatment	Rate	Unit Code*		
1	Untreated			14.29 b	8248 b
2	Vapam HL	30	gal/a IR	15.52 a	10732 a
3	Strike 80 CP	8.5	gal/a IR	15.74 a	10757 a
4	Vapam HL	40	gal/a BC	14.82 ab	9890 a
LSD P=.10				0.9538	1437.18
Standard Deviation				0.7358	1108.76
CV				4.88	11.19
Grand Mean				15.0906	9906.56
Treatment Prob(F)				**0.0747**	**0.0342**

BC = Broadcast, IR = In-row.

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

Summary

Broadcast Vapam fumigation was more effective than in-row fumigation with Vapam or Strike for Russet Burbank. In-row Vapam and Strike were generally similar. Broadcast Vapam was the only application method to improve late-season vigor over the non-fumigated check. However, all three fumigation treatments had higher total yields than the check. Vapam (either application method) produced higher marketable yield than Strike or the check and broadcast Vapam produced more US#1 tubers than Strike or the check. Broadcast Vapam also resulted in more large tubers (> 10 oz) and a higher average tuber weight than other treatments. All of these factors combined resulted in higher dollar per cwt (both processing and fresh) and higher dollar per acre values than all other treatments.

All three fumigation efforts were similar in increasing yield for Russet Norkotah 296. Vigor was improved by Strike in-row and broadcast Vapam in early August. All treatments improved vigor in late August and increased total and marketable yield compared to the non-fumigated check. Grade was generally similar for all treatments. Dollar return per acre was improved with all three fumigation treatments over the check and all three were similar.

MATERIALS AND METHODS

Site Preparation

The trial was established at the Miller Research Experimental Farm near Acequia, ID in the West field. The previous crops were winter barley (2016), sugar beet (2015), and potato (2014).

The trial area was harrowed twice and disked twice in 2016 following the harvest of the winter barley. The trial area was ripped on October 11. Fumigation treatments were applied as described below.

Soil samples were collected in the spring (March 20, 2017). A total of 25 soil cores (0-12") were collected from the trial area. The soil was analyzed by Stukenholtz Laboratory.

Additional details relating to the trial site are in Appendix 1.

Fumigation

All fumigation applications were made by professional fumigation applicators.

The broadcast application of Vapam (Vapam HL) was made on October 18 by Chris Etherington (Turnin' Dirt). Vapam was injected 9 inches deep on the ripper shanks at a rate of 40 gallons per acre. Potato rows were marked out on November 15.

In-row fumigation with Vapam (Vapam HL) was performed on October 24 by Brian Hansen (Hansen Farms). Fumigant was applied at a rate of 30 gallons per acre and rows were marked out at the same time.

In-row fumigation with Strike (Strike 80CP) was performed on November 15 by Trident personnel. Strike was applied at a rate of 7.5 gallons per acre and rows were marked out at the same time.

Planting

Certified seed (cv. Russet Burbank and Russet Norkotah TX-296) for this trial was purchased from a commercial grower. Russet Burbank seed had been cut and treated (6% MZ, 1 lb/cwt) by the grower. Russet Norkotah TX-296 (hereafter referred to as Norkotah 296) was cut and treated (6% MZ, 0.75 lb/cwt + Maxim MZ, 0.25 lb/cwt) by the grower. Details on seed size and planting density are given in Appendix 1.

Potato seed was planted on May 1-2 with a modified ACME commercial potato planter. Starter fertilizer was applied in the row two inches to the side and just below where the seed pieces were dropped. Row spacing was 34 inches, plant spacing within the row was 12 inches, and seed pieces were planted to a depth of 6-7 inches. Plots were four rows wide (11.33 feet) and 29 feet long with a 7-foot border between plots. Drive rows were established within the plot so that foliar applications could be made to the trial without driving through the plot area. Two rows were established on the east and west sides of the trial and between reps 2 and 3 as drive rows for sprayer travel. Treatments were established in a randomized complete block design with four replications (Figure 1).

Trial Maintenance

Prior to emergence, dry fertilizer was spread over the trial area. Fertilizer was incorporated and potato rows were re-hilled with a Lilliston cultivator. Potato tubers in the plot border areas were lifted with a modified two-row potato harvester, and then plot borders were cut using a Maschio roto-tiller. Herbicides were applied by ground application and incorporated with irrigation. Details on fertilizers and maintenance pesticide applications are provided in Appendix 2.

Plant Vigor

Vigor was rated as a percentage of healthy canopy remaining on August 10 and 22. Ratings were made by two individuals and averaged.

Verticillium Colonization

Stem sections were collected to quantify the colonization of *Verticillium dahliae* in plants. The top 7.5 cm (3 inches) of the main stem were collected from 5 different plants in each plot. Stems were surface sterilized in 0.5% NaOCl (10% bleach) for 10 sec. Stems were then allowed to air dry for several weeks. All stems were combined and ground into a fine powder using a Wiley mill on December 7-8.

Five 10-mg subsamples were spread on NPX media using an Anderson sampler. Plates were incubated for 21 days at room temperature.

Nematode Sampling

Soil was sampled for the presence of nematodes on August 17. Ten soil cores were collected from each plot from between plants in the root zone. Soil samples were submitted to Dr. Saad Hafez at the University of Idaho in Parma for evaluation.

Tuber Yield

Tubers were harvested on September 28 using a specially modified two-row Lockwood 4620 harvester. Tubers from rows 2 and 3 of each plot were lifted and cleaned by the harvester and crew riding on the machine. The tubers were dropped into a basket hanging from the end of the delivery boom. The basket was suspended by an electronic load cell scale which weighed all tubers harvested from the center two plot rows. A fresh-pack cardboard box (50 lb capacity) was placed in the hanging basket in order to obtain a sample (45-50 lb) for determining potato grade and tuber black scurf/silver scurf. The remaining tubers were dropped in a pile on the ground so that tuber rot could be recorded. The weight in pounds was converted to cwt/acre.

Tuber Grade and Quality

USDA standards were used in grading the tuber samples collected at harvest. Tubers were graded on November 21 (Norkotah 296) and 27 (Burbank). Tubers were separated into US#1, US#2, cull, and undersize categories and then individually weighed. Weights were then used to determine the percentage of yield in the following grade categories: < 4 oz, 4-6 oz. US#1, 6-10 oz. US#1, 10-14 oz. US#1, >14 oz. US#1, culls, 4-10 oz. US#2, and >10 oz. US#2. The percentage of tubers in various fresh-pack carton sizes was also determined as described below in "Economic Return."

Economic Return

The gross economic value for Russet Burbank was estimated using mock processing and fresh pack contracts. For processing, the marketable yield (all US#1 and US#2 tubers greater than 4 oz) was determined. Incentives were calculated based on the percentage of US#1 tubers and the percentage of US#1 and US#2 tubers >10 oz.

For US#1 tubers, an incentive of $0.01/cwt was awarded for each percentage point over 65% with a cap set at 85%. A disincentive of -$0.01/cwt was deducted for each percentage point below 65%. For the percentage of tubers > 10 oz, an incentive of $0.02 was awarded for every percentage point over 21% with a cap set at 36%. A disincentive of -$0.02 was deducted for every percentage point down to 0 where the value was -$0.42. Incentives or disincentives were added to or subtracted from a base contract price of $8.13/cwt of marketable tubers.

For fresh pack, the price for each sample was determined using values from the National Potato and Onion Report issued on Thursday, October 5, 2017 by the USDA Agricultural Marketing Service Fruit and Vegetable Program. Dollar values were assigned to the grade sample based on the following table:

Item	Tuber Category	Value/cwt		Item	Tuber Category	Value/cwt
10# Film	5.0-6.4 oz., US1	$12.00		80 ct	9.5-10.7 oz., US1	$19.00
40 ct	18-22 oz., US1	$18.00		90 ct	8.5-9.5 oz. , US1	$19.00
50 ct	14.7-18 oz., US1	$18.00		100 ct	6.4-8.5 oz. , US1	$19.00
60 ct	12.4-14.7 oz., US1	$19.00		US#2	6-10 oz	$19.00
70 ct	10.7-12.4 oz., US1	$19.00		US#2	>10 oz	$19.00

The percentage of tubers in each Tuber Category was multiplied by the price (Value/cwt column) and the resulting values were totaled to obtain a dollar value per cwt. These values were then multiplied by the marketable yield in hundredweight per acre to obtain the dollar value per acre.

The fresh pack contract calculations do not take into account the packer margin or shipping costs which can be highly variable and will reduce the amount paid to the grower. As a result, the values calculated for fresh pack are typically higher than what is calculated for processing. Even though the growers would receive less than what is reported, the values are reflective of quality differences which may have been influenced by treatments.

Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) using Agricultural Research Manager (ARM) 2017. When the treatment effect was significant ($P<0.10$; see "Treatment Prob (F)" at the bottom of each data column in the tables), mean separation was performed using Tukey's HSD. Means followed by the same lowercase letter are not statistically different when compared to each other. If the treatment variances were not homogeneous as determined by Bartlett's test for homogeneity, means were transformed prior to analysis. Back-transformed data are listed in the results.

Appendix 1. Site Description

Trial Location

City:	Rupert	Latitude of LL Corner °:	42.65285 N
State:	ID	Longitude of LL Corner °:	113.58664 W
Postal Code:	83350	Altitude of LL Corner, Unit:	4170 FT
Country:	USA	Angle y-axis to North °:	0

Directions: Travel northeast from Rupert, Idaho on State Highway 24 for 2.8 miles. Turn east (right) on 200 North and travel just over two miles to the Miller Research Office located at 426 East 200 North. The trial is located in the southwest corner of the potato field just one quarter mile to the north of the office.

Crop Description

Solanum tuberosum – Potato		Russet Norkotah TX-296		Russet Burbank	
		Planting Date:	May 1, 2017	Planting Date:	May 2, 2017
Planting Depth:	6 in	Planting Rate:	2267 lb/acre	Planting Rate:	3044 lb/acre
Row Spacing:	34 in	Avg. Seed Size:	2.36 oz	Avg. Seed Size:	3.16 oz
Plant Spacing :	12 in	Emergence:	May 22	Emergence:	May 22

Site and Design

Plot Width:	11.33 ft	Site Type:	Field
Plot Length:	29 ft	Experimental Unit:	1 Plot
Plot Area:	329 ft^2	Tillage Type:	Conventional-till
Replications:	4	Study Design:	Randomized Complete Block (RCB)
% Slope:	0.0	Untreated Arrangement:	Single control randomized in each block

Previous Crop

Year	Crop	Pesticides
2016	Winter barley	Affinity Broadspec, Starane Ultra, Aproach, Grizzly Z, Palisade
2015	Sugar beet	Mustang Maxx, Roundup PowerMax, Chlorpyrifos 4E-AG
2014	Potato	Vapam, Tricor 4F, Outlook, Prowl H_2O, Ultra Flourish, Leverage 360, Luna Tranquility, Bravo WS

Soil Description

% Sand:	80	% OM:	0.97	Texture:	Loamy sand
% Silt:	12	pH:	7.3	Soil Name:	Tindahay
% Clay:	8	CEC:	9.2	Fert. Level:	Good
				Soil Drainage:	Excellent

Analyzed By: Agvise Laboratories; 604 Highway 15 West; PO Box 510; Northwood, ND 58267

Cultural Practices

Tillage – McFarlane harrow; August 3 and 5, 2016
Tillage – Disk; August 9 (dry) and 23 (volunteer barley), 2016
Tillage – Ripper; October 11, 2016
Tillage – Row mark-out; November 15, 2016
Hilling – Lilliston Cultivator; May 10, 2017
Crop Destruct: Lifted Potatoes- 4-Row Lifter on October 9; Tillage – McFarlane Harrow; October 16, 2017

Initial Soil Fertility (sampled March 20, 2017; West Pivot east side sample)

Salts (mmhos/cm)	0.3	Organic N (lb/acre)	60	Calcium (meq/100 g)	5.4
Chlorides (ppm)	33	Ammonium-N (ppm)	4.5	Magnesium (meq/100g)	2.2
Sodium (meq/100g)	0.2	Nitrate-N (ppm)	6	Sulfate-S (ppm)	5
Excess Lime (%)	0.4	Phosphorus (ppm)	14	Zinc (ppm)	2.1
		Potassium (ppm)	145	Iron (ppm)	7.1
				Manganese (ppm)	6.9
				Copper (ppm)	0.7
				Boron (ppm)	1.05

Analyzed by Stukenholtz Laboratory; 2924 Addison Ave. E., PO Box 353; Twin Falls, ID 83303-0353

Appendix 2. Maintenance Applications

Fertilizers Applied

Date	Fertilizer	Composition	Rate/acre	Method	Units Applied
04 May 17	Ammonium Phosphate	11-37-0	22.5 gal	Planter/In-row	29 N, 100 P_2O_5
09 May 17	Urea (dry)	46-0-0	163 lb	Tyler air cart	75 N
	MAP	11-52-0	192.3 lb		21 N, 100 P_2O_5
	KCl	0-0-60	125 lb		75 K_2O
	K_2SO_4	0-0-50-18 S	200 lb		100 K_2O, 36 S
	Blu-Min Zinc Sul	0-0-0-18S-33Zn	30 lb		5 S, 10 Zn
09 Jun 17	UAN	32-0-0	5.37 gal	Fertigation	19 N
17 Jun 17	UAN	32-0-0	6.50 gal	Fertigation	23 N
22 Jun 17	UAN	32-0-0	6.64 gal	Fertigation	23.5
27 Jun 17	UAN	32-0-0	7.63 gal	Fertigation	27
28 Jun 17	K_2SO_4	0-0-50-18 S	190 lb	Tyler air cart	95 K_2O, 34 S
03 Jul 17	UAN	32-0-0	7.77 gal	Fertigation	27.5 N
10 Jul 17	UAN	32-0-0	7.63 gal	Fertigation	27 N
16 Jul 17	UAN	32-0-0	7.63 gal	Fertigation	27 N
23 Jul 17	UAN	32-0-0	7.63 gal	Fertigation	27 N
28 Jul 17	UAN	32-0-0	6.21 gal	Fertigation	22 N
02 Aug 17	UAN	32-0-0	4.80 gal	Fertigation	17 N
10 Aug 17	UAN	32-0-0	5.60 gal	Fertigation	20 N

Total Units Applied

N	385	S	75
P_2O_5	200	Zn	10
K_2O	270		

Maintenance Pesticides

Date	Product	Active Ingredient	Rate/acre	Target
15 May 17	Tricor 4F	metribuzin	0.75 pt	Weeds
	Outlook	dimethenamid-p	15 fl oz	Weeds
	Prowl H_2O	pendimethalin	1.5 pt	Weeds
15 Jun 17	Ridomil Gold SL	mefenoxam	6.1 fl oz	Pink rot
28 Jun 17	Ridomil Gold SL	mefenoxam	6.1 fl oz	Pink rot
	Movento	spirotetramat	4.0 fl oz	Aphids, psyllids
	Luna Tranquility	fluopyram + pyrimethanil	11.2 fl oz	Early blight, white mold
	Bravo WS	chlorothalonil	1.0 pt	Early blight, late blight
13 Jul 17	Endigo ZC	λ-cyhalothrin + thiamethoxam	4.5 fl oz	Loopers, aphids, CPB
	Movento	spirotetramat	4.0 fl oz	Aphids, psyllids
	Luna Tranquility	fluopyram + pyrimethanil	11.2 fl oz	Early blight, white mold
	Bravo WS	chlorothalonil	1.0 pt	Early blight, late blight
01 Aug 17	Bravo WS	chlorothalonil	1.5 pt	Early blight, late blight

Appendix 3: Weather Data

Precipitation and irrigation was recorded on site with a Watchdog Digital Rain (3665RD, Spectrum Technologies). Air temperature and relative humidity was recorded on-farm using a Watchdog 2000 Series weather station.

Trial Summary

			Air Temperature (F)						
	Precip	Irrigation	Max T		Min T			RH (%)	
Month	(in)	(in)	Absolute	Average	Absolute	Average	Avg T	Max	Min
May-17	1.05	0.24	91	71	30	42	56	100	8
Jun-17	0.66	7.26	100	80	41	51	66	100	9
Jul-17	0.00	9.70	100	92	50	58	75	94	12
Aug-17	0.06	6.62	99	89	38	53	70	100	7
Sep-17	1.77	1.75	98	74	32	45	59	100	11
Oct-17	0.28	0.00	68	57	23	32	44	100	10
Total	3.54	25.57							

Long Term Averages

		Temperature (F)		
	Precip			
Month	(in)	Max	Min	Average
May	1.13	69.5	39.6	54.6
Jun	0.80	78.7	47.1	62.9
Jul	0.26	88.1	53.0	70.5
Aug	0.33	87.4	51.6	69.5
Sep	0.55	77.0	43.0	60.0
Total	3.07			

Long term averages were taken from the Minidoka Dam, ID US USC00105980 NOAA weather station, “Summary of Monthly Normals, 1981-2010.”

May	Precipitation (in)		Air Temperature (F)			RH (%)	
Date	Rain	Irrigation	Max	Min	Avg	Max	Min
1-May	0	0	60	38	49	84	18
2-May	0	0	63	45	53	70	32
3-May	0	0	72	40	55	84	8
4-May	0	0	81	39	61	91	20
5-May	0	0	83	48	66	76	19
6-May	0	0	73	46	57	93	37
7-May	0.02	0	59	42	49	93	51
8-May	0	0	67	41	52	76	34
9-May	0	0	72	39	57	88	25
10-May	0	0	77	40	60	87	19
11-May	0	0	85	42	65	92	19
12-May	0	0	75	42	57	77	27
13-May	0	0	56	37	46	87	28
14-May	0	0	66	32	49	95	14
15-May	0	0	63	42	52	74	27
16-May	0.2	0	61	38	49	97	27
17-May	0.16	0	39	33	35	100	81
18-May	0	0.24	56	30	44	100	51
19-May	0.04	0	66	46	54	95	33
20-May	0.12	0	75	42	59	94	25
21-May	0.23	0	72	49	59	97	32
22-May	0	0	77	46	62	96	25
23-May	0	0	81	47	64	86	19
24-May	0	0	73	48	63	87	18
25-May	0	0	67	43	55	59	25
26-May	0.28	0	61	44	50	97	49
27-May	0	0	74	39	57	100	21
28-May	0	0	81	42	63	94	17
29-May	0	0	85	47	66	85	9
30-May	0	0	89	50	69	76	11
31-May	0	0	91	57	73	64	9
Absolute	1.05	0.24	91	30		100	8
Average			71	42	56		

June	Precipitation (in)		Air Temp (F)			RH (%)	
Date	Rain	Irrigation	Max T	Min T	Avg T	Max	Min
1-Jun	0	0	74	56	65	87	33
2-Jun	0	0.40	78	48	64	92	25
3-Jun	0	0.40	92	49	71	86	16
4-Jun	0	0	91	57	72	72	19
5-Jun	0	0	77	52	64	80	18
6-Jun	0	0.48	92	46	70	89	11
7-Jun	0	0.44	96	55	76	75	14
8-Jun	0	0	85	55	71	67	18
9-Jun	0.14	0.54	63	48	55	94	43
10-Jun	0	0	66	48	56	86	33
11-Jun	0	0	73	45	56	88	31
12-Jun	0.08	0	57	46	51	95	37
13-Jun	0.4	0.67	62	43	51	100	48
14-Jun	0	0	74	41	60	99	28
15-Jun	0	0	79	48	65	95	27
16-Jun	0	0	79	53	66	87	42
17-Jun	0	0.69	74	55	64	67	29
18-Jun	0	0	80	51	67	84	33
19-Jun	0	0	93	55	73	88	19
20-Jun	0	0.6	93	58	76	89	26
21-Jun	0	0	82	62	72	83	22
22-Jun	0	0.53	78	54	66	58	16
23-Jun	0	0	79	47	65	75	25
24-Jun	0	0	84	47	66	89	26
25-Jun	0	0.72	92	49	73	88	11
26-Jun	0	0	100	59	75	64	9
27-Jun	0	0.67	82	60	71	85	25
28-Jun	0	0	79	56	68	81	30
29-Jun	0	0.5	77	46	63	90	25
30-Jun	0	0	84	51	68	88	19
Absolute	0.62	6.64	100	41		100	9
Average			80	51	66		

July	Precipitation (in)		Air Temp (F)			RH (%)	
Date	Rain	Irrigation	Max T	Min T	Avg T	Max	Min
1-Jul	0	0.58	90	50	71	84	19
2-Jul	0	0	93	55	76	85	19
3-Jul	0	0.6	93	54	75	85	20
4-Jul	0	0	96	57	77	88	26
5-Jul	0	0.61	92	60	77	76	33
6-Jul	0	0	98	59	78	87	16
7-Jul	0	0	97	59	80	85	19
8-Jul	0	0.69	98	64	80	72	19
9-Jul	0	0	97	63	79	73	26
10-Jul	0	0.61	92	62	78	84	19
11-Jul	0	0	87	58	72	83	31
12-Jul	0	0.65	92	56	75	84	19
13-Jul	0	0	98	57	77	88	16
14-Jul	0	0.67	97	60	78	87	19
15-Jul	0	0	100	64	80	79	19
16-Jul	0	0.66	87	60	72	79	28
17-Jul	0	0	86	58	71	84	26
18-Jul	0	0.64	94	52	73	90	19
19-Jul	0	0	90	54	73	94	28
20-Jul	0	0	85	59	72	89	27
21-Jul	0	0.59	88	54	71	87	19
22-Jul	0	0	89	54	71	83	19
23-Jul	0	0.67	95	57	76	84	19
24-Jul	0	0	92	58	75	88	19
25-Jul	0	0	91	69	76	72	28
26-Jul	0	0.55	89	65	74	86	33
27-Jul	0	0	87	60	74	87	26
28-Jul	0	0.67	89	58	73	88	19
29-Jul	0	0	95	54	75	85	12
30-Jul	0	0	95	55	75	85	19
31-Jul	0	0.68	94	54	75	91	19
Absolute	0	8.87	100	50		94	12
Average			92	58	75		

August	Precipitation (in)		Air Temp (F)			RH (%)	
Date	Rain	Irrigation	Max T	Min T	Avg T	Max	Min
1-Aug	0	0	92	60	75	90	12
2-Aug	0	0.64	90	49	71	95	15
3-Aug	0	0	84	53	69	77	11
4-Aug	0	0.76	91	47	69	94	7
5-Aug	0	0	99	48	74	95	9
6-Aug	0	0	94	58	76	87	9
7-Aug	0	0	89	55	72	78	29
8-Aug	0	0.72	94	56	75	76	19
9-Aug	0	0	96	58	75	93	19
10-Aug	0	0.64	83	60	71	88	38
11-Aug	0	0	89	56	70	97	19
12-Aug	0	0	95	50	71	99	13
13-Aug	0	0.72	94	53	73	97	9
14-Aug	0.08	0	94	52	73	96	9
15-Aug	0	0.44	89	52	70	92	19
16-Aug	0	0	90	54	71	83	19
17-Aug	0	0	89	53	72	87	9
18-Aug	0	0	88	49	68	94	15
19-Aug	0	0.56	89	51	69	96	19
20-Aug	0	0	86	52	69	97	19
21-Aug	0	0.60	79	53	65	98	38
22-Aug	0	0	87	49	67	100	22
23-Aug	0	0	89	48	69	100	19
24-Aug	0	0.48	79	53	66	86	7
25-Aug	0	0	86	38	65	94	8
26-Aug	0	0.56	96	55	71	79	19
27-Aug	0	0	89	56	73	84	16
28-Aug	0	0	95	49	72	99	15
29-Aug	0	0	82	55	66	79	19
30-Aug	0	0.56	88	48	66	98	19
31-Aug	0	0	87	57	70	92	27
Absolute	0.08	6.68	99	38		100	7
Average			89	53	70		

September	Precipitation (in)		Air Temperature (F)			RH (%)	
Date	Rain	Irrigation	Max	Min	Avg	Max	Min
1-Sep	0	0.44	85	52	69	82	19
2-Sep	0	0	95	48	70	93	19
3-Sep	0	0	98	50	73	88	13
4-Sep	0	0	93	52	72	87	11
5-Sep	0	0	89	56	70	79	18
6-Sep	0	0.48	90	48	67	87	12
7-Sep	0	0	87	48	67	87	19
8-Sep	0	0	90	54	72	88	19
9-Sep	0	0	88	60	73	77	27
10-Sep	0	0	85	61	72	81	29
11-Sep	0	0	90	49	69	93	19
12-Sep	0	0	84	50	68	88	27
13-Sep	0	0	80	59	69	82	41
14-Sep	0.56	0	81	53	61	96	33
15-Sep	0.04	0	57	44	52	94	39
16-Sep	0	0	60	37	49	96	31
17-Sep	0	0	73	43	56	88	12
18-Sep	0	0	69	42	55	94	35
19-Sep	0	0	54	40	47	99	55
20-Sep	0.2	0	67	37	51	98	29
21-Sep	0.52	0	47	42	44	100	88
22-Sep	0.04	0	50	36	44	95	57
23-Sep	0.12	0	54	32	43	100	50
24-Sep	0	0	57	35	46	97	34
25-Sep	0	0	62	36	48	96	31
26-Sep	0	0	68	34	49	99	31
27-Sep	0	0	71	35	52	100	19
28-Sep	0	0	74	39	54	92	23
29-Sep	0	0	77	41	58	90	13
30-Sep	0	0	61	45	53	83	48
Absolute	1.48	0.92	98	32		100	11
Average			74	45	59		

Northwest Potato Research Consortium
A Cooperative Effort of the Potato Commissions of ID, OR, & WA
February Quarterly Report FY 2017-18 – Final Report

TITLE: Use of metconazole for improved yields and fungicide resistance management.

PERSONNEL: Jeff Miller, Trent Taysom, Cheryn Clayton, Shane Hansen, Scott Anderson; Miller Research; Rupert, ID 83350.

REPORTING PERIOD: February 2018

ACCOMPLISHMENTS:
The trial is complete. Full results are presented in the report below. This report was written with the Results and Discussion first, followed by the Materials and Methods. While this is not standard, it allows the readers to focus first on what we learned. The reader can then view the Methods if interested.

RESULTS:

1. As a fungicide, Quash was generally no more effective than Bravo for early blight control.
2. White mold incidence was low and this trial was not effective in measuring control.
3. Total yield was significantly increased when Quash was used at the first or second application (at row closure or two weeks later) in a four application program.
4. The yield increase was observed using both the 2.5 oz and 4.0 oz rate. The 4.0 oz rate did not increase yield over the low rate.
5. The yield increase was observed for programs using a single application and for those using two applications. Two applications did not improve yield compared to a single application when applied early in the program.
6. Metconazole (formulated as Quash) is a group 3 fungicide (triazole) and provides a different mode of action than the group 7 (SDHI) or group 11 (strobilurin) fungicides for early blight/brown leaf spot control. Use of metconazole in conjunction with group 7 or 11 fungicides will help with fungicide resistance management.
7. The yield increase will more than offset the cost of using the product.
8. MRL issues may still limit the use of this product for potatoes grown in the PNW.

PUBLICATIONS:

Miller, J.S., Gevens, A.J., Gudmestad, N.C., Taysom, T.W., Wharton, P.S., Welch, L., and Walston, A. 2017. Effect of metconazole on disease control and yield in potatoes. *Am. J. Potato Res*. 94:235-236.

PRESENTATIONS & REPORTS:

1. Miller J. Potato disease management. Invited presentation given at the 2018 Simplot Grower Solutions NW Regional Training at Idaho Falls, ID on February 13, 2018.
2. Miller, J. Avoiding pesticide resistance. Invited presentation at the Far West Agribusiness Association Winter Conference in Boise, ID on December 13, 2017.

3. Miller J. Potato disease management. Invited presentation given at the 2017 Simplot Grower Solutions NW Regional Training at Idaho Falls, ID on February 7, 2017.
4. Miller, J. Managing foliar diseases of potato. Invited presentation given at the 49th Annual Idaho Potato Conference at Pocatello, ID on January 18, 2017.
5. Miller, J.S., Taysom, T.W., Clayton, C., Miller, T.D., and Anderson, D.S. 2017. Effect of metconazole on disease control and yield in potatoes. Poster presented at the 2017 Potato Expo in San Francisco, CA on January 4-6, 2017.
6. Miller, J. Managing early blight and brown leaf spot of potato. Invited presentation given at the 43rd Annual Hermiston Farm Fair at Hermiston, OR on November 30, 2016.
7. Miller, J. Improving Efficiency of Product Usage in Potato. Invited presentation given at the 2016 Syngenta Potato Partners program in San Diego, CA on November 16, 2016.

INTRODUCTION

Our hypothesis is that the low rate of Quash (2.5 oz/a) is effective in reducing early blight/brown leaf spot and will increase yield similar to the high rate (4.0 oz/a) if applied relatively early in the season. A field trial was conducted to test this hypothesis as outlined below.

Treatments

Trt	App 1 (A)	App 2 (B)	App 3 (C)	App 4 (D)
1				
2	Bravo WS (1.5)	Bravo WS (1.5)	Bravo WS (1.5)	Bravo WS (1.5)
3	**Quash** (2.5) Bravo WS (1.0)	Bravo WS (1.5)	Bravo WS (1.5)	Bravo WS (1.5)
4	**Quash** (4.0) Bravo WS (1.0)	Bravo WS (1.5)	Bravo WS (1.5)	Bravo WS (1.5)
5	Bravo WS (1.5)	**Quash** (2.5) Bravo WS (1.0)	Bravo WS (1.5)	Bravo WS (1.5)
6	Bravo WS (1.5)	Bravo WS (1.5)	**Quash** (2.5) Bravo WS (1.0)	Bravo WS (1.5)
7	Bravo WS (1.5)	Bravo WS (1.5)	Bravo WS (1.5)	**Quash** (2.5) Bravo WS (1.0)
8	**Quash** (2.5) Bravo WS (1.0)	**Quash** (2.5) Bravo WS (1.0)	Bravo WS (1.5)	Bravo WS (1.5)
9	**Quash** (4.0) Bravo WS (1.0)	**Quash** (4.0) Bravo WS (1.0)	Bravo WS (1.5)	Bravo WS (1.5)
10	Bravo WS (1.5)	Bravo WS (1.5)	**Quash** (2.5) Bravo WS (1.0)	**Quash** (2.5) Bravo WS (1.0)

Rate per acre given in parentheses as either oz (Quash) or pints (Bravo WS).

RESULTS AND DISCUSSION

General Observations

Miller Research has evaluated metconazole (formulated as Quash) for the control of early blight and brown leaf spot for the past six years. Quash is easy to work with and mixes well. No phytotoxicity has been observed during this time frame. The effects of Quash have been fairly well documented and these results help solidify previous observations.

Greening and NDVI

One of the most visual effects that we have seen with Quash is a deeper green look to the canopy. It usually takes a few weeks after application to see this effect. On July 7 first symptoms of greening were observed (data not shown). Treatments 4 and 9 were the primary plots exhibiting greening at that time. On July 19 a more thorough evaluation was made by rating crop vigor/greening on a 1-3 scale. A 1 represented normal crop vigor as was observed in the untreated check and a 3 represented the darkest green color. By this time two foliar application had been made. Treatments with Quash applied at the A timing (treatments 3, 4, 8, and 9) were the only treatments that had a different color than the untreated check (Table 1). However, only the high rate of Quash (treatments 4 and 9) and two applications of the low rate (treatment 8) had statistically different color than the check at that time. On a follow up evaluation the next day, all treatments that had Quash applied to that point had a different color rating than the check. The lone exception was the 2.5 oz rate at the B timing which was only slightly higher. By July 28 it appeared that the color change observed on July 20 was beginning to dissipate. Treatments that had received Quash at the B timing (treatments 5, 8, and 9) had the highest color rating at that point. On August 11, following all applications, all treatments exhibited a color change compared to the check. However, treatments 4, 5, 8, 9, and 10 were the only treatments that had significantly different color than the check. The two applications of the high rate (treatment 9) had the highest color rating on that date.

An NDVI (Normalized Vegetative Index) reading was measured to try to quantify more fully the canopy color. This was done using a hand held GreenSeeker (Trimble Navigation Limited) which was held approximately 24” over either row two or row three of every plot. The Greenseeker device measures the reflectance of NIR red and infrared light off of the canopy surface and reports the value as a ratio of the two wavelengths of light. A value of 1 would be more lush and green and a value of 0 would be no green. Hence, the higher the value the greater the canopy health. Although we could visually see color differences in the field, differences in NDVI values were not observed (Table 1). This would indicate that although some plots were darker green they were not necessarily healthier.

These crop color data suggest that the greening effect observed with Quash takes time to develop and begins to dissipate as time increases from the application of Quash. When we made two application of Quash, the color change lasted longer. Even treatment 10 which had two later applications of Quash (at C and D) had a trend for darker green color. It also appears that the high rate of Quash may have a greater greening effect than the low rate and lasted longer. Treatment 8 had begun to fade by August whereas treatment 9 was still showing darker green color at that time.

Disease Control

Early blight and brown leaf spot, hereafter referred to as early blight, developed later than usual for our area. By August 14, the untreated check was only exhibiting around 5% severity

but increased significantly to 35% on August 30 (Table 2). All treatments significantly reduced early blight over the untreated check on all rating dates. Treatments that received Quash early at application A tended to have lower early blight than other treatments. Rate of application did not appear to be significant as two applications of the 2.5 oz rate (treatment 8) was just as effective as the two applications of the 4 oz rate (treatment 9). In addition, one application of the 2.5 and 4 oz rates (treatments 3 and 4, respectively) was statistically similar to two applications (treatments 8 and 9).

White mold incidence was light and only averaged 12 lesions per 58 row feet in the untreated check (Table 3). Differences among treatments were not observed for disease incidence and severity. Other trials with Quash have shown some efficacy with white mold in the past. Although white mold control is listed on the label, we have not observed Quash to work as well as other premium fungicides like Endura, Omega, or Luna Tranquility.

Crop Vigor

The untreated check was among the lowest for late season crop vigor (table 3). Only Quash at the high rates (treatments 4 and 9) and two early application at the low rate (treatment 8) had significantly higher crop vigor than the untreated check.

Total Yield

In past trials plots treated with Quash have almost always had the highest yields. Yields in this trial were relatively high for southern Idaho. Treatments where Quash was applied at either the A or B timings had the highest yields. Two applications at timings A and B yielded similarly to only one application at A. Two applications of Quash at timings C and D also yielded similarly to the highest performers. The higher rate did not provide an advantage over the lower rate in total yield, but yields of the high rate were among the top numerically. These data are consistent to what was observed in a similar trial in 2015. Applications at the early timings provided the highest yields. It is interesting to note that in 2015 the application of Quash at the C and D timings did not yield as well as was observed in this trial.

Resistance Management

We did not evaluate fungicides sensitivity as part of our research. However, it is important to emphasize the principles related to using Quash as part of an integrated disease control program. Currently, most growers are applying FRAC group 7 fungicides (succinate dehydrogenase inhibitors – SDHI) and/or FRAC group 11 fungicides (strobilurins) to control early blight and brown leaf spot.[1] Resistance to both of these fungicide classes in the *Alternaria solani* population have been shown to be widespread in southern Idaho.[2,3] Quash is a triazole fungicide, and is group 3. Using a triazole fungicide (like Quash) will diversify the modes of action to which the A. solani populations are exposed. This should help in slowing the development of fungicide resistance.

[1] The "groups" referred to here are the mode of action groups established by the Fungicide Resistance Action Committee, or FRAC. (http://www.frac.info/)

[2] Fairchild, K.L., Miles, T.D., and Wharton, P.S. 2013. Assessing fungicide resistance in populations of *Alternaria* in Idaho potato fields. Crop Protection 49:31-39.

[3] Pasche, JS, and Gudmestad, NC. 2008. Prevalence, competitive fitness and impact of the F129L mutation in *Alternaria solani* from the United States. Crop Protection 27:427-435.

Economic Benefit

At the beginning of 2017, we were quoted approximate prices for Quash from distributors in southern Idaho. Our purchase price for Quash would have translated to approximately $20/acre/application for the 2.5 oz rate and $30/acre/application for the 4.0 oz rate. Regardless of the cost, the average increase in total yield for 2.5 oz applied early (timing A or B) was 38 cwt/acre compared to the Bravo WS only program. The dollar return from the additional 38 cwt would more than cover the cost of the Quash application.

In the past, growers have expressed some hesitation to diversify fungicide treatments. It is difficult to add an additional mode of action to a fungicide application if the only reason for doing so is fungicide resistance management. The added fungicide should provide a return on that investment. While the actual numbers may change, multiple trials with Quash have shown a large enough yield increase to pay for the added cost of the product.

Based on other fungicide trial work we have done, we recommend growers use a group 7 fungicide such as Luna Tranquility or Endura mixed with a protectant product for the first two foliar applications (timings A and B). This approach provides the most effective early blight and white mold control. Adding or replacing the protectant with Quash to the program will further diversify the modes of action and increase the yield.

Other Considerations

A major limitation to using Quash relates to maximum residue limits (MRLs). At the time of writing this report, MRLs have not been established for all Pacific Rim countries. If growers are considering using Quash in a fungicide program, it is imperative that they check with the buyer to be sure that Quash is on an approved use list.

We have been asked if the results of this trial would translate to other geographic regions. Research trials done in the Midwest have not always shown the same yield response as we have observed.[4] Trends for yield increase have been noted, but the magnitude of the response was not as great as what was observed in Idaho with a four-application program. At the 2017 Potato Expo results from a previous trial (similar to this) were presented at the poster session. A grower from Chile indicated that they had observed similar results with metconazole. (In Chile, metconazole on potatoes is sold as Caramba.) At the 2018 Potato Expo, a consultant from Alberta, Canada indicated that he observed increased yields due to metconazole application in strip trials in 2016. However, in 2017 he did not measure a yield increase. We do not know how the consultant's application approach relates to the strategy that we employed in this trial.

Summary

Quash applications resulted in a change in crop color in the form of canopy greening. This effect occurs a few weeks after application and dissipates following the last application. Early blight control was greatest when Quash was applied early. The high rate did not improve control over the low rate. White mold pressure was low. Yields were the highest when Quash was applied early. This is similar to what was observed in a similar trial in 2015. Two applications of Quash at C and D increased yield in 2017 but not in 2015. With respect to yield, one application of Quash at the first or second applications at the low label rate (2.5 oz) will provide a positive return on investment.

[4] Miller, J.S., Gevens, A.J., Gudmestad, N.C., Taysom, T.W., Wharton, P.S., Welch, L., and Walston, A. 2017. Effect of metconazole on disease control and yield in potatoes. *Am. J. Potato Res*. 94:235-236.

Table 1. Visually Observed Greening Evaluation and NDVI Reading

Description					Greening				NDVI
Rating Unit					1-2	1-2	1-2	1-2	Number
Date					19 July	20 July	28 July	11 August	11 August
Trt	Treatment	Rate	Unit	Code*					
1	Check				1.00 b	1.00 c	1.00 d	1.00 d	0.863 a
2	Bravo WS	1.5	pt/a	ABCD	1.00 b	1.00 c	1.00 d	1.13 cd	0.865 a
3	Quash	2.5	oz wt/a	A	1.13 b	1.35 b	1.25 cd	1.25 bcd	0.868 a
	Bravo WS	1.0	pt/a	A					
	Bravo WS	1.5	pt/a	BCD					
4	Quash	4.0	oz wt/a	A	1.50 a	1.87 a	1.50 bc	1.38 bc	0.870 a
	Bravo WS	1.0	pt/a	A					
	Bravo WS	1.5	pt/a	BCD					
5	Quash	2.5	oz wt/a	B	1.00 b	1.23 bc	1.75 ab	1.50 b	0.870 a
	Bravo WS	1.0	pt/a	B					
	Bravo WS	1.5	pt/a	ACD					
6	Quash	2.5	oz wt/a	C	1.00 b	1.00 c	1.00 d	1.25 bcd	0.868 a
	Bravo WS	1.0	pt/a	C					
	Bravo WS	1.5	pt/a	ABD					
7	Quash	2.5	oz wt/a	D	1.00 b	1.00 c	1.00 d	1.00 d	0.870 a
	Bravo WS	1	pt/a	D					
	Bravo WS	1.5	pt/a	ABC					
8	Quash	2.5	oz wt/a	AB	1.63 a	1.74 a	2.00 a	1.38 bc	0.870 a
	Bravo WS	1	pt/a	AB					
	Bravo WS	1.5	pt/a	CD					
9	Quash	4.0	oz wt/a	AB	1.75 a	2.00 a	2.00 a	1.88 a	0.873 a
	Bravo WS	1	pt/a	AB					
	Bravo WS	1.5	pt/a	CD					
10	Quash	2.5	oz wt/a	CD	1.00 b	1.00 c	1.25 cd	1.50 b	0.868 a
	Bravo WS	1	pt/a	CD					
	Bravo WS	1.5	pt/a	AB					
LSD P=.10					0.291	0.263 - 0.315	0.338	0.295	0.0088
Standard Deviation					0.242	0.086t	0.281	0.245	0.0073
CV					20.13	6.37t	20.4	18.52	0.84
Grand Mean					1.200	1.342t	1.375	1.325	0.8683
Treatment Prob(F)					**0.0001**	**0.0001**	**0.0001**	**0.0009**	0.7639

*A = June 26; B = July 10; C = July 24; D = August 7

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the automatic square root transformation of x+0.5 (Jul-20). Back transformed means are given in the table.

Table 2. Early Blight Severity

Description				% Early Blight		
Rating Date				Aug-14	Aug-23	Aug-30
Trt	Treatment	Rate	Code*			
1	Check			5.2 a	12.8 a	35 a
2	Bravo WS	1.5 pt/a	ABCD	1.2 bc	3.8 cde	15 bcd
3	Quash	2.5 oz wt/a	A	0.9 bc	3.3 def	12 cde
	Bravo WS	1.0 pt/a	A			
	Bravo WS	1.5 pt/a	BCD			
4	Quash	4.0 oz wt/a	A	0.7 bc	2.3 f	10 de
	Bravo WS	1.0 pt/a	A			
	Bravo WS	1.5 pt/a	BCD			
5	Quash	2.5 oz wt/a	B	1.4 b	3.5 def	15 bc
	Bravo WS	1.0 pt/a	B			
	Bravo WS	1.5 pt/a	ACD			
6	Quash	2.5 oz wt/a	C	1.3 bc	5.0 bc	18 b
	Bravo WS	1.0 pt/a	C			
	Bravo WS	1.5 pt/a	ABD			
7	Quash	2.5 oz wt/a	D	1.4 b	4.5 bcd	16 bc
	Bravo WS	1 pt/a	D			
	Bravo WS	1.5 pt/a	ABC			
8	Quash	2.5 oz wt/a	AB	0.6 c	2.3 f	9 e
	Bravo WS	1 pt/a	AB			
	Bravo WS	1.5 pt/a	CD			
9	Quash	4.0 oz wt/a	AB	1.0 bc	3.0 ef	11 de
	Bravo WS	1 pt/a	AB			
	Bravo WS	1.5 pt/a	CD			
10	Quash	2.5 oz wt/a	CD	1.4 bc	5.3 b	17 b
	Bravo WS	1 pt/a	CD			
	Bravo WS	1.5 pt/a	AB			
LSD P=.10				0.83 - 2.13	1.46	4.07 - 6.09
Standard Deviation				0.15t	1.21	3.11t
CV				42.08t	26.62	13.53t
Grand Mean				0.36t	4.55	23.02t
Treatment Prob(F)				**0.0009**	**0.0001**	**0.0001**

*A = June 26; B = July 10; C = July 24; D = August 7

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

t=Mean descriptions are reported in transformed data units, and are not de-transformed. Data were transformed using the automatic log transformation of x+1(Aug 14) and the automatic arcsine square root % transformation (Aug-30). Back transformed means are given in the table.

Table 3. Late Vigor, White Mold Incidence and Severity

Description Rating Unit Rating Date				Vigor % Sep-6	White Mold Incidence # Lesions Sep-14	 Severity 0-3 Sep-14
Trt	Treatment	Rate	Code*			
1	Check			10 d	12 a	1.70 a
2	Bravo WS	1.5 pt/a	ABCD	21 cd	19 a	2.90 a
3	Quash	2.5 oz wt/a	A	22 bcd	4 a	1.88 a
	Bravo WS	1.0 pt/a	A			
	Bravo WS	1.5 pt/a	BCD			
4	Quash	4.0 oz wt/a	A	32 abc	14 a	1.95 a
	Bravo WS	1.0 pt/a	A			
	Bravo WS	1.5 pt/a	BCD			
5	Quash	2.5 oz wt/a	B	19 cd	24 a	2.50 a
	Bravo WS	1.0 pt/a	B			
	Bravo WS	1.5 pt/a	ACD			
6	Quash	2.5 oz wt/a	C	10 d	10 a	2.88 a
	Bravo WS	1.0 pt/a	C			
	Bravo WS	1.5 pt/a	ABD			
7	Quash	2.5 oz wt/a	D	20 cd	26 a	2.80 a
	Bravo WS	1 pt/a	D			
	Bravo WS	1.5 pt/a	ABC			
8	Quash	2.5 oz wt/a	AB	42 a	8 a	1.80 a
	Bravo WS	1 pt/a	AB			
	Bravo WS	1.5 pt/a	CD			
9	Quash	4.0 oz wt/a	AB	38 ab	13 a	2.83 a
	Bravo WS	1 pt/a	AB			
	Bravo WS	1.5 pt/a	CD			
10	Quash	2.5 oz wt/a	CD	21 cd	6 a	1.20 a
	Bravo WS	1 pt/a	CD			
	Bravo WS	1.5 pt/a	AB			
LSD P=.10				15.39	13.69	1.160
Standard Deviation				12.77	11.37	0.963
CV				54.3	82.52	42.93
Grand Mean				23.53	13.78	2.243
Treatment Prob(F)				**0.0156**	0.1547	0.1640

*A = June 26; B = July 10; C = July 24; D = August 7

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

Table 4. Total Yield

Description Rating Unit				Total Yield cwt/acre
Trt	Treatment	Rate	Code*	
1	Check			701 c
2	Bravo WS	1.5 pt/a	ABCD	705 bc
3	Quash	2.5 oz wt/a	A	751 a
	Bravo WS	1.0 pt/a	A	
	Bravo WS	1.5 pt/a	BCD	
4	Quash	4.0 oz wt/a	A	752 a
	Bravo WS	1.0 pt/a	A	
	Bravo WS	1.5 pt/a	BCD	
5	Quash	2.5 oz wt/a	B	736 ab
	Bravo WS	1.0 pt/a	B	
	Bravo WS	1.5 pt/a	ACD	
6	Quash	2.5 oz wt/a	C	715 bc
	Bravo WS	1.0 pt/a	C	
	Bravo WS	1.5 pt/a	ABD	
7	Quash	2.5 oz wt/a	D	713 bc
	Bravo WS	1 pt/a	D	
	Bravo WS	1.5 pt/a	ABC	
8	Quash	2.5 oz wt/a	AB	749 a
	Bravo WS	1 pt/a	AB	
	Bravo WS	1.5 pt/a	CD	
9	Quash	4.0 oz wt/a	AB	768 a
	Bravo WS	1 pt/a	AB	
	Bravo WS	1.5 pt/a	CD	
10	Quash	2.5 oz wt/a	CD	737 ab
	Bravo WS	1 pt/a	CD	
	Bravo WS	1.5 pt/a	AB	
LSD P=.10				33.7
Standard Deviation				28.0
CV				3.82
Grand Mean				732.7
Treatment Prob(F)				**0.0234**

*A = June 26; B = July 10; C = July 24; D = August 7

Means followed by same letter do not differ significantly (P=0.10, LSD). Mean comparisons performed only when ANOVA Treatment Prob (F) is significant at the pre-determined mean comparison level (<0.10). Significant values are bolded.

MATERIALS AND METHODS

Trial Establishment

The trial was established at the Miller Research Experimental Farm near Acequia, ID. The previous crops were winter barley (2016), sugarbeet (2015), and potato (2014).

The trial area was harrowed on August 3 and 5, 2016 and then disked on August 9 (dry) and 22 to kill volunteer grain. The field was ripped to a depth of 12 inches on October 11. On October 18, metam sodium fumigant (Vapam HL) was injected 9 inches deep on the ripper shanks at a rate of 40 gallons per acre. Potato rows were marked out on November 15.

Soil samples were collected in the spring (March 20). A total of 25 soil cores (0-12") were collected from the trial area. The soil was analyzed by Stukenholtz Laboratory.

Planting

Potato seed was obtained from a commercial grower who had cut and treated the seed with 6% MZ dust. The average seed size was 3.16 oz/seed piece.

Potato seed was planted on May 4 with an ACME bulk potato planter. Starter fertilizer was applied in the row two inches to the side and just below where the seed pieces were dropped. Row spacing was 34 inches, plant spacing within the row was 12 inches, and seed pieces were planted to a depth of 6-7 inches. This translates to a planting rate of 3044 lb/acre. Plots were four rows wide (11.33 feet) and 29 feet long with a 7-foot border between plots. Drive rows were established within the plot so that foliar applications could be made to the trial without driving through the plot area. These two rows were established on the east and west sides of the trial and between reps 2 and 3. Treatments were established in a randomized complete block design with four replications (Figure 1).

Fungicide Applications

Fungicides were applied using the Miller Research ground plot sprayer (a small self-propelled tractor with a hydrostatic drive). The first application was made just prior to row closure. Three additional applications were made on approximately a 14-day schedule.

Fungicides were mixed in a three-gallon capacity stainless steel tank. A Teflon-coated laboratory magnet was placed inside the tank. The tanks were loaded on a rack on the spray tractor which had a second magnet located under the tank. The magnet on the rack was turned with a hydraulic motor which caused the magnet inside the tank to turn. This allowed for constant agitation of the spray mixtures during application. Spray tanks were pressurized with compressed air. The spray boom consisted of eight TeeJet XR 11002 VS flat fan nozzles spaced 18 inches apart. Sprayer speed was measured at 3.7 mph and this resulted in a spray volume of 12.3 gallons per acre.

Details on application conditions, crop conditions, and application equipment are provided in Appendix 1.

Trial Map

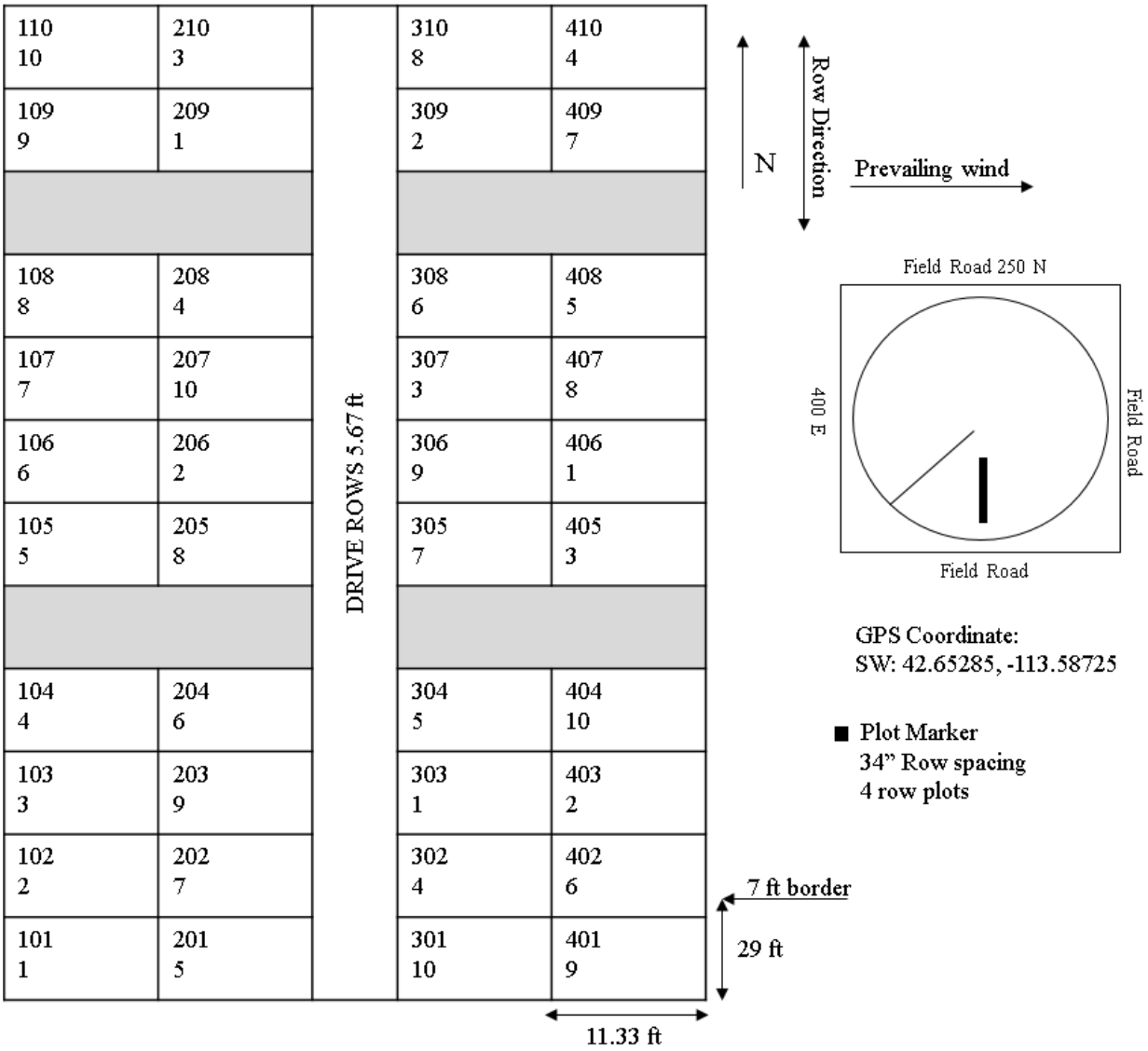

Figure 1. Trial map for the Potato Research Consortium Metconazole trial conducted at Miller Research near Acequia, ID in 2017. The number on the first line in each plot is the plot number and the number on the second line is the treatment number. Plots in replication 1 are labeled in the 100's, replication 2 in the 200's, etc.

Early Blight/ Brown Leaf Spot

Each plot was visually assessed for the percentage of foliage with early blight (caused by *Alternaria solani*) and brown leaf spot (caused by *A. alternata*) symptoms on August 17, 24, and 30. Assessments were made by two people evaluating the center two rows and the ratings were averaged.

Crop Vigor

Plants were rated for general vigor on September 6. The health of the plants was visually estimated using a 0-100 scale where 0 represents dead plants and 100 represents completely healthy plants. Ratings were made by two individuals and averaged to obtain the final rating.

White Mold

White mold (caused by *Sclerotinia sclerotiorum*) was evaluated on September 14. The number of visible lesions in one row (29 row feet) of each plot was counted. The severity of each infection was graded on a 1-3 scale where one represented a small lesion (less than 2 inches in length), 2 represented a moderate lesion (2-5 inches in length), and 3 represented a severe lesion (greater than 5 inches in length.

Stem lesions affected with black dot and gray mold can sometimes appear similar to white mold. The presence of sclerotia in lesions was used to verify that lesions were caused by *S. sclerotiorum* when lesion identity was in doubt.

Tuber Yield

Tubers from two plot rows were harvested using a specially modified two-row Lockwood 4620 harvester on September 26. Tubers were lifted and cleaned by the harvester and crew riding on the machine, and then dropped into a basket hanging from the end of the delivery boom. The basket was suspended by an electronic load cell scale which weighed all tubers harvested from the two center rows. The weight in pounds was converted to cwt/acre.

Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) using Agricultural Research Manager (ARM) 2017. When the treatment effect was significant ($P<0.10$; see "Treatment Prob (F)" at the bottom of each data column in the tables), mean separation was performed using Fisher's protected LSD. Means followed by the same lowercase letter are not statistically different when compared to each other. If the treatment variances were not homogeneous as determined by Bartlett's test for homogeneity, means were transformed prior to analysis. Back-transformed data are listed in the results.

Appendix 1. Application Data

Environmental Data

	A	B	C	D
Application Description:	Row close (RC)	A + 14	B + 14	C + 14
Application Date:	June 26	July 10	July 24	August 7
Appl. Start Time:	4:17 PM	8:35 AM	11:21 AM	4:08 PM
Appl. Stop Time:	4:36 PM	8:47 AM	11:34 AM	4:28 PM
Application Method:	Spray	Spray	Spray	Spray
Application Timing:	Row closure	Fixed interval	Fixed interval	Fixed interval
Application Placement:	Foliar	Foliar	Foliar	Foliar
Applied By:	Taysom, T.	Taysom, T.	Taysom, T.	Taysom, T.
Air Temperature (F):	93	78	88	86
% Relative Humidity:	32	58	40	48
Wind Velocity (mph):	2.5	0	4	2
Wind Direction:	E	N/A	W	N
Dew Presence:	N	N	N	N
Soil Temperature (F):	74	66	67	74
Soil Moisture:	85	75	85	80
% Cloud Cover:	60	0	20	70
Next Moisture:	June 27	July 10 (5:00 PM)	July 26	July 8

Crop Stage (BBCH) at Application

	A	B	C	D
Stage Majority:	39	43	45	47
Diameter (in):	32	34	34	34
Height (in):	23	24	24	24
Crop coverage (%):	95	100	100	100

Application Equipment

Appl. Equipment:	MR Sprayer 1
Equipment Type:	Tractor-mounted sprayer
Operation Pressure (psi):	20
Nozzle Type:	Teejet flat fan
Nozzle Size:	XR 11002VS
Nozzle Spacing (in):	18
Nozzles/Row:	2
% Coverage:	100
Boom ID:	1A
Boom Length (ft):	12
Boom Height (in):	10-12 (above canopy)
Ground Speed (mph):	3.70
Carrier:	Water
Water Hardness (ppm CaCO3):	250
Spray Volume:	12.3
Mix Size (gal):	1.5
Spray pH:	7.8 (pre-mix)
Propellant:	Compressed air
Tank Mix:	No

Northwest Potato Research Consortium

A Cooperative Effort of the Potato Commissions of ID, OR, & WA

February Quarterly Report FY 2017-18

TITLE: Evaluation of phosphorous acid fungicide programs for improved pink rot management and assessment of mefenoxam resistance in pink rot pathogen populations in the PNW.

PERSONNEL:

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REPORTING PERIOD: February 2018

ACCOMPLISHMENTS:

The field trials for objectives 1 and 2 have been completed and the mefenoxam sensitivity testing from objective 3 has been completed for the 2017 growing season. The results from the field test were discouraging from the standpoint that a significant reduction in pink rot was not observed with treatments which have shown significant reductions in the past. (In a nearby trial which was sponsored by Syngenta, significant differences were observed among treatments and selected treatment results are shown below for comparison purposes.)

Additional tests are underway to challenge inoculate tubers harvested from the field plots to see how tubers respond to a more uniform pathogen exposure. These results will be added to an updated final report in the future.

The following report was written with the Results and Discussion first, followed by the Materials and Methods. While this is not standard, it allows the readers to focus first on what we learned. The reader can then view the Methods if they are interested.

RESULTS:

1. Pink rot incidence ranged from 1-5% for the various treatments in both trials.
2. Phosphite (=phosphorous acid) programs were generally similar to the untreated check for pink rot incidence.
3. Significant differences were observed for timing in relation to irrigation (Table 2). When assessing disease incidence by weight, applications which were made 12 hours before application had the lowest incidence.
4. These results indicate that the timing between application and irrigation may not be critical for the performance of phosphorous acid.
5. Mefenoxam resistance was common in southern Idaho.
6. Some mefenoxam resistance was observed in the Columbia Basin.
7. Foliar phosphite fungicide programs did not provide protection against Pythium leak based on a post-harvest challenge assay.

PUBLICATIONS:
None to date.

PRESENTATIONS & REPORTS:
1. Miller, J., Taysom, T. and Hansen, S. Miller Research Potato Pest Management Seminar. Held at Rupert, ID on January 30, 2018.
2. Miller, J., Olsen, N. Kinzer, K. Getting a Head Start – Be Proactive about Foliar Blight Diseases. Potato Grower: June 2016, pp.28-29.
3. Miller J. Potato disease management. Invited presentation given at the 2018 Simplot Grower Solutions NW Regional Training at Idaho Falls, ID on February 13, 2018.
4. Miller, J. Using all the available tools to manage pink rot and leak. Invited presentation at the WA/OR Potato Conference in Kennewick, WA on January 24, 2018.
5. Miller, J. Avoiding pesticide resistance. Invited presentation at the Far West Agribusiness Association Winter Conference in Boise, ID on December 13, 2017.
6. Miller, J. Managing pink rot and Pythium leak. Invited presentation given at the Bingham Cooperative CHS Grower Meeting at Blackfoot, ID on March 1, 2017.
7. Miller J. Managing pink rot and Pythium leak. Invited presentation given at the McCains's 2017 Potato Grower Meeting at Burley, ID on February 27, 2017.
8. Miller, J. Managing pink rot and early blight. Invited presentation given at the 25th Annual Shoshone Bannock Tribes Agricultural Resources Management Grower Meeting at Fort Hall, ID on February 23, 2017.

RESULTS AND DISCUSSION

Timing of Program Initiation

Pink rot incidence was statistically similar for all timing programs (Table 1). Despite inoculation, the untreated check only had 4.2% incidence (based on number of tubers infected) or 2.6% incidence (based on weight of tubers infected). The phosphite program which was started early (full emergence) had the lowest incidence. It was discouraging that the normal program (starting when the largest tubers were dime-size) did not result in any type of reduction (statistical or numerical).

Tuber number was numerically higher with all phosphite fungicide programs (Table 1). Total yield was highest for the early program.

Due to the lack of statistical differences, it is not possible to draw conclusions about the efficacy of the different programs.

Table 1. Effect of Seasonal Phosphite Program Timing

	Total Yield		Pink Rot Incidence	
Description	cwt/acre	Tuber #	Tuber #	Weight
Trt Treatment				
1 Untreated check	629 a	436 a	4.2 a	2.6 a
2 Early (Full emergence)	650 a	460 a	2.3 a	1.4 a
3 Normal (Dime size tubers)	631 a	457 a	4.8 a	3.4 a
4 Late (Row closure	617 a	465 a	3.8 a	2.2 a
LSD P=.10	55.0	57.68	3.320	2.106
Standard Deviation	42.5	44.50	2.561	1.625
CV	6.72	9.79	67.62	67.88
Grand Mean	632.0	454.56	3.788	2.394
Treatment Prob(F)	0.7452	0.8090	0.5797	0.4498

Early = Applications started at full emergence; June 3, June 17, and July 1
Normal = Applications started at dime-size tubers; June 14, June 28, and July 12
Late = Applications started at row closure; June 27, July 12, and July 25

Timing of Application Relative to Irrigation

When pink rot incidence was calculated based on tuber number, treatments differences were not statistically different (Table 2). However, statistical differences were observed when incidence was measured according to tuber weight. When phosphite fungicides were applied 12 hours before irrigation, pink rot was significantly lower compared to the untreated check. Applications made 48 and 6 hours before irrigation were statistically similar to the 12-hour treatment. We expected greater treatment separation based on previous studies. However, these data indicate that irrigation does not need to be 48 hours after application to see the greatest performance of phosphite fungicides. The data from the 12-hour interval show that adequate control can be obtained otherwise.

Total yield and tuber number were statistically similar for all treatments (Table 2). Tuber number was numerically higher for phosphite fungicide treatments, but yields were very similar.

Table 2. Effect of Application Timing Prior to Irrigation

Description	Total Yield		Pink Rot Incidence (%)	
	cwt/acre	Tuber #	Tuber #	Weight
Trt Treatment				
1 Untreated check	629 a	436 a	4.2 a	2.6 a
5 48 hours pre-irrigation	637 a	469 a	3.7 a	2.0 ab
3 24 hours pre-irrigation	631 a	457 a	4.8 a	3.4 a
6 12 hours pre-irrigation	609 a	444 a	1.3 a	0.6 b
7 06 hours pre-irrigation	637 a	454 a	3.4 a	2.1 ab
LSD P=.10	46.3	46.78 - 48.07	2.162	1.596
Standard Deviation	36.8	0.04t	1.715	1.267
CV	5.85	1.36t	49.22	60.03
Grand Mean	628.6	2.66t	3.485	2.110
Treatment Prob(F)	0.8000	0.7354	0.1041	**0.0917**

Disease Pressure in the Field

Another pink rot trial was conducted on our farm, but was irrigated by center pivot. (The trial was sponsored by Syngenta Crop Protection and shared here by permission). Outside of the irrigation, the trials were conducted similarly. In the center pivot trial, the check showed over 6% incidence (Table 3). All treatments in the trial significantly reduced pink rot. Orondis Gold combined with the phosphite fungicide Resist 57 provided the greatest reduction.

This indicates that the potential for disease reduction was present under our growing conditions. It is not certain why the fungicide programs under the solid set were not as effective. Previous work sponsored by the Idaho Potato Commission showed that pink rot pressure was generally higher when plants were irrigated less frequently with more water, compared to those irrigated more frequently with less water. Under these conditions, fungicide applications of metalaxyl (formulated as Metastar) were more effective when irrigation was with less water, but applied more frequently (similar to a pivot). That may be why the disease control was greater under the pivot than under the solid set.

Table 3. Selected results from a pink rot trial sponsored by Syngenta Crop Protection. This trial was irrigated by a center pivot, not solid set.

Description			% Pink Rot
Trt Treatment	Rate Unit	Code*	
1 Check			6.3 a
3 Orondis Gold	10.8 fl oz/acre	A	0.9 cde
6 Ridomil Gold Bravo	2.5 pt/acre	BC	1.4 cd
7 Orondis Gold	10.8 fl oz/acre	A	0.5 de
Resist 57	8 pt/acre	BCD	
8 Ranman	6.5 fl oz/acre	A	3.1 b
LSD P=.10			0.86 - 3.44
Standard Deviation			0.18t
CV			38.03t
Grand Mean			0.47t
Treatment Prob(F)			**0.0001**

Code: A = in-furrow at planting, B = dime size tubers, C = B + 14 days, D = C + 14 days.

Mefenoxam Sensitivity of Phytophthora erythroseptica

In southern Idaho, Phytophthora erythroseptica was recovered from samples representing only 6 fields in Idaho and 5 in the Columbia Basin (Table 1). A total of 80 and 16 isolates were obtained, respectively, from these samples with each isolate representing a different tuber. In Idaho, most were resistant to mefenoxam (88%). The few isolates which were sensitive came from 4 of the 6 fields. This indicates that some sensitive isolates are still present. Testing was also done to see if the isolates were sensitive to phosphite fungicides and none showed resistance. In the Columbia Basin, resistance to mefenoxam was observed in two different samples.

This indicates that mefenoxam efficacy is still somewhat limited in southern Idaho, and may be losing efficacy in some places in the Columbia Basin. More work needs to be done in this area.

Table 4. Results for mefenoxam sensitivity testing for Phytophthora erythroseptica in the PNW for 2017.

	# Isolates	# Fields	% Resistant to Mefenoxam	% Resistant to Phosphite
Southern Idaho	80	6	88	0
Columbia Basin	16	5	38	--

An effort was made to get more isolates for evaluation. Carrie Wohleb distributed extension materials which 1) let growers know that some populations of *Phytophthora erythroseptica* have developed resistance to mefenoxam; and 2) notified them that they could have tubers with pink rot tested. Seven issues of the *WSU Potato Pest Alerts* featured pink rot. These were sent to 780 subscribers:
July 7, 2017 (9)
July 21, 2017 (11)
Aug. 4, 2017 (13)
Aug.18, 2017 (15)
Sept. 1, 2017 (17)
Sept. 8, 2017 (18)
Sept. 15, 2017 (19)

Efficacy of Fungicide Programs on Pythium Leak

There was no statistical significance among phosphite fungicide treatments for Pythium leak incidence (p-value=0.62; Table 5). There was no evidence that the timing of phosphite fungicide applications help in the protection against Pythium leak disease (Table 6).

Table 5. ANOVA table for Pythium leak inoculation trial on tubers harvested from plots receiving phosphite fungicide treatments at different timings.

	Degrees of freedom (Df)	Deviance	Residual Df	Resid. Dev	Pr (>Chi)
Treatment	3	1.76	614	740.31	0.62
Rep	7	20.88	607	719.43	0.0039
NULL	NA	NA	617	742.06	NA

Table 6. Incidence of Pythium leak when tubers from plants receiving different foliar phosphite fungicide programs were challenged with *Pythium ultimum*.

Description	Leak Incidence (%)
Trt Treatment	
1 Untreated check	27 a
2 Early (Full emergence)	26 a
3 Normal (Dime size tubers)	29 a
4 Late (Row closure	32 a

Early = Applications started at full emergence; June 3, June 17, and July 1
Normal = Applications started at dime-size tubers; June 14, June 28, and July 12
Late = Applications started at row closure; June 27, July 12, and July 25

MATERIALS AND METHODS

Site Preparation

The trial was established at the Miller Research Experimental Farm near Acequia, ID in the West field. The previous crops were winter barley (2016), sugar beet (2015), and potato (2014).

The trial area was harrowed twice and disked twice in 2016 following the harvest of the winter barley. The trial area was ripped on October 11. On October 27, metam sodium fumigant (Vapam HL) was injected 9 inches deep on the ripper shanks at a rate of 40 gallons per acre. Potato rows were marked out on November 15.

Soil samples were collected in the spring (March 20, 2017). A total of 25 soil cores (0-12") were collected from the trial area. The soil was analyzed by Stukenholtz Laboratory.

Additional details relating to the trial site are in Appendix 1.

Inoculation

The trial was inoculated just prior to planting, and again just prior to hilling. For each inoculation, cultures of *Phytophthora erythroseptica* (which were sensitive to mefenoxam) were blended with water in a blender. The resultant suspension of agar with *P. erythroseptica* mycelia and oospores was further diluted in order to have enough inoculum to treat the entire trial. The inoculum applied to the soil was estimated to be 1×10^4 oospores/ml and 4.2×10^3 oospores/ml for the planting and hilling inoculations, respectively.

The final suspension was sprayed as a 10" banded spray over the top of the potato hills at a rate of 11 ml/row ft, which translates to 2.75×10^4 and 1.17×10^4 oospores/row ft for the planting and hilling inoculations, respectively.

The first inoculation was done immediately prior to the planting of the treated potato seed and inoculum was incorporated into the soil with the potato planter. The second inoculation was done immediately prior to hilling and inoculum was incorporated into the soil with the Lilliston cultivator.

Planting

Certified seed (cv. Russet Norkotah TX-296) for this trial was purchased from a commercial potato grower. The seed had been cut and treated (6% MZ bark, 1 lb/cwt and Maxim MZ, 0.25 lb/cwt) by the grower. The average seed piece weight was 2.36 oz/seed piece.

Potato seed was planted on April 25 with a modified ACME bulk potato seed planter. Starter fertilizer was applied in the row two inches to the side and just below where the seed pieces were dropped. Row spacing was 34 inches, plant spacing within the row was 12 inches, and seed pieces were planted to a depth of 6-7 inches. This translates to a planting rate of 2267 lb/acre. Plots were four rows wide (11.33 feet) and 29 feet long with a 7-foot border between plots. Drive rows were established within the plot so that foliar applications could be made to the trial without driving through the plot area. Two rows were established on the north and south sides of the trial and between reps 2 and 3 as drive rows for sprayer travel. Treatments were established in a randomized complete block design with four replications (Figure 1).

Foliar Applications

Foliar phosphite applications (Resist 57, 10 pt/acre) were made according to the schedule shown in Table 7. Applications were made using the Miller Research ground plot sprayer (a small self-propelled tractor with a hydrostatic drive).

Products were mixed with water in 3-gallon capacity stainless steel tanks. A Teflon-coated laboratory magnet was placed inside the tank and the tank was loaded on the sprayer. A second magnet on the sprayer located under the tank was turned with a hydraulic motor which caused the magnet inside the tank to turn. This allowed for constant agitation of the spray mixture during application. The spray tank was pressurized with compressed air and connected to the spray manifold.

The spray boom consisted of eight TeeJet XR 11002 VS flat fan nozzles spaced 18 inches apart. Sprayer speed was measured at 3.70 mph and this resulted in a spray volume of 12.3 gallons per acre.

Table 7. Actual treatment dates for phosphorous acid applications.

Trt	Full Emergence	Dime-size Tubers	Row Closure (RC)	RC + 14 days	RC + 28 days	Description
1.						Check
2.	June 3	June 17	July 1			Early, 48 pre
3.		June 14	June 28	July 12		Normal, 24 pre
4.			June 27	July 12	July 25	Late, 48 pre
5.		June 13	June 27	July 11		Normal, 48 pre
6.		June 15	June 29	July 13		Normal, 12 pre
7.		June 15	June 29	July 13		Normal, 6 pre

Phytotoxicity

Plants were evaluated for phytotoxicity on June 16, July 7, 20, and 28. Additionally, a portion of the trial was evaluated during each application. Any sign of leaf burn or curling was noted.

Tuber Yield

Tubers were harvested on September 18 using a specially modified two-row Lockwood 4620 harvester. Tubers from rows 2 and 3 were lifted and cleaned by the harvester and crew riding on the machine. The tubers were dropped into a basket hanging from the end of the delivery boom. The basket was suspended by an electronic load cell scale which weighed all tubers harvested from the center two plot rows. After weighing, tubers were dropped on the ground. The weight in pounds was converted to cwt/acre. The tubers were evaluated for pink rot a few hours later after the moisture on tuber skins had dried.

Pink Rot

After the plot weight was obtained, tubers were dropped on the ground and allowed to air dry. It is important to evaluate the tubers when they are dry because infected tubers remain moist longer than healthy, sound tubers, making them easier to identify. This is especially true for tubers in the early stages of decay. All harvested tubers were visually inspected for pink rot symptoms on September 18. The total number of tubers and the number of tubers with pink rot symptoms was recorded. All tubers with pink rot symptoms were weighed. Pink rot incidence was estimated as the percentage of tubers infected by number and the percentage of the harvested weight infected.

Statistical Analysis

Data from the Miller Research field experiments were analyzed by analysis of variance (ANOVA) using Agricultural Research Manager (ARM) 2017. When the treatment effect was significant ($P<0.10$; see "Treatment Prob (F)" at the bottom of each data column in the tables), mean separation was performed using Fisher's protected LSD. Means followed by the same lowercase letter are not statistically different when compared to each other. If the treatment variances were not homogeneous as determined by Bartlett's test for homogeneity, means were transformed prior to analysis. Back-transformed data are listed in the results.

Mefenoxam Sensitivity Tests

Tubers putatively affected with *P. erythroseptica* were submitted to either Washington State University (Rachel Bomberger and Carrie Wohleb) or Miller Research (Jeff Miller). *P. erythroseptica* could not be isolated from all tubers. When *P. erythroseptica* was isolated, radial growth was tested on agar amended with mefenoxam. At Miller Research isolates were also tested for sensitivity to phosphite (Resist 57). If isolate growth was not restricted at 10 ppm mefenoxam, then the isolate was considered resistant.

Post-Harvest Pythium Leak Assay

A subset of tubers harvested from the field trial at Miller Research on September 18 were cured at 55°F at the Kimberly Research and Extension center. Twenty tubers from each rep of the timing treatments were taken and warmed to 70°F on October 2 (experiment 1) and 31 in the walk-in incubator and bin 4 (experiment 2), respectively. Tubers were bruised for 90 sec in a cement mixer and inoculated with $4.6x10^5$ oospores/ml and $3.2x10^5$ oospores/ml at a rate of 3 ml/lb. Tubers were then returned to incubate until evaluated four days post inoculation. Percent severity was recorded, however percent incidence was analyzed using logistic regression in R.

Final Report
February 15, 2018

Title: Identifying a potential pathogenic cause of the disease symptoms and early plant senescence in the Columbia Basin potatoes in 2016.

Personnel: Kylie D. Swisher, USDA-ARS, TTFVRU Prosser, WA

Cooperators: Tim Waters, WSU Extension, Pasco, WA; Carrie Wohleb, WSU Extension, Moses Lake, WA

Reporting Period: 2017-18

Summary of accomplishments:

1. *Grafting symptomatic field tissue:*

   Showed that the symptoms identified in commercial and research plots across the Columbia Basin during the 2017 field season were not graft-transmissible to healthy recipient plants in the greenhouse.

   Implications of results: Since the symptoms of crinkly, distorted leaves, terminal purpling, and stem blistering were not graft-transmissible, it suggests that the cause of the 2017 field symptoms was not pathogenic in nature. This information is what growers need to know in order to continue the quest to identify a causal agent and prevent these symptoms in the future.

2. *Molecular diagnostics:*

   Showed that the symptoms identified in commercial and research plots across the Columbia Basin during the 2017 field season were not associated with any common viral, bacterial, or fungal pathogen.

   Implications of results: Since there was no pathogen identified by targeting known pathogens, or by targeting universal fungal pathogens, this provides further evidence and supports the grafting results, indicating that the 2017 field symptoms were not pathogenic in nature.

Activities or experiments conducted:

A total of 75 symptomatic potato plants were collected or received from eight different commercial or research fields across the Columbia Basin in July and August of 2017. Symptoms included purpling of upper leaf terminals, leaf distortion on newer growth, including leaf crinkling, warping, and holes, and bumps or blisters along the stems, often accompanied by necrotic lesions (see Figure 1). Symptoms were seen across entire fields; there did not appear to be any edge effect as often seen with insect-vectored pathogens which occur when the insect migrates into a field. Standing back from several fields, it was difficult to see the symptoms, but once in the field, it was near impossible to find a plant that did not have one or more of the symptoms listed above. These symptoms were observed in at least five different potato cultivars, including Ranger Russet, Alturas, Umatilla Russet, Clearwater and Challenger. Symptoms were

worse in certain cultivars, especially Umatilla Russet. Interestingly, an organic commercial potato field was observed to have the same symptoms, indicating that chemical damage was likely not the cause.

Figure 1. Symptoms observed across the Columbia Basin during the 2017 growing season. Pictures on the left, top and bottom: Crinkly, warped, distorted leaves. Pictures in the middle, top and bottom: Purple terminals, negative for BLTVA phytoplasma. Pictures on the right, top and bottom: Stem blistering, bumps, and necrotic lesions.

1. Greenhouse grafting:

A subset of the plant tissue received or collected from the field was grafted to healthy plants (grown from greenhouse-grown minitubers) in the greenhouse in Prosser. The goal here was to observe if symptoms from the field samples could be transmitted to healthy plants. This would indicate whether the symptoms were caused by a pathogen that could go systemic or by other factors such as an environmental stimulus, insect feeding damage, or even an insect toxin, all of which cannot go systemic. A total of 48 grafted plants were generated in the greenhouse, each with two or three scions originating from the commercial or research field plants (see Figure 2). Plants were grafted and observed on a weekly basis. Notes were taken to indicate how long the grafted scions survived, and whether any symptoms appeared on the recipient plants. Control plants were also grafted each time field-collected samples were

Figure 2. Example of grafting conducted in the greenhouse. Three scions were grafted to the greenhouse-grown recipient plant.

grafted. Controls consisted of greenhouse-grown scions grafted to greenhouse-grown recipient plants. These controls enable the identification of any symptoms generated by the greenhouse conditions.

2. Molecular diagnostics:

All samples collected or received from the field were subjected to molecular laboratory diagnostic assays to identify any pathogen(s) present in the samples. Two samples were taken from the leaf tissue of each plant for diagnostics to ensure that detection of a pathogen would not be missed. Additionally, tubers were collected or received from three different field sites and sampled for pathogens by molecular methods. Nucleic acids were extracted from each leaf and tuber sample using the standard Dellaporta protocol. Extracts were then subjected to standard polymerase chain reaction (PCR) analysis or reverse transcription-PCR analysis for the detection of the following pathogens: phytoplasmas (universal target), '*Candidatus* Liberibacter solanacearum' (Lso), *Tobacco rattle virus, Potato mop top virus, Alfalfa mosaic virus, Tomato spotted wilt virus, Potato leafroll virus, Potato virus Y,* and fungal pathogens (universal target).

Results:

1. Greenhouse grafting:

Overall, the symptoms of crinkly, distorted leaves, purpling terminals, and stem blistering and necrosis did not transmit from grafted scions to the recipient plants, as no symptoms were seen that correlated across all plants. Nearly all recipient plants with symptomatic scions originating from the field showed normal plant growth similar to the control plants (see Figure 3).

Figure 3. All images depict recipient plants with scions grafted six and a half weeks prior. Red arrows point to the scions visible on each recipient plant.

Some observations were made on recipient plants that did not appear normal or healthy. Specifically, two plants showed purpling of leaves and stems, and developed aerial tubers. These plants were confirmed positive for BLTVA phytoplasma in the laboratory. A different plant showed possible leaf deformity, but a control plant showed similar leaf deformity suggesting that the symptoms was not be due to the field sample. Another plant showed purpling of terminal leaves on the recipient plant, but when the recipient plant was tested in the

laboratory, it was negative for BLTVA phytoplasma. One plant showed aerial tuber formation at the site of grafting, but with no other visible symptoms, it was assumed that the aerial tuber developed as a result of the grafting process and disruption of the phloem. Lastly, one plant showed swelling of nodes, but this was different from the stem blisters and necrotic lesion from the field samples, and was assumed to be unrelated to the field symptoms.

2. Molecular diagnostics:

There were no pathogens identified in the laboratory that correlated to the field symptoms. Two samples collected mid-July from a commercial field, and two samples collected at the beginning of August from a research plot were positive for BLTVA phytoplasma (the causal agent of potato purple top). Two of these four samples were grafted in the greenhouse and produced the classic BLTVA symptoms, including purpling of leaf and stem tissue and aerial tuber formation. No other field samples, many of which showed classic purple top symptoms were positive for phytoplasma. Comparison of the purple terminals from BLTVA-infected and BLTVA-free plants was done, but there was no obvious distinction in symptoms (see Figure 4).

Figure 4. Comparison of purple terminals from a BLTVA-positive plant (right) and a BLTVA-negative plant (left).

No samples, leaves or tubers, were positive for '*Candidatus* Liberibacter solanacearum' (Lso), *Tobacco rattle virus, Potato mop top virus, Alfalfa mosaic virus, Tomato spotted wilt virus,* or *Potato leafroll virus.* Numerous foliar samples were positive for *Potato virus Y*, including PVY^{NTN} and $PVY^{N:O}$/$PVY^{N\text{-}Wilga}$ strains. There was no correlation between symptomatic samples with leaf distortion, purpling terminals, and stem blistering, and those positive for specific strains of PVY.

Numerous foliar samples also showed a PCR product when a universal fungal ITS region was targeted. Five of these samples (different cultivars and different symptoms) were subject to molecular cloning and sequencing of the unknown fungal pathogen(s). Sequencing of three clones from each sample did not identify any pathogen that was correlated with the symptoms of leaf distortion, purple top, or stem blistering/necrosis. Common fungal pathogens were however identified, including Altenaria species and *Sclerotinia sclerotiorum*.

Conclusions drawn from these results:

The field symptoms from 2017 did not appear to be graft-transmissible in our hands, and no pathogen was correlated to the symptoms using molecular analyses in our laboratory. Based on this, we must conclude that the symptoms were not pathogenic in nature. Despite the above-ground symptoms, there did not appear to be symptoms in the tubers sampled, and it appeared that tuber yield was good at the time of sampling. These observations indicate that the symptoms may not cause quality or quantity losses for growers.

Problems to address in the future:

Grafting analyses was performed using standard technique. However, a small percentage of scions died after the grafting process (within the first two weeks). While this should be enough time for transmission of some pathogens, it is possible that the grafting technique failed on these samples. Performing additional grafting on samples with these same symptoms, if they appear again in 2018, will be necessary to be 100% confident that these symptoms are truly not pathogenic in nature.

Molecular analyses did not identify any pathogen directly associated with the symptoms of leaf distortion, purple top, or stem blistering and necrosis. While this likely means that the plant symptoms were not actually associated with a pathogen, it is possible that the pathogen(s) was missed using the PCR methods listed above. Again, performing these analyses on a second set of samples, assuming these symptoms appear in 2018, will be necessary to validate our results from this first year of study.

Continued research from the 2017-2018 year:

Tubers were collected from three different commercial fields in the Columbia Basin during the 2017 field season. After the tubers were sampled for molecular testing in the laboratory, they were stored in the potato storage building at the Prosser research station. In the next couple of weeks, we plan to plant these tuber samples in the greenhouse so we can observe the plants that grow from these apparently healthy tubers. While it is a long shot, if any plants emerge and show similar foliar symptoms to those of the 2017 season, it will provide important information about the nature of the causal agent of these field symptoms.

Publications:

None

Presentations:

K.D. Swisher. Symptomatic (crinkly) potatoes. AgriNorthwest. Kennewick, WA. February 6, 2018.

Annual Progress Report
Annual reports due: February 15th

TITLE: Identification and characterization of elicitors to maximize defense system against powdery scab in potato roots

PERSONNEL:
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REPORTING PERIOD: January, 2017 – December, 2017

ACCOMPLISHMENTS:

The goal of this project is to identify an effective elicitor derived from the powdery scab pathogen or from infected potato root tissues. We hypothesize that a specific elicitor (a plant defense inducer) for the powdery scab disease can evoke a strong, effective defensive response in potato roots. Identification of a specific elicitor and characterization of the recognition system of the elicitor will eventually allow us to develop economical and effective methods to control powdery scab.

Since there were not any useful bioassays using potatoes for the elicitor screening, during FY2015-17, we have dedicated to establish the methods to monitor early stress responses in potato cells, e.g., cytosolic calcium elevation, production of reactive oxygen species (ROS), extracellular pH elevation, and changing in expression of defense-related genes. Those results were published as methodology articles (Moroz et al., 2017a; Moroz et al., 2017b). For cytosolic calcium measurement, we have produced transgenic potatoes expressing calcium sensor, aequorin, and those results will be reported elsewhere (a manuscript is currently in preparation). We also established molecular detection methods for powdery scab pathogens, *Spongospora subterranea* f. sp. *Subterranea* (*Sss*) and Potato mop top virus (PMTV). Such quantitative methods for the pathogen detection was needed for preparing inoculum in this project. One of the methods we established, i.e., on-site molecular detection, was reported in a video journal (DeShields et al., 2018) and a couple of others are currently in preparation.

We are currently working on setting some disease infection methods for powdery scab in a petri dish and greenhouse trial, which is important to reproduce the disease in the lab. We have also dedicated to isolate defense inducers, i.e., elicitors from infected potato tissues, and obtained some preliminary results. In this report, we describe some results through trial and error in CY2017. For designing infection methods, PI Tanaka has been advised by Co-PIs, Dennis Johnson and Charles Brown with technical supports and materials for testing. We are hoping that we can show more results in next round of reports.

RESULTS:

Cytosolic calcium measurement

We have successfully generated transgenic Désirée potato, in which a calcium sensor, aequorin, is expressing in cytosolic area in the potato cells. Using those potato plants, we have tested elicitor-induced calcium response based on the previous protocol (Tanaka et al., 2013) with some modification. As shown in Figure 1, flgII-28, systemin, and chitin elicitors induced calcium response in different potato tissues, leaves, nods, internodes, and roots. The results suggested that potato can response to elicitors in a comparable manner as other plants, e.g., Arabidopsis. Leaves and nodes showed highest responses, whereas roots responded very weak. Those results indicate that this calcium measurement assay is unsuitable to test soilborne and root-infecting pathogens. But it is still useful as a phenotypic marker when we perform isolation of new elicitors (defense inducers) in aerial potato tissues.

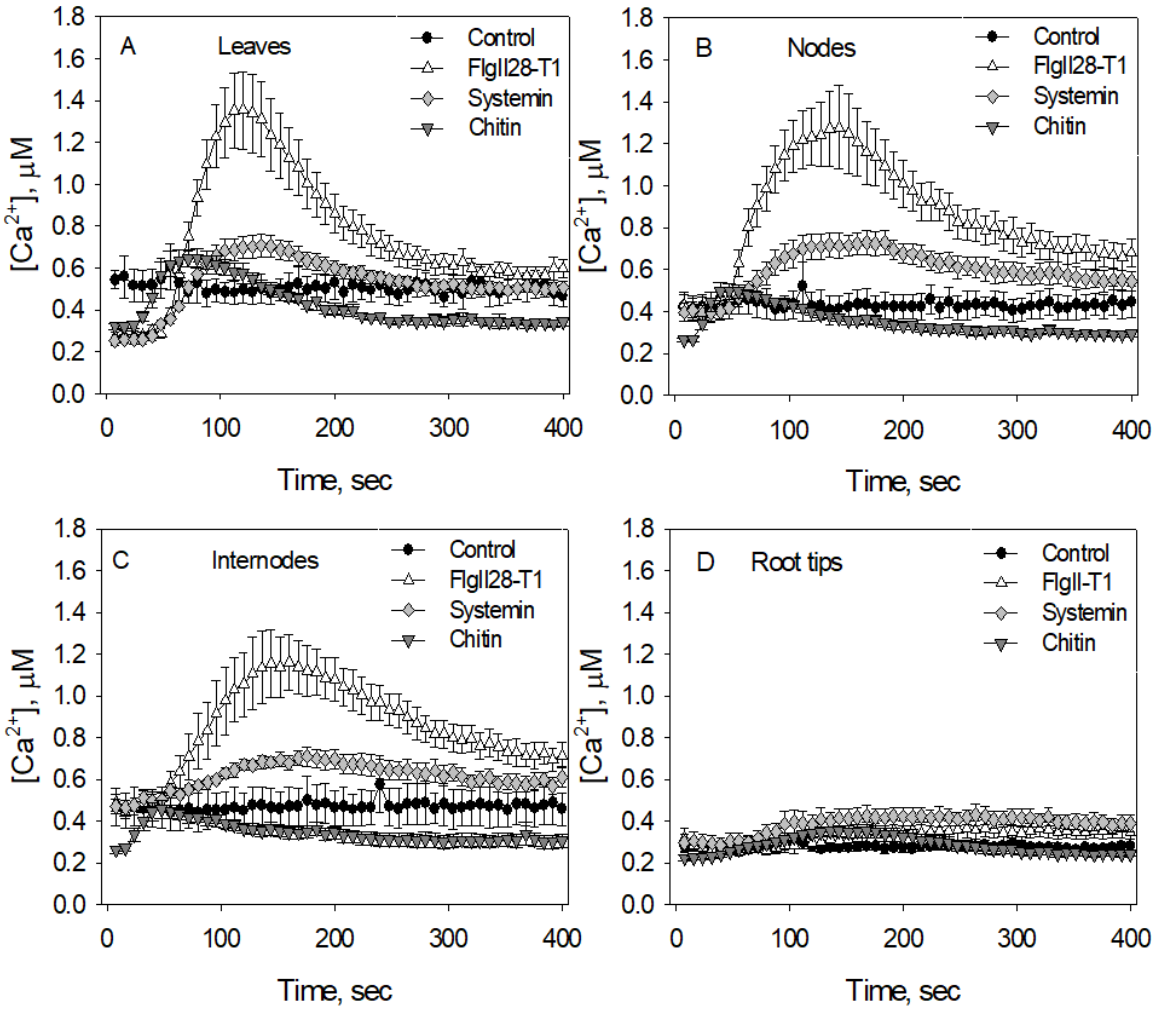

Figure 1. Effects of various elicitors on the dynamic changes in cytosolic calcium concentrations in different potato tissues. Individual samples from leaves (5 mm diameter disc), nodes, internodes, and root tips (5 mm long) was subjected to measurement of cytosolic calcium concentrations.

Explore an infection method for powdery scab

Given that there is no available in vitro culture system for *Sss*, we are required to set up disease infection methods for powdery scab either in a petri dish or a greenhouse trial. Stable source of inoculum and infected tissues are crucial to obtain substantial amounts of starting materials for the elicitor isolation.

As an *in vitro* infection system, we tried a method using hairy root potato, which is induced by *Agrobacterium rhizogenes* (Qu and Christ, 2007). After induction of the hairy roots, we infected the roots with suspended *Sss* pathogen from the plant callus harboring the pathogen. As shown in Figure 2, we have been trying a hairy root system to obtain galls in the petri dish. We have successfully induced hairy roots of Russet Burbank, Mesa, and Shepody, however, gall formation has not been observed yet. Now we are troubleshooting with modifying conditions, e.g., temperature, nutrition in the root growth medium, etc.

We have also performed an alternative trial in a growth chamber in which we used sandy soil containing *Sss* inoculum and relatively low temperature (~18°C). Shepody potatoes were used for the experiment. As shown in Figure 3, we successfully reproduced powdery scab disease on the tuber skin. This inoculation method has been triplicated with the typical scabby symptom. We also confirm *Sss* existence using ELISA and qPCR. However, we are never successful to induce the root galls. This growth-chamber-based method is at least useful in the future to test effect of elicitor candidates isolated in this project. Moreover, although it might not be efficient, this method will help to boost amounts of inoculum.

Figure 2. Procedure for *S. subterranea* infection using a hairy-root system. Hairy roots were induced by *A. rhizogens*. After two weeks of hairy-root growth, spore balls of *Sss* were applied over the roots, and incubated further six weeks. Note that any galls have not been observed on the hairy roots yet.

Figure 3. Growth chamber trials. Pictures represent roots and tubers from growth chamber trials. Shepody potato was planted in soil containing *Sss* inoculum, except for control (top left). *Sss* inoculum successfully caused scabby skin on the tubers, whereas no root galls were observed.

Isolation of active compounds (elicitor candidates) for *Sss*-infected potato tissues

Since there is no success for in vitro infection system, we decided to use potato suspension cultures as a starting material for the extraction of active compounds that cause early defense responses (e.g., extracellular alkalinization). Several extractions have been attempted, e.g., homogenization, boiling, acid, and acetone extractions. As a negative control, we will use extract from non-infected potato cells. After several trials, we concluded to use an acid-based extraction method (Figure 4), in which TFA (trifluoroacetic acid) were used for acidification and reverse-phased chromatography were employed to fractionation of mixed compounds. Finally, several fractions containing active compounds were purified by several fractionation steps. All procedures were performed under aseptic conditions. As shown in Figure 5, we monitored the putative elicitor's activity using extracellular alkalinization assay (Moroz et al., 2017a). As a result, purified fraction named as CSss30 (eluted by 30% acetonitrile from infected cells), MSCSss30 (eluted by 30% acetonitrile from supernatant of infected cells), and CSss40 (eluted by 40% acetonitrile from infected cells) were strongly induced plant defense response (based on extracellular alkalinization assay).

For further characterization, we submitted those purified fractions to mass spectrometry analysis in the WSU core facility (Tissue Imaging and Proteomics Laboratory https://labs.wsu.edu/tipl/) and other facility (http://sams.ucalgary.ca/). Currently, we are analyzing those omics data (Table 1) and narrowing down the candidates. Subsequently, we will synthesize candidate elicitors, and screen by testing with several assays we established in the project, i.e., alkalinization assay, ROA assay, calcium assay, and measurement of defense-related gene expressions. At the same time, for further purification, we also fractionated the extract, including putative active elicitors by high-performance liquid chromatography (HPLC).

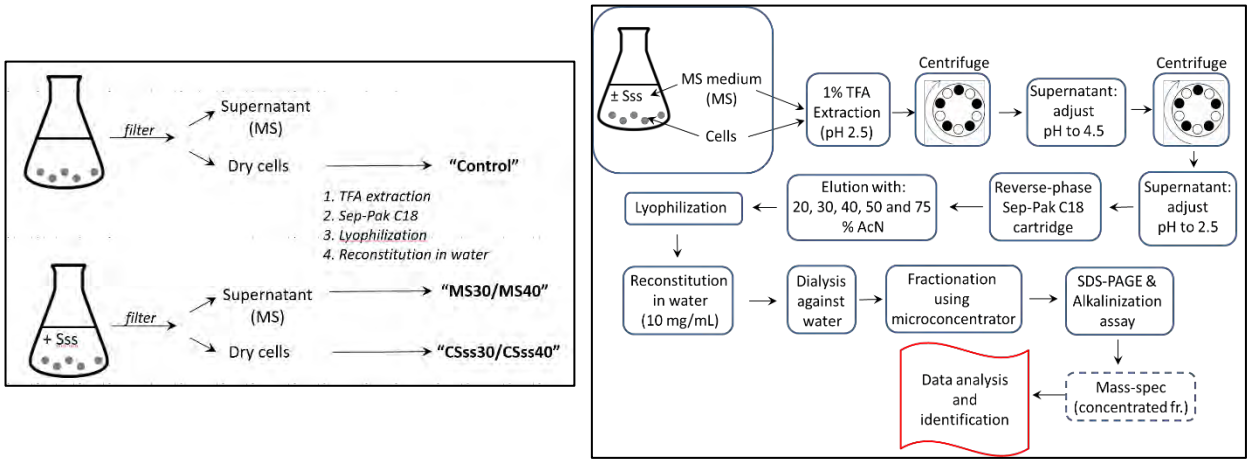

Figure 4. Experimental design for elicitor isolation from infected potato cells. Left panel represents the origin of each sample. Lower panel shows procedure of fractionation and purification steps to isolate active compounds that induce plant defense responses, i.e. elicitors.

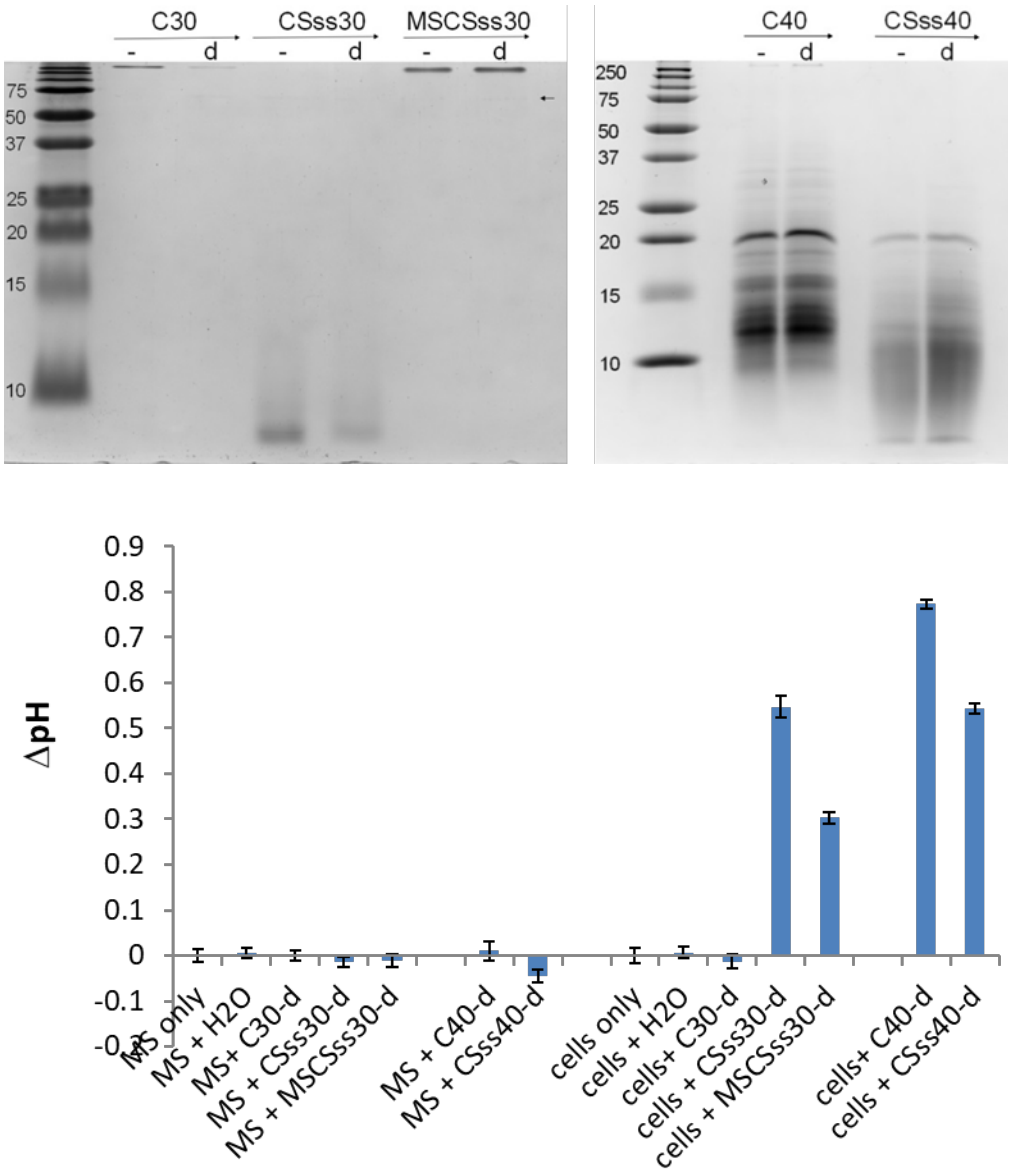

Figure 5. Elicitor candidate fractions from infected potato tissues. Top panels represent SDS-PAGE images of each fractions (see Figure 4 in detail). Lower panel shows activity of the fractions on extracellular alkalinization, which is a indicator of early plant defense response.

Table 1. Mass spectrometry results. Note that this is a list of top 32 out of 17,200 candidates.

Protein name	Percentage sequence coverage	Peptide sequence	Best Peptide identification probability	Best Mascot Ion Score
Uncharacterized protein OS=Solanum tuberosum GN=102592822 PE=4 SV=1	27.80%	CLEQFSSEK	99.70%	32.9
Uncharacterized protein OS=Solanum tuberosum GN=102592822 PE=4 SV=1	27.80%	IQTPAYPSAAK	98.70%	21.6
Uncharacterized protein OS=Solanum tuberosum GN=102595943 PE=3 SV=1	10.60%	AAVPSGASTGIYEALELR	99.70%	67.7
Uncharacterized protein OS=Solanum tuberosum GN=102595943 PE=3 SV=1	10.60%	YGQDATNVGDEGGFAPNIQENKEGLELLK	99.70%	31
Uncharacterized protein OS=Solanum tuberosum GN=102584758 PE=3 SV=1	15.10%	AVEVASQVNQWAEK	99.70%	44
Uncharacterized protein OS=Solanum tuberosum GN=102584758 PE=3 SV=1	15.10%	QIVDNVYK	99.00%	24.8
Uncharacterized protein OS=Solanum tuberosum GN=102584758 PE=3 SV=1	15.10%	STDELNSLSSQIVEVVFADGSPSGGPR	99.70%	47
Uncharacterized protein OS=Solanum tuberosum GN=102584758 PE=3 SV=1	15.10%	VSSESQFLER	99.70%	69.8
Uncharacterized protein OS=Solanum tuberosum GN=102600321 PE=4 SV=1	6.67%	ISLSEVVDEAGVGGEVER	99.70%	47.5
Uncharacterized protein OS=Solanum tuberosum GN=102600260 PE=4 SV=1	37.50%	EVLPAVQR	99.00%	33.6
Uncharacterized protein OS=Solanum tuberosum GN=102600260 PE=4 SV=1	37.50%	MIGGVLVER	99.70%	41.6
Uncharacterized protein OS=Solanum tuberosum GN=102600260 PE=4 SV=1	37.50%	NKEGIEEVIAR	99.70%	48.4
Uncharacterized protein OS=Solanum tuberosum GN=102600827 PE=4 SV=1	44.40%	EKVNNMVLFDK	99.70%	35.9
Uncharacterized protein OS=Solanum tuberosum GN=102600827 PE=4 SV=1	44.40%	GTYDKLITEAPK	99.70%	52.6
Uncharacterized protein OS=Solanum tuberosum GN=102600827 PE=4 SV=1	44.40%	LITPSVLSDR	99.70%	51.9
Uncharacterized protein OS=Solanum tuberosum GN=102600827 PE=4 SV=1	44.40%	LITPSVLSDRLR	99.70%	27.1
Uncharacterized protein OS=Solanum tuberosum GN=102600827 PE=4 SV=1	44.40%	MVSAHASQQIYTR	99.70%	101
Uncharacterized protein OS=Solanum tuberosum GN=102600827 PE=4 SV=1	44.40%	VNNMVLFDK	99.70%	69.7
Uncharacterized protein OS=Solanum tuberosum GN=102600827 PE=4 SV=1	44.40%	VNNMVLFDKGTYDK	99.50%	22.4
Dirigent protein OS=Solanum tuberosum GN=DIR1 PE=2 SV=1	20.90%	EMAVIGGSGLFR	99.70%	25.8
Dirigent protein OS=Solanum tuberosum GN=DIR1 PE=2 SV=1	20.90%	GYVEASTHSWDFK	99.70%	55.6
Dirigent protein OS=Solanum tuberosum GN=DIR1 PE=2 SV=1	20.90%	TGDATVQYDAYVLHY	99.70%	49.2
Uncharacterized protein OS=Solanum tuberosum GN=102605779 PE=4 SV=1	16.50%	AGIVPVAYR	99.70%	30.1
Uncharacterized protein OS=Solanum tuberosum GN=102605779 PE=4 SV=1	16.50%	SLISYNVAPAHWSFGQTYTGAQFH	99.70%	84.9
Uncharacterized protein OS=Solanum tuberosum GN=102605779 PE=4 SV=1	16.50%	TGWQPMSR	99.00%	29.8
Pectin acetylesterase OS=Solanum tuberosum PE=3 SV=1	28.90%	ALNSLGPSSTR	99.70%	36.4
Pectin acetylesterase OS=Solanum tuberosum PE=3 SV=1	28.90%	AVGDWYYER	99.70%	38.1
Pectin acetylesterase OS=Solanum tuberosum PE=3 SV=1	28.90%	CSSAQLQTMQAFR	99.70%	40.3
Pectin acetylesterase OS=Solanum tuberosum PE=3 SV=1	28.90%	GAVCLDGSPPAYHFDK	99.70%	37
Pectin acetylesterase OS=Solanum tuberosum PE=3 SV=1	28.90%	IFSAVMEDFLAK	99.70%	43
Pectin acetylesterase OS=Solanum tuberosum PE=3 SV=1	28.90%	NAQNAILAGCSAGSLAAILHCDR	99.70%	57.6
Pectin acetylesterase OS=Solanum tuberosum PE=3 SV=1	28.90%	TVSFSGILSNK	99.70%	26.3

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DeShields JB, Bomberger RA, Woodhall JW, Wheeler DL, Moroz N, Johnson DA, Tanaka K (2018) On-site molecular detection of soil-borne phytopathogens using a portable real-time PCR system. J Vis Exp. doi: 10.3791/56891

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- Moroz N, Fritch KR, Marcec MJ, Tripathi D, Smertenko A, Tanaka K (2017) Extracellular alkalinization as a defense response in potato cells. *Front. Plant Sci.* 8: 32 doi:10.3389/fpls.2017.00032

PRESENTATIONS & REPORTS:

- Demonstration Workshop at WA-OR Potato Conference. January 24, 2018. Kennewick, WA. "On-site detection of pathogens from soil" organized by DeShields JB, Bomberger RA, Woodhall JW, Blua M, and Tanaka K.
- Tanaka K (2017) Classic yet new method for diagnosis of powdery scab disease. Washington-Oregon Potato Conference. Kennewick, WA, USA. January 26, 2017 (Invited oral)
- DeShields JB, Bomberger RA, Moroz N, Tanaka K (2017) Rapid, accurate, and on-site molecular detection of soilborne potato pathogens. Pioneering Ideas in Agriculture (DuPont Pioneer Plant Science Symposia Series). March 17, 2017. Pullman, WA, USA (Poster)
- Fritch KR, Moroz N, Tanaka K (2017) Development of aequorin luminescence-based cytosolic calcium measurement in potato. WSU SURCA, March, 27, 2017. Pullman, WA, USA (Poster)
- Moroz N, Fritch KR, Marcec MJ, Tripathi D, Smertenko A, Tanaka K (2017) Extracellular alkalinization as a defense response in potato cells. WSU MPS Retreat. March 4, 2017. Pullman, WA, USA (Poster)
- Moroz N, Tripathi D, Fritch KR, Tanaka K (2016) Extracellular alkalinization assay: a fast and reliable method to detect the defense response in potato. APS Pacific Division Meeting. June 28-30, 2016. La Conner, WA, USA (Poster)

Annual Progress Report

Developing methods for an early warning detection system for foliar potato pathogens

Personnel: James Woodhall, Phill Wharton, Kasia Duellman

Reporting Period: 2017-18

Summary of accomplishments

- TaqMan assays designed for four foliar pathogens.
- Funding secured for a spore trap network of up to 16 samplers.
- High throughput DNA extraction method developed.
- ASO-PCR technology evaluated for late-blight characterisation in plant and air sample material without the need for culturing.
- For three weeks, results from three spore samplers at the end of the 2017 season were processed and industry informed within 24 hours after collection.

Activities or experiments conducted

This project focuses on the development and validation of a spore trapping network for Idaho. Essentially this is in two phases, method development and field validation. The method development phase which involves developing and technical validation of qPCR assays for *Phytophthora infestans, Alternaria solani, Alternaria alternata, Botrytis cinerea* and *Sclerotinia sclerotium*. This also involves developing a high-throughput method for DNA extraction using a ThermoFisher Kingfisher ML particle processer. The final aspect of method development is adapting the ASO-PCR method of Gagnon and co-workers (2016).

Field validation activities included deploying spore traps at Parma, Kimberly and Aberdeen and regular testing of those samples. In addition, small trials were planted adjacent to the samplers at Parma and Aberdeen which were monitored weekly for disease. At Parma, this trial included untreated plots, plots applied with fungicides (Luna Tranquillity) and plots which were misted 8 hours a day to encourage disease development. In addition, leaf samples were taken on a weekly basis from one plot and tested for the presence of foliar pathogens. Twice during the growing season leaves were taken from all plots for testing with qPCR.

Results

Method development
Assays were successfully designed or developed for all pathogens in the project except *Alternaria solani*. For all assays, they were tested against 15 samples of DNA from closely related pathogens and a range of potato pathogens to ensure specificity. They were also evaluated for sensitivity with pure DNA extracts and sensitivity was appropriate for qPCR. Two assays were evaluated for *Alternaria solani* and both did not demonstrate specificity against other *Alternaria* species. Therefore, additional DNA sequencing will be undertaken to determine appropriate species

specific primer sites. Four sequencing primers have been designed as well as a small working collection of 12 *Alternaria* isolates to generate sequence.

ASO-PCR was undertaken on a working collection of ten *Phytophthora infestans* isolates which had been characterises using conventional methods. Five markers were used from Gagnon et al. (2016) and concordant results were obtained for all the isolates. Further isolates/markers will be tested with this method in Spring 2018. A rapid DNA extraction method was developed. This involved using CTAB based buffer with various additives to cope with PCR inhibitory chemicals potentially found in spore samples (such as humic acid from soil dust).

Field validation

All spore samples collected between June and September at all sites were tested for *Phytophthora infestans* using qPCR. No samples tested positive for this pathogen and the pathogen was not observed in the sentinel plots or near any spore samplers in 2017. The grey mould pathogen, *Botrytis cinerea*, was detected in the spore samplers. At Parma, the spore levels appeared to increase as the temperature cooled in September (Figure 1). Preliminary results indicate white mould was consistently detected each week although daily levels could change by up to a factor of ten. White mould was observed at Aberdeen in trial plots adjacent to the sampler and it was detected in the sampler itself. Further work is required to elucidate the trends with this pathogen and if it related to weather conditions. The spore samples remain to be tested with the *Alternaria* primers at time of writing.

Leaf material taken weekly was testing for *Phytopthora infestans*, *Botrytis cinerea* and *Alternaria alternata*. No *Phytophthora infestans* was detected. *Alternaria alternata* DNA levels in leaves appeared to accumulate as the season progress. *Botrytis cinerea* DNA levels appeared to vary widely over the season.

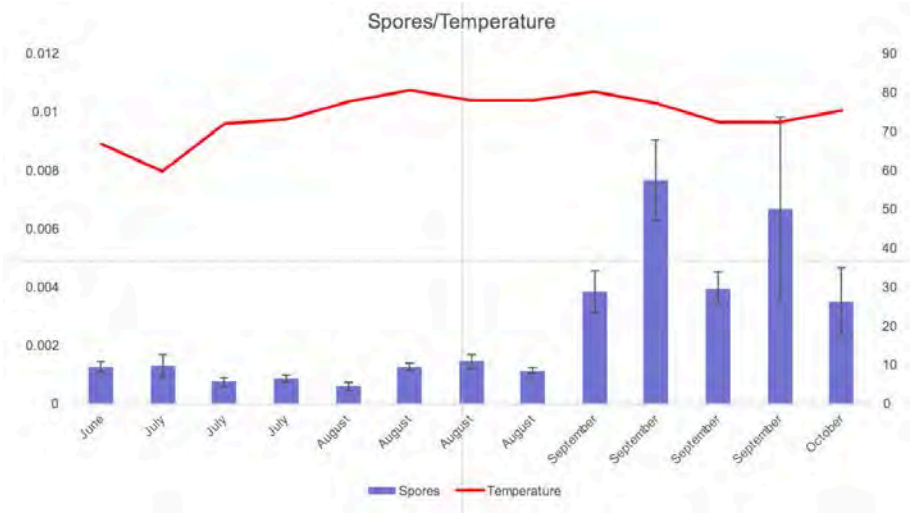

Figure 1. Average weekly *Botrytis cinerea* DNA levels from spores collected at Parma in summer 2017.

Further work

An *A. solani* assay will be developed over winter, spring 2018. Further spore traps will arrive in February 2018 and will be deployed by mid-May 2018. Weekly collection and testing of the samples will take place from the end of May 2018 to September 2018. Results will be disseminated within 24 to 28 hours of collection. Initially results will be disseminated to industry partners by email but eventually a secure website will be developed. In addition to spore trap data, weather data will be utilised to forecast disease risk. Initially this will be based on the Agrimet network but the programme is looking to invest in more sophisticated weather stations which can be deployed in potato growing areas.

Publications

Researchers encouraged by spore sampling results – Capital Press, 19th September 2017

Spore samplers give farmers an early warning system against crop diseases – Capital Press, 2nd February, 2017

Industry support boosts Idaho spore sampling network – Capital Press, 24th April, 2017

Presentations & Reports

Parma R&E Center field day. Approximately 40 people in the disease and spore sampling session. 2 hours.

Kimberly R&E Center field day. Approximately 50 people saw a 20 minute demonstration of spore trapping.

Wharton, P.S., and Woodhall, J., 2017. Molecular tools for pathogen detection and disease diagnosis. 2017 University of Idaho Potato Conference, Pocatello, Idaho, January, 2017.

Spore trapping for potato diseases. J. Woodhall. Idaho Grower Shippers Association annual meeting, Sun Valley, ID, August 30, 2017.

Annual Progress Report

Title: Enhancement of the *Ny$_{tbr}$* protective capabilities against the recombinant strains of PVY

Personnel: Aymeric Goyer, Department of Botany and Plant Pathology, Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, OR, 541-567-8321 ext. 112, aymeric.goyer@oregonstate.edu

Reporting Period: 2017-18

Summary of accomplishments:

- We built tools (i.e. DNA constructs) to disable or boost the genes targeted in this project. We made two disabling and one boosting constructs per gene.
- We established a protocol that enables to introduce DNA constructs with high efficiency in the variety Premier Russet. This variety had not been transformed before, so we needed to establish an efficient protocol.
- We obtained 34 regenerated plantlets with the boosting construct of one gene.
- These accomplishments are essential for the successful continuation of the project.

Activities or experiments conducted:

- To design DNA constructs, we used webtools available at the following link http://cfans-pmorrell.oit.umn.edu/CRISPR_Multiplex/. This enabled us to select an appropriate vector and design primers. DNA was amplified by PCR and PCR products were assembled into the vector pDIRECT_22c by Golden Gate assembly. Golden Gate reaction was introduced into *E. coli*, and colonies were screened for the presence of the vector. DNA constructs were verified by sequencing.
- We tested various transformation methods to establish an efficient transformation protocol for Premier Russet. Various hormones and hormone concentrations were tested to induce callus formation and regeneration of shoots. We established a protocol that enable to regenerate plantlets that contain the DNA construct with 30-40% success in less than 3 months.
- We used the boosting construct of one gene to transform Premier Russet. We obtained 34 shoots that were transferred to root inducing medium to regenerate plantlets.

Results:

As described above, we have built the essential tools needed to make progress toward our long-term goals. We have obtained regenerated plantlets that carry the boosting DNA construct of one of the targeted gene. We are on schedule with the objectives of the project.

Publications:

None to report at this time

Presentations & Reports:

None to report at this time

Quarterly Report – February 2018

TITLE: New sources of PVY resistance

PERSONNEL: Alexander Karasev (UI), Joseph Kuhl (UI), Vidiasagar Sathuvalli (OSU)

REPORTING PERIOD: Quarterly, February 2018

ACCOMPLISHMENTS/RESULTS:
The ultimate objective of this project is to identify new sources of resistance to PVY suitable for introgression into the most popular potato cultivars grown in the Pacific Northwest (PNW). Among them, possible recessive (often strain-specific) resistance to PVY in potato found in other solanaceous crops, like tomato. We are testing the susceptibility to PVY strains of four progeny of the Yukon Gem x Norkotah cross established previously in tissue culture. These four lines were found resistant to the NE-11 strain challenge. Two of the progeny lines were found also resistant to PVY^{NTN} and $PVY^{N\text{-}Wi}$. These two potato lines are being used in further crossings, to resolve the genetic basis of this recessive resistance to a broad range of PVY strains. We focus on the strains most common in the PNW. Besides the HR phenotype, we are scoring virus replication in inoculated and in systemically infected upper leaves.

Three potato cultivars have been screened for the expression of strain-specific resistance: Dark Red Norland, Chieftain, and Payette Russet. Both Red Norland and Chieftain expressed hypersensitive resistance (HR) response to PVY^{O} and PVY^{NTN} strains indicating presence of two genes Ny_{tbr} and Nz_{tbr} in their genetic background. These two *N* genes provided partial resistance to PVY^{O} and PVY^{NTN} in both Dark Red Norland and in Chieftain. Payette Russet was tested with twelve different isolates (9 strains) of PVY and found resistant to all of them (Table 1). This resistance is expressed as immunity or extreme resistance, since no virus replication is detected in inoculated leaves, and no local lesions suggesting HR reaction were observed.

Table 1. ELISA testing of the inoculated and upper inoculated potato leaves after mechanical inoculation with PVY isolates representing different virus strains. Numerator, positives; denominator, number of inoculated plants.

Isolate (strain)	**Payette**		**Desiree**		**Maris Bard**	
	Inoculated leaves	Systemic infection	Inoculated leaves	Systemic infection	Inoculated leaves	Systemic infection
Tb60 (**O**)	0/6	0/6	6/6	3/6	6/6	3/6
N1 (**N-Wi**)	0/6	0/6	6/6	5/6	6/6	6/6
Alt (**N:O**)	0/6	0/6	6/6	6/6	6/6	6/6
HR1 (**NTN**)	0/6	0/6	6/6	6/6	6/6	6/6
ID20 (**NE-11**)	0/6	0/6	6/6	5/6	6/6	4/6
ID269 (**O5**)	0/6	0/6	6/6	3/6	6/6	3/6
Tam13 (**SA-N**)	0/3	0/3	0/3	0/3	0/3	0/3
Tam15 (**SA-N**)	0/3	0/3	0/3	0/3	0/3	0/3
Tam17 (**SA-N**)	0/3	0/3	0/3	0/3	0/3	0/3
Poha2 (**C-Poha**)	0/3	0/3	0/3	0/3	0/3	0/3
Poha6 (**C-Poha**)	0/3	0/3	0/3	0/3	0/3	0/3
HI14 (**C2**)	0/3	0/3	0/3	0/3	0/3	0/3

Two experiments were conducted with progenies of the cross between a CIP accession Maria Huanca and Russet Norkotah. A segregating population has been maintained in tissue culture, and plants were screened against PVY^{N-Wi} and PVY^{NTN}. 66 plants in total were analyzed for the induction of HR, local infection in inoculated leaves, and systemic infection using ELISA and RT-PCR. The project is on schedule.

Cassandra Funke, a graduate student (M.S.) who conducted all the potato cultivar screenings defended her thesis on October 9, 2017, and graduated in December 2017 with a M.S. degeree in Plant Sciences.

PVY Screening (HAREC):
23 advanced Tri-state and Regional selections were planted along with PVY inoculated rows to evaluate selections for their field resistance to PVY. The trial was harvested on Sep 16 2017 and ten tubers from each plot were collected and were evaluated for their PVY infection. The percent PVY infection is presented in the Table 2. PhD student Ryan Graebner is working on PVY resistance from Castle Russet. Two populations segregating for resistance from Castle Russet was developed and phenotypic evaluations were carried out with the help of Chuck Brown's research group. Currently Ryan is working on the data analysis and mapping of the PVY resistance. The project is on schedule

PUBLICATIONS:

1. Funke, C.N., Nikolaeva, O.V., Green, K.J., Tran, L.T., Chikh-Ali, M., Quintero-Ferrer, A., Cating, R., Frost, K.E., Hamm, P.B., Olsen, N., Pavek, M.J., Gray, S.M., Crosslin, J.M., and Karasev, A.V. 2017. Strain-specific resistance to *Potato virus Y* (PVY) in potato and its effect on the relative abundance of PVY strains in commercial potato fields. Plant Dis. 101: 20-28.
2. Green, K.J., Chikh-Ali, M., Hamasaki, R.T., Melzer, M.J., and Karasev, A.V. 2017. *Potato virus Y* (PVY) isolates from *Physalis peruviana* are unable to systemically infect potato or pepper and form a distinct new lineage within the PVY^{C} strain group. *Phytopathology* 107: 1433-1439.

PRESENTATIONS & REPORTS:

1. Karasev, A.V., Funke, C.N., Frost, K.E., and Olsen, N. 2017. Strain-specific resistance to PVY in potato and its effect on prevalence of PVY strains in the field. Annual Meeting of the Potato Association of America. Fargo, ND, July 24, 2017.
2. Karasev, A.V. and Chikh-Ali, M. 2017. Changing strain composition of PVY in the Columbia Basin –What is happening and why? 44th Annual Hermiston Farm Fair. Hermiston, OR, November 29, 2017.
3. Karasev, A.V., Singh, M., Woodell, L., Whitworth, J., and Duellman, K. 2018. PVY Management. 50th Annual Idaho Potato Conference. Pocatello, ID, January 17, 2018.

Table 2. Field PVY infection of clones from regional and tri-state trials performed at HAREC, Hermiston. The green highlighted clones are field resistant to PVY

S.No.	Clone	Trial	PVY Field %
1	Atlantic	Reg. Chip	90
2	Snowden	Reg. Chip	100
3	AC01144-1W	Reg. Chip	65
4	AOR09034-3	Reg. Chip	55
5	NDA081453CAB-2C	Reg. Chip	100
6	NDTX081648CB-13W	Reg. Chip	95
7	OR09256-2	Reg. Chip	100
8	Ranger Russet	Ctrl	95
9	Russet Burbank	Ctrl	70
10	Russet Norkotah	Ctrl	90
11	A06030-23	Reg. Russet	100
12	A07061-6	Reg. Russet	30
13	A08009-2TE	Reg. Russet	30
14	A08433-4VR	Reg. Russet	0
15	AO03123-2	Reg. Russet	65
16	AO06191-1	Reg. Russet	30
17	AOR06070-1KF	Reg. Russet	95
18	AOR07781-5	Reg. Russet	0
19	CO08065-2RU	Reg. Russet	100
20	CO08155-2RU/Y	Reg. Russet	10
21	CO08231-1RU	Reg. Russet	100
22	TX08352-5Ru	Reg. Russet	90
23	A07098-4	Tri-State Russet	45
24	A071012-4BF	Tri-State Russet	55
25	A07705-4	Tri-State Russet	50
26	A07769-4	Tri-State Russet	35
27	A08422-2VRsto	Tri-State Russet	0
28	A08422-4VRsto	Tri-State Russet	5
29	A08510-1LB	Tri-State Russet	0
30	A10021-5TE	Tri-State Russet	10
31	AOR06576-1	Tri-State Russet	0
32	AOR07821-1	Tri-State Russet	75
33	POR12NCK50-1	Tri-State Russet	95

Quarterly Report – February 2018

TITLE: Monitoring the PVY strain composition in seed potato in the PNW

PERSONNEL: Alexander Karasev (UI), Kasia Duellman (UI), Debra Inglis (WSU), Chris Benedict (WSU Extension)

REPORTING PERIOD: Quarterly, January 2018

ACCOMPLISHMENTS/RESULTS:

This project addresses the strain composition of PVY isolates circulating in seed potato fields in both Idaho and in (Western) Washington. Hence, it tracks the PVY strain composition in these two states only, and specifically focuses on seed potato. The numbers on the PVY strain breakdown here may deviate from average numbers we currently observe in Othello and Hermiston, since Idaho seed lots make up only about 15% of seed lots planted in Columbia Basin. We have reliable data for the Idaho WGO since 2012, but the first two years of data were collected in Brawley, CA, and are less robust than the data collected in Hawaii, from the 2013/2014 season on-ward.

We have completed the analysis of the 2017 WGO data for Idaho. Total number of mosaic samples analyzed – 830, with the overall PVY infection rate of 81%. The following PVY strain breakdown was recorded: PVY^{O} at 3.1%, $PVY^{N\text{-}Wi}$ at 68.3%, PVY^{NTN} at 22.8%, $PVY^{N:O}$ at 0.6%, and PVY-NE11 at 0.7%. Mixed or undefined samples were at 4.5% of all PVY-positives. No new or unusual PVY recombinant (identifiable by RT-PCR) strain types were found in the 2017 WGO. However, three isolates typed as N-Wi were found to have no identifiable serotype (reactive only with polyclonal antibodies), one isolate typed as NTN was found to have an N/AST-serotype, and one isolate typed as NTN was found to have a mixed O+N serotype. Both NTN with N/AST serotype and NTN with O+N serotype were reported previously from potato production areas overseas (Brazil and Middle East, respectively), but were not so far found in the U.S. All these PVY isolates with unusual serotypes have been transmitted to a laboratory host, and are being subjected to the whole genome sequencing, to identify the nature of the unusual serology. PVS infection stood at 60% of all tested mosaic samples covering most of the 19% difference between collected mosaic samples and the PVY-positive samples. Only 8.6% of the collected mosaic samples had neither PVY nor PVS, perhaps carrying other viruses or representing our "error-margin" for visual symptoms. The most prevalent strain in all submitted samples by far is $PVY^{N\text{-}Wi}$, followed by PVY^{NTN}. PVY^{O} ticked slightly up in the Idaho WGO, to a little over 3% of all PVY-positives.

We have sequenced the whole genomes for two PVY isolates collected at the 2016 WGO testing site in Hawaii. One of those lacked an identifiable serotype (neither O nor N), despite being a clear N-Wi isolate. Another had an O5 serotype with also an N-Wi genome. The sequences determined pointed to the locations of the epitopes recognized by the SASA-O and 1F5 monoclonal antibodies.

Potato plantings from five direct market farms within the Washington Seed Potato Isolation District were sampled for PVY. Samples were taken of 12 different varieties from both symptomatic and asymptomatic plants. Initial testing with Agdia Immunostrip test kits showed that 36% of samples tested positive for PVY. Further lab testing by Karasev confirmed that PVY-O and $PVY^{N\text{-}Wi/NTN}$ were the dominant strains. Preparations have been made for sampling production fields in Idaho during summer, and for sampling retail stores and seed potato fields in Western Washington. Multiple samples have been submitted to the UI-Moscow lab for typing to strain directly by collaborators from ID and WA, or indirectly through a network of crop consultants. The most prevalent strain in all submitted samples by far is $PVY^{N\text{-}Wi}$, followed by PVY^{NTN}. Further testing from these farms will occur in 2018. The project is on schedule.

Objective for Sub-Project 1-3. Collect seed potato samples from western Washington garden store outlets that service customers who have small plantings near commercial specialty potato production fields. Grow-out the seed samples, test for PVY by ELISA, and type positives to strain using our serotype-specific ELISA and RT-PCR methodology.

A total of 31 seed potato tuber samples (certified and non-certified) from seven northwestern Washington garden store outlets (in Burlington, Mount Vernon, Seattle, Sedro Wooley, and Stanwood) were collected in Spring 2017. Whenever possible, sample selection was based on the presence of shallow, suberized canoe-shaped cracks (which have been associated with PVY), or evidence of tuber necrosis. The seed tubers were grown-out in three replicates in a greenhouse test at WSU Mount Vernon NWREC, and all foliage, whether symptomatic or asymptomatic, was tested for PVY by ELISA. Positive samples were sent to Karasev laboratory at U of I for PVY strain identification.

Cultivars acquired in the survey included Adirondack Blue, All Blue, Bintje, Cal White, French Fingerling, Huckleberry Gold, Kennebec, Modoc, Norkotah, Pontiac, Red LaSoda, Red Norland, Rose FinnApple, Russet Burbank, Russet Goldrush, Russian Banana, Viking Purple, and Yukon Gold. Nine of the cultivars were sold as Washington-certified seed potatoes, while five were Idaho-certified, and three were Montana-certified. The other cultivars either were either not certified or not listed as being certified.

Altogether 21.5% (20/93) of the replicated samples (5 in Rep A, 8 in Rep B, and 7 in Rep C) proved positive by both their symptomology and via ELISA. Percent recovery by strain was: PVY^{O} at 45% (9/20), PVY^{NTN} at 5% (1/20), $PVY^{N\text{-}Wi}$ at 45% (9/20), and PVY^{O}+$PVY^{N\text{-}Wi}$ at 5% (1/20). No new or unusual PVY strain types were found in this northwestern Washington survey in 2017. However, 65% (13/20) of the positive samples were acquired from organic seed production sources compared to 5% (1/20) from conventional seed production sources; 30% (6/20) were from unknown cropping system sources. The findings of higher detection of PVY in organic seed sources, and an approximate equivalent ratio of PVY^{O} to $PVY^{N\text{-}Wi}$ (45%:45%), are similar to those reported by Beissinger in 2016, and McMoran in 2016 following his survey of Skagit Co. organic and conventional potato fields, respectively.

Of particular interest, 39.8% (37/93) of the seed tubers that were planted, were cracked. Of the plants grown-out from these cracked seed tubers, 27% (10/37) were PVY positive i.e. 3 for PVY^{O}; 1 for PVY^{NTN}; and 6 for $PVY^{N\text{-}Wi}$. These rates of seed transmission from cracked tubers

are quite high. Also of interest, foliage was asymptomatic or only mildly symptomatic in the greenhouse grow-out for 6 of 9 PVY^{O} plants as well as for 7 of 9 PVY^{N-Wi} plants.

The survey confirmed that: (i) garden store outlets can be a source of PVY in western Washington—an educational program on the risks of providing non-certified seed potatoes to local gardeners could be helpful; (ii) PVY^{N-Wi} remains an important constituent of the region's PVY strain composition; (iii) organic potato growers need better access to certified seed potatoes that are virus-free so as to not also be a source of PVY in the region; (iv) PVY^{O} and PVY^{N-Wi} are not necessarily symptomatic in greenhouse grow-out tests, and laboratory testing is essential for virus confirmation; (v) rates of seed transmission from cracked tubers can be relatively high.

PUBLICATIONS:

1. Green, K.J., Brown, C.J., Gray, S.M., and Karasev, A.V. 2017. Phylogenetic study of recombinant strains of *Potato virus Y*. Virology **507**: 40-52.
2. Green, K.J., Brown, C.J., and Karasev, A.V. 2018. Genetic diversity of potato virus Y (PVY): sequence analyses reveal ten novel PVY recombinant structures. Archives of Virology **163**: 23-32 (http://dx.doi.org/10.1007/s00705-017-3568-x).
3. Beissinger, A. 2016. Proactive approaches for managing *Potato virus Y* in western Washington. Washington State University, Department of Plant Pathology, M.S. Thesis, 123 p.
4. Beissinger, A., Benedict, C., and Inglis, D. 201x. Potential sources of *Potato virus Y* in western Washington. WSU Extension Technical Bulletin: 2017-2022. x p. (accepted Sep 20, 2017).
5. Beissinger, A., Goldberger, J.R., Benedict, C.A., and Inglis, D.A. 2017. Seed potatoes, virus management, and the non-adoption of an agricultural innovation. Rural Sociol. (published online Aug 9, 2017; doi: 10.1111/ruso.12181)
6. Beissinger, A. and Inglis, D.A. 201x. Comparison of two detection methods for *Potato virus* Y^{N-Wi} at four potato growth stages. Plant Health Progress: (accepted pending revisions, Dec 11, 2017; resubmitted Jan 30, 2018).

PRESENTATIONS & REPORTS:

1. Karasev, A.V. and Chikh-Ali, M. 2017. Changing strain composition of PVY in the Columbia Basin –What is happening and why? 44th Annual Hermiston Farm Fair. Hermiston, OR, November 29, 2017.
2. Karasev, A.V., Singh, M., Woodell, L., Whitworth, J., and Duellman, K. 2018. PVY Management. 50th Annual Idaho Potato Conference. Pocatello, ID, January 17, 2018.Benedict, C. 2018. The Bridge Between the Garden and the Farm: A Focus on PVY. Whatcom County Master Gardener Advanced Training. Bellingham, WA. February 8, 2018.
3. Inglis, D.A., Gundersen, B., and Beissinger, A. 2016. Evidence that tuber cracking in potato can be caused by *Potato virus Y*. Ann. Mtg. Pacific Div. Amer. Phytopathol. Soc. 106:S4.199, La Conner, WA (abstract and poster presentation).

4. Inglis, D.A., Gundersen, B., Beissinger, A., and Karasev, A.V. 2017. Reactions of five fresh market potato varieties with three *Potato virus Y* strains. Ann. Mtg. Amer. Phytopath. Soc., S10:x, San Antonio, TX (abstract and poster presentation).
5. Inglis, D.A. Feb 24, 2017. Potato research findings in 2016. 35th Annual Western Washington Potato Workshop. Mount Vernon, WA (presentation).
6. Inglis, D.A., Gundersen, B., Beissinger, A., and Karasev, A.V. Mar 7, 2017. Interactions of three PVY strains with five fresh market potato varieties. USDA NIFA SCRI CAPS Team meeting on Potato Necrotic Virus project at annual multi-state WERA89 meeting, "Potato Virus and Virus-Like Disease Management," San Diego, CA (abstract and presentation).
7. Inglis, D.A. Mar 14, 2017. Information for small market growers about important potato diseases in western Washington and their control. Cloud Mountain Center Farm Roundtable. Everson, WA (presentation).
8. Inglis, D.A. Oct 16, 2017. Implications of planting seed potato tubers infected with *Potato virus Y*. WSU Mount Vernon NWREC Brown Bag seminar (presentation).
9. Inglis, D.A. Nov 29, 2017. Implications of planting seed potato tubers infected with *Potato virus Y*. Ann. Hermiston Farm Fair, Hermiston, OR (presentation).

Quarterly Report – February 2018

TITLE: Monitoring the PVY strains in Othello and Hermiston trials

PERSONNEL: Alexander Karasev (UI), Ken Frost (OSU), Mark Pavek (WSU, collaborator)

REPORTING PERIOD: Quarterly, February 2018

ACCOMPLISHMENTS/RESULTS:

This project addresses the strain composition of PVY isolates circulating in commercial potato fields in the Columbia Basin. As such, this monitoring tracks the PVY strains coming from multiple states in the U.S., and to a lesser degree several provinces in Canada. About 35% of all seed lots analyzed came from Montana, about 15% from Idaho, the rest from multiple other states. The numbers on the PVY strain breakdown (Fig. 1 & 2) reflect broadly the PVY strain situation in the U.S., or at least in the western part of the U.S., since potato seed lots coming from the East Coast represent a small portion of the seed lots planted in the Columbia Basin.

317 samples were collected in the Othello field in the 2017 season, on June 6. Initial testing suggested that 81% of mosaic-flagged plants were PVY-positive this time. Strain typing indicated the following breakdown: 8.6% PVY^{O}, 2.3% $PVY^{N:O}$, 68.8% PVY^{N-Wi}, 0.8% NE-11, 17.6% PVY^{NTN}, 1.9% mixed or unclassified, out of all PVY-positive samples. The current season data saw PVY^{O} strain stabilizing at about 6-8% level relative to two previous years, while PVY^{NTN} increased slightly to ca. 17% level after a slight decline last year.

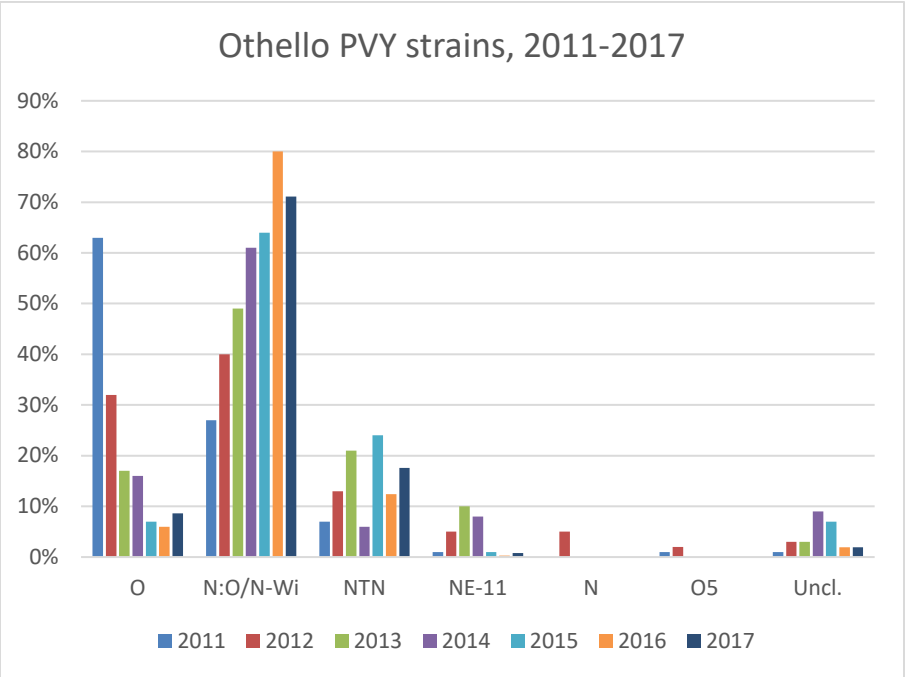

Fig. 1. The breakdown of the PVY strains as determined in Othello, WA, over the period of 7 years, relative to all PVY-positives collected in each year.

The same samples collected based on mosaic symptoms exhibited 60% level of infection with PVS, slightly below the last year level of 66%. The 2017 data have been analyzed, tabulated, and summarized on Fig.1. A manuscript was published in 2017 (Funke et al. 2017) in a peer review journal *Plant Disease*. This publication was selected as Editor-in-Chief's pick of the month of January 2017, and granted limited free access during that month. Multiple PVY isolates collected from the Othello seed lot trials were used for a comprehensive study of the phylogeny of PVY recombinants (Green et al. 2017). This study concluded that the origin of PVY recombinants is polyphyletic – in other words, these recombinants were originated from different parents, multiple times.

In the seed lot trial in Hermiston, OR, 147 symptomatic (i.e. mosaic) plants were sampled in June 2017. PVY was detected in approximately 90% (132/147) of the symptomatic plant samples. PVY strains detected in Hermiston were PVY-N:O/N-Wi (80.2%), PVY-NTN (15.3%), PVY-O (3.8%) and PVY-N (0.8%). Over the last 7 years, the changes to PVY composition in Hermiston, OR (Fig. 2) have been consistent with the changes observed in Othello, WA (Fig. 2).

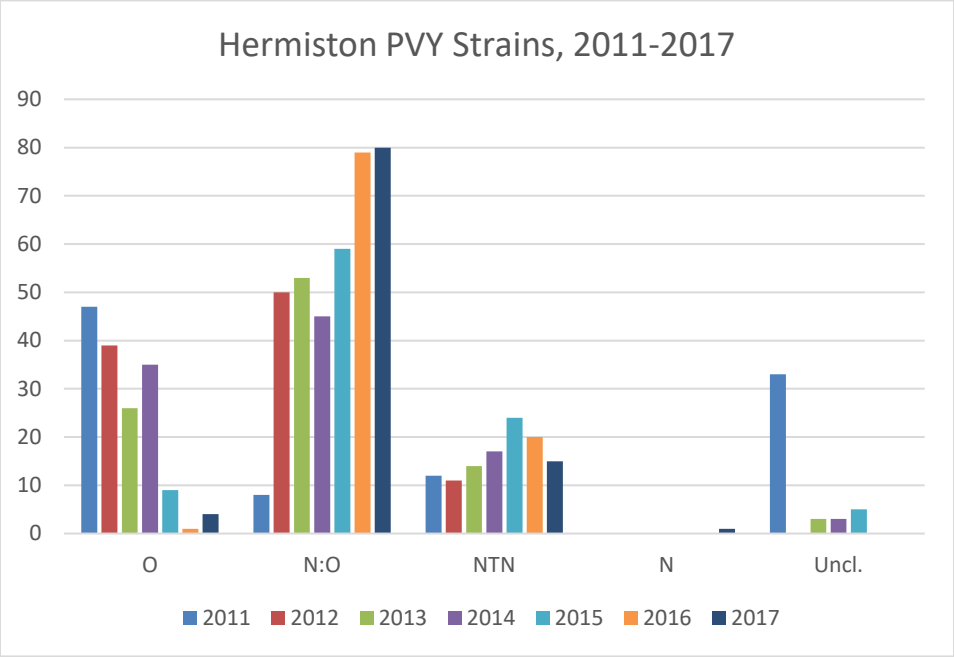

Fig. 2. The relative abundance of PVY strains as determined in Hermiston, OR over the period of 7 years, relative to all PVY-positives collected in each year.

Several isolates collected in the field in 2017 are being studied for their serological, molecular and biological properties. Preparations are being made on personnel training, detection and typing methods' refinement, to have everything ready for the next season's testing. Two isolates of PVY collected in the Othello trial plots have been sequenced, to refine the epitope recognized by an O-specific antibody. A manuscript was published in September 2017 (Green et al. 2018) in a peer review journal *Archives of Virology*. The project is on schedule.

PUBLICATIONS:

1. Funke, C.N., Nikolaeva, O.V., Green, K.J., Tran, L.T., Chikh-Ali, M., Quintero-Ferrer, A., Cating, R., Frost, K.E., Hamm, P.B., Olsen, N., Pavek, M.J., Gray, S.M., Crosslin, J.M., and Karasev, A.V. 2017. Strain-specific resistance to *Potato virus Y* (PVY) in potato and its effect on the relative abundance of PVY strains in commercial potato fields. Plant Disease **101**: 20-28.
2. Green, K.J., Brown, C.J., Gray, S.M., and Karasev, A.V. 2017. Phylogenetic study of recombinant strains of *Potato virus Y*. Virology **507**: 40-52.
3. Green, K.J., Brown, C.J., and Karasev, A.V. 2018. Genetic diversity of potato virus Y (PVY): sequence analyses reveal ten novel PVY recombinant structures. Archives of Virology **163**: 23-32 (http://dx.doi.org/10.1007/s00705-017-3568-x).

PRESENTATIONS & REPORTS:

1. Karasev, A.V. and Chikh-Ali, M. 2017. Changing strain composition of PVY in the Columbia Basin –What is happening and why? 44th Annual Hermiston Farm Fair. Hermiston, OR, November 29, 2017.
2. Karasev, A.V., Singh, M., Woodell, L., Whitworth, J., and Duellman, K. 2018. PVY Management. 50th Annual Idaho Potato Conference. Pocatello, ID, January 17, 2018.

Quarterly Report – February 2018

TITLE: Validation of the tools to detect PMTV and TRV in the PNW

PERSONNEL: Alexander Karasev (UI), Kasia Duellman (Kinzer) (UI), Ken Frost (OSU)

REPORTING PERIOD: Quarterly, February 2018

ACCOMPLISHMENTS/RESULTS:

The objective of this project is to validate the ELISA and immunocapture (IC)-RT-PCR based methodologies for the PMTV and TRV detection in tuber tissues against the current industry standard, direct RT-PCR on tuber extracts. Both ELISA and IC-RT-PCR tests were developed previously with NPRC funding, but tested only on foliar tissue from laboratory, experimental hosts, like *Nicotiana benthamiana* or tobacco.

Over 200 tubers have been secured that tested PMTV-positive by RT-PCR. Close to 50 tubers were identified as TRV-positive, again using RT-PCR. These came in several batches received from Idaho Falls, both PMTV-positive, and TRV-positive. In a few cases, tubers had both PMTV and TRV. All tubers were subjected to testing using our own ELISA-based tests, developed through the support of the Consortium, and also using RT-PCR and IC-RT-PCR. Those positive for any of the viruses were planted after sprouting, to monitor virus movement and distribution in the plant. All these tubers and plants are being used for testing using different detection methodologies, with direct comparison to each other. We are trying to establish how close the probability of PMTV and TRV detection is relative to the current industry standard. Preliminary, raw data suggest that the ELISA protocol developed is the most reliable tool for PMTV detection. Several commercial kits were also compared to the developed detection tools, and the best detection methodology identified.

Both PMTV and TRV are now established in the lab in model plants, as a result of prior funding by the Consortium. We can now maintain and handle these viruses in the lab without dependence on field samples. In addition to ELISA and RT-PCR based tests, we started the development of real-time RT-PCR test, amenable to a multiplex format which includes both PMTV and TRV, and also PVY. The project is on schedule.

PUBLICATIONS:

N/A.

PRESENTATIONS & REPORTS:

1. Tran, L.T., Nikolaeva, O.V., and Karasev, A.V. 2017. Development of an immunocapture-reverse transcription (RT)-PCR methodology for detection of *Potato mop-top virus* in tuber and leaf tissue. 101st Annual Meeting of the Potato Association of America. Fargo, ND, July 24, 2017.

2. Karasev, A.V., Woodell, L., and Whitworth, J. 2018. Necrotic viruses, TRV and PMTV. 50th Annual Idaho Potato Conference. Pocatello, ID, January 18, 2018.

Annual/Final Report Format

TITLE: **Identifying and exploiting susceptibility genes in potato to build resistance against *Meloidogyne chitwoodi***

PERSONNEL: Cynthia Gleason, Department of Plant Pathology, Washington State University, Phone: 509-335-3742, email: cynthia.gleason@wsu.edu

REPORTING PERIOD: 2017

ACCOMPLISHMENTS:

This proposal focuses on the Columbia root-knot nematode (CRKN, *Meloidogyne chitwoodi*). Because this nematode infects potato tubers, it causes visual blemishes that can severely affect potato market value. There are no commercially available, CRKN-resistant potato cultivars. In an effort to develop nematode resistance to CRKN in potato, my group has studied the molecular underpinnings of the potato-nematode interaction. We are particularly interested in how the nematode is manipulating the plant during the susceptible interaction. We hypothesize that the CRKN secretes proteins (called effectors) that are targeting key plant "susceptibility" processes that facilitate nematode infection. Our long-term goal is to generate new, durable potato resistance to CRKN through mutation of key "susceptibility" gene(s) using new non-GMO technology.

In our efforts to achieve this long-term goal, the Gleason lab has reached several milestones in the past year. First, we performed RNA-sequencing of two isolates of nematodes (Race 1 and Race 1 Roza) on resistant and susceptible potato lines at different time points following inoculation. This was significant because it gave us a wealth of data about the genes that are differentially expressed during the plant-nematode interaction. Using bioinformatic tools and the Kamiak cluster of high performance computers, we were able to find nematode genes that are specifically up-regulated in expression at 8 days post-inoculation (early infection) and that are likely secreted by the nematode. This has been a major achievement because we now have a list of potential nematode effectors for future characterization. Thus, in the past year, we have gone from knowing virtually nothing about the CRKN and its secreted proteins to a list of potential effector candidates.

Another major achievement is that we have made progress on the potato side of the pathosystem. We have made a potato cDNA library, which can be screened with the nematode effectors candidates. The library will expedite the future experiments where we plan to fish out the plant proteins that interact with the nematode effectors.

The last major accomplishment of the past year focused on two potential "susceptibility" genes in plants. The susceptibility (S) genes are define as plant genes that provide some critical resource or process for the nematode to be successful in parasitism. We have cloned two susceptibility gene candidates from Arabidopsis and potato. We generated constructs for RNAi silencing, and introduced these into Arabidopsis to generate stably transformed lines. We have also developed a potato root transformation protocol for the introduction of these RNAi constructs into potato. Overall, the accomplishments in the last year have set-up the foundation for our continued research into nematode effectors and their plant targets. It has kept us on track to meet our long-term goal of engineering nematode resistance against the CRKN in potato.

EXPERIMENTS CONDUCTED:

1) Performed RNA-sequencing of two isolates of nematodes (Race 1 and Race 1 Roza) on resistant and susceptible potato lines at different time points following inoculation.

The goal of this project was to compare gene expression in the pre-parasitic juvenile nematodes to parasitic nematodes in both the resistant (PA99N82-4) (1) and susceptible (Russet Burbank) potatoes. We collected and isolated RNA from pre-parasitic juveniles of two nematode pathotypes: Race 1 and Roza. In addition, we collected RNA samples from Russet Burbank and PA99N82-4 infected tissues at 4 and 8 days post-inoculation (dpi). The RNA was sent to Novogene for sequencing. We now have Illumina HiSeq PE150 sequencing data for the pre-parasitic juveniles and resistant-infected and susceptible-infected plants at 4 and 8 days post-inoculation (dpi).

2) Finalized the transcriptome analysis of the CRKN during the early stage of parasitism in susceptible potato.

Our goal in this experiment was to identify nematode genes that are differentially regulated in the Race 1 parasitic juveniles (J2) after Russet Burbank infection. Therefore, we compared our sequencing data from the pre-parasitic Race 1 J2 to the Race 1 J2 inside the potato roots at 8 dpi. Dr. Lei Zhang, a post-doctoral research scientist in the lab, used a TopHat and Cufflinks pipeline (2), and at least 332 unique transcripts IDs were significantly up-regulated (> 2-fold) at 8 dpi. Subsequent analyses using different pipeline settings indicated that there were no more than 1479 transcript IDs differentially expressed at 8 dpi. The transcript IDs are not necessarily full-length gene sequences and their coding regions are unknown as well. Therefore, we used the program Transdecoder to identify the predicted coding regions for all of the transcripts found in J2 and 8 dpi samples. This resulted in 17,542 coding sequences, of which 12,688 are full-length sequences (start and stop codons). From this data, we could extract full-length coding sequences from 73% of the transcript IDs up-regulated at 8 dpi. Because nematode effectors are secreted by the nematode (3), we then used the software programs SignalP and TMHMM to detect classical secretion signal peptides and transmembrane domains in the predicted protein sequences encoded by the up-regulated transcripts.

3) Cloned potential S genes from Arabidopsis and potato

Based on a previous publication, an F-box protein from Arabidopsis (At2g44130) was identified as a possible root-knot susceptibility gene (4). We have also focused on a second putative susceptibility gene from potato, *PMR4*, which encodes a protein involved in callose synthesis (5). We have performed Gateway cloning to clone segments of potato *PMR4* and the F-box protein ortholog into the RNAi vector pK7GWIWG2(II). The constructs were introduced into *Agrobacterium tumefaciens* for whole plant transformation and *Rhizobium rhizogenes* for potato hairy root transformation. We have established a successful potato "hairy root" transformation assay and have generated hairy roots expressing a control construct expressing the green fluorescent protein. For Arabidopsis, we have transformed wild-type plants (Col-0) by standard floral dip transformation protocol using the *Agrobacterium tumefaciens* carrying the RNAi constructs for both the PMR4 and F-box proteins.

RESULTS:

1) **RNA-sequencing of two isolates of nematodes (Race 1 and Race 1 Roza) on resistant and susceptible potato lines at different time points following inoculation.**

Overall, we have received nearly 9 Gigabytes of sequencing data. This experiment has a lot of power because it allows us to analyze both nematode and plant responses in both resistant and susceptible interactions. The data generated from this experiment can be used to ask questions about the resistance response, such as what nematode protein(s) are recognized in the resistant plant or what sort of resistant signaling cascade is triggered by the avirulent nematodes. With this resource, we have data that can be used for research on potato-nematode interactions that extends beyond the scope of this proposal, and we anticipate that this will lead to new research questions with impact for the NW potato industry. However, because for this proposal we are specifically interested in studying the early susceptible interaction, we have focused our attention to the susceptible response at 8 dpi.

2) Transcriptome analysis of the CRKN during the early stage of parasitism in susceptible potato.

For this proposal, we only focus on the RNA-seq data for the pre-parasitic Race 1 J2 and the Russet Burbank infected with Race 1 at 8 dpi. First, we determined how many sequencing reads could map to the *M. chitwoodi* draft genome for each of our samples. For the J2 samples, there were 48,072,828 paired reads that mapped to the *M. chitwoodi* Race 1 draft genome. For the 8 dpi samples, there were 3,813,280 aligned pairs to the *M. chitwoodi* genome. This data tells us that our pre-parasitic J2 sample is pretty good and mostly aligns to the genome as expected. The 8 dpi reads tells us that we are still getting nematode reads from the sample, the majority of the reads are probably from potato. Nevertheless, such a reduction in reads in mixed tissues (i.e. nematode-infected potato roots) is expected, and it aligns with previous publications performing similar experiments. By changing the stringency of the bioinformatics pipeline, we found 1479 transcript IDs up- regulated at 8 dpi. Using the predicted full-length sequence, we ran SignalP and TMHMM analyses to detect predicted secreted sequences. From the SignalP analysis, we had 218 genes with predicted secretion peptide sequences. Of those, 167 genes also did not any predicted transmembrane domains. The next step in the pipeline will be to perform a BLAST search of the 167 sequences against the non-redundant (nr) database of NCBI to see if there are conserved proteins domains in these sequences and to develop a prioritized list of effector candidates. A preliminary BLAST search has indicated that there are number of putative esophageal gland cell secretory proteins in our candidate list. We are now at the starting point for effector characterization. Future experiments will include in situ hybridization to determine where the effector candidates are made in the nematodes and plant transformation with the effector candidate genes to see if the genes play roles in parasitism.

3) Cloned potential S genes from Arabidopsis and potato

The F-box protein from Arabidopsis At2g44130 and *PMR4* RNAi constructs have been introduced into Arabidopsis. We currently have T1 seeds. The next step is to select the T1 seeds on media containing the antibiotic kanamycin. We have also obtained the *PMR4* mutant Arabidopsis seed from the Salk Institute. The seed is being bulked so that we can perform nematode bioassays. We are particularly interested in the mutant in Arabidopsis *PMR4* because it does not make the callose synthase GLUCAN SYNTHASE-LIKE5 (AtGSL5) and lacks wound and stress-induced callose deposition (6). Previous work on the mutant has shown the plants are more resistant to powdery mildew due to its hyperactive defense pathways (7). PMR4 may be regulating plant defense responses. We will be interested to see if this mutant is affected in nematode susceptibility and if so, to investigate its mode of action.

PUBLICATIONS: None

PRESENTATIONS & REPORTS:

• Presentation - Washington-Oregon Potato Conference, Kennewick, WA 2018

• Presentation - Columbia Basin Crop Consultants Association 2018 Short Course, Moses Lake, WA 2018

• Article - Potato Progress: "New Approaches for nematode resistance," Vol XVII, No. 3, 2017

1. Brown CR, Mojtahedi H, Zhang LH, Riga E. Independent resistant reactions expressed in root and tuber of potato breeding lines with introgressed resistance to Meloidogyne chitwoodi. Phytopathology. 2009;99(9):1085-9.
2. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature Protocols. 2012;7(3):562-78.
3. Mitchum MG, Hussey RS, Baum TJ, Wang X, Elling AA, Wubben M, et al. Nematode effector proteins: an emerging paradigm of parasitism. New Phytol. 2013;199(4):879-94.
4. Curtis RH, Pankaj, Powers SJ, Napier J, Matthes MC. The Arabidopsis F-box/Kelch-repeat protein At2g44130 is upregulated in giant cells and promotes nematode susceptibility. Mol Plant Microbe Interact. 2013;26(1):36-43.
5. Sun K, Wolters AM, Vossen JH, Rouwet ME, Loonen AE, Jacobsen E, et al. Silencing of six susceptibility genes results in potato late blight resistance. Transgenic Res. 2016;25(5):731-42.
6. Jacobs AK, Lipka V, Burton RA, Panstruga R, Strizhov N, Schulze-Lefert P, et al. An Arabidopsis Callose Synthase, GSL5, Is Required for Wound and Papillary Callose Formation. Plant Cell. 2003;15(11):2503-13.
7. Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H, Somerville SC. Loss of a callose synthase results in salicylic acid-dependent disease resistance. Science. 2003;301(5635):969-72.

Annual Report for NPRC

Date: February 15, 2018

Title: Functional genomics of *Solanum sisymbriifolium*

Prepared by: Louise-Marie Dandurand, Joe Kuhl, Allan Caplan, Fangming Xiao, Chuck Brown, Inga Zasada.

Optimizing gene transfer protocols for *S. sisymbriifolium* and potato: LT becomes more resistant to bacteria as it ages.

Problem-Transcriptome analysis assigns functions to sequences based on their similarity to genes that have already been identified. Verifying that these assignments are correct requires demonstrating that any alteration in their expression alters the sensitivity of the plants to infection. In order to make these genetic alterations possible, we have developed protocols to introduce foreign genes into LT using a process called *Agrobacterium tumefasciens-mediated* transient expression or agroinfection. In the course of optimizing this process, we observed that *S. sisymbriifolium* becomes more resistant to bacterial infection as it acquires more leaves. This detail about the plant's defenses against bacterial pathogens may later help us define related defenses that LT uses against nematode and fungal pathogens.

Progress–In the months since our last report we have continued to add to our previous work:

- In 2017, we expanded our previous studies through measurements of salicylic acid levels and analysis of expression of several defense related genes. These studies showed that neither the amounts of this signaling molecule nor the tested genes correlated with the changes in bacterial resistance that we observed. Thus, the novel mechanism of age-related resistance remains to be discovered. A paper including these expanded studies was submitted in December, 2017 to Physiological and Molecular Plant Pathology for review and publication.

Characterization and analysis of the *S. sisymbriifolium* genome

Problem-In early 2015, we set out to produce the world's first database for the LT genome. RNA was extracted from stems, buds, leaves, roots, and infected roots, copied into DNA, and sequenced using two different "short-read" protocols. Each read corresponded to 200-500 nucleotides taken from each portion of every gene. Every one of the resulting 8.4 million reads were then repeatedly compared to every other read to reconstruct what the original genes might have been. This computational analysis resolved the 8,400,000 bits into approximately 102,226 "potential genes". However, a laborious assessment of this output showed that a large proportion of these "potential genes" were in fact, chimeric, and not at all like genes that could be found in our plant, or in any other. We therefore made a new transcriptome using Single-Molecule Real-time (**SMRT**) technology that produced the sequence of each gene from beginning to end and did not need any kind of *in silico* assembly.

Progress–We have completed the assembly of a **SMRT** transcriptome for *S. sisymbriifolium*. This database offers researchers a searchable catalog of all of the genes expressed in the leaves, stems, buds, and roots of healthy *S. sisymbriifolium* plants. We have amassed a series of experiments establishing the accuracy of the sequencing, the completeness of the library, and shown that this compares favorably to the available transcriptomes of plants like tomato and potato.

- In addition to the summary that we provided in our previous report concerning the kinds of genes found in our transcriptome, we have searched for explanations as to how this plant might have acquired some of the genes that it seems to have. Early on, measurements that we made of the size of the *S. sisymbriifolium* genome showed it contained more DNA in its nucleus than a tetraploid potato. This expansion of the genome could have occurred through a massive increase in non-coding and transposon sequences, or by a duplication of an ancestral genome, or by a blending of two independent genomes through an ancient hybridization event. Using a several different computer programs designed to find evidence supporting one or more of these options, we found genetic signatures consistent with the hypothesis that large portions of the genome had been duplicated relative recently (on an evolutionary time-scale). This genome duplication would have allowed some copies of pathogen-resistance genes to acquire new functions while the remaining copies continued to perform their original role. It is our hypothesis that this event therefore contributed to the novel abilities that *S. sisymbriifolium* has shown.

- Two RNA-seq experiments have been sequenced and are currently being analyzed. The first experiment focused on *in vitro* prepared LT roots with 5 treatments and four replicates each, for a total of 20 libraries for sequencing. Treatments include: time zero (roots prior to inoculation), 3 days post inoculation and mock, and 10 days post inoculation and mock. We have begun to analyze this information seeking genes and pathways that showed significant up- or down regulation during nematode infection. Based on early analysis, eighteen genes (associated with defense response in other systems) were identified with differential expression between infected and uninfected root tissue. Primers were designed and semi-quantitative PCR conducted on cDNA from infected and uninfected roots. Results indicated that 4 of the genes had no discernable difference, while the remaining eleven genes were all differentially expressed. Interestingly 11 of the fourteen genes were expressed higher in uninfected roots than in infected roots, consistent with our RNAseq analysis. The 4 exceptions could possibly be less reliable markers for infection and consequently, not induced or repressed to the same degree in all infections. Another set of primers were designed for differentially expressed genes that were of unknown function. Three of these appear to have significantly increased expression in infected roots. The second experiment involved hydroponically grown plant roots treated with salicylic acid, jasmonic acid, wounding and uninfected mock-treatments. Four replicates of each treatment were collected for a total of 16 sequencing libraries. We have used a variety of bioinformatics programs to reduce the numbers of

genes showing significant (≥ 4-fold increase or decrease in expression and a P-value that this was due to chance alone of ≤ 0.01) changes in expression in response to each treatment. Perhaps not unexpectedly, a significant percentage of these genes showing these parameters have no homolog in the current data sets taken from other plants. Our next goal is to map the activities of the genes that *do* have features like those in other plants into biochemical pathways in order to identify which pathways are most likely responsible for conferring resistance, and which pathways are changed only to repair damage and form feeding syncytia.

- We are currently revising a publication that will be submitted in February, 2018 to G3, a journal of the Genetics Society of America. The data files for the transcriptome described in this manuscript has been uploaded on the National Center for Biotechnology Information (NCBI). Our goal is to submit our findings regarding the RNAseq analysis of the treated *S. sisymbriifolium* transcriptome by Summer, 2018.

- A publication describing a protocol that we developed to efficiently isolate high quality RNA from both nematodes and nematode-infected roots was published in 2017 in the Journal of Nematology.

Comparative analysis of single cell transcriptome of *S. sisymbriifolium* and *S. tuberosum*

Comparative analysis of single cell transcriptome of *S. sisymbriifolium* and *S. tubersoum*

Problem - The nematode infected cells are a small fraction of the entire root, so that when mRNA is isolated and analyzed, transcript changes occurring in the few infected cells are overshadowed by the expression of housekeeping functions and secondary responses in uninfected cells. Our approach has been to use microaspiration to collect cell content samples from infected cells. Our recent developments in the fluorescent labelling of *G. pallid*a J2s by using PKH26 prior to inoculation help to locate the infected nematode inside the plant root without killing the nematode and plant.

Progress -

- A total of 43,916 genes were obtained in both the plant samples. Out of these, 2473 genes were significantly (P ≤ 0.05) differentially expressed. These genes were further studied for identification by comparing the gene sequences to https://solgenomics.net/ and NCBI.
- Resistant genes (R genes) such as BS2 and R3a were more highly expressed in *S. sisymbriifolium* compared to *S. tuberosum*. In potato, the expression of these genes was negligible. R3a expression has been studied previously for its role in nematode effector-triggered cell death in *Nicotiana benthamiana*. BS2 has sequence similarity with Rx and Gpa which are known for nematode resistance.
- Other pathogenesis related genes such as 6-phosphogluconate dehydrogenase, cinnamyl alcohol dehydrogenase, NADH-quinone oxydoreductase, nucleoporins, S-adenosyl methionine decarboxylase and Transcription factor AHRD V1 was identified from the differentially expressed list of genes. Many fold higher expression was shown in *S.*

sisymbriifolim compared to *S. tuberosum*. These genes have been reported for their role in pathogen resistance in tomato, potato and other solanaceous plant species.

Differential Expression of *Globodera pallida* in the roots of *Solanum tuberosum* and *Solanum sisymbriifolium*

Problem - *G. pallida* effector genes help the nematode establish feeding sites inside the plant by modulating many aspects of plant cell morphogenesis and physiology, including defense responses. The suppression of these effectors is necessary for a successful resistant reaction. Therefore, we are studying the effector genes of infected *G. pallida* collected from the *S. sisymbriifolium* and *S. tuberosum* roots in the early stage of infection. This will help our understanding of the immune reaction of *S. sisymbriifolim* in suppressing *G. pallida* immediately after infection.

Progress

- From our RNAseq data, a total of 21,989 *G. pallida* genes was obtained. Out of these, 41 were significantly ($P \leq 0.05$) different when infected in *S. tuberosum* and *S. sisymbriifolium*. Out of these 41 genes, 22 were uniquely expressed in *S. sisymbriifolium*. 9 genes were uniquely expressed in *S. tuberosum*.
- The results showed that several parasitism related genes highly expressed in *G. pallida* when infected in potato were not expressed when they were infected in litchi tomato. This list includes effector genes namely, a Rbp-4 homologue and a *Globodera rostochiensis* 1106 effector family ortholog. An uncharacterized gene with a signal peptide (character of secretory effectors) was also found to be not expressed when infected in litchi tomato.
- Other than secretory protein coding genes, the differentially expressed genes were characterized for their functions using metabolic pathway analysis and found that these genes have roles in the formation of the cytoskeleton, immunity and defence, stress responses, and sex determination.
- Cellulase, Hydroxyacyl-coenzyme A dehydrogenase, heat-shock protein coding genes and esophageal-gland-specific pectate lyase were up-regulated in *G. pallida* infected in *S. sisymbriifolium*.
- Specific primers were synthesized for Rbp-4, the *G. rostochiensis* 1106 effector orthologue and for another unknown secretory protein and were successfully amplified from the cDNA derived RNA isolated from the juveniles of *G. pallida*. *G. rostochiensis* 1106 effector cloning has been done for localization of this gene to confirm the secretory character of this gene coding protein.
- The gene *G. rostochiensis* 1106 effector orthologue was localized by using in-situ hybridization and found to be expressed in the glands of *G. pallida*, which further indicates its function as an effector.
- Primers have been developed to the 1106 effector, and studies to silence to determine its function in *G. pallida* have been initiated.
- A manuscript is currently under preparation on the *G. pallida* differential transcriptome.

Determination of LT genes that can recognize nematode effectors.

Progress – Using the reference transcriptome described above,

- We have begun screening for amino acid sequences (motifs) that are commonly found in proteins associated with pathogen defense processes. So far, we have found 327 with 1 or more amino acid motifs frequently found in "R" proteins (proteins responsible for sensing the presence of pathogens).

- Independently, we have used a set of nucleotide primers designed to copy those motifs into DNA by means of PCR. We have begun to clone these short (approx. 600 nucleotide) fragments so that they can be sequenced and compared to the 327 R-like sequences that we found in our reference transcriptome.

- We have generated a LT expression cDNA library from LT roots. Since LT plants defend PCN infection through the root tissues, this library will be used to find LT proteins capable of interacting with nematode effectors in a yeast two-hybrid screen. From those LT proteins we will identify resistance genes against PCN.

- It may be possible to evaluate whether any of the full-length copies of potential R-genes are responsible for detecting nematodes by using yeast two-hybrid screens to test whether any interact with any of the *Globodera* effectors that are being cloned.

- We have cloned and characterized one *Globodera* effector (GpRHA1B) that is a ubiquitin E3 ligase. We found that GpRHA1B can suppress defense signaling (HR cell death) mediated by multiple resistance proteins, and, significantly, this cell death suppression is dependent on its ubiquitin ligase activity, which is responsible for protein ubiqutination and degradation. This suggests that PCN may use GpRHA1B effector to destroy defense/resistance protein(s) for parasitism. Thus, we have begun to use GpRHA1B as bait for immune signaling component, including resistance gene, hunting in LT plants.

- Using a newly developed immunoprecipitation-based Mass spectrometry technique, we have identified three putative virulence targets in susceptible potato plants. Our *in vitro* ubiquitination assay indicates GpRHA1B can ubiquitinate these proteins and *in planta* expression assay showed GpRHA1B can promote their degradation in plant cells.

- Significantly, we have recently identified one Globodera effector (Gp0400) that is a putative thioredoxin protein. We found that transient expression of Gp0400 can trigger HR cell death (defense signaling in other words) in the model plant Nicotiana benthamiana. This result strongly suggests that Gp0400 is an avirulence effector that can elicit a defense response in plants and is a good candidate for resistance gene hunting in LT plants. Thus, this Gp0400 effector will be used to identify, by yeast two-hybrid screen and immunoprecipitation-based Mass spectrometry, resistance gene(s) and/or immune signaling component(s) in LT plants.

- We have cloned and characterized one Globodera effector (GpRHA1B) that is a ubiquitin E3 ligase. We found that GpRHA1B can suppress defense signaling (HR cell death) mediated by multiple resistance proteins, and, significantly, this cell death suppression is dependent on its ubiquitin ligase activity, which is responsible for protein ubiqutination and degradation. This suggests that PCN may use the GpRHA1B effector to destroy defense/resistance protein(s) for parasitism. Thus, we have begun to use GpRHA1B as bait for immune signaling components, including resistance genes, in LT plants. In addition, this GpRHA1B effector will also be used to identify, by yeast two-hybrid screens, the possible virulence target(s) in susceptible potato plants.

- Using a newly developed immunoprecipitation-based Mass spectrometry technique, we have identified three putative virulence targets in susceptible potato plants. Our *in vitro* ubiquitination assay indicates GpRHA1B can ubiquitinate these proteins and *in planta* expression assay showed GpRHA1B can promote their degradation in plant cells.

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Presentations and Reports

Casavant, N.C., Kuhl, J.C., Xiao, F.-m., Caplan, A.B., and Dandurand, L.-M. 2017. Assessment of *Globodera pallida* RNA Extracted from *Solanum* Roots. **J. Nematol**. 49: 12-20.

Kooliyottil, R., L.M. Dandurand, J.C. Kuhl, A. Caplan, and F. Xiao. 2017. Microaspiration of *Solanum tuberosum* root cells at early stages of infection by *Globodera pallida*. Plant Methods. 13 (1): 68

Presented:

Kooliyottil R., and Dandurand L. M., Caplan A., Xiao F., Kuhl J. 2017. Early infection transcriptome analysis of *Globodera pallida* infected in the susceptible *Solanum tuberosum* and resistant *Solanum sisymbriifolium.* Society of Nematologists annual meeting, Williamsburg, VA August 12-17, 2017.

Kud J, Wang[1] W., Fan[1] Y., Duarte A.*, Dandurand L.M., Xiao F. Gl*obodera pallida* effector RHA1B manipulates plant immunity through its E3 ubiquitin ligase activity. Society of Nematologists annual meeting, Williamsburg, VA August 12-17, 2017.

Wixom A., Casavant N.C., Kuhl J.C., Xiao F., Dandurand L.M., Caplan A.B. More than just the prickles: Investigating the protective responses of *Solanum sisymbriifolium* through *in-silico* and *ex-silico* methods. 14th Solanaceae Conference, Valencia, Spain. Sept. 3-6, 2017.

Submitted:

Wixom, A.Q., Casavant, N.C., Kuhl, J.C., Xiao, F.-m., Dandurand, L.-M., and Caplan, A.B. *Solanum sisymbriifolium* plants become more recalcitrant to *Agrobacterium* transfection as they age. (submitted to **Physiological and Molecular Plant Pathology**).

In preparation:
Wixom, A.Q., Casavant, N.C., Kuhl, J.C., Xiao, F.-m., Dandurand, L.-M., and Caplan, A.B. Assessment of an organ-specific *de novo* transcriptome of the nematode trap-crop, *Solanum sisymbriifolium*.. (submitted to **G3**).

Report for NPRC

Date: February 15, 2018

Title: Eradication strategies for PCN: Use of Trap Crops

Prepared by: Louise-Marie Dandurand, and Pam Hutchinson

The goal of this research is to develop and deploy trap crops as an eradicative tool for the pale cyst nematode (PCN), *Globodera pallida*. Non-host trap crops which stimulate egg hatching but do not support nematode reproduction can provide a strategy to eradicate PCN, since hatched juveniles have limited food reserves and die if they do not successfully parasitize plant roots. We have identified a trap crop species (*Solanum sisymbriifolium* or litchi tomato, LT) that stimulates suicide hatch, is a non-host and we will evaluate its efficacy in laboratory, greenhouse, and field studies. In addition, we are developing management strategies for this crop.

The progress for each objective is as follows:

Objective 1: **Herbicide management.**

Progress - 2017 trial results and comparisons with past trial results. Herbicide management recommendations are included in the summary. Future trials needs are also included.

In 2017, three replicated herbicide tolerance and control research trials were conducted in the Shelley LT grower field: Preemergence-applied; postemergence-applied; and end of season kill.

The objectives of the trials were to:

1. Determine which preemergence and postemergence herbicides applied during the season were a) tolerated by the LT so could be used for weed control in the trap crop; b) would kill the trap crop if it was found in rotational crops or non-crop situations.
2. Which herbicide/herbicide combinations could be applied at the end of the growing season to kill the trap crop so that a) the trap crop can be chopped then plowed/disked into the soil; b) no regrowth will occur; and c) no viable seed is produced, therefore, no volunteers will be present in subsequent years.

The grower (Bart Wattenberger) planted the LT May 25, 2017 and preemergence herbicide trial treatments were applied approximately one week after planting. The postemergence trial treatments were applied July 20, 2017 when the LT was approximately 6 inches tall, and the end-of-season kill treatments were applied August 8 and 15, 2017. LT was 36+ inches tall when the end-of-season treatments were applied.

2017 Preemergence Trial
Linex (linuron), metribuzin, Chateau (flumioxazin), and Reflex (fomesafen), as well as the high rate of Prowl H2O (2.1 pt/A) provided 94 to 100% control of Litchi tomato (Figure 1). Matrix, Sonalan, Matrix + Sonalan, Betamix, and Devrinol at 2 or 4 lb/A did not kill the LT although some stunting did occur with all those treatments except Betamix and Sonalan alone (Figure 1.)

In contrast to past trial years, Dual Magnum and Eptam did not kill the LT in 2017 (Figure 1 and Table 1).

Table 1. Control of litchi tomato with preemergence-applied herbicides: Aberdeen R&E Center 2013-2014 and Shelley, ID 2016-2017

	2013	2014	2016	2017
Herbicide rate/A	---------- % Litchi tomato control ----------			
Metribuzin 0.5 lb ai	97	100	-	100
Chateau 1.5 oz	100	-	-	100
Linex 1.5 pt	55	0	100	98
Matrix 1.5 oz + Linex 1.5 pt	-	-	100	94
Eptam 4.5 pt	68	20	100	0
Reflex 1 pt	35	15	-	89
Outlook 16 fl oz	52	50	87	21
Prowl H2O 2.1 pt	75	85	83	98
Prowl H2O 1.5 pt	-	-	20	43
Dual Magnum 1.4 pt	18	35	-	0
Matrix 1.5 oz	0	0	0	0
Sonalan 2.5 pt	0	0	0	0
Matrix 1.5 oz + Sonalan 2.5 pt	-	-	17	0
Betamix 1.5 pt	-	-	0	0
Devrinol 2 lb	-	-	-	0
Devrinol 4 lb	-	-	-	0
Matrix PRE + POST (1.5 oz + 1 oz)	-	-	-	0

Herbicides were applied preemergence to litchi tomato and sprinkler incorporated with 0.4 to 0.6 in irrigation water.

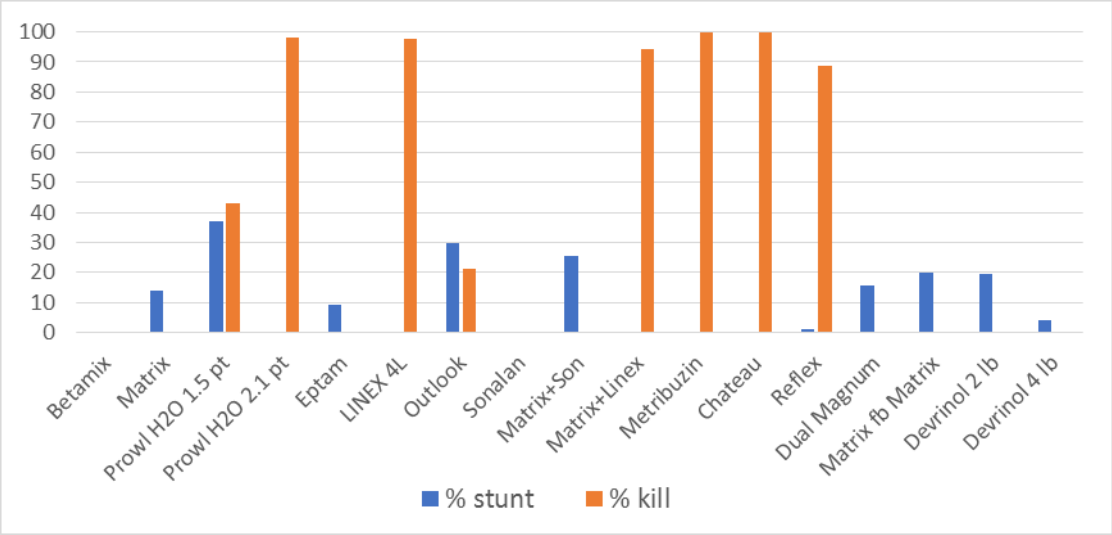

Figure 1. Effect of preemergence-applied herbicides on Litchi tomato six weeks after application in a 2017 Shelley, ID field trial. NOTE: Matrix fb Matrix was 1.5 oz preemergence + 1 oz/A postemergence.

2017 Postemergence Trial

As in past trials, Starane Ultra (fluroxypyr), Roundup (glyphosate), Plateau (imazapic), and Milestone (aminopyralid) controlled LT when applied postemergence (Figures 2, 3, and 4). Dicamba and Raptor (imazamox) provided partial control. Otherwise, LT tolerance was greatest for Matrix, Linex, Nortron, Prowl H2O, and Eptam, followed by Buctril (bromoxynil), Sharpen, Betamix, metribuzin, 2,4-D, Stinger, Aim (carfentrazone), and Harmony (thifensulfuron). Eptam applied postemergence has caused relatively more injury in past trials (Figures 3 and 4).

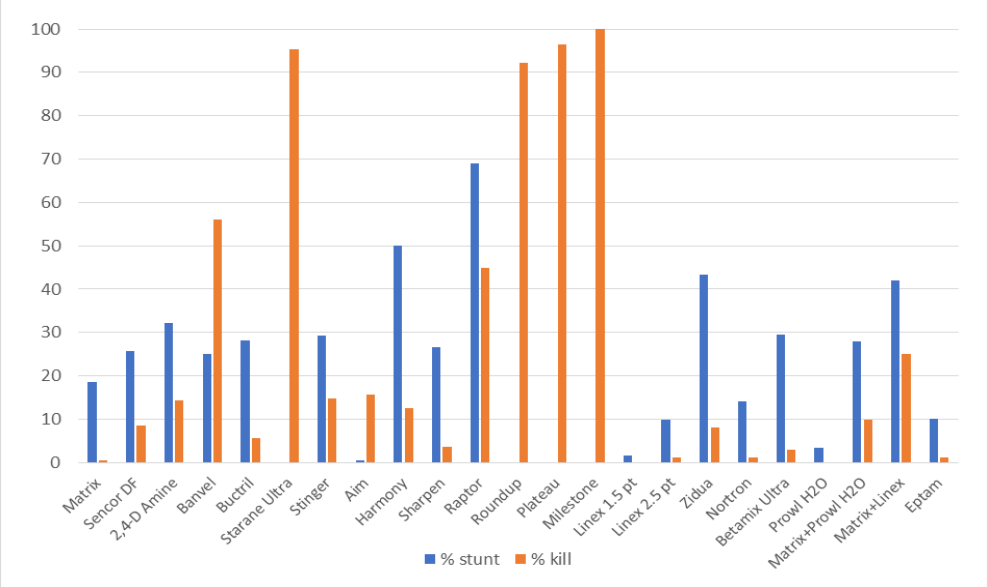

Figure 2. Effect of postemergence-applied herbicides on Litchi tomato 5 wks after application in a 2017 Shelley, ID field trial.

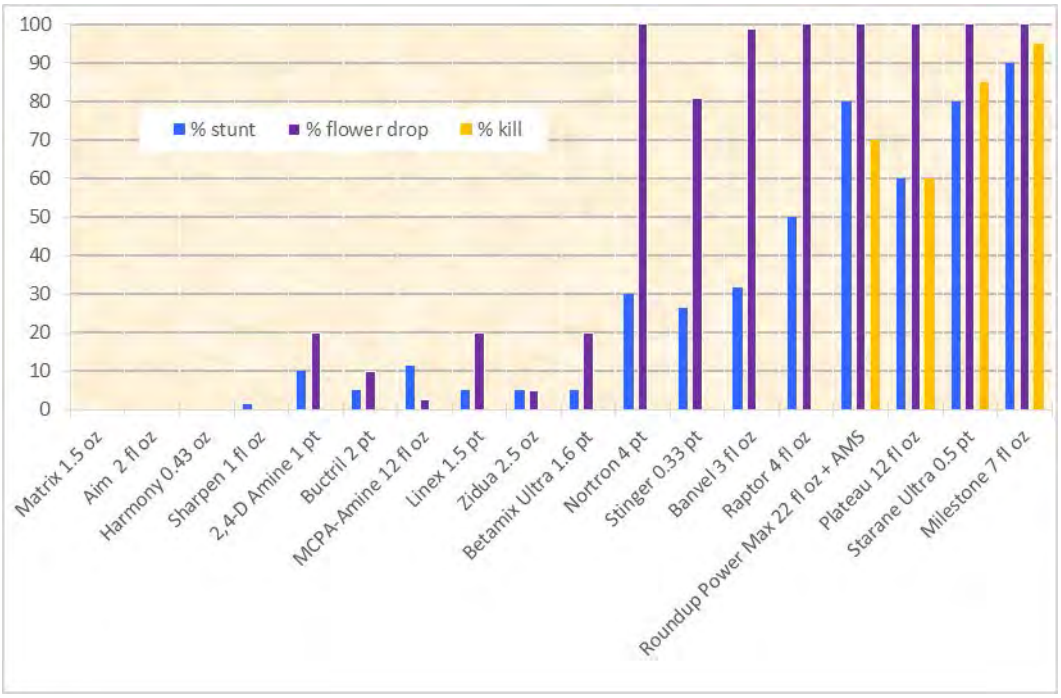

Figure 3. 2016 Litchi tomato postemergence herbicide trial in Shelley, ID
Tolerance and control with postemergence-applied herbicides 5 weeks after application.

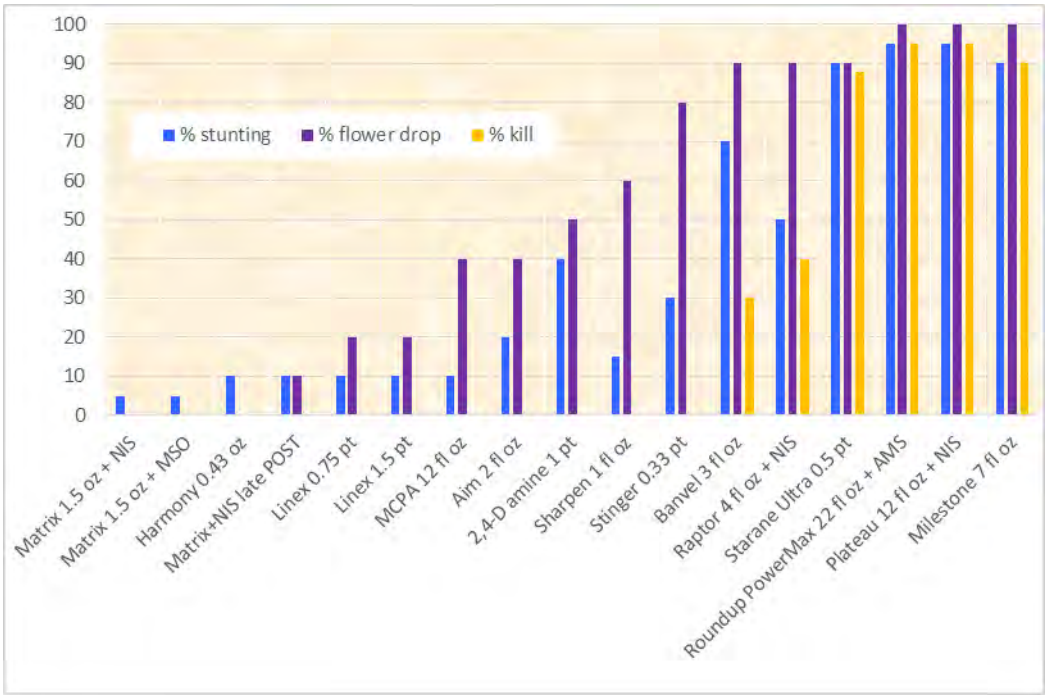

Figure 4. 2015 Litchi tomato postemergence herbicide trial in Shelley, ID
Tolerance and control with postemergence-applied herbicides 1 month after application,

2017 End-of-season Kill Trial. Roundup + Starane kill/desiccation was less than 60% at 2 WAT, and 80% at 3 WAT. Rely alone or with Vida provided greater than 75% kill/desiccation regardless of rating time. Kill/desiccation by any Reglone treatment became relatively less over time because new growth occurred after treatment. Metribuzin in this trial did not provide effective kill as it did when in the 2017 preemergence trial. The difference could be due to spray volume of the end-of-season treatments = 35 GPA compared with 17.5 GPA spray volume for preemergence treatments.

Management and future research recommendations: Weed control in LT can be provided safely by preemergence-applied Matrix, Sonalan, Matrix + Sonalan, or Betamix. Devrinol was safe to LT in 2017 and should be included in future trials to confirm tolerance. Premergence-applied Dual Magnum and Prowl H2O safety has been variable in this and past trials, however, inclusion in future trials is warranted. Eptam variability seems to be too great and should not be used preemergence for weed control in LT. Postemergence-applied herbicides Matrix, MCPA, Nortron, and Eptam could be used safely in LT for weed control. Linex postemergence has been safe even though preemergene-applied Linex kills LT. Harmony safety has been somewhat variable and should be included in future trials. Safety to postemergence-applied Prowl H2O seems to be rate dependent so lower rates should be tested.

Greenhouse tests could provide some information as to why results are variable with some of these herbicides. Herbicides such as 2,4-D and Sharpen could possibly be used to cause flower drop/prevent flowering and subsequent berry production. A specific flower-drop trial should be conducted.

LT can be controlled in rotation crops and non-crop situations with preemergence application of Linex, metribuzin, Chateau and/or Reflex and postemergence-applied Starane Ultra, Roundup, Plateau, or Milestone. Further testing is needed for tank-mix control options.
The trap crop can be killed at the end of the season with two applications of Roundup one week apart or one application of Roundup + Starane Ultra, however, kill is relatively slow and chopping/flailing the LT should be held off until 3 weeks after application in order to take advantage of the full effect of these herbicides. Rely alone or tank-mixed with Vida provided the quickest kill and there was little or no growth after application whereas, the LT plants continued to grow after Reglone application(s) even though initially desiccated. Further testing of Rely and Vida, in particular, should be conducted as well as to find more tank mixtures which are effective.

Objective 2: **Trap crop influence on *G. pallida* populations.**
Progress –
Field Trial 2016: In 2016, no reproduction of *G. pallida* was observed on a susceptible potato subsequent to planting the trap crop, LT (Table 2).

Table 2. Effect of litchi tomato on remaining *G. pallida* encysted eggs at the end of one growing season, and subsequent impact on reproduction (final population/initial population – P_f/P_i) of *G. pallida* on potato.

	Litchi tomato - Field		Potato bioassay subsequent to litchi tomato - greenhouse			
Crop	**Remaining eggs cyst^{-1} 12 weeks**	***Viability (%)***	**Crop**	**Progeny cysts kg soil^{-1}**	**Encysted eggs Eggs cyst^{-1}**	**P_f/P_i eggs (g soil)$^{-1}$**
Fallow	**162 ± 19**	**71.4 ± 3**	**Potato**	**19.8 ± 9**	**187.1 ± 17**	**1.4 ± 0.4**
S. sisymbriifolium	**80.7 ± 7.9**	**66.4 ± 5.6**	**Potato**	**0.0 ± 0.0**	**NA**	**0.0 ± 0.0**
P-value	**0.002**	**0.49**		**0.0006**	**0.0001**	**0.0001**

Field Trial 2017: One experiment is being conducted in Shelley Idaho in a cooperator's field in microplot trials.

1. To establish the optimal length of time needed for exposure of PCN to litchi tomato for maximum population decline Litchi tomato was planted in microplot trials. Cysts were removed after exposure to litchi tomato for 6, 9, and 12 weeks. Viability, and hatching of eggs from these cysts are currently being assessed. The greatest reduction in encysted eggs occurred at 12 week (33% fewer eggs after exposure to litchi tomato compared to bare soil). Bioassays indicated a significant decrease in multiplication of G. pallida on potato subsequent to litchi tomato. When cysts were exposed to litchi tomato for 6 weeks,

PCN cyst reproduction on potato was reduced by 80%, and after 9 weeks, an 87% reduction in cyst numbers was observed. The bioassay of the cysts exposed to litchi tomato for 12 weeks is underway.

Other trap crop experiments:

1. European *S. sisymbriifolium* varieties: The two European varieties of litchi tomato Diamond and White Star from P.H. Petersen, Germany are being evaluated for trap crop quality against the Idaho PCN population. Although, biomass of the three varieties was not significantly different, spine length and numbers were much greater in the two European variety compared to the variety developed by Dr. Chuck Brown, S. sisymbriifolium synthetic cross II, (LTsynII) which is the variety that has been used in all studies conducted on LT at the University of Idaho. Hatching assays indicated no significant difference in the ability of the three varieties to cause hatch of the nematode. However, the host assay showed that G. pallida could reproduce on the two European varieties, albeit at very low levels whereas no cysts were found on the variety developed by Dr. Chuck Brown.

2. Litchi tomato compared to Innovator, a potato variety with a high level of resistance to *G. pallida*:

   An experiment was established to compare the impact of the trap crop *Solanum sisymbriifolium* or litchi tomato (LT), a resistant potato (*cv.* Innovator), or a susceptible potato (*cv.* Desiree) on subsequent multiplication of *Globodera pallida* on susceptible potato (Desiree). Soil was initially infested with *G. pallida* encysted eggs at one of two rates: 5 eggs/g soil, or 20 eggs/g soil, and then pots were planted with either the susceptible potato cv 'Desiree', resistant potato 'Innovator, or LT, or exposed to a bare soil treatment. After 16 weeks growth under greenhouse conditions, tops of each plant was removed, infested pots were incubated in a cold room for 8 weeks, after which susceptible potato 'Desiree' was planted into all pots. Cyst counts were estimated after the first round (first 16 weeks) by extraction of soil from an additional set of pots. At the termination of the second round, cysts were extracted from all treatments and reproduction on potato was assessed. (Egg counts from these cysts are in progress).

   As can be seen from Table 3, LT significantly decreased reproduction of *G. pallida* on a subsequent susceptible potato crop whether *G. pallida* was applied at a low or high rate. The resistant potato Innovator slowed the increase of *G. pallida* but not as at both *G. pallida* initial rates greatly on a subsequent susceptible potato crop. Although, reproduction rates on a susceptible potato crop was less following the resistant potato compared to either bare soil or Desiree, reproduction rates were remain much higher than

those after litchi tomato. Increasing the initial *G. pallida* rate resulted in a subsequent increase in multiplication of *G. pallida* on Desiree for all crop rotations tested except for S. sisymbriifolium. For *S. sisymbriifolium*, reproduction on susceptible potato was not influenced by the initial rate of *G. pallida* inoculum.

Litchi tomato decreased the reproduction rate of *G. pallida* on a subsequent susceptible potato more than the resistant potato 'Innovator'. Egg population under Innovator appears to remain viable which contributes to high multiplication rate on a subsequent susceptible crop. Reproduction on Desiree after litchi tomato is negligible, indicating that eggs viability may have been compromised.

Table 3. Impact of a resistant potato (Innovator) or the trap crop, *Solanum sisymbriifolium* or litchi tomato (LT) on *Globodera pallida* reproduction on a subsequent susceptible potato (Desiree).

Exposure to different crops (First round)			**Rotation on potato (Second round)**	
Plant	**Initial rate Eggs/g soil**	**Extracted Cysts/root ball**	**Rotation crop Desiree**	**Extracted Cysts/root ball**
Desiree	5	357	Desiree - Desiree	622
Innovator	5	3	Innovator - Desiree	90
LT	**5**	**0**	**LT - Desiree**	**7**
Bare soil	5	0	Bare soil - Desiree	208
Desiree	20	293	Desiree - Desiree	1282
Innovator	20	1	Innovator - Desiree	222
LT	**20**	**0**	**LT - Desiree**	**5**
Bare Soil	20	0	Bare soil - Desiree	619

Manuscripts:

Dandurand, L.M., M. J. Morra, I. A. Zasada, W. S. Phillips, I. Popova, C. Harder. 2017. Control of *Globodera spp.* using *Brassica juncea* seed meal and seed meal extract. Journal of Nematology. 49:437-445.

Annual Report – February 15, 2018

Title: Molecular diet analysis of insects known to vector pathogens of potato

Personnel: Project Leaders: **Kenneth Frost**, Assistant Professor & Extension Plant Pathologist, Oregon State University, kenneth.frost@oregonstate.edu, phone: 541-567-8321 ext. 105; **Robert Cating**, Plant Pathologist and Plant Disease Diagnostician, Oregon State University, robert.cating@oregonstate.edu, phone: 541-567-8321 ext. 120; **Sandy DeBano**, Associate Professor – Invertebrate Ecologist, Department of Fisheries and Wildlife, Oregon State University, sandy.debano@oregonstate.edu, phone: 541-567-6337.

Reporting Period: July 1, 2016 – February 15, 2018

Summary of accomplishments:

1) We have identified several primer sets that target plant DNA and have had success amplifying target sequences extracted from insects. We are currently using the trnL (UAA) intron and the internal transcribed spacer region (i.e. ITS-S2F and ITS4R) to amplify plant DNAs.
2) As a proof of concept, we sequenced 96 plant and insect samples using the Illumina MySeq system.
3) We are developing a pipeline for automated post-processing of the sequence data, including reference database development
4) We have conducted no-choice feeding assays with Lygus bugs, thrips, and leafhoppers and will send sample from these experiments for sequencing this in February 2018.
5) We have increased our capacity to study insect diet, both in terms of the molecular methods we used as well as the computational capacity to examine the sequence data generated from these studies. Additional data will be forthcoming as our additional experiments are sequenced.

This report represents the first 1.5 years of this project.

Activities or experiments conducted:

2016

As proof of concept and to test primer sets designed to target chloroplast DNA, a population of thrips were collected from dandelion, *Taraxacum officinale*, flowers around the HAREC in non-crop areas. From the collection of thrips, DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) methodology. Quantity and quality of the extracted DNA was assessed by scanning 1.5 µl of each sample in a NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc. Waltham, MA). Extracted DNAs were used as template in polymerase chain reaction that used primer sets that targeting the chloroplast trnL (UAA) intron. Amplicons generated from PCR reactions were cloned into a plasmid (pGEM, Promega Madison, WI) and plasmids were transformed into E. coli. Bacterial colonies from the resulting library were selected and plasmids were purified and inserts were sequenced. The BLAST was used to compare insert sequences to sequence present in GenBank.

2017

Plant species mixtures. Five common weedy plant species were used including bull thistle (*Cirsium vulgare* (Savi) Ten.), field bindweed (*Convolvulus arvaensis* L.), Russian olive (*Elaeagnus angustifolia* L.), bittersweet nightshade (*Solanum dulcamrar* L.), and white clover (*Trifolium repens* L.). Samples of each plant species were collected from locations at the HAREC, and several other locations (i.e. Riverside Park and the corner of NE 8th and E Oregon) in Hermiston, OR.

DNA extraction of plant samples was attempted on both fresh and dried plant samples. Nucleic acid extraction of dry plant samples resulted in lower DNA concentrations for both 10 and 100 mg of plant material than fresh plant material. A fresh weight of 50 mg of plant material yielded the highest concentration and quality of DNA so individual plant species and plant species mixtures were created to total 50 mg fresh plant tissue. As a proof of concept and to determine if relative abundance of plant species in a mixture could be inferred from sequence data obtained using the metabarcoding procedures, each plant species (50 mg) and three mixtures of each plant species in equal, highly skewed, and slightly skewed amounts (Table 1) of the five plant species were created based on fresh weight. Nucleic acids from each of the plant samples were then extracted using the Dellaporta method (REF.) and subjected to PCR and metabarcoding.

Insect Field Collection. In June 2017, Lygus bugs and western flower thrips were collected from two field sites in Umatilla County of northeastern Oregon. The insects were collected by sweep net sampling or aspirating from specific plant hosts in both crop and non-crop areas around potato. At least 10 individual thrips and 5 lygus bugs were collected from each plant species at each location.Insects for each insect-plant host-pairing were collected from the same plant species in at least two locations for "proof of concept" metabarcoding experiments. DNA was extracted from groups of 5 Lygus individuals and 10-15 thrips individuals using a modified CTAB extraction protocol.

Amplicon library preparation and sequencing. For all plant and insect samples collected, the chloroplast trnLgene was amplified using PCR with dB49863 and cA49325 (Table 2) primers that included adaptors for Illumina indexing and sequencing. Amplicons from the PCR reaction were visualized in an agarose gel, stained with gel red (Figure 1), and purified using Quiagen kit. PCR products from each of the five plant species, individually, and the three plant species mixtures were prepared for sequencing. Sample indexing PCR was performed by Oregon State University's Center for Genome Research and Biocomputing OSU-CGRB and sequences were obtained using the MiSeq Protocol resulting in paired 300-bp reads.

Work-flow for Miseq metabarcode sequences. The sequence data were downloaded from the OSU-CGBR server to local machine and fastqc was used to check the quality of the raw reads. Trimmomatic (version 0.36) was used to trim pair-end adapters and reads were organized into folders by samples identification. Paired- reads were assembled with COPE () and cutadapt () was used to remove the primer sequences. Sequences were converted from fastq to fasta and all fasta files from each study were pooled together. Identical sequences were dereplicated, clustered excluding chimearas and Megan6 was used to produce an OTU table. A python script was written to import trnL sequences and retrieve taxonomic information associated with each sequence from GenBank. The trnL sequences and taxonomic information were concatenated together to be used as a reference database. We are currently working to create a reference database that contains plant species specific to the Columbia Basin. QIIME was used to assign

taxonomy to each OTU in the OTU table using the blastn method. Quantification of sequence abundance, data visualization, and statistical analysis were conducted in R.

The following additional experiments have been conducted and nucleic acids have been extracted from all of the samples generated from those experiments using a modified CTAB extraction protocol. Also, trnL amplicon libraries have been generated using the dB49863 and cA49325 primers, but none of the trnL libraries have been sent for sequencing at this time.

Gut clearance time experiments. Our preliminary work suggested that plant DNA could be detected from lygus bug (*Lygus Hesperus*), Western flower thrips (*Frankliniella occidentalis*), and beet leafhopper (*Circulifer tenellus*). All three insects have different feeding behaviors which may impact the time plant meals can be detected in the insect gut (Backus 1988). Lygus spp. have a "lacerate and flush" feeding style, where repeated insertion and withdrawal of a stylet into plant cells macerates and discharge of saliva extra-orally digests the plant tissues and cell contents. Saliva is then used to flush the digested cell contents into the sucking mouthpart. Thrips generally have a punch and suck feeding style meaning the insect breaks up plant cells with their mouthparts (i.e., punch) prior to ingesting the plant juices (i.e., suck). Leafhoppers are sheath-feeding insects that generally feed on the vascular tissue of the plant (i.e., phloem) but ingest cell contents from other plant tissues during numerous short duration tests probes prior to settling and feeding for longer duration on the vascular tissue (Backus 1988). To determine the length of time a plant species remains detectable and identifiable in the insect gut, the following gut passage experiment were conducted in 2017:

Lygus. Lygus bugs (*Lygus spp.*) were collected in several locations around the HAREC and were placed in a cage and given access to either green bean plants or water. Lygus bugs were sampled after 0, 12, 24, 72, and 168 hours of access to each diet treatment. This experiment was repeated four times and Lygus bugs were sampled every three days until there were no remaining insects in the cages (13 days for replicates 2 and 3; 43 days for replicate 4, and 28 days for replicate5).

Leafhopper. Similar experiments were conducted using beet leafhoppers (*Circulifer tenellus* Baker) and aster leafhoppers (*Macrosteles* spp.) caught around the HAREC. Leafhoppers were caged on beet (*Beta vulgaris*) plants or water and sampled after 0, 12, 24, 72, and 168 hours of access to their respective diet.

Thrips. Thrips caught from potato, white clover, and puncture vine flowers were caged on green bean, water or a green 5% sucrose solution and sampled after 0, 12, 24, 72, and 168 hours of access to their respective diet treatment.

Brown marmorated stink bug (BMSB). Since BMSB were easy to maintain in culture and produces eggs that are not exposed to plant materials and has a lacerate-and-flush feeding style similar to lygus bugs, we decided to use BMSB for a proof of concept feeding experiment. Following eclosion, 1st instar individuals were given access to choice and no-choice diets consisting of seven treatments, 1) no food, 2) carrot, 3) sugar snap pea, 3) apple, 4) blueberry, 5) blueberry & apple, 6) carrot & sugar snap pea, and 7) all four foods. Currently, a sequential no-choice feeding experiment with three diets is being conducted.

Results:

2016

We identified several primer sets that target plant DNA and have had success amplifying target sequences extracted from insects. From thrips collected from dandelion, we detected DNA sequences that matched sequences from the genera *Taraxicum* (50%), Pstacia (25%), Solanum (6%), and Medicago (6%). Approximately 13% of the sequences did not match known sequences in GenBank.

2017

Plant species mixture. The proof of concept experiment to examine if changing the relative abundance of a specific plant species based on fresh tissue weight revealed that (Table 4, Figure 1) all species that were put in the mixture were detected, at some level, by sequencing. Other sequences were also present in low abundance, less than 2% of the total sequences, suggesting the existence of some background contamination or sequencing errors. There is not a direct relationship between relative abundance of tissue fresh weight and relative abundance of sequences obtained after sequencing. Using this metabarcoding method, we cannot infer relative abundance of plant materials based on sequence information; only the presence absence of plant is currently all that can be inferred. These results are not surprising given the PCR amplification step is known to introduce bias into the methodology. There are currently some new methods being used to account for bias in the PCR amplification step – we may adopt some of those methods.

Insect Field Collections. We have learned that we can detect plant DNA in plant feeding insects with multiple feeding behaviors. In total, we have detected plant materials in Thrips (punch-and-suck), lygus (lacerate and flush), aphid (phloem-feeding), leafhopper (phloem-feeding) and psyllid (phloem-feeding). Here we only report our work on thrips and lygus.

Plant sequences obtained from nucleic acid extracts of insects collected from a single plant host were often diverse, and often represented multiple plant hosts (Figure 2 & 3). There are multiple reasons that this may have occurred. Multiple insects were sampled from a single host, but we do not know if or for how long each individual may have fed on the host plant; each insect may have had a different feeding history. If the plant hosts on which the insects were collected is not a preferred host, the insects might have been transitioning to a preferred host, test-probing any green tissue they encountered on the way. The gut passage and sequential feeding studies that have been completed, but are not yet sequenced will reveal

Thrips are known to be highly polyphagous. The relative abundance of dandelion and potato sequences amplified was high from Thrips collected from dandelion and potato flowers (Figure 2). This might suggest that the thrips collected from those hosts had been feeding on those hosts for longer periods of time. There was a greater diversity of plant sequences amplified from thrips collected on white clover and onion. Lygus spp. are very mobile and highly polyphagous. There was a high diversity of plant sequences detected in lygus spp. despite being collected on a single host plant (Figure 3). This suggest that the individual lygus bugs may have been moving host-to-host looking for a preferred host on which to settled and feed.

Publications:
Frost, K.E. Exploring the landscape of plant viruses using plant feeding insects [proceedings]. Proceedings of the 2016 Washington-Oregon potato Conference, January 27 & 28, 2016 Three Rivers Convention Center, Kennewick, WA.

Presentations & reports:
Molecular diet analysis and program research update 2018. Washington State Potato Commission. January 17, 2018. Pasco, WA.

Aster Yellows in Vegetable Crops. Pacific Northwest Vegetable Association Conference & Trade Show. November 11, 2016. Kennewick, WA.

Exploring the landscape of plant viruses using plant-feeding insects. Washington Oregon Potato Conference, January 27, 2016. Kennewick, WA.

Insect vectors in vegetables, new approaches. Pacific Northwest Vegetable Association Conference & Trade Show. November 19, 2015. Kennewick, WA.

Tables and Figures:

Table 1. Number of milligrams of fresh tissue from each plant species in each mixtures based on fresh tissue weight.

Mixture	Bull thistle	Bindweed	Russian Olive	Nightshade	White Clover
Bull thistle	50	0	0	0	0
Bindweed	0	50	0	0	0
Russian Olive	0	0	50	0	0
Nightshade	0	0	0	50	0
White clover	0	0	0	0	50
Equal	10	10	10	10	10
Highly Skewed	46	1	1	1	1
Slightly Skewed	15	12.5	10	7.5	5

Table 2. Primer sequences used for amplification of plant barcode regions

Primer	Primer Seq. (5'-3')	ExpectedAmplicon Size (bp)
trnL(c)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGAAATCGGTAGACGCTACG	~400-600
UAA(h)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATTGAGTCTCTGCACCTATC	
ITS86	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGAATCATCGAATCTTTGAA	
ITS4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC	

Note: Symbol Key: W = A or T. Full length primer sequences for plant barcode library preparation (i.e. highlighted portion of the sequence) with Illumina adaptor sequences.

Table 3. Host plant species from which Lygus and Thrips were collected (i.e. insect-host plant species pairing) prior to nucleic acid extraction, trnL library preparation, and sequencing.

Insect	# Replicates	# Individuals	Plant Species
Lygus spp.	3	5	Potato (*Solanum tuberosum* L.)
	2	5	Russian Thistle (*Kali tragus* L.)
	2	5	Pigweed (*Amaranthus spp*.)
	2	5	Lamb's quarter (*Chenopodium album* L.)
Thrips spp.	3	10-15	Potato (*Solanum tuberosum* L.)
	2	10-15	White Clover (*Trifolium repens* L.)
	2	10-15	Common Dandelion (*Taraxacum officinale* L.)
	2	10-15	Onion (*Allium cepa* L,)

Table 4. Average percent (SD) of sequences of each plant species from Illumina MiSeq sequencing of plant species mixtures.

Mixture	Not mixed	Equal[a]	Highly Skewed	Slightly Skewed
Bull thistle	98.7	17.7 (5.1)	73.4 (1.4)	31.7 (5.6)
Bindweed	98.2	5.5 (2.4)	2.7 (0.6)	7.1 (1.6)
Russian Olive	99.4	54.4 (12.5)	12.8 (3.3)	39.9 (10.1)
Nightshade	99.5	17.0 (4.0)	6.9 (2.3)	14.6 (2.6)
White clover	99.9	1.6 (0.7)	0.4 (0.2)	1.0 (0.4)

[a]Other sequences represented 3.8, 3.8, and 5.7 percent of the equal, high skewed, and slightly skewed plant mixtures, respectively.

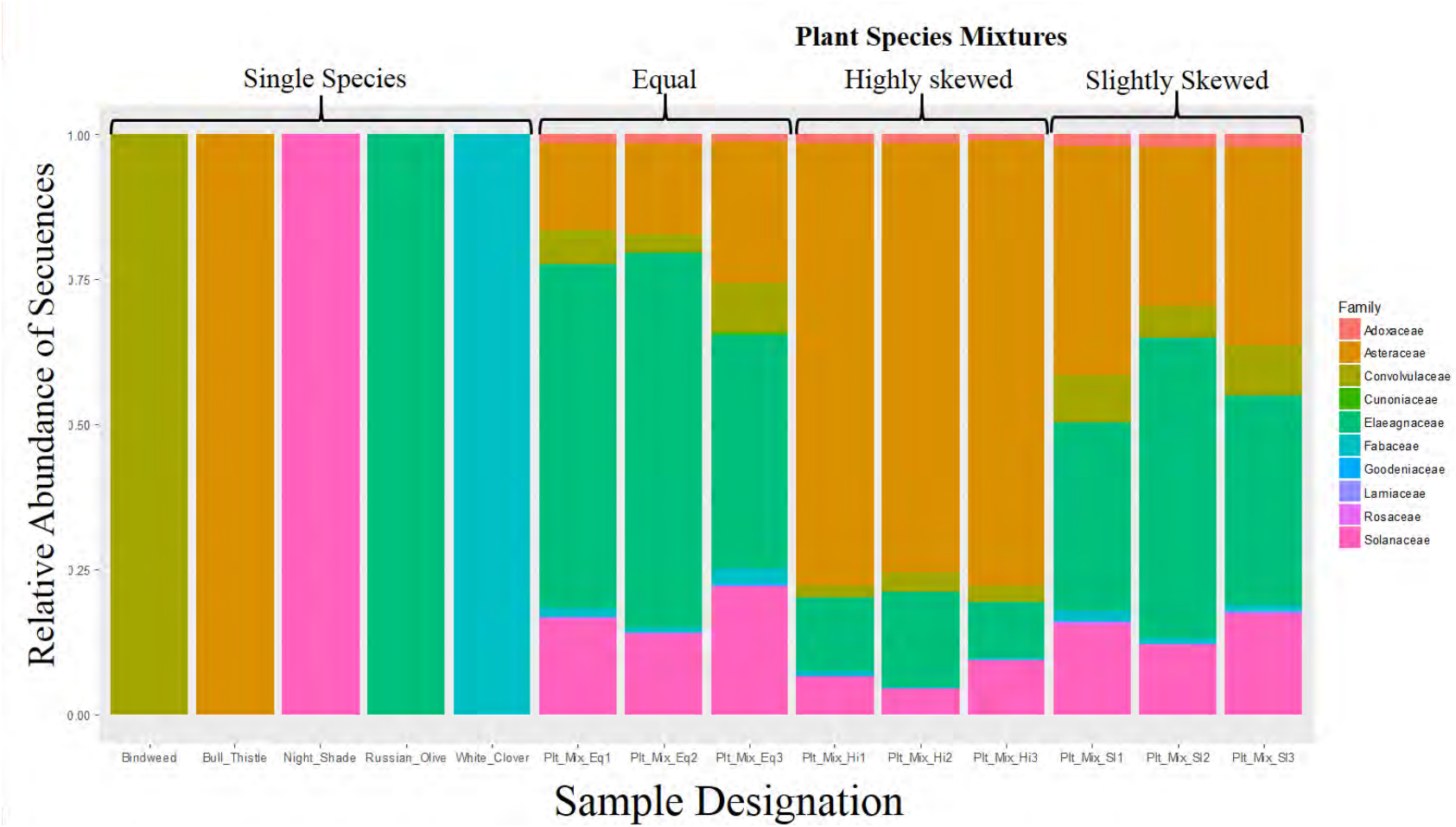

Figure 1. Relative abundance of sequences and assigned families for plant species mixture experiment. Only one replicate of each Individual plant species was sequenced and plant species mixtures were run in triplicate.

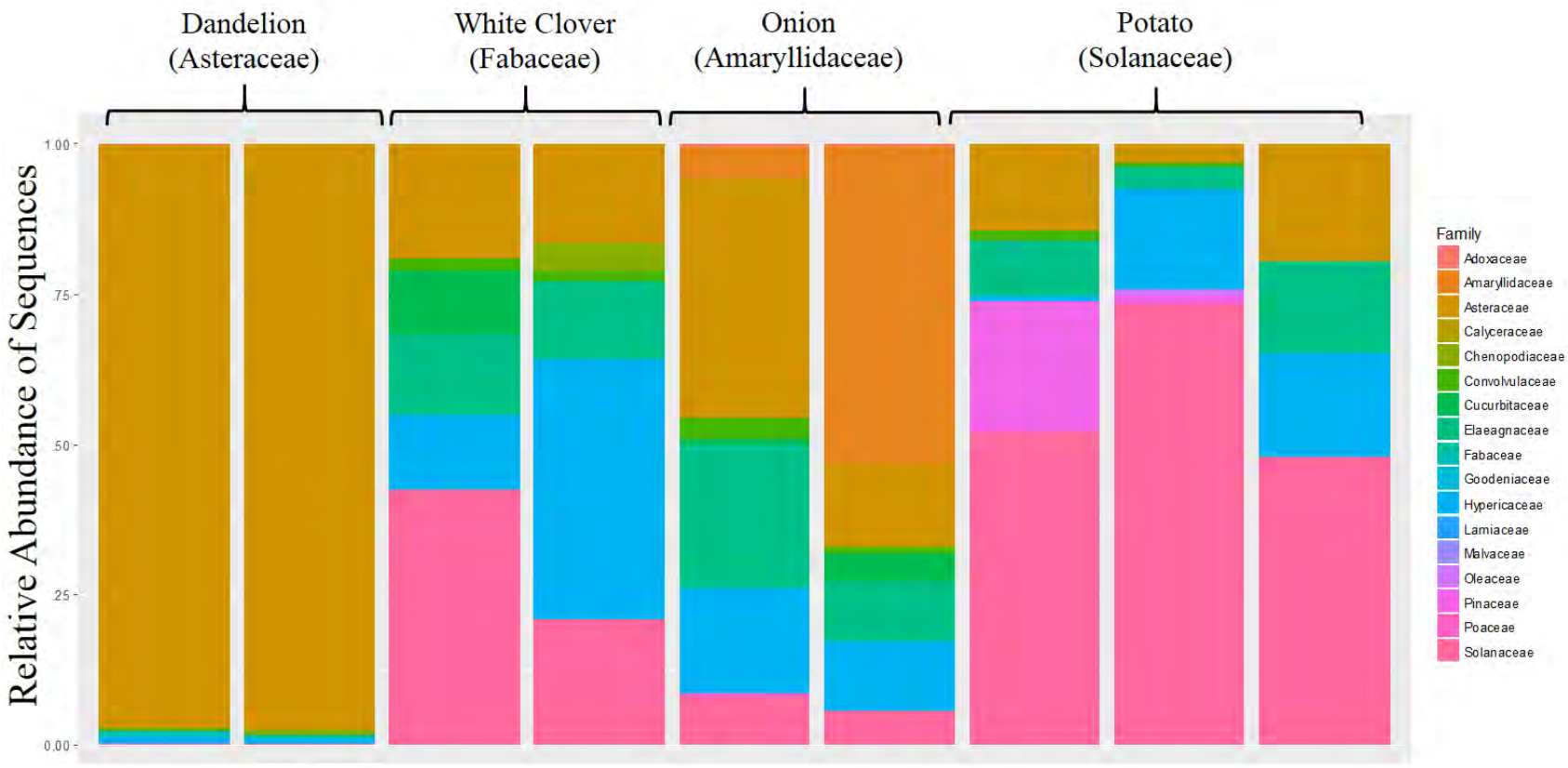

Figure 2. Relative abundance of sequences and assigned families of amplicons from thrips species collected from different plant species, dandelion, white clover, onion, and potato. Multiple thrips individuals (10-15) were in each sample and thrips collected from each plant species were run in duplicate or triplicate.

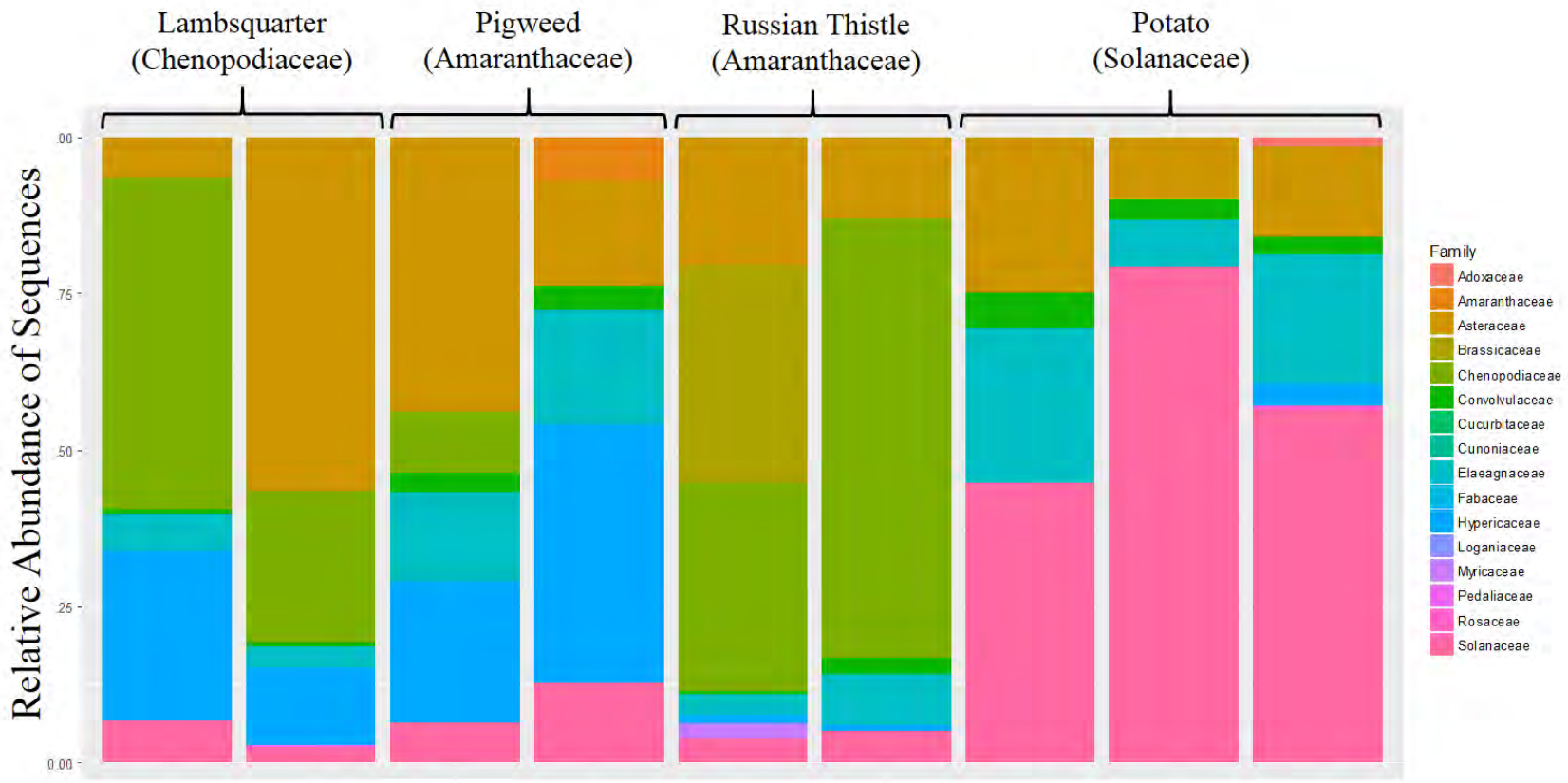

Figure 3. Relative abundance of sequences and assigned families of amplicons from lygus bugs collected from different plant species, lambsquarter, pigweed, Russian thistle, and potato. Multiple thrips individuals (10-15) were in each sample and thrips collected from each plant species were run in duplicate or triplicate.

TITLE: Evaluation of Management Programs for Potato Psyllids and Other Potato Pests

YEAR INITIATED: 2017-18 **Current Year** 2017-18 **Terminating Year** 2019-20

PERSONNEL:

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COOPERATORS
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REPORTING PERIOD: 2017-2018

SUMMARY OF ACCOMPLISHMENTS:
Psyllid pressure was again surprisingly low in 2017. In open field trials, we observed minor, but statistically different treatment effects on psyllid eggs and nymphs, with Brigade, Exirel and Movento tending to have lower numbers than the untreated control. However, Brigade, a synthetic pyrethroid led to significantly higher numbers of thrips.

Brigade and Torac significantly reduced populations of beneficial insects.

Sleeve cage trials with Liberibacter-infected psyllids did not demonstrate that insecticides significantly reduced the transmission of Liberibacter. However, there was a trend for numerically lower levels of zebra chip disease symptoms with Agrimek and Brigade. Additional replication of these tests would be needed to confirm these results.

ACTIVITIES OR EXPERIMENTS CONDUCTED:
Our research team conducted a series of experiments to evaluate insecticides for psyllid management and their effect on other pest and beneficial insects and to assess different plot designs and sampling strategies to help improve the efficiency of psyllid research trials.

Open field insecticide trials with small versus large plots and different sampling schemes were conducted at Eltopia, WA, Pasco, WA and Ontario, OR. Sleeve cage trials to evaluate insecticides were conducted in Kimberly, ID and Hermiston, OR.

Pasco, WA Trial
Determining the Efficacy of Insecticides on Potato Psyllid Relative to Plot Size

Materials and Methods:
A trial for determining the efficacy of the insecticides Agri-Mek (abamectin), Brigade (bifenthrin), Movento (spirotetramat), and Torac (tolfenpyrad) was performed at the WSU research farm in Pasco, WA. The trial was arranged on a randomized complete block design with four replications. Each replication was established with two plots per treatment to evaluate the efficacy of sampling from small plots (four rows or 11.33 ft x 25ft) versus large plots (eight rows or 22.66 ft x 25ft).
Potato (cv. Ranger Russet) was planted on April 18th, 2017. Treatments were made starting July 14th, 2017, and in 14 day intervals to follow (July 28, August 11, and August 28th, respectively). Treatments were applied using a three-point, tractor-mounted sprayer applying the insecticide at 20 gallons of water per acre. The boom was outfitted with 11002 Teejet XR Air induction nozzles, spraying at 25 PSI.

Evaluations were made of the perimeter rows of each four row plot, using three different sampling methods, described as follows:

- An inverted leaf blower with an organza fabric bag was used on the outside rows, and the contents of the bag were place into a one-gallon Ziploc bag, and brought back to the WSU Franklin County lab to be evaluated under magnification.
- Ten leaves were randomly selected from the outside rows of each plot, and placed in a one-gallon Ziploc bag. Samples were brought back to the lab to be evaluated under magnification.
- Five plants were randomly selected from the outer row and assertively shaken over a bucket. The insects were enumerated and recorded, and then the contents of the bucket emptied.

Each evaluation method was performed to determine efficacy, and then every seven days until harvest. Winged and wingless aphids, Lygus, beet leafhopper, Colorado potato beetle adults and larvae, and two-spotted spider mites were enumerated along with potato psyllid eggs, nymphs, and adults.

Pasco, WA Trial – Results and Discussion:
Each sample set was analyzed as a factorial experiment, comparing treatments and plot size. The number of psyllids this growing season was too low to discern any difference in this trial. Wingless aphids collected by vacuum sampling were summed for the season and are reported below in Figure 1. Torac provided the best level of season-long aphid control. Movento provided a greater level of control than the untreated check and Agri-Mek, but not better than Brigade. Similar results were observed with the bucket sampling method (Figure 2) and the leaf sampling method (Figure 8). The beneficial insects, big eyed bugs and nabids, also showed significant differences between treatments. Plots treated with Movento and Agri-Mek contained higher numbers of beneficial insects, while the Brigade and Torac treated plots reduced their populations statistically lower than the untreated check (Figure 3).

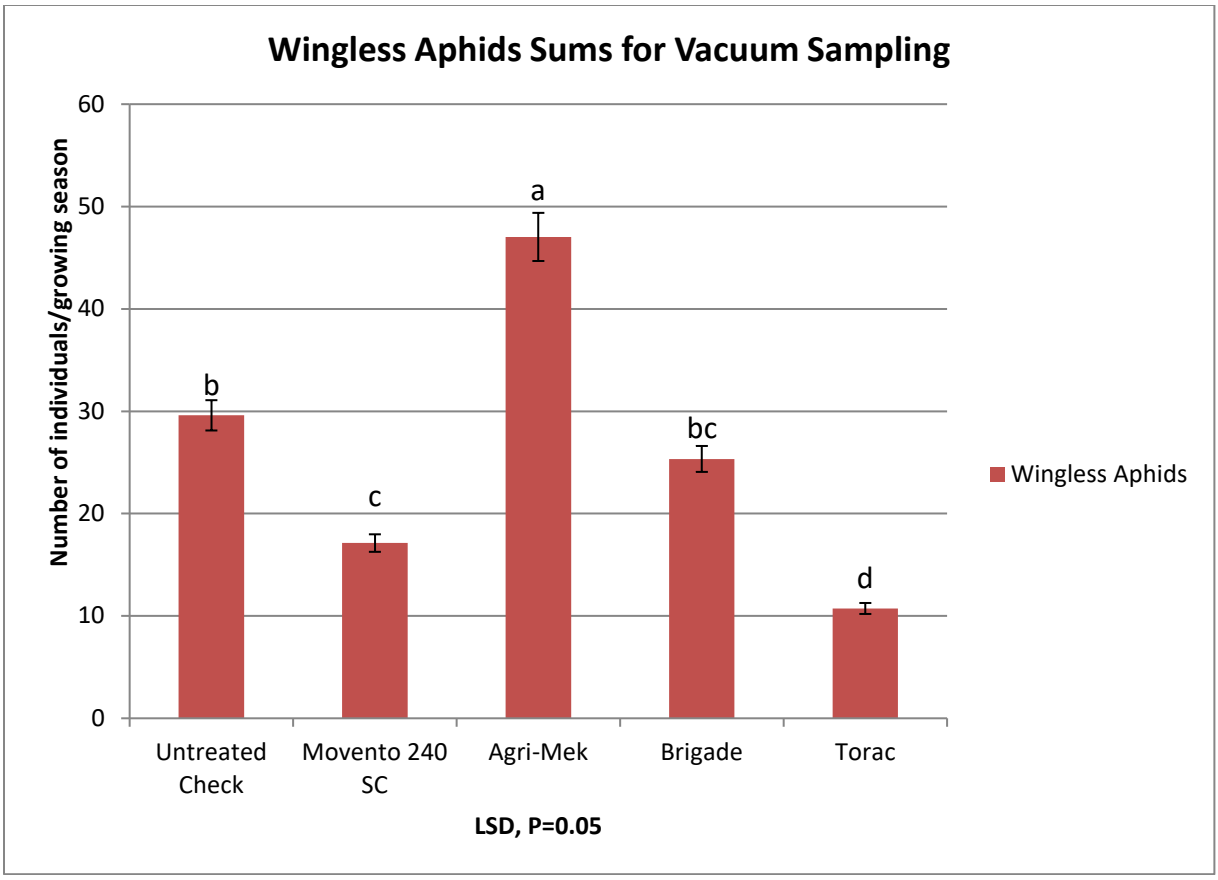


Figure 1: The sum of wingless aphid collected for the season via vacuum sampling. Means with the same letter show no significant difference P=0.05(Student-Newman-Keuls).

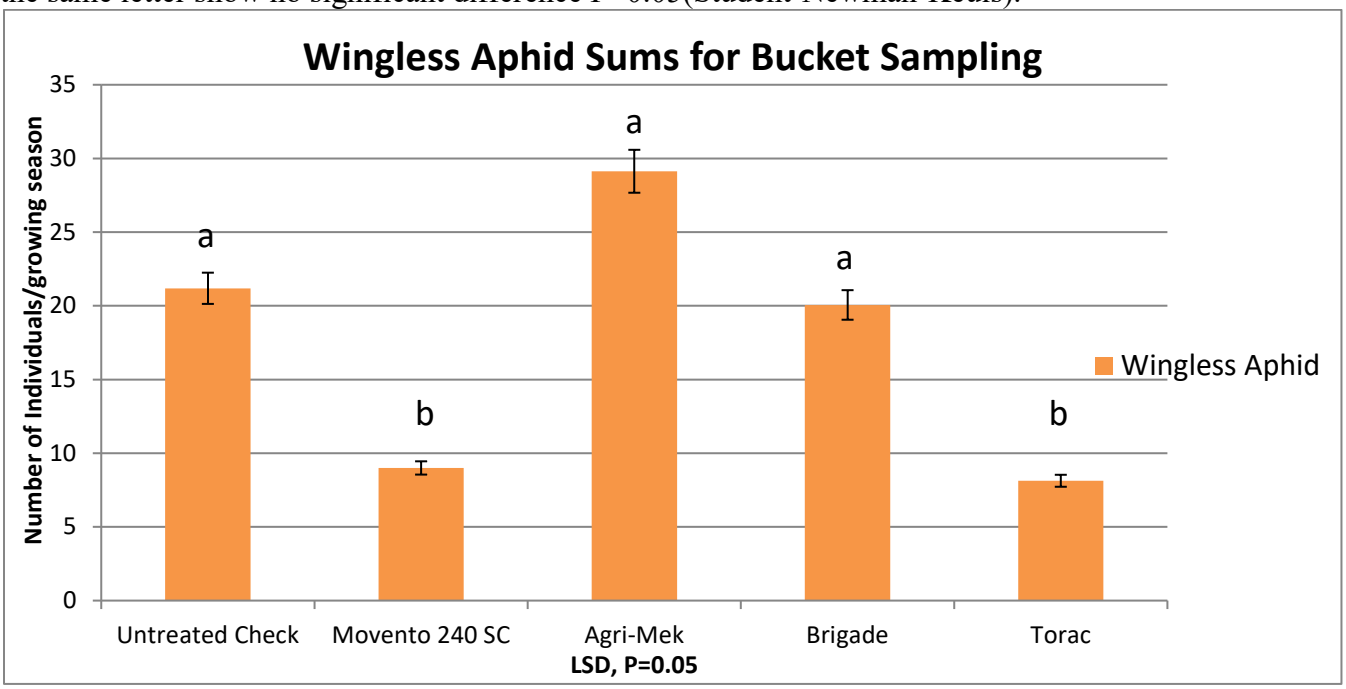


Figure 2: Wingless aphids sum for the season via the bucket sampling method. Means with the same letter are not statistically different. P=0.05 (Student-Newman-Keuls)

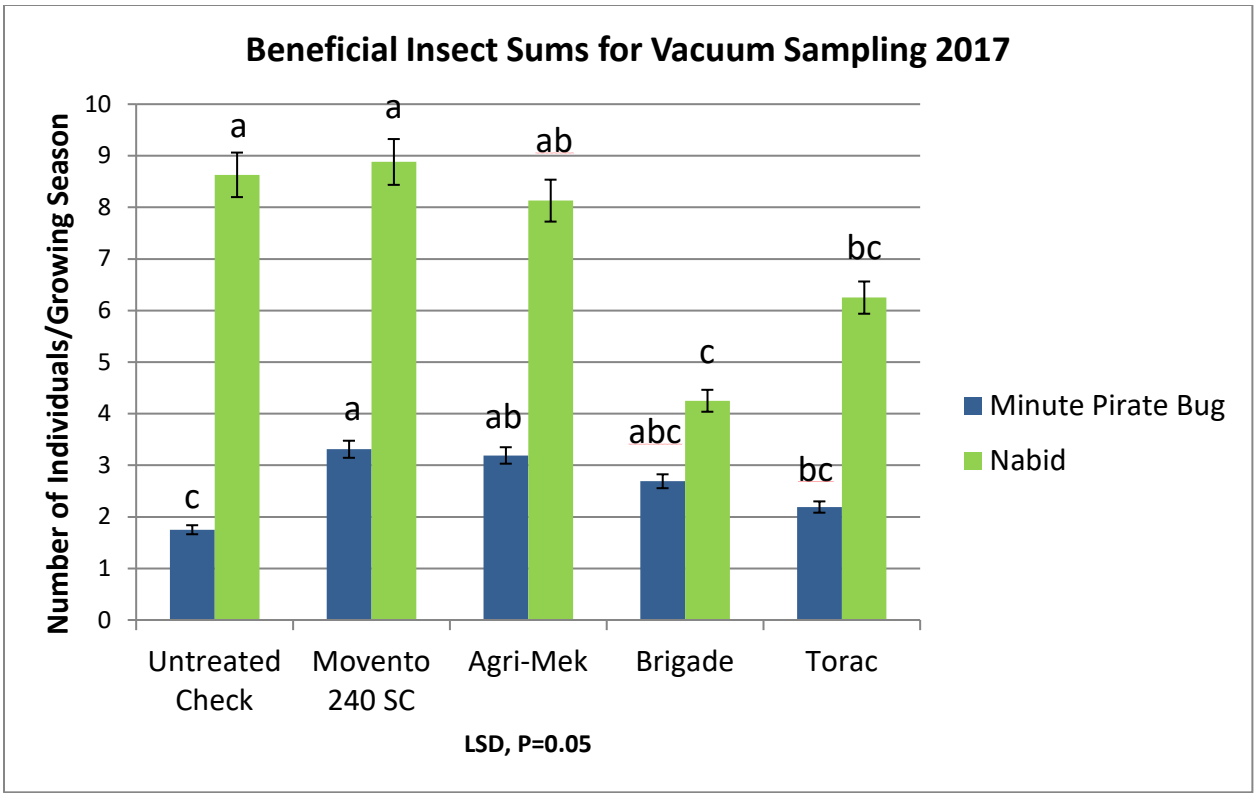


Figure 3: Beneficial insect sums for the season via the vacuum sampling method. Means with the same letter are not statistically different. (Student-Newman-Keuls)

The second factor of the analysis was plot size. The small plots were compared to the larger plot per each sample set, including the sums of each insect collected throughout the season. After the third application, the mean of winged and wingless aphids and Lygus bugs were significantly different between plot sizes for the vacuum sampling method, where larger plots contained more insects (Figure 4). The same collection date (August 23) for leaf sampling showed significant difference between plot sizes for Lygus bugs where, again, more insect were collected in the larger plots (Figure 5). The sums of insects for vacuum sampling exhibited significant differences in every species, with the exception of the ones whose populations were too low to analyze, which included psyllids (Figure 6). Bucket and leaf sampling only showed differences in the sums of Lygus bugs.

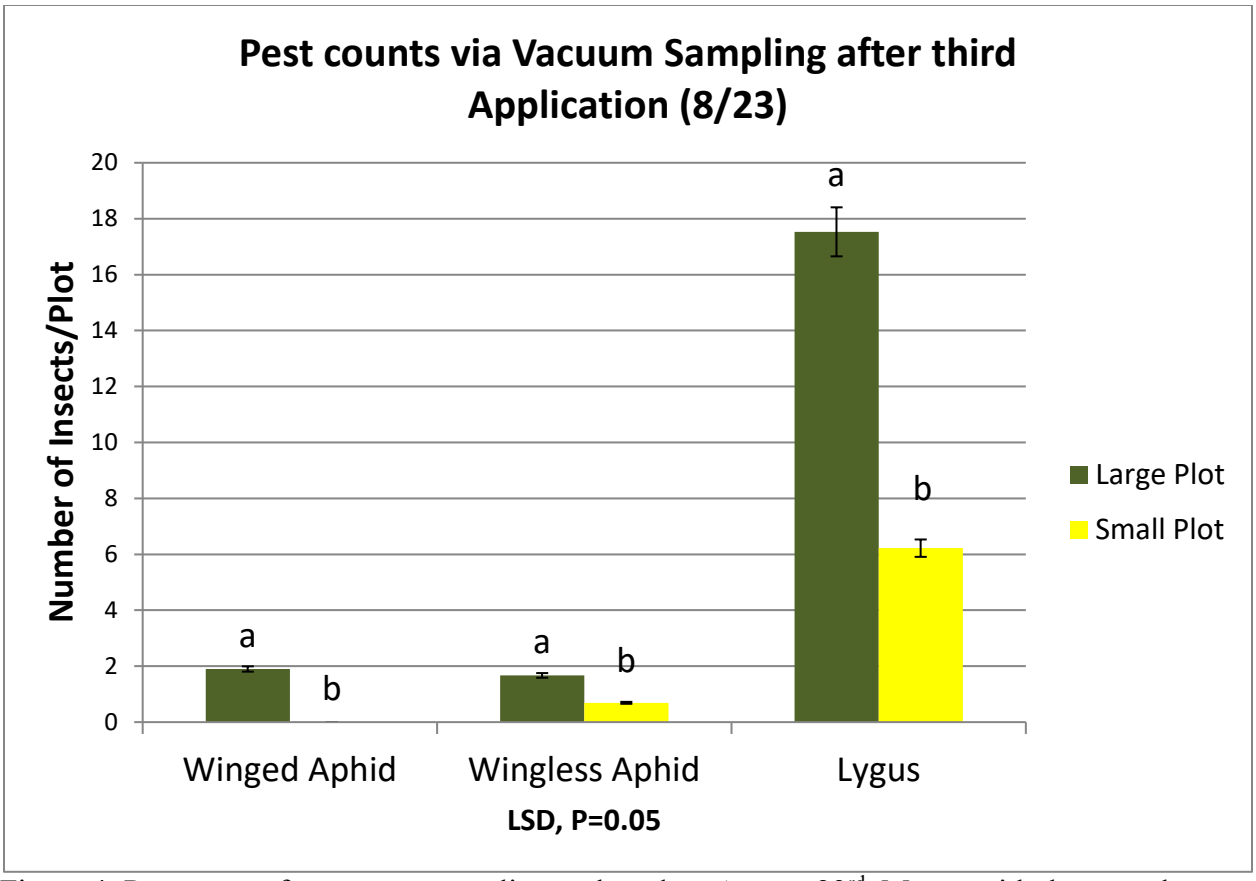


Figure 4: Pest counts for vacuum sampling gathered on August 23rd. Means with the same letter are not significantly different. (Student-Newman-Keuls)

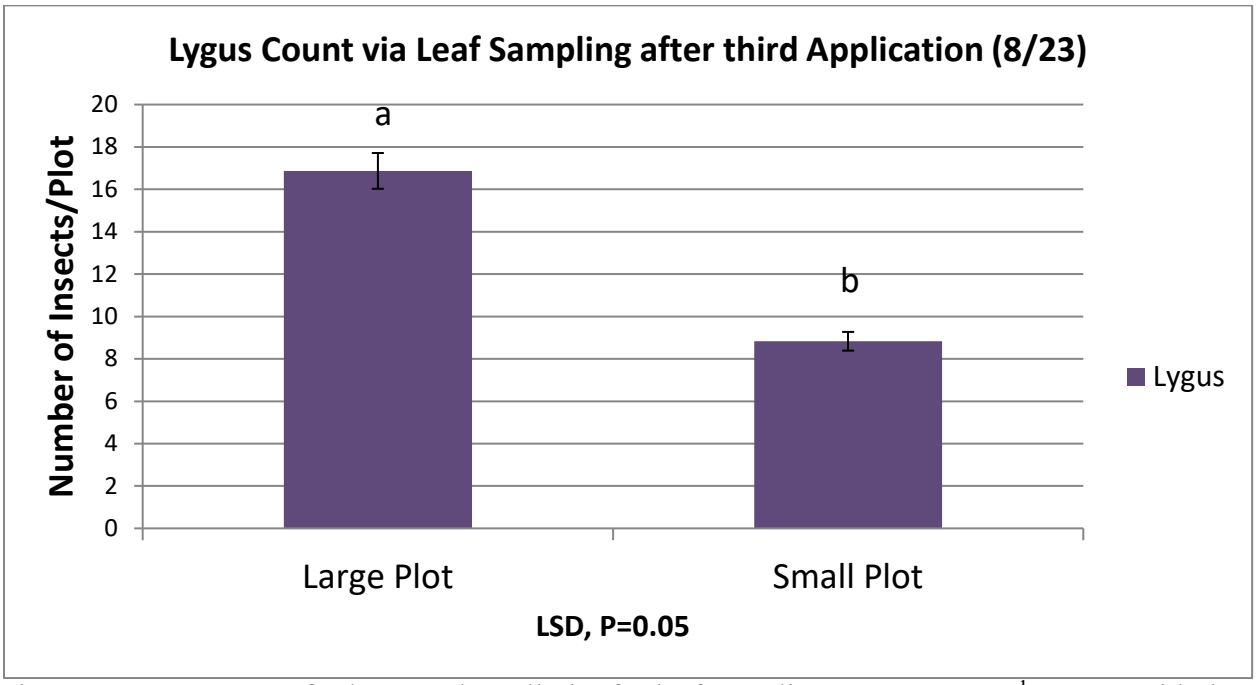


Figure 5: Lygus means for large and small plot for leaf sampling on August 23rd. Means with the same letter are not significantly different. (Student-Newman-Keuls)

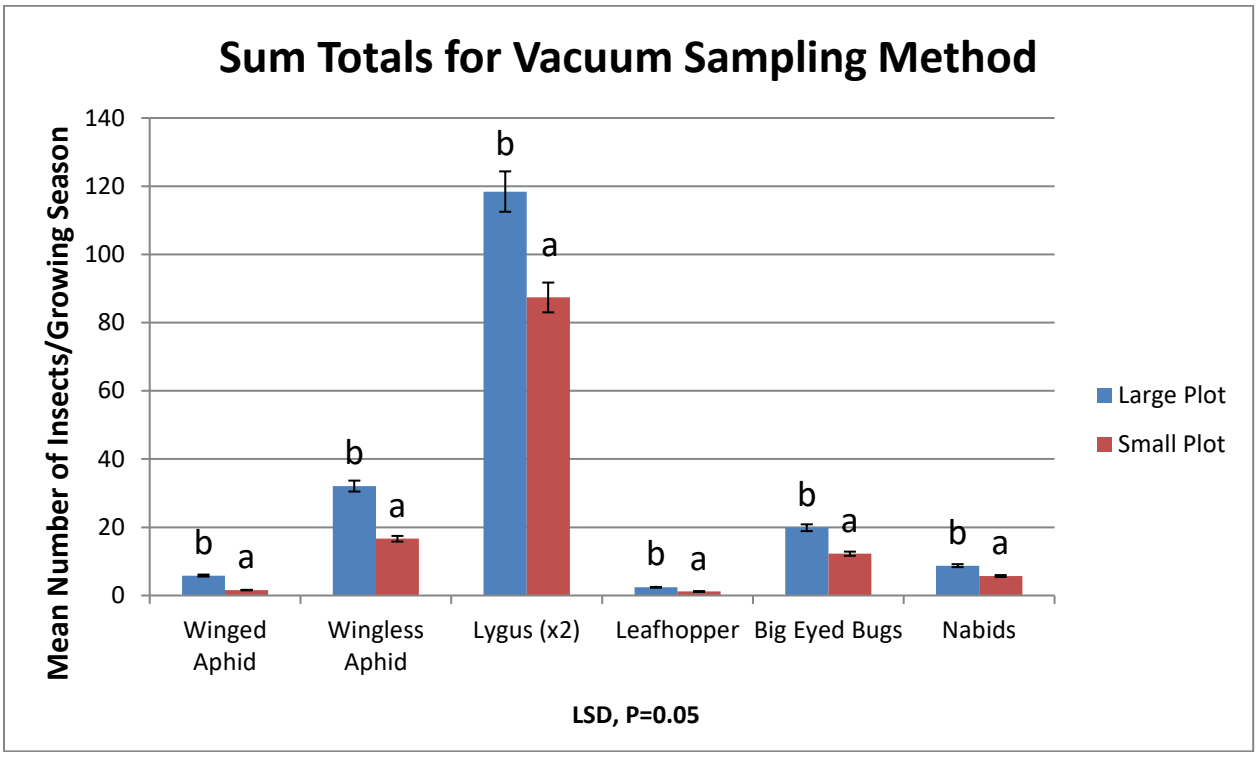


Figure 6: Sum of insects for the season for vacuum sampling. Means with the same a letter are not significantly different. (Student-Newman-Keuls)

The final comparison was with a single collection method leaf sampling. In this experiment, the larger plots of 22.66 ft x 25ft had 20 leaves randomly sampled and the smaller plots of 11.33ft x 25ft had 10. The factorial ANOVA was performed to determine if this sampling method is affected by the plot size. Although a significant difference could be seen in the leaf samples when comparing the treatments against each other (Figure 8), there was no statistical difference between the plot size comparisons extracted from the same data (Figure 7).

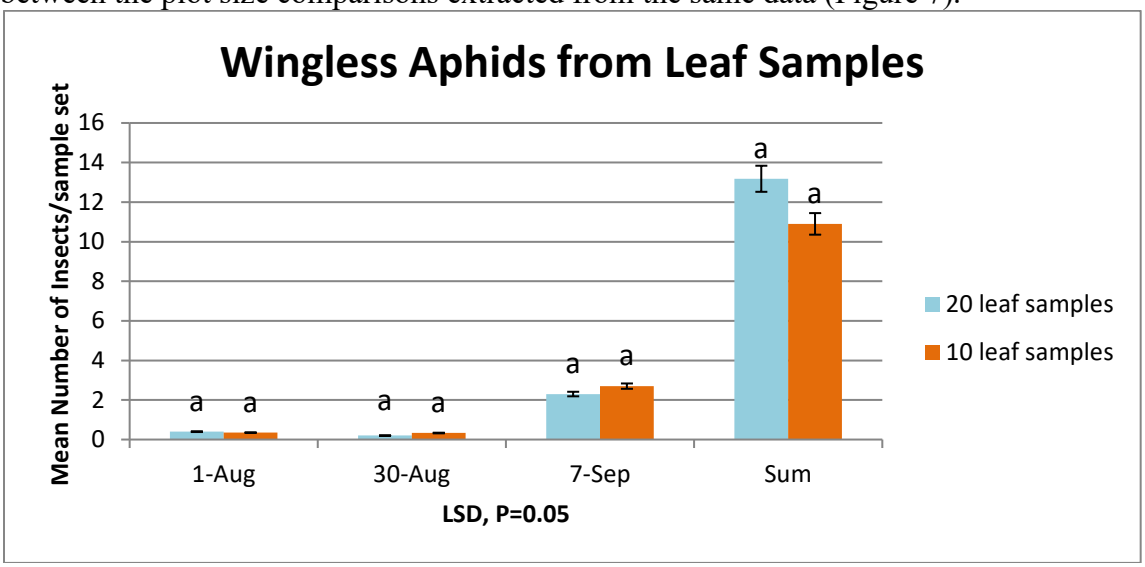


Figure 7: Wingless aphid data from leaf samples analyzed by plot size. Means with the same letter are not significantly different. (Student-Newman-Keuls)

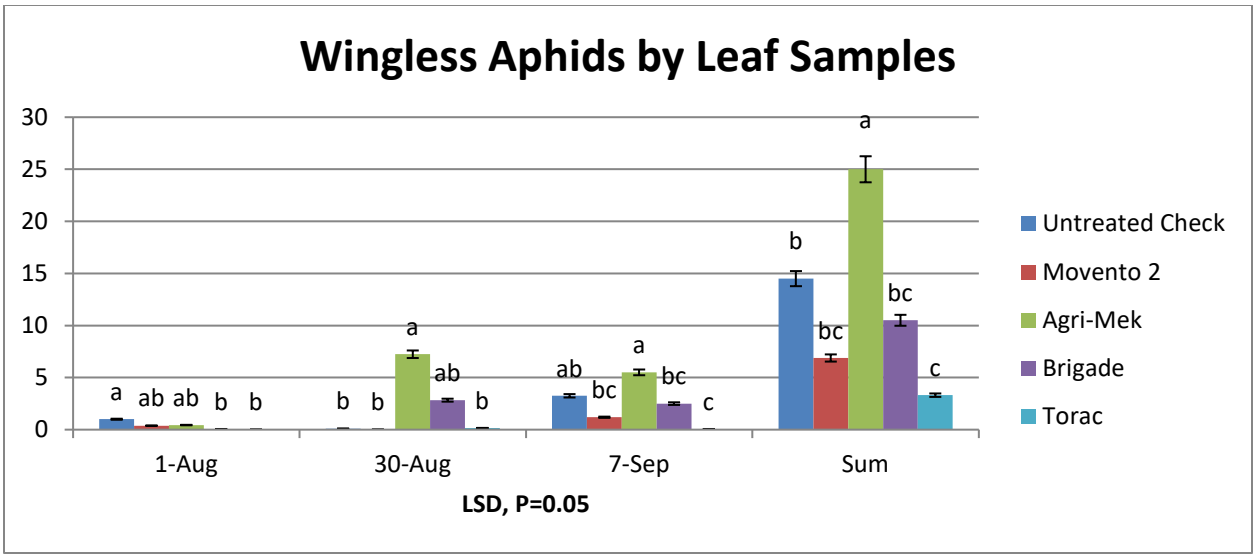


Figure 8: Wingless aphid data from leaf samples analyzed by treatment. Means with the same letter are not significantly different. (Student-Newman-Keuls)

Conclusions:

The data on aphids demonstrated that Torac was statistically better overall at managing aphid populations than the other insecticides tested. Movento proved to be much softer on the beneficial insect populations than the other insecticides and still provided good aphid control. Both Torac and Brigade were detrimental to beneficial insect populations.

When comparing sampling from smaller to larger plot sizes, the biggest difference was in the vacuum sampling method where larger plots contained on average more pest insects. It is likely that the increased number of exterior plants, or greater area allowed for an increased opportunity to collect insects that occur in a patchy distribution on larger plots compared to nearby small plots. The finding that the same numbers of insects on average were collected from 20 and 10 leaf samples contradicts earlier research with potato psyllids.

Eltopia, WA Trial
Potato Psyllid Control Efficacy – Intensive sampling trial - 2017
Materials and Methods

During the summer of 2017, the staff at the Agriculture Development Group, Inc. conducted a research trial investigating the efficacy of Movento, Abamectin, Brigade, and Exirel for the control of potato psyllids. The experimental design for this trial was a RCBD with 4 replications and plot sizes of 23ft x 25ft (i.e. two sides of four rows for total eight rows). Umatilla potato variety was planted in April, 2017. Applications for this trial were made with a multi-boom sprayer calibrated to apply treatment sprays at 25 gallons per acre. Only the middle two rows of each four rows were assessed for insect pest severity ratings to minimize the potential of overlap spray at the edge rows.

Potato psyllid, green peach aphids, and western flower thrips were observed in this trial but the psyllid pressure was very low. Three applications A, B, and C were applied on July 12, July 26 and August 9 at 14 days intervals. Evaluations for psyllids, thrips, and aphid severity were conducted on a weekly basis until 14 days after C applications (DAC), by examining the number of psyllid adults, nymphs, and eggs, total winged + wingless aphid, or thrips on 20 random leaves from the middle 4 rows within each plot under microscope.

Eltopia, WA Intensive Sampling Trial Results and Discussion

Unfortunately, the extremely low psyllid population masked the treatment effect and we only observed slightly reduced study total psyllid eggs by Movento, when compared with the untreated control.

Significant treatment effects were only observed on thrips at 14 days after B application (DAB), and 7 DAC, where Movento and Abamectin performed as well as Exirel (Graph 1). Brigade on the other hand, promoted thrips population by more than 50% compared with the untreated control. Although not statistically significant, all treatments appeared to promote aphid population, especially by Abamectin and Brigade, with 43% and 129% higher study total than untreated control.

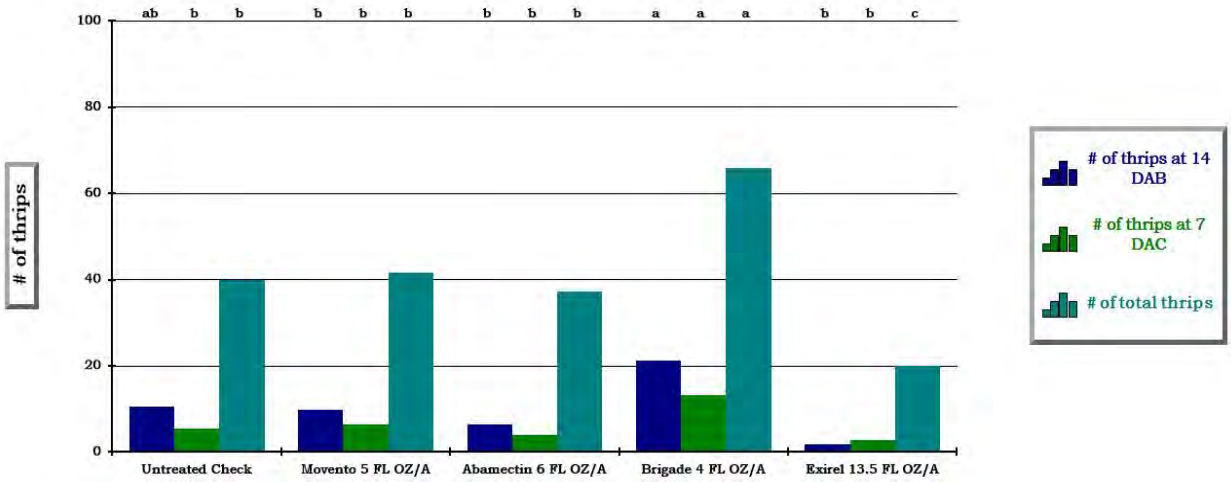


Figure 1. Treatment effect on thrips at 14 or 7 days after B or C application (DAB and DAC) in the intensive sampling trial

Potato Psyllid Control Efficacy- Standard Sampling Trial - 2017
Materials and Methods

During the summer of 2017, the staff at the Agriculture Development Group, Inc. conducted a research trial investigating the efficacy of Movento, Abamectin, Brigade, and Exirel for the control of potato psyllids. The experimental design for this trial was a RCBD with 4 replications and plot sizes of 11.5ft x 25ft (i.e. four potato rows). Umatilla potato variety was planted in April, 2017, applications for this trial were made with a multi-boom sprayer calibrated to apply treatment sprays at 25 gallons per acre. Only the middle two rows were assessed for insect pests to minimize the potential of overlap spray at the edge rows.

Potato psyllid, green peach aphids, and western flower thrips were observed in this trial but the psyllid pressure was very low. Three applications A, B, and C were applied on July 12, July 26 and August 9 at 14 days intervals. Evaluations for psyllids, thrips, or aphid severity were conducted at weekly basis until 14 days after C applications (DAC), by examining the number of psyllid adults, nymphs, and eggs, total winged + wingless aphid, or thrips on 10 random leaves from the middle 2 rows within each plot under microscope.

Eltopia, WA Standard Sampling Trial Results and Discussion
Overall, no significant treatment effect was observed on psyllids, thrips, or aphids. Relatively, Movento showed the best control efficacy on thrips with around 25% less study total thrips, and Abamectin showed the best control efficacy on aphids with 33% less study total aphid (Graph 1). Unfortunately, the extremely low psyllid population masked the treatment effect.

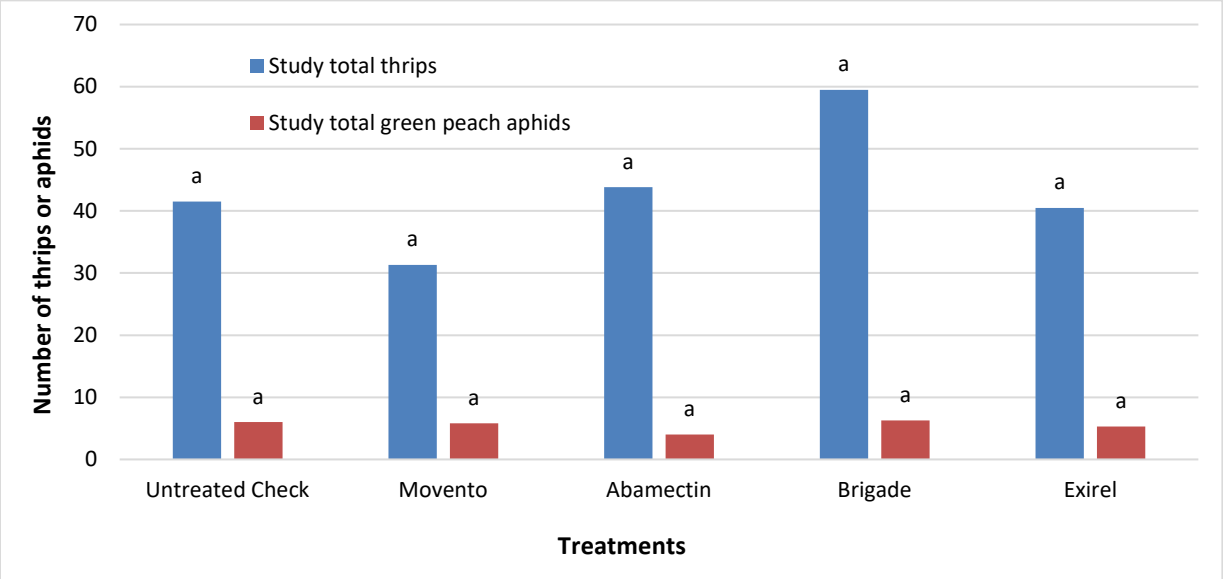


Figure 2. Treatment effect on total counted thrips and green peach aphids.

Table 1. Impact of sample size and insecticide on potato psyllid, thrips and green peach aphid. Ranked by decreasing number of thrips.

Trt	Treatment	Rate	Appl	20 leaf sample by pest and life stage									
				Psyllid life stage by number									
No.	Name	Of Application	Code	adult		nymph		egg		thrips		green peach aphid	
4	Brigade	4 fl oz/a	ABC	0.0	a	0.0	a	0.8	a	66.0	a	8.0	a
2	Movento	5 fl oz/a	ABC	0.0	a	0.0	a	0.3	a	41.5	b	4.3	a
1	Untreated Check			0.0	a	0.0	a	0.5	a	40.0	b	3.5	a
3	Abamectin	6 fl oz/a	ABC	0.0	a	0.0	a	0.5	a	37.3	b	5.0	a
5	Exirel	13.5 fl oz/a	ABC	0.0	a	0.0	a	1.0	a	20.0	c	3.8	a
				10 leaf sample by pest and life stage									
4	Brigade	4 fl oz/a	ABC	0.3	a	0.0	a	0.0	a	59.5	a	6.3	a
3	Abamectin	6 fl oz/a	ABC	0.0	a	0.0	a	0.0	a	43.8	a	4.0	a
1	Untreated Check			0.0	a	0.0	a	0.3	a	41.5	a	6.0	a
5	Exirel	13.5 fl oz/a	ABC	0.0	a	0.0	a	0.0	a	40.5	a	5.3	a
2	Movento	5 fl oz/a	ABC	0.3	a	0.0	a	0.0	a	31.3	a	5.8	a

This table shows the Brigade flare thrips with significantly more thrips than the untreated check when the sample size was 20 leaves per plot. WSU's Dr. Tim Waters has demonstrated this effect in onions. This is the first evidence that a pyrethroid insecticide can flare thrips in potatoes. It is particularly important to note that while the same trend was seen of more thrips in the Brigade treatment as compared to the untreated check when the sample size was ten leaves per plot the difference was not significant. It is noteworthy that this is the first data showing efficacy of Exirel against thrips in potatoes.

Potato Psyllid Efficacy Trial – Ontario, OR

Materials and Methods:

A trial for determining the efficacy of the insecticides Agri-Mek (abamectin), Brigade (bifenthrin), Exirel (cyazapyr), and Movento (spirotetramat) was conducted at the OSU Malheur Experiment Station. The trial was arranged on a randomized complete block design with four replications of each treatment and plot size. Small plots were four rows or 12 ft x 25ft). Large plots were eight rows or 24 ft x 25ft. Ranger Russet potatoes were planted on April 24, 2017. Treatments were made at a 14 day interval starting August 4, 2017, August 18 and September 1). Treatments were applied with a CO2 powered backpack sprayer applying insecticides at 20 gallons of water per acre and 30 PSI.

Table 1. Insecticides used in field trial at the Malheur Experiment Station, Ontario, OR.

Treatment Products	Active Ingredient	Rate (fl oz/a)	Timing
Control	-	-	-
Movento	Spirotetramat	5	ABC
Agrimek SC	Abamectin	3.5	ABC
Brigade	Bifenthrin	4	ABC
Exirel	Cyazypyr	13.5	ABC

Evaluations were made using two different sampling methods, as follows:

- Leaf samples were randomly selected from the middle canopy of plants in the interior rows of each plot, and placed in a one-gallon Ziploc bag. Samples were brought back to the lab for evaluation under magnification. Intense samples consisted of 20 leaves per plot and standard samples consisted of 10 samples per plot.
- An inverted leaf blower with an organza fabric bag was used on the outside rows, and the contents for each sample were place into one-gallon Ziploc bags, and evaluated with the use of dissecting microscopes. Intense samples were collected over a 3 minute interval and standard samples were collected over a 90 second interval.

Sample collection began 3 days after the first insecticide application and every 7 days thereafter.

Results

Overall insect populations were relatively low. Few potato psyllids were detected in this trial. Results were comparable between small and large plot samples (see figures below). The more intensive sampling regimens detected more insects and mites and tended to have less variation than the standard sampling regimens.

There were no significant differences in numbers of adult potato psyllids among the treatments (Figure 1). However, there were significant differences in potato psyllid eggs among treatments (Figure 2). Agrimek did not have an effect on potato psyllid eggs as there was no difference between the Agrimek treatment and the untreated control. Egg numbers were significantly lower in the Brigade, Exirel and Movento treatments compared with the untreated control. The same pattern was observed with the potato psyllid nymphs (Figure 3).

In large plot samples, two-spotted spider mite populations were significantly lower in the Agrimek, Brigade and Movento treatments than in either the Exirel treatment or the untreated control (Figure 4). In the small plots, Agrimek and Movento performed the best. Exirel and Brigade had significantly lower populations than the untreated control, but higher than either Agrimek or Movento.

Thrips populations also differed among treatments (Figure 5). In both the small and large plots, there were significantly more thrips in the Brigade treatment than in any of the other treatments, including the untreated control. Agrimek, Exirel and Movento performed equally well, with the three treatments having significantly fewer thrips than the untreated control.

Although we observed some statistical differences among treatments for psyllid eggs and psyllid nymphs, their biological importance is uncertain. The differences were consistent between the large and small plots but the averages only ranged from 0 to 2.3 per sample. Many leaves had no psyllid eggs or nymphs. The trial results support other trial results showing that pyrethroids (e.g., Brigade) flare thrips populations.

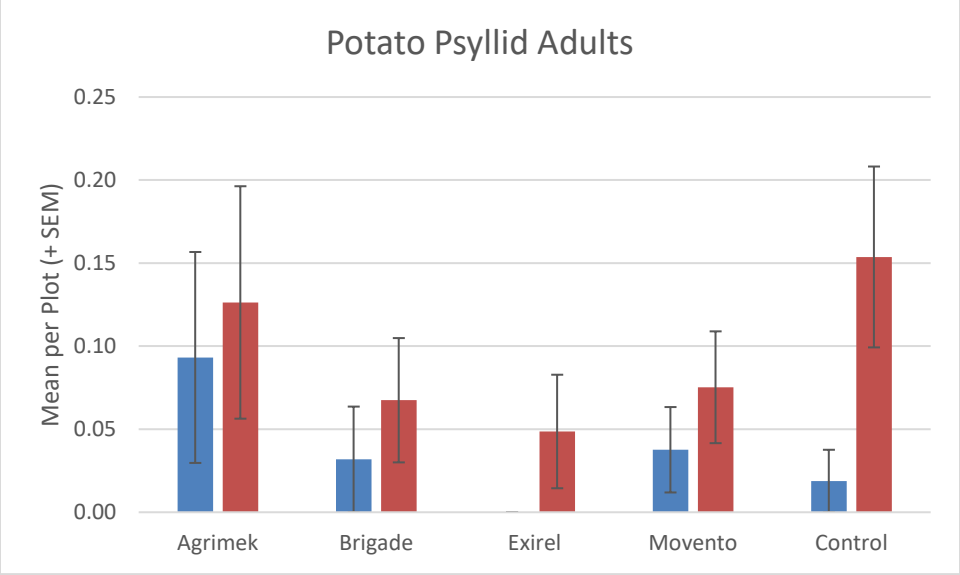


Figure 1. Mean number of adult potato psyllids by insecticide treatment in small plot (blue) and large plots (red) in efficacy trial at Ontario, OR, 2017, There were no treatment differences.

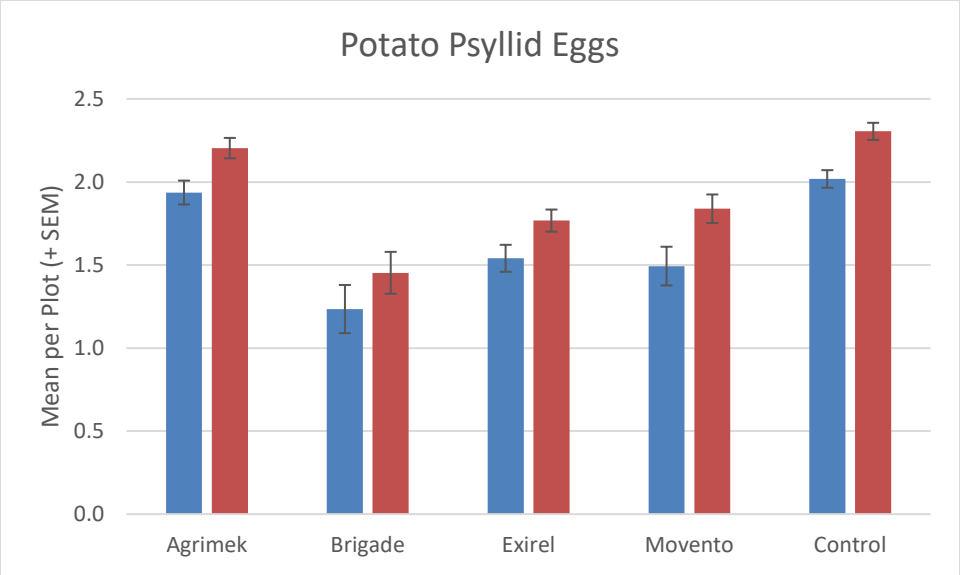


Figure 2. Mean number of potato psyllid eggs by insecticide treatment in small plot (blue) and large plots (red) in efficacy trial at Ontario, OR, 2017, Brigade, Exirel and Movento had significantly fewer eggs than the untreated control or the Agrimek treatment.

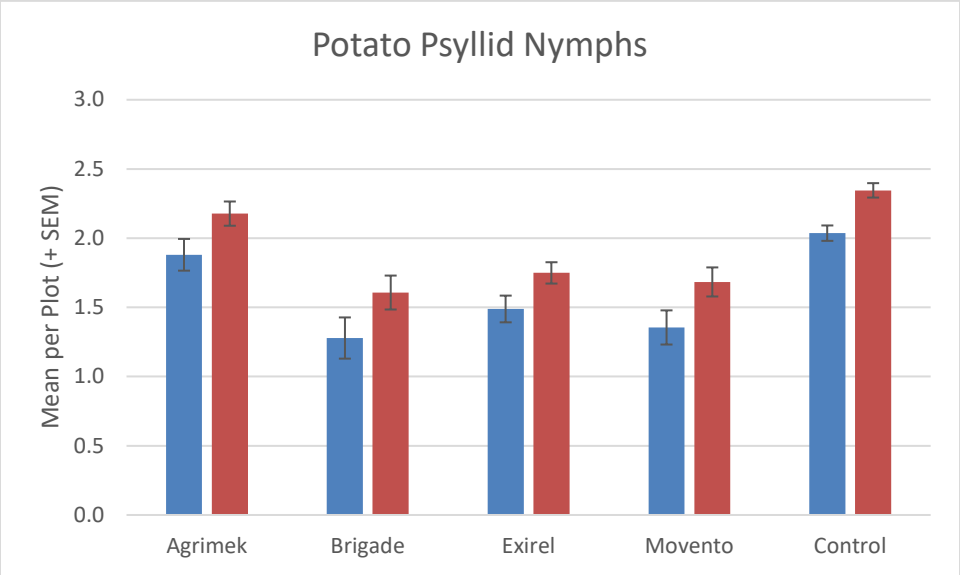


Figure 3. Mean number of potato psyllid nymphs by insecticide treatment in small plot (blue) and large plots (red) in efficacy trial at Ontario, OR, 2017, Brigade, Exirel and Movento had significantly fewer nymphs than the untreated control or the Agrimek treatment.

Kimberly, ID Trial
Sleeve-cage trials comparing effects of inoculation density of potato psyllids on incidence and severity of zebra chip disease

Field plots were established at the University of Idaho Kimberly Research & Extension Center, Kimberly, ID. Two-row by 5-foot long plots were planted with 'Russet Burbank' seed pieces on 2 May 2018, using 36-inch between-row spacing and 12-inch within-row spacing. Plots were arranged in a randomized complete block design with six replicates. Each plot was treated with one of six different insecticide treatments, including an untreated check (Table 1).

Table 1. List of insecticide treatments and application dates.

Treatment	Application rate		Application dates
Untreated Check			N.A.
Coragen (Colorado potato beetle check)	5.0	fl oz/a	June 30
Movento	5.0	fl oz/a	June 14, June 28, July 13, July 27, August 9, August 23
Abamectin	6.0	fl oz/a	June 14, June 28, July 13, July 27, August 9, August 23
Brigade	4.0	fl oz/a	June 14, June 28, July 13, July 27, August 9, August 23
Exirel (HGW86)	13.5	fl oz/a	June 14, June 28, July 13, July 27, August 9, August 23

One treatment consisted of a Colorado potato beetle "check" in which Coragen was applied to limit defoliation from beetles while having little effect on potato psyllids. Sprays were applied using a CO_2-powered sprayer at ca. 30 PSI, using 17 gallons per acre, and Teejet flat spray nozzles (8002VS), with one nozzle centered over each row.

Plots were inoculated with bacteriliferous potato psyllids from a laboratory colony within 24 hours after the second, fourth, and fifth sprays, on June 29, July 28, and August 10, respectively. A sleeve cage was attached to an individual leaf of a plant in the upper third of the canopy, and glass vials holding psyllids were opened within each cage. Each inoculated plant was flagged so that it could be tracked for subsequent inoculations and harvest. Five to six days after inoculation, the leaf (with sleeve cage attached) was excised from each plant. Two inoculation rates were used in each plot: 2 psyllids per plant or 4 psyllids per plant. In each case, a balanced sex ratio was used (i.e., 1 female + 1 male or 2 females + 2 males).

On September 7, plants were mechanically vine killed. Each inoculated plant was hand-harvested on September 29. On October 12-13, after storage at room temperature, each tuber was rated for zebra chip disease (ZC) symptoms by slicing tubers and conducting a visual rating using a 0-3 scale. Tubers were rated either as 0 (no symptoms), 1.5 (some evidence of mild symptoms near

the stem end), or 3 (severe symptoms visible through much of the tuber). PCR tests on a subset of samples confirmed visual ratings. For each plot and inoculation density, the percentage number of tubers with severe ZC symptoms as well as the mean ZC rating were calculated. These parameters were compared among insecticide treatments using Analysis of Variance.

For the two-psyllid inoculations, no significant differences were observed among insecticide treatments with respect to the percentage of tubers with severe ZC symptoms, nor for mean ZC rating (Table 2). Both parameters tended to be numerically lowest for the Abamectin and Brigade treatments, though the differences were not significant. Similarly, for the four-psyllid inoculations, no significant differences were observed among insecticide treatments with respect to the percentage of tubers with severe ZC symptoms, nor for mean ZC rating (Table 2). However, again both Abamectin and Brigade treatments tended to exhibit numerically lower values. ZC incidence and ratings tended to be higher for the four-psyllid relative to the two-psyllid inoculations, suggesting that disease symptoms are positively related to level of inoculation pressure. Moreover, similarity between the two- and four-psyllid inoculation densities in terms of responses among insecticide treatments suggests that any differences among treatments are robust, though subtle. More replication may well results in statistically significant differences among treatments. In addition, greater inoculation density likely would result in greater ZC incidence and severity, which might also contribute to observation of separation among treatments.

Table 2. Comparison among treatments of zebra chip (ZC) severity ratings.

	2 psyllids		4 psyllids	
	Percent of tubers with severe ZC symptoms (ZC rating of 3)	Mean ZC rating (0-3 scale)	Percent of tubers with severe ZC symptoms (ZC rating of 3)	Mean ZC rating (0-3 scale)
UTC	8.4	0.47	35.0	1.10
CPB Check (Coragen)	10.8	0.49	44.7	1.39
Movento	17.4	0.75	59.5	1.81
Abamectin	3.0	0.18	13.6	0.41
Brigade	1.2	0.11	14.3	0.48
Exirel (HGW86)	10.6	0.50	20.5	0.69
F	1.37	0.76	0.28	1.87
P-value	0.270	0.581	0.920	0.135

Hermiston, OR Sleeve Cage Trial

Methods

Plots were 4 rows wide x 25' feet long; 34" row spacing (25,000 plants/a). Experiment was set up as a Randomized Complete Block Design (RCBD) with four replications per treatment. Normal commercial production practices were followed throughout the season (e.g. fertilization, herbicide, fungicide, etc).

Individual plants were selected for infestation with Lso-infected potato psyllids in each replicate. Plants were inoculated with Lso-infected potato psyllids from a laboratory colony by placing a mesh satchel containing 4 adult psyllids on one leaf. Psyllids were released at two, four, and six weeks after the first insecticide application, immediately following the 2nd, 3rd, and 4th insecticide applications. Additionally, 10 leaves were randomly selected from other plants in each plot on 3rd and 23rd August to monitor natural psyllid populations. Infested plants were taken to yield. Plants were harvested on the 2nd week of September. Each tuber was rated for ZC symptoms by slicing tubers and conducting a visual rating using the 0-3 Texas rating scale. PCR tests on a subset of samples confirmed visual ratings. For each plot and inoculation density, the percentage of tubers with severe ZC symptoms as well as mean ZC ratings were calculated.

Data Analysis

Data was analyzed within sampling dates using ANOVA followed by Student-Newman-Keuls multiple comparisons all data analyses were performed using ARM 2017.

Results and Discussion

The caged mortality portion of the trial showed that Agri-mek and Brigade were the most effictive against potato psyllid adults across infestation dates (**Table 2**). While the first release had generally low efficacy for all treatments, the second and third infestations both showed 40% efficacy increased. The amount of PP eggs (**Table 3**) laid on caged leaves was more sporadic throughout release dates overall showing the Agrimek treatment plots with the lowest number of PP eggs followed by Brigade and Exirel; The untreated check and Movento plots presented the highest PP egg counts. Finally, the PP nymphs (**Table 4**) on caged leaves were relatively low, but Movento, Agri-mek plots, and the untreated check had the highest potato psyllid nymph counts while Brigade and Exirel showed the best control. No treatments showed significant yield differences (**Table** 5). ZC symptoms negligent.

Uncaged leaves showed Exirel, Movento, Agri-mek performing the best against both potato psyllid eggs and nymphs. High numbers occurred in the untreated check, and Brigade performed even worse than the untreated check. Yield showed no significant differences among treatments (Data no shown).

Table 1. List of products, rates and time of treatment applications, Hermiston, OR 2017.

Treatments	Products	Rate (fl oz/a)	Timing
T1	UTC	-	-
T2	Movento	5	ABCD
T3	Agrimek	6	ABCD
T4	Brigade	4	ABCD
T5	Exirel	13.5	ABCD

A First Application; B Second Application – 14 days after A; C Third Application – 14 days after B; D Fourth Application – 14 days after C

Table 2. Mean (±SE) number of dead PP adults, Hermiston, OR 2017.

	Days After Treatment (DAT)		
Treatments	**2-Aug**	**17-Aug**	**31-Aug**
UTC	0.00±0.00	0.00±0.00	0.25±0.25
Movento	0.00±0.00	0.50±0.29	0.00±0.00
Agrimek	0.50±0.50	1.00±0.41	1.00±0.41
Brigade	0.25±0.25	1.00±0.41	1.00±0.41
Exirel	0.25±0.25	0.50±0.29	0.75±0.48

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls). Total number of potato psyllids=4 per plant.

Table 3. Mean (±SE) number of Potato psyllid eggs, Hermiston, OR 2017.

	Days After Treatment (DAT)		
	2-Aug	**17-Aug**	**31-Aug**
UTC	0.00±0.00	0.75±0.48	9.50±8.19
Movento	0.75±0.75	2.50±1.50	9.25±5.57
Agrimek	0.75±0.75	0.75±0.48	2.50±1.55
Brigade	5.00±4.36	0.25±0.25	2.75±1.11
Exirel	0.00±0.00	1.25±0.75	6.75±4.82

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls). Total number of potato psyllids=4 per plant.

Table 4. Mean (±SE) number of Potato psyllid nymphs, Hermiston, OR 2017.

	Days After Treatment (DAT)		
	2-Aug	**17-Aug**	**31-Aug**
UTC	0.00±0.00	0.25±0.25	2.75±0.95
Movento	0.00±0.00	1.25±1.25	2.50±1.50
Agrimek	0.00±0.00	0.75±0.48	2.50±2.50
Brigade	0.00±0.00	0.00±0.00	1.00±0.71
Exirel	0.00±0.00	0.00±0.00	0.50±0.50

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls).
Total number of potato psyllids=4 per plant.

Table 5. Mean yield of plants infested with Lso-infected potato psyllids Hermiston, OR 2017.

	Yield					
	CULLS	**2-4 OZ**	**4-6 OZ**	**6-10 OZ**	**>10 OZ**	**TOTAL**
UTC	0.04	0.31	0.74	3.23	4.51	8.83
Movento	0.04	0.25	0.43	1.41	4.25	6.38
Agrimek	0.08	0.45	0.45	2.00	4.85	7.82
Brigade	0.05	0.69	0.43	1.60	4.34	7.10
Exirel	0.07	0.20	0.59	1.64	4.41	6.91

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls).

PUBLICATIONS:

Reitz, S.R. 2018. Potato Pest Management in with specific reference to the Pacific Northwest (USA) in the Wake of Recent Emerging Pests. In: Stuart Wale (ed.), Integrated pest management strategies in potato cultivation. In: Achieving sustainable cultivation of potatoes, Vol.2, Production and Storage, Crop Protection and Sustainability. In press.

Rondon, S. I. 2018. Insecticide effects on potato psyllid in potato. Arthropod Management Tests.

PRESENTATIONS & REPORTS:

Wenninger, E. 2018. Effects of Potato Psyllid Density and Time of Infection on Zebra Chip Disease Development both at Harvest an During Storage. Washington-Oregon Potato Conference.

Wenninger, E. 2018. Insect Management. Idaho Potato Conference.

Annual Progress Report/Final Report

Title: Understanding the pest status of Lygus in the Pacific Northwest

Personnel: Silvia Rondon (PI); Josephine Antwi (Postdoctoral scholar)

Reporting period: 2016-2017

Summary of accomplishments:
The goal of our study was to determine the role of Lygus (*Lygus* spp.) in potatoes including the role of Lygus in the epidemiology of the phytoplasma known as BLTVA, a.k.a. Beet Leafhopper Transmitted Virescence Agent or purple top disease. Therefore, since summer 2014-2015, producers have been observing high numbers of Lygus bugs on commercial potato fields, low numbers of beet leafhoppers (*Circulifer tenellus* Baker), but high incidence of BLTVA; beet leafhoppers are important potato pests and known to vector BLTVA. In recent years, Lygus were linked to fields with high incidence of BLTVA (34% of Lygus collected from our monitoring program tested positive for BLTVA in 2016; 28% in 2017), which prompted us to investigate if Lygus were indeed a vector and/or carrier of BLTVA; not much was known about how and if Lygus fed on potatoes. Results from our studies have determined: (1) the basis of Lygus feeding and feeding damage on potatoes; (2) ability of Lygus to carry BLTVA; (3) we are currently evaluating the efficiency of and the nature of Lygus/potato interaction; (4) Lygus and Lygus damage vertical distribution, which is higher in the upper third of a potato plant followed by the middle and lower sections; (5) Lygus/specie composition using DNA barcoding, since overlapping physical traits and the fact that the same species exhibit morphological variations across a geographic range makes it difficult to identify *Lygus* to species level; three species were identified in the region: *Lygus hesperus* (Knight), *Lygus elisus* L., and *Lygus keltoni* L; (6) population dynamics of Lygus bugs, where weekly average of Lygus (season-long) were highest mid-season (late June-early July) and lowest at the beginning and at the end of the season; (7) across potato varieties, Lygus abundance was the highest on Ranger and Alturas throughout the season compared to Umatilla, Russets, or Clearwater; (8) Preliminary GIS information shows specific areas in the lower Basin more prone to the presence of Lygus; (9) effect of Lygus densities on yield were not statistically different from the control, however, numerical differences were observed; more than 50 Lygus/plant reduced numerically yield; (10) insecticides were tested for efficacy against Lygus on potatoes to determine which insecticides best control this pest under standard growing conditions in the Columbia Basin: Vydate, Permethrin, Beleaf, Asana, and Sevin were tested; Vydate provided good control and it was easier to control nymphs compared to adults.

<u>Activities or experiments conducted/Results</u>: (2016-2017)

Hypothesis: Lygus is a competent vector of BLTVA in potato.

Lygus bugs were collected from potato fields and used to establish a colony at the OSU HAREC Irrigated Agricultural Entomology Program Lab. Lygus in colony were fed with fresh organic green beans and maintained at 78^0C on a 12:8 hr (L:D). Lygus from the colony were used in all greenhouse and field studies. A beet leafhopper colony was also established; they were collected from weeds around potato fields. They were fed with radishes and potatoes following Murphy et al. (2014), and Rondon and Murphy (2016) protocol.

Transmission studies. In fall 2015 and 2016, greenhouse experiments were carried out to determine the ability of Lygus bugs to vector BLTVA to potatoes. Periwinkle-BLTVA positive were used to graft potato plants, and used as a source of BLTVA infection for transmission experiments. Plants were tested for BLTVA thru PCR (Crosslin et al. 2006), and used in the transmission experiment. Four wks after planting, 10 Lygus (same aged) were allowed to feed on infected plants for 1 wk before transferring them to 4 wk-old uninfected potatoes ("clean" plants). Uninfected plants exposed to "hot" Lygus (hot=Lygus infected with BLTVA) showed clear purple top symptoms; however, not all plants tested showed the desired bands via PCR. In a second round of experiments, Lygus were allowed to feed on caged-BLTVA-infected plants in the field (infection confirmed via PCR) for 1 wk, and then placed on uninfected plants in the greenhouse. Data was recorded at 8, 12 and 16 wks after exposure to Lygus following same procedure as described above. Similar experiments were conducted using beet leafhoppers used as a control.

In general, results suggest that the efficiency of transmission of BLTVA by Lygus when compared to beet leafhopper is relatively poor. Although "clean" potato plants infected with "hot" Lygus showed typical purple top foliar symptoms, they tested negative for the pathogen; in contrast, none of the "clean" plants infested with "hot" beet leafhoppers showed foliar symptoms but 16% of them were BLTVA positive. BLTVA found in potato plants and insects, beet leafhopper, and Lygus, were sequenced and all belong to the same BLTVA strain or phytoplasma group. However, we are still trying to understand the lack of correlation between foliar symptoms, and PCR results unless it is related to timing of infection, which unfortunately will require new set of experiments. Moreover, since phytoplasma distribution in the plant is disproportionately with higher titers in sink organisms (e.g. auxiliary buds) and to lesser extent in petioles (unpublished data), it is unclear how Lygus feeding behavior correlated with BLTVA distribution; Christensen et al. (2004) observed similar titer distribution in other phytoplasma system. In the meantime, Lygus and Lygus damage vertical distribution is higher in the upper third of a potato plant followed by the middle and lower sections (Figure 1). Thus, phytoplasma distribution information in combination with distribution and feeding behavior of Lygus in potato plants could provide us a better picture of the relationship insect-pathogen.

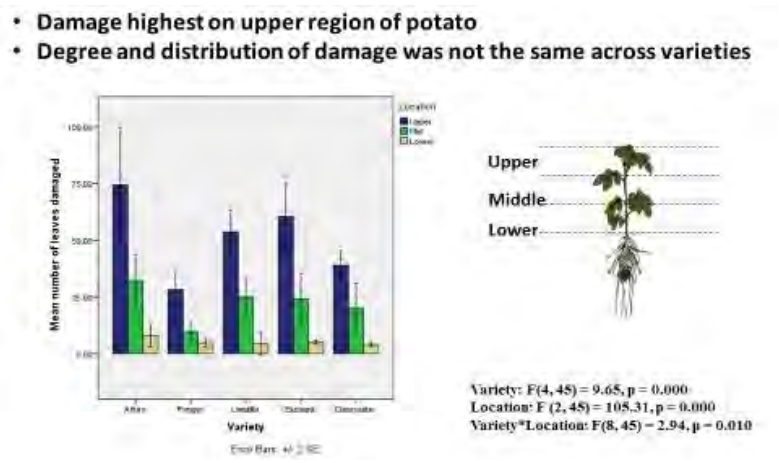
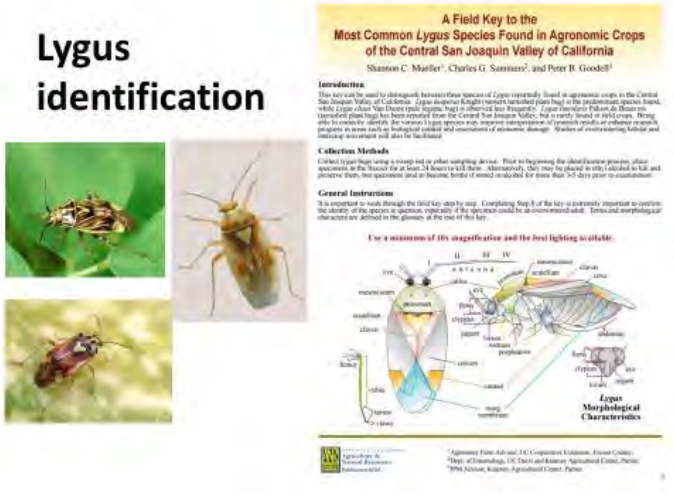


Figure 1. Lygus damage above ground.

BLTVA and Lygus; Lygus species composition

Some questions remain such as where the phytoplasma is located in Lygus and/or if there are differences between Lygus species. To answer the first part of the question, we will conduct a series of experiments in the spring-summer 2018, where infected Lygus will be dissected to determine where the phytoplasma might be present. Cooper et al. (2014) using *Bactericera cockerelli* and Candidatus *Liberibacter* conducted similar studies. As for Lygus/specie composition, overlapping physical traits and the fact that the same species exhibit morphological variations across a geographic range makes it difficult to identify *Lygus* to species level; in general we treat them as a "Lygus complex". Thus, we used DNA barcodes in combination with morphological characters to identify *Lygus* species on potatoes. Lygus collected from our entire signature monitoring statewide trap were used. Three species were identified in the region: *Lygus hesperus* (Knight), *Lygus elisus* L., and *Lygus keltoni* L. Interspecific genetic distances among *Lygus* species were relatively low, ranging from 1 to 1.5%. Neighbor joining tree clustered *L. hesperus* and *L. elisus* into two separate clades, with *L. keltoni* forming a subclade within *L. hesperus* clade. Statistical parsimony analysis indicates that none of the species shares a single haplotype, yet haplotype clusters did not correspond to species identity. This study was conducted to demonstrate the utility of integrating morphology and molecular markers in identifying morphologically similar species like *Lygus* bugs. Specie differentiation could lead to speciation that could explain differences in insect-pathogen interrelationship.



Hypothesis: Lygus cause economic damage to potato crops.

Population dynamics. In mid-April 2016 and 2017, five potato varieties (Alturas, Ranger Russet, Umatilla Russet, Russet Burbank, and Clearwater Russet) were planted in the field, and were arranged in a randomized complete block design. The entire plot was surrounded by a wide strip of alfalfa (20 X 40 ft) with a 10 ft space between the alfalfa strip and the potato plot to attract Lygus. To assess population abundance of Lygus across varieties, Lygus bugs were sampled weekly using an inverted leaf blower. In general, weekly average of Lygus (season-long) was highest mid-season (late June-early July) and lowest at the beginning and at the end of the season (Figure 2). Across varieties, Lygus abundance was the highest on Ranger and Alturas throughout the season. Preliminary GIS information shows specific areas in the lower Basin more prone to the presence of Lygus (data not shown).

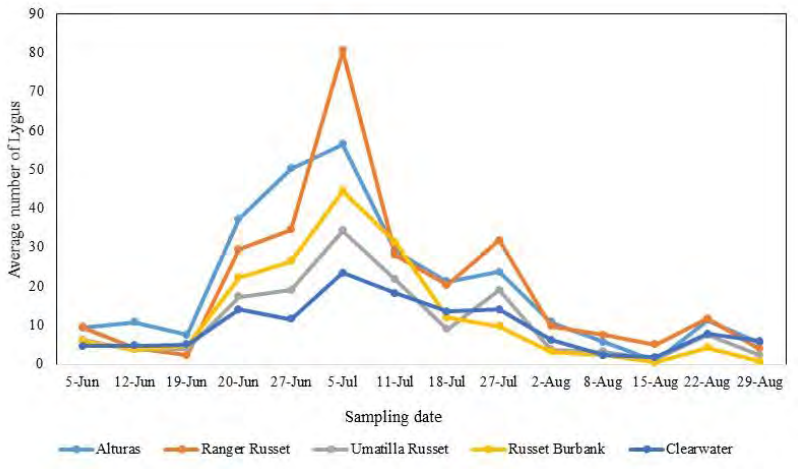


Figure 2. Population dynamics of Lygus, Hermiston OR 2016-2017.

Lygus damage. In the same field study, to assess Lygus damage, 5 plants per plot per variety were randomly selected every 4 weeks. Those plants were exposed to natural populations of Lygus. Plants were divided into 3 levels: upper, middle and lower canopy where damage was estimated in each section. Throughout the season, damage was higher in the upper canopy, followed by middle, and lower canopy (Figure 1). Not surprisingly, foliage damage increased as the season progressed. A follow-up experiment was conducted under controlled environment in the greenhouse to confirm vertical distribution of Lygus on potato plants. As in the field experiment, Lygus damage was more common in the upper canopy compared to lower canopy.

Lygus effect on yield. To measure the effect of Lygus density on yield, we set up insect hoop cages to cover single plants/ plot/ variety; all varieties listed above were included. Additionally, we set up walk-in (6 X 6 X 6 ft) to cover 12 plants; only Ranger Burbank's was included (we did not have enough funds to add walking cages in all varieties). In both, hoop and walk-in cages, prior to introducing Lygus, each cage was sprayed to exclude other insects. Three densities of Lygus were used for the single/individual hoop cages: 3, 10, and 30 Lygus/per plant. Densities for the walk-in cages included 50, 100, and 200 Lygus per cage. Control cages (0 Lygus) were sprayed periodically to ensure they remain insect-free. In both studies, the single plant/individual hoop cages and walk-ing cages, effect of Lygus densities on yield were not statistically different from the control (Figures 3 and 4, respectively), however, numerical differences were observed. Figure 4 shows the effect of density on yield; a release of 50 Lygus/plant increases yield. This singularity has been observed in other systems and it is known as "compensation" (Gagic et al. 2016); however, > 50 Lygus/plant will reduce yield. Other varieties should be tested. Producers have observed that Umatilla's are more affected than any other variety.

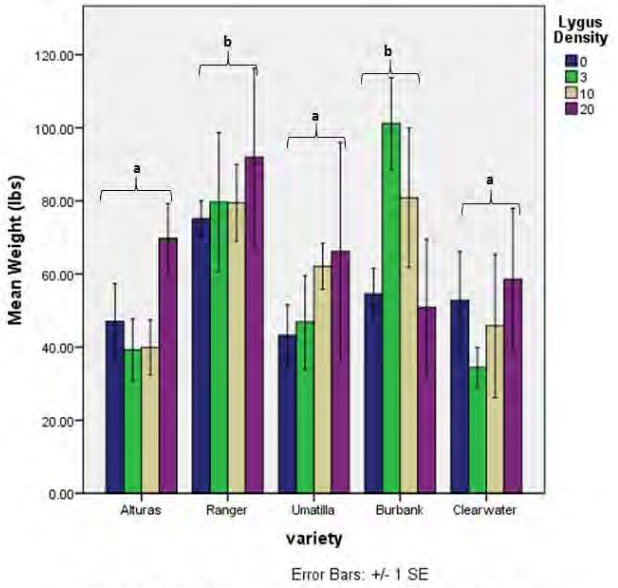


Figure 3. Effect of Lygus density on yield. Lygus density had no effect on yield. Same letters on top of varieties indicates that yield on those varieties were not statistically different.

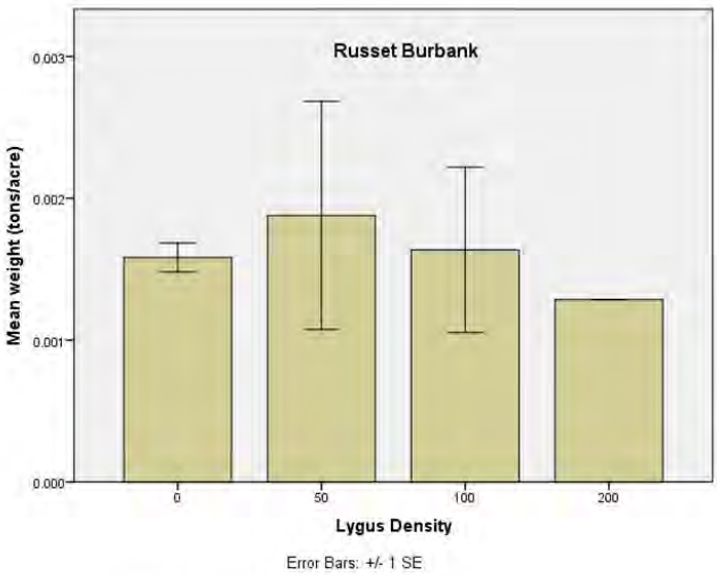


Figure 4. Effect of Lygus density on yield in walking cages

<u>Hypothesis:</u> Insecticides differ in their ability to control Lygus on potatoes.

Insecticides were tested for efficacy against Lygus on potatoes to determine which insecticides best control this pest under standard growing conditions in the Columbia Basin. Vydate, Permethrin, Beleaf, Asana, and Sevin were tested. Vydate provided good control and it is easier to control nymphs compared to adults (Rondon and Thompson 2016).

Publications:

Peer reviewed

Antwi J., and S.I. Rondon. 2018. Population dynamics of Lygus in northeastern Oregon and its relationship with purple top disease. To be submitted to J. Econ. Entomol.

Antwi J., and S.I. Rondon. 2018. Molecular and morphological identifications reveal species composition of Lygus (Hemiptera: Miridae) bugs in potato fields in the Lower Columbia Basin of the United States. Submitted to Environ. Entomol. (under review).

Rondon, S.I., J. Antwi, and R. Cooper. 2017. Lygus bugs on potatoes in the Pacific Northwest. EM 9173. https://catalog.extension.oregonstate.edu/em9173.

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Non-refereed, Proceeding, Abstracts

Thompson, I.D. and S.I. Rondon. 2018. Chemical control of the Lygus complex in potatoes. *In* 77th annual Pacific Northwest Insect Management Conference. 8-9 Jan. Portland, OR. Section V. Pp 49.

Rondon. 2017. Lygus in potatoes. *In* 76th annual Pacific Northwest Insect Management Conference. 9-10 Jan. Portland, OR. Section V. Pp 41.

Thompson, I.D. and S.I. Rondon. 2017. Controlling Lygus in potatoes. *In* 76th annual Pacific Northwest Insect Management Conference. 9-10 Jan. Portland, OR. Section V. Pp 44-45.

Rondon, S.I. and J. Antwi. 2017. Managing Lygus on potatoes in the Columbia Basin: should we care?. *In* Proceed. WA/OR Annual conference. 26-28 Jan. Kennewick, WA. Pp 56.

Antwi, J., S.I. Rondon, K. Frost, A. Goyer, and R. Cating. 2016. Taking a second look at Lygus in the Pacific Northwest. *In* 75th annual Pacific Northwest Insect Management Conference. 11-12 Jan. Portland, OR. Section V. Pp 54-57.

Reports

Observations of Lygus bugs in the Basin. Potato Update, 10, 16 pp 1-2.

Several notes were also in the Potato Update http://oregonstate.edu/dept/hermiston/trap-reports

September/October 2016: Potato Country, "Finding fixes for potential problems". http://reader.mediawiremobile.com/ColumbiaMediaGroup/issues/108533/viewer?page=8

Antwi, J. and, S.I. Rondon. 2016. Elucidating the role of Lygus. *In* Annual Northwestern Consortium Reports. http://www.oregonspuds.com/images/publications/ Consortium_reports_ January_2016rev.pdf

Antwi, J., S.I. Rondon, P.B. Hamm, R. Cating, and A. Goyer. 2015. Elucidating the role of Lygus. *In* Annual Northwestern Consortium Reports. http://www.oregonspuds.com/images/publications/Consortium_reports_January_2015rev.pdf

Presentations:

Rondon, S.I. 2017. Managing Lygus on potatoes in the Columbia Basin: should we care? WA-OR Potato Conference. 24-26 Jan. Kennewick, WA (320 participants).

R Rondon S.I. 2017. Lygus bugs: Identification, biology, and management. Potato Field Day, Hermiston Agricultural & Research Extension Center, Hermiston, OR. Number of attendees ~100.

Rondon, S.I. 2016. Potato growers: don't take Lygus bugs lighly. 30 Nov. Farm Fair. Hermiston, OR (190 participants).

Rondon, S.I. 2016. The role of *Lygus sp.* in the epidemiology of BLTVA in potatoes in the pacific Northwest. 25th International Congress of Entomology, Orlando, FL.

Rondon, S.I. 2016. Searching for *Lygus* bugs in potatoes in the Pacific Northwest. Potato Field Day, WSU Irrigated Agriculture Research & Extension Center, WSU, Othello, WA. Number of attendees ~70

Rondon, S.I. 2016. Searching for *Lygus* bugs in potatoes in the Pacific Northwest. Potato Field Day, Hermiston Agricultural & Research Extension Center, Hermiston, OR. Number of attendees ~120.

Rondon, S.I. 2016. Taking a second look at Lygus bugs. 2016 Pacific Northwest Insect Management Conference, Portland, OR.

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Gagic V., L.G.A. Riggi, B. Ekbom, G. Maksher, A. Rusch, and R. Bommarco. 2016. Interactive effects of pests increase seed yield. Ecol. Evol. 6(7): 2149-2157.

Murphy A.F., S.I. Rondon, R. Marchosky, J. Buckham, and J. Munyaneza. 2014. Evaluation of beet leafhopper transmitted virescence agent damage in the Columbia Basin. Am. J. Pot. Res. 91 (1): 101-108.

Rondon, S.I. and A.F. Murphy. 2016. Monitoring and controlling the beet leafhopper *Circulifer tenellus* in the Columbia Basin. Am. J. Pot. Res. 93(1): 80-85. DOI 10.1007/s12230-015-9491-3.

TITLE: Management of Lygus and Thrips in Pacific Northwest Potatoes.

YEAR INITIATED: 2017. **CURRENT YEAR:** 2017-18. **TERMINATING YEAR** 2019.

PERSONNEL & COOPERATORS:

Principal Investigator:

Alan Schreiber, Agriculture Development Group, Inc., 509 266 4348, aschreib@centurytel.net , 2621 Ringold Road, Eltopia, WA, 99330,

Key Findings from This Research Effort

1. Insecticides with greatest efficacy against Lygus were, in decreasing order of efficacy, Vydate CLV, Beleaf, Comoran, Transform and Lannate.
2. In a cage study, potatoes kept free of Lygus had significantly greater yields than potatoes with low and high level of Lygus. This is the second year of data from Schreiber showing that Lygus can have a significantly negative impact on potato production.
3. In a trial targeting thrips, products with the greatest efficacy against Lygus were, in decreasing order of efficacy, Hero, Lannate, Beleaf and Vydate
4. Insecticides with greatest efficacy against thrips were, in decreasing order of efficacy, Lannate, Vydate, Beleaf, Beleaf tank mixed with Mustang Max, Dimethoate, Torac and Mustang Max. Thrips pressure was relatively low in this trial.
5. In a trial targeting thrips, all insecticides reduced psyllid level to zero as compared to the untreated check. Overall psyllid levels were quite low. Products in the trial were Lannate, Vydate CLV, Beleaf, Mustang Max, Dimethoate, Torac, Abamectin, Hero, Movento, Asana and Cormoran.
6. In a trial targeting thrips, products with the greatest efficacy against Lygus were, in decreasing order of efficacy, Beleaf, Torac and Movento. A treatment of three applications of Mustang Max had a total of 350% more aphids than the untreated check, once again demonstrating the ability of a pyrethroid insecticide to flare aphid populations.
7. A trial targeting psyllids with an objective of determining if increasing sampling size would improve researchers ability to detect treatment differences examined impact of various insecticide programs on psyllids and other potato insect pests. In this trial, we found that a psyllid managemet program based on pyrethroid insecticides flared thrips. This is the first evidence that pyrethroids exacerbate thrips populations in potatoes.
8. The flaring of thrips by a pyrethroid insecticide was detected by a more intensive sampling method. The same treatment made to potatoes using the industry standard sampling method which was less intensive did not detect the same treatment difference. This result suggests that current leaf sampling method may not be sufficiently rigorous for research purposes.

Potato Lygus Control Efficacy Trial-2017

Materials and Methods

During the summer of 2017, the staff at the Agriculture Development Group, Inc. conducted a research trial investigating the efficacy of multiple insecticides (treatment list in Table 1) for the control of western lygus bug in potato. The experimental design for this trial was a RCB with 4 replications and plot sizes of 11.5ft x 25ft with four potato rows. Umatilla potato variety was planted in April, 2017, and three applications A, B, and C were applied on July 13th, July 27th, and August 10th by a multi-boom sprayer calibrated to apply treatment sprays at 25 gallons per acre. Only the middle two rows were assessed for insect pest severity ratings to minimize the potential of overlap spray at the edge rows.

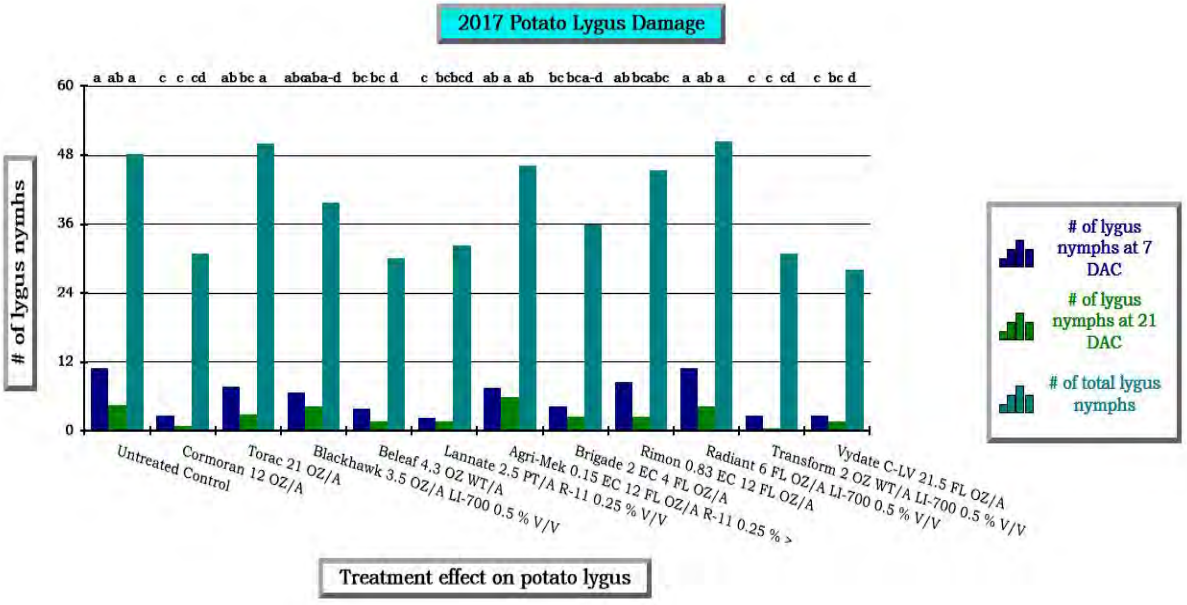
Evaluations for lygus nymphs and adults severity were made at 0, 7, 14, and 21 days after each applications by examining the number of lygus caught in the net by 15 sweepings (sweep net method) from the middle 2 rows of each plot. Collected data was input into ARM for statistical analysis where ANOVA and mean separation tables were generated.

Table 1. Treatment list and application details.

Trt No.	Treatment Name	Form Conc	Form Type	Description	Rate	Rate Unit	Appl Code	Appl Description
1	Untreated Control							
2	Cormoran		EC	Adama	12	oz/a	ABC	14 Day Intervals
3	Torac		EC	Nichino	21	oz/a	ABC	14 Day Intervals
4	Blackhawk	36	WG	Dow	3.5	oz/a	ABC	14 Day Intervals
	LI-700	80	SN	DOW	0.5	% v/v	ABC	14 Day Intervals
5	Beleaf	0.5	SG	WSPC	4.3	oz wt/a	ABC	14 Day Intervals
6	Lannate	2.4	SN	WSPC	2.5	pt/a	ABC	14 Day Intervals
	R-11	80	SN	WSPC	0.25	% v/v	ABC	14 Day Intervals
7	Agri-Mek 0.15 EC	0.15	EC	WSPC	12	fl oz/a	ABC	14 Day Intervals
	R-11	80	SN	WSPC	0.25	% v/v	ABC	14 Day Intervals
8	Brigade 2 EC	2	EC	WSPC	4	fl oz/a	ABC	14 Day Intervals
9	Rimon 0.83 EC	0.83	EC	WSPC	12	fl oz/a	ABC	14 Day Intervals
10	Radiant	1	SC	Dow	6	fl oz/a	ABC	14 Day Intervals
	LI-700	80	SN	DOW	0.5	% v/v	ABC	14 Day Intervals
11	Transform	50	WG	Dow	2	oz wt/a	ABC	14 Day Intervals
	LI-700	80	SN	DOW	0.5	% v/v	ABC	14 Day Intervals
12	Vydate C-LV	3.77	SN	WSPC	21.5	fl oz/a	ABC	14 Day Intervals

Results and Discussion

Treatments started to show significant control effect on lygus nymphs towards the later season in late August. Cormoran, Lannate, Transform, and Vydate performed the best lygus control at 7 DAC (Aug 17) with less than 3 nymphs/plot, followed by Beleaf and Brigade at around 4/plot (dark blue bars in graph). By 21 DAC, lygus population started natural decreasing but there was still a significant residual effect from Cormoran and Transform with >300% less lygus nymphs than untreated control (green bars in graph). Although not statistically significant, earlier data collected at 7 and 14 days after A application (DAA) showed that Brigade and Rimon actually resulted in similar to or even better lygus nymph control than treatments like Cormoran, Lannate, Transform, and Vydate. However, both Brigade and Rimon could not keep up the good control like the four superior treatments did after application B, indicating some sort of fast resistance build-up in the nymphs' system. Furthermore, although not one of the best performers, Beleaf consistently performed a medium control efficacy throughout the whole study period. As a result, the study total nymphs were also significantly controlled by Beleaf (30 in total), beside Cormoran (31 in total), Lannate (32 in total), Transform (31 in total), and Vydate (28 in total), compared to the 48 total in untreated (Table 2).



On the other hand, it appears that treatments had different effect on adults and nymphs, where adult stage consistently showed less susceptibility with no significant separation among all treatments at all dates or for study totals. This may be also caused by the greater variation of the adult data which can be found in the ANOVA and mean separation table, likely due to the higher mobility of flying adults.

Table 2. Mean separation table for comparison of 11 insecticidal program for control of lygus nymphs in potatoes.

Trt No.	Treatment Name	Rate of Application		Number of Applications	Total number of Lygus Adults		Total number of Lygus Nymphs	
12	Vydate C-LV	21.5	fl oz/a	ABC	33.5	a	28.0	d
5	Beleaf	4.3	oz wt/a	ABC	39.0	a	30.0	d
2	Cormoran	12	oz/a	ABC	32.5	a	30.8	cd
11	Transform	2.0	oz wt/a	ABC	29.8	a	30.8	cd
6	Lannate	2.5	pt/a	ABC	29.0	a	32.3	bcd
8	Brigade 2 EC	4	fl oz/a	ABC	46.5	a	36.0	a-d
4	Blackhawk	3.5	oz/a	ABC	37.3	a	39.8	a-d
9	Rimon 0.83 EC	12	fl oz/a	ABC	46.3	a	45.3	abc
7	Agri-Mek 0.15 EC	12	fl oz/a	ABC	41.0	a	46.3	ab
1	Untreated Control				37.3	a	48.3	a
3	Torac	21	oz/a	ABC	33.8	a	50.0	a
10	Radiant	6	fl oz/a	ABC	37.3	a	50.5	a

Potato Lygus Control Efficacy - 2017

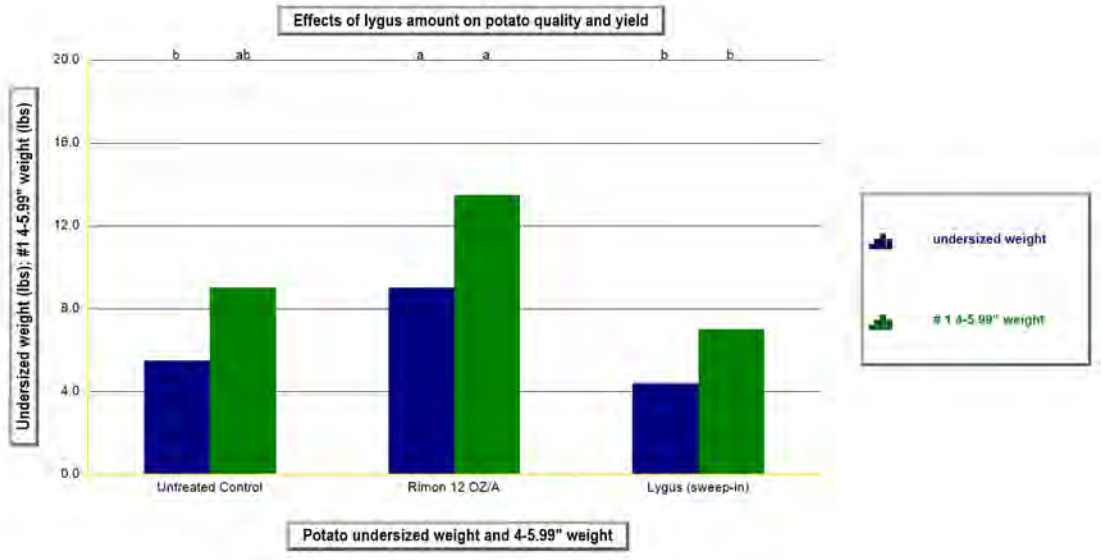
Materials and Methods

The staff at the Agriculture Development Group, Inc. started a research trial at Eltopia, WA in April 2017 to determine how many lygus it takes to damage potatoes and better clarify the damage lygus does on potatoes. The experimental design for this trial was a RCB with 4 replications and plot sizes of 4.4 ft x 9 ft. Applications for this trial were made with a backpack sprayer calibrated to apply treatment sprays at 20 gallons per acre. The potatoes established for this trial were not treated with any maintenance fungicides except Success (only aiming on Colorado Potato Beetle, which was noticed on August 9) in order to prevent the possibility of interfering with the lygus population. The potatoes were planted on April 6, and the cages were set up (see attached photo) on July 12 before the first application of Rimon. The applications for Rimon were made on July 13, July 27, and August 10 inside the cage by lifting up one side of the cage.

Results and Conclusions

Twenty lygus bugs were caught from alfalfa field and swept into the cages for treatment 3 on July 28, August 3, August 10, and August 17. This trial was harvested on September 18. Rimon treatment showed significantly more weight for undersized potatoes, which is 64% higher than untreated check. For potato size 4-5.99" (Graph 1), Rimon treatment had significantly (93%) more weight compared to treatment with lygus swept in. Although not statistically different, Rimon treatment showed 28% and 38% increase of total weight and net weight (Graph 2), respectively, compared to untreated check. The results suggested that less lygus resulting from Rimon applications could contribute to improved potato size and total yield.

Graph 1. Effects of different levels of lygus amounts on potato undersized weight and weight for size 4-5.99".



Graph 2. Effects of different levels of lygus amounts on potato total yield and net yield (total yield – cull weight - # 2 weight).

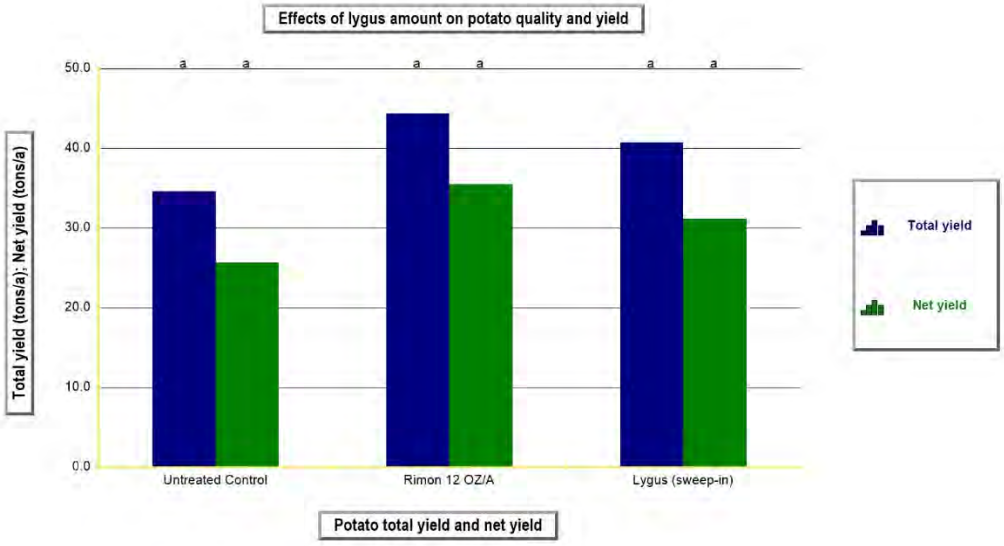


Photo 1. Potato plots covered with cages on July 12, 2017.

Photo 2. Lygus captured from alfalfa field for sweeping into the cages for treatment 3 on July 28.

Potato Thrips Control Efficacy Trial - 2017

Material and Methods

A potato insecticide trial was initiated in April 2017 at the Agricultural Development Group, Inc. to evaluate the effects of multiple insecticides on control of thrips on potato (Table 1). The experimental design was a RCB with 4 replications with the plot size of 11.5 ft x 25 ft. Applications for this trial were made by tractor-mounted multi-boom calibrated to apply treatment spray at 25 gallons/acre. The potato planted for this trial were not treated with any other insecticide in order to prevent the possibility of interfering with the existing trial's objective.

The potato was planted on April 21, 2017. The applications were made on July 18, July 31, and August 14. The total number of western flower thrips (*Frankliniella occidentalis*), potato psyllids (*Bactericera cockerelli*), western plant bug (Lygus hesperus), and green peach aphids (Myzus persicae) were counted 6 times starting on July 25 with 7 days interval, for each plot with 2 plants per plot, and each plant got 5 beats (2 subsamples per plot).

Table 1. Treatment list and application details.

Trt No.	Treatment Name	Form Conc	Form Type	Description	Rate	Unit	Appl Code
1	UTC						
2	Abamectin	0.15	EC	WSPC	3.5	fl oz/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
3	Dimethoate	2.67	EC	WSPC	1	pt/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
4	Hero	1.24	EC	WSPC	8	fl oz/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
5	Lannate LV	29	SN	WSPC	3	pt/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
6	Mustang Max	0.8	EC	WSPC	4	fl oz/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
7	Movento	2	SC	WSPC	5	fl oz/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
8	Vydate CLV	2	SN	WSPC	3.675	pt/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
9	Beleaf	50	SG	WSPC	2.8	oz wt/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
10	Asana		SL	WSPC	8	fl oz/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
11	Beleaf	50	SG	WSPC	2.8	oz wt/a	ABC
	Mustang Max	0.8	EC	WSPC	4	fl oz/a	ABC
	R-11	100	SL	WSPC	0.25	% v/v	ABC
12	Cormoran		EC	Adama	12	oz/a	ABC
13	Torac		EC	Nichino	12	oz/a	ABC

Results and Conclusion

Investigated insect pressure was generally low throughout the season, but created some complex results. Results are presented in Tables 2, 3, 4, and 5 for better interpretation of the results. Hero showed the best control on lygus with 69% fewer thrips than the untreated control followed by Lannate LV, Vydate C-LV, and Beleaf (all around 50% reduction), while remaining treatments did not show any significant reduction in thrips numbers (Table 2).

Table 2. Comparison of 12 Insecticidal Programs for Control of Lygus, Thrips, Psyllids and Green Peach Aphid on Potato ranked by efficacy against lygus.

Trt No.	Treatment Name	Rate of Application	# of Application	Total Average per 10 Leaves							
				Lygus		Thrips		Psyllids		Green peach aphid	
4	Hero	8 fl oz/a	ABC	**4.6**	**d**	4.5	a	0.0	a	20.5	cde
5	Lannate LV	3 pt/a	ABC	**7.3**	**cd**	2.3	a	0.0	a	39.5	cde
9	Beleaf	2.8 oz wt/a	ABC	**7.3**	**cd**	2.4	a	0.0	a	3.9	e
8	Vydate C-LV	3.675 pt/a	ABC	**7.9**	**bcd**	2.3	a	0.0	a	24.5	cde
10	Asana	8 fl oz/a	ABC	**10.5**	**abc**	5.8	a	0.0	a	44.8	cd
12	Cormoran	12 oz/a	ABC	**10.6**	**abc**	6.1	a	0.0	a	26.8	cde
3	Dimethoate	1 pt/a	ABC	**10.8**	**abc**	3.0	a	0.0	a	84.9	ab
2	Abamectin	3.5 fl oz/a	ABC	**11.3**	**abc**	4.4	a	0.0	a	52.3	bc
6	Mustang Max	4 fl oz/a	ABC	**12.0**	**abc**	3.3	a	0.0	a	116.3	a
11	Beleaf/Mustang M	2.8 4 oz wt/a	ABC	**13.4**	**ab**	2.5	a	0.0	a	4.6	e
13	Torac	12 oz/a	ABC	**14.4**	**a**	3.0	a	0.0	a	12.6	de
1	UTC			**15.0**	**a**	8.3	a	8.5	a	30.9	cde
7	Movento	5 fl oz/a	ABC	**15.1**	**a**	4.9	a	0.0	a	19.9	cde

Notes: Values within each column with the same letters are not significant different based on F test of LSD at P<0.05.

Overall thrips numbers were low in this trial making it difficult to draw significant conclusions regarding insecticidal control of thrips. Although no statistical differences were noticed among treatments for thrips control, most treatments reduced thrips population by the end of the study (Graph#1). Compared to untreated, overall total thrips numbers were reduced by Dimethoate (64%), Lannate LV (72%), Mustang Max (60%), Vydate C-LV(72%), Beleaf (71%), Beleaf+Mustang Max (70%), and Torac (64%) was better than abamectin (47%), Hero (46%), Movento (41%), and Asana (30%) (Table 3).

Table 3. Comparison of 12 Insecticidal Programs for Control of Lygus, Thrips, Psyllids and Green Peach Aphid on Potato ranked by efficacy against thrips.

Trt	Treatment	Rate of	# of	Total Average per 10 Leaves							
No.	Name	Application	Apps	Lygus		**Thrips**		Psyllid		Green peach aphid	
5	Lannate LV	3 pt/a	ABC	7.3	cd	**2.3**	**a**	0.0	a	39.5	cde
8	Vydate C-LV	3.675 pt/a	ABC	7.9	bcd	**2.3**	**a**	0.0	a	24.5	cde
9	Beleaf	2.8 oz wt/a	ABC	7.3	cd	**2.4**	**a**	0.0	a	3.9	e
11	Beleaf/Mustang M	2.8 and 4 oz wt/a	ABC	13.4	ab	**2.5**	**a**	0.0	a	4.6	e
3	Dimethoate	1 pt/a	ABC	10.8	abc	**3.0**	**a**	0.0	a	84.9	ab
13	Torac	12 oz/a	ABC	14.4	a	**3.0**	**a**	0.0	a	12.6	de
6	Mustang Max	4 fl oz/a	ABC	12.0	abc	**3.3**	**a**	0.0	a	116.3	a
2	Abamectin	3.5 fl oz/a	ABC	11.3	abc	**4.4**	**a**	0.0	a	52.3	bc
4	Hero	8 fl oz/a	ABC	4.6	d	**4.5**	**a**	0.0	a	20.5	cde
7	Movento	5 fl oz/a	ABC	15.1	a	**4.9**	**a**	0.0	a	19.9	cde
10	Asana	8 fl oz/a	ABC	10.5	abc	**5.8**	**a**	0.0	a	44.8	cd
12	Cormoran	12 oz/a	ABC	10.6	abc	**6.1**	**a**	0.0	a	26.8	cde
1	UTC			15.0	a	**8.3**	**a**	8.5	a	30.9	cde

Notes: Values within each column with the same letters are not significant different based on F test of LSD at P<0.05.

As with other insect pests in this trial, psyllid pressure was low. The only treatment with psyllids was the untreated check while all other treatments had no psyllids indicating a six treatment program with the 11 active ingredients would provide acceptable control of the insect pests in a low pressure situation (Table 4).

Table 4. Comparison of 12 Insecticidal Programs for Control of Lygus, Thrips, Psyllids and Green Peach Aphid on Potato ranked by efficacy against psyllid.

Trt No.	Treatment Name	Rate of Application	Number of Applications	Total Average per 10 Leaves							
				Lygus		Thrips		**Psyllid**		Green peach aphid	
5	Lannate LV	3 pt/a	ABC	7.3	cd	2.3	a	**0.0**	**a**	39.5	cde
8	Vydate C-LV	3.675 pt/a	ABC	7.9	bcd	2.3	a	**0.0**	**a**	24.5	cde
9	Beleaf	2.8 oz wt/a	ABC	7.3	cd	2.4	a	**0.0**	**a**	3.9	e
11	Beleaf/Mustang M	2.8 and 4 oz wt/a	ABC	13.4	ab	2.5	a	**0.0**	**a**	4.6	e
3	Dimethoate	1 pt/a	ABC	10.8	abc	3.0	a	**0.0**	**a**	84.9	ab
13	Torac	12 oz/a	ABC	14.4	a	3.0	a	**0.0**	**a**	12.6	de
6	Mustang Max	4 fl oz/a	ABC	12.0	abc	3.3	a	**0.0**	**a**	116.3	a
2	Abamectin	3.5 fl oz/a	ABC	11.3	abc	4.4	a	**0.0**	**a**	52.3	bc
4	Hero	8 fl oz/a	ABC	4.6	d	4.5	a	**0.0**	**a**	20.5	cde
7	Movento	5 fl oz/a	ABC	15.1	a	4.9	a	**0.0**	**a**	19.9	cde
10	Asana	8 fl oz/a	ABC	10.5	abc	5.8	a	**0.0**	**a**	44.8	cd
12	Cormoran	12 oz/a	ABC	10.6	abc	6.1	a	**0.0**	**a**	26.8	cde
1	UTC			15.0	a	8.3	a	**8.5**	**a**	30.9	cde

Notes: Values within each column with the same letters are not significant different based on F test of LSD at P<0.05.

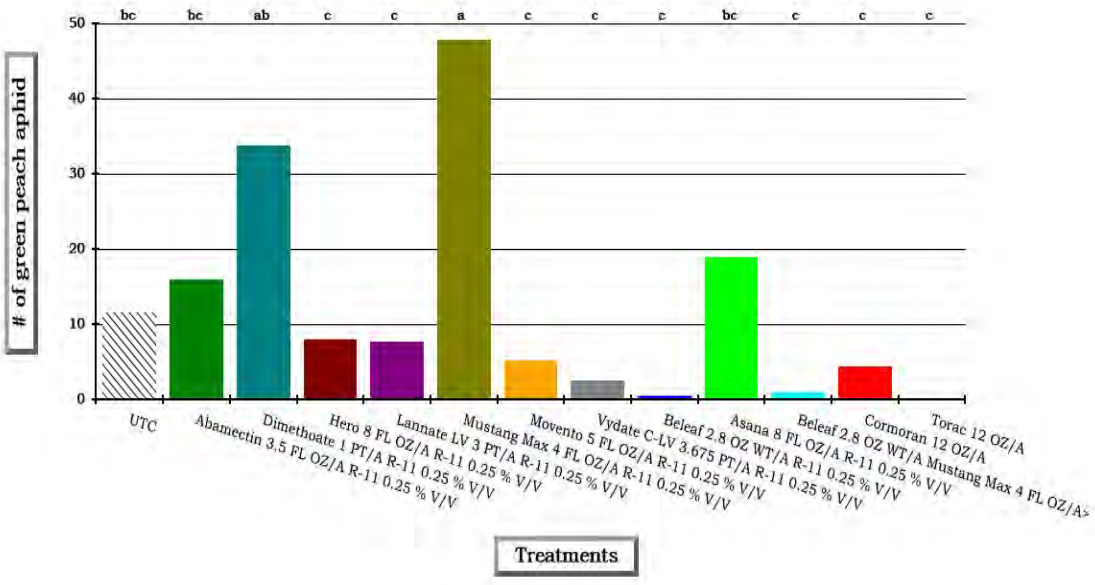
Finally, aphid data showed significant treatment effects at 8 and 14 days after C application (DAC) the third of three applications, where Vydate C-LV, Beleaf, Beleaf + Mustang Max, and Torac resulted in 78 to 97% reduction of aphids at 8 DAC (Graph 1), compared to untreated control (Table 5). However, Vydate lost its effect at 14 DAC with higher than control aphid counts, as well as most treatments except Beleaf, Beleaf + Mustang Max, and Torac (Graph 2).

Table 5. Comparison of 12 Insecticidal Programs for Control of Lygus, Thrips, Psyllids and Green Peach Aphid on Potato ranked by efficacy against green peach aphid.

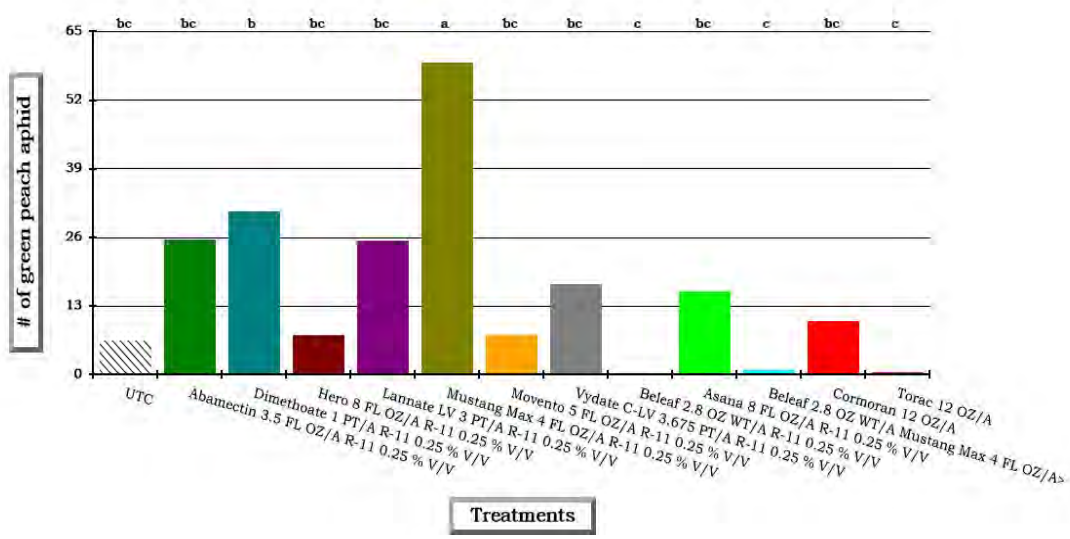
Trt No.	Treatment Name	Rate of Application	Number of Applications	Total Average per 10 Leaves							
				Lygus		Thrips		Psyllids		Green peach aphid	
9	Beleaf	2.8 oz wt/a	ABC	7.3	cd	2.4	a	0.0	a	**3.9**	**e**
11	Beleaf/Mustang M	2.8 and 4 oz wt/a	ABC	13.4	ab	2.5	a	0.0	a	**4.6**	**e**
13	Torac	12 oz/a	ABC	14.4	a	3.0	a	0.0	a	**12.6**	**de**
7	Movento	5 fl oz/a	ABC	15.1	a	4.9	a	0.0	a	**19.9**	**cde**
4	Hero	8 fl oz/a	ABC	4.6	d	4.5	a	0.0	a	**20.5**	**cde**
8	Vydate C-LV	3.675 pt/a	ABC	7.9	bcd	2.3	a	0.0	a	**24.5**	**cde**
12	Cormoran	12 oz/a	ABC	10.6	abc	6.1	a	0.0	a	**26.8**	**cde**
1	UTC			15.0	a	8.3	a	8.5	a	**30.9**	**cde**
5	Lannate LV	3 pt/a	ABC	7.3	cd	2.3	a	0.0	a	**39.5**	**cde**
10	Asana	8 fl oz/a	ABC	10.5	abc	5.8	a	0.0	a	**44.8**	**cd**
2	Abamectin	3.5 fl oz/a	ABC	11.3	abc	4.4	a	0.0	a	**52.3**	**bc**
3	Dimethoate	1 pt/a	ABC	10.8	abc	3.0	a	0.0	a	**84.9**	**ab**
6	Mustang Max	4 fl oz/a	ABC	12.0	abc	3.3	a	0.0	a	**116.3**	**a**

Notes: Values within each column with the same letters are not significant different based on F test of LSD at P<0.05.

Graph 1. Treatment effect on green peach aphids at 8 days after C application (DAC).



Graph 2. Treatment effect on green peach aphids at 14 days after C application (DAC).



Most treatments was able to suppress the total aphid number below untreated control (Table 5), except Abamectin, Dimethoate, Lannate LV, Mustang Max, and Asana. It is important to note that the treatment with the greatest number of aphids was not the untreated check but rather was Mustang Max with nearly 400% the number of aphids as the check, once again demonstrating the propensity of a pyrethroid insecticide to flare aphids. Dimethoate, had nearly three times the number of aphids as the check. Abamectin and Asana also had more aphids than the untreated check.

Overall, different products exhibited different characteristics/control effect on certain insect species. Beleaf and Vydate showed the broadest species control with decent control efficacy. Hero was very selective on lygus control, Torac was very selective on aphid control, and Lannate was efficient on both thrips and lygus control. Abamectin, Dimethoate, Asana, Movento, and Mustang Max showed relative less control effect on the four investigated species.

Potato Thrips Control Efficacy - 2017 Second Trial

Materials and Methods

During the summer of 2017, the staff at the Agriculture Development Group, Inc. conducted a research trial investigating the efficacy of multiple insecticides at different rates (Table 1) for the control of potato psyllids. The experimental design for this trial was a RCB with 4 replications and plot sizes of 11.5ft x 25ft with four potato rows. Umatilla potato variety was planted in April, 2017, and applications for this trial were made with a multi-boom sprayer calibrated to apply treatment sprays at 25 gallons per acre. Only the middle two rows were assessed for insect pest incidence and severity ratings to minimize the potential of overlap spray at the edge rows. Potato psyllid (*Bactericera cockerelli*) and western flower thrips (*Frankliniella occidentalis*) were observed in this trial and the pressure was very low at beginning but slowly increased to a moderate level by the end of the season.

Six applications A, B, C, D, E, and F were applied from June 29th to September 7th at 14 days interval. Evaluation of psyllids/thrips severity were conducted at 0, 7, 14, and 21 days after each applications by examining the number of psyllid adult and eggs, or thrips on 10 random leaves from plants in the middle 2 rows of each plot using microscope.

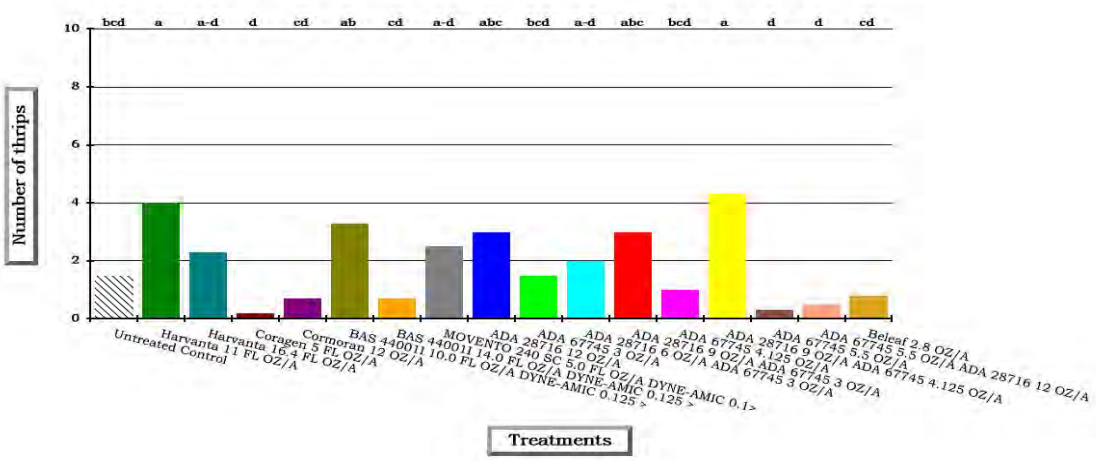
Table 1. Treatment list and application details.

Trt No.	Treatment Name	Form Conc	Form Type	Description	Rate	Rate Unit	Appl Code	Appl Description
1	Untreated Control							
2	Harvanta		SL	ISK	11	fl oz/a	ABCDEF	10-14 D Intervals
3	Harvanta		SL	ISK	16.4	fl oz/a	ABCDEF	10-14 D Intervals
4	Coragen		SC	ISK	5	fl oz/a	ABCDEF	10-14 D Intervals
5	Cormoran		EC	Adama	12	oz/a	ABCDEF	10-14 D Intervals
6	BAS 44001I	50	DC	BASF	10.0	fl oz/a	AB	A 1st psyllid; B 10-21 DA A
	DYNE-AMIC	901	OL	BASF	0.125	% v/v	AB	A 1st psyllid; B 10-21 DA A
7	BAS 44001I	50	DC	BASF	14.0	fl oz/a	AB	A 1st psyllid; B 10-21 DA A
	DYNE-AMIC	901	OL	BASF	0.125	% v/v	AB	A 1st psyllid; B 10-21 DA A
8	MOVENTO 240 SC	240	SC	BASF	5.0	fl oz/a	AB	A 1st psyllid; B 10-21 DA A
	DYNE-AMIC	901.0	OL	BASF	0.125	% v/v	AB	A 1st psyllid; B 10-21 DA A
9	ADA 28716		EC	Adama	12	oz/a	ABCDEF	10-14 D Intervals
10	ADA 67745		WG	Adama	3	oz/a	ABCDEF	10-14 D Intervals
11	ADA 28716		EC	Adama	6	oz/a	ABCDEF	10-14 D Intervals
	ADA 67745		WG	Adama	3	oz/a	ABCDEF	10-14 D Intervals
12	ADA 28716		EC	Adama	9	oz/a	ABCDEF	10-14 D Intervals
	ADA 67745		WG	Adama	3	oz/a	ABCDEF	10-14 D Intervals
13	ADA 67745		WG	Adama	4.125	oz/a	ABCDEF	10-14 D Intervals
14	ADA 28716		EC	Adama	9	oz/a	ABCDEF	10-14 D Intervals
	ADA 67745		WG	Adama	4.125	oz/a	ABCDEF	10-14 D Intervals
15	ADA 67745		WG	Adama	5.5	oz/a	ABCDEF	10-14 D Intervals
16	ADA 67745		WG	Adama	5.5	oz/a	ABCDEF	10-14 D Intervals
	ADA 28716		EC	Adama	12	oz/a	ABCDEF	10-14 D Intervals
17	Beleaf		SG	Adama	2.8	oz/a	ABCDEF	10-14 D Intervals

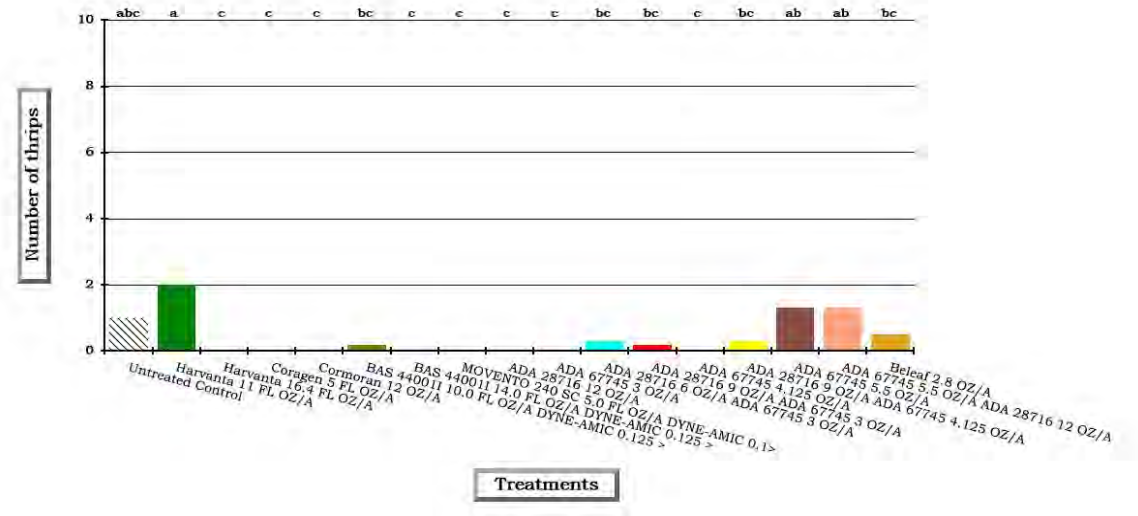
Results and Conclusion

Treatments started to show significant effect on 8 days after C application (DAC) (August 4th) (Graph 1). While no treatment statistically reduced the thrips compared to untreated control, population of thrips was actually increased by half of the treatments on the list, except Coragen, Cormoran, BAS 4400 at higher rate (14 fl oz/a), treatments with higher rates (>4 oz/a) of ADA 67745, and Beleaf. Although such promoting effect on thrips generally disappeared at 8 days after E application (DAE) (Graph 2) where most treatments basically eliminated thrips, apparently but unclearly why that Harvanta and treatments with the highest rate of ADA 67745 (5.5 oz/a) still had more thrips than untreated plots.

Graph 1. Treatment effect on thrips at 8 days after C application (DAC).

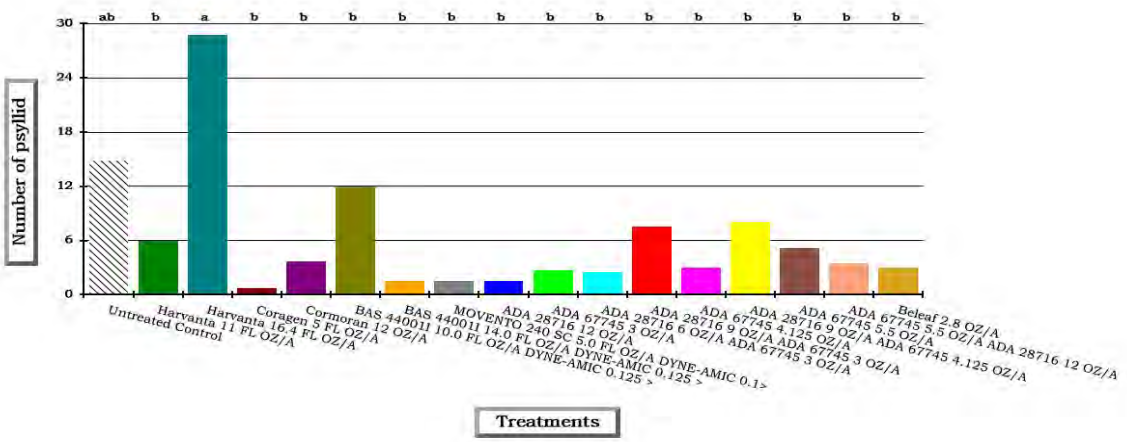


Graph 2. Treatment effect on thrips at 8 days after E application (DAE).



Different results were observed on psyllid with significant treatment separation later in the season at 8 days after F application (DAF) (Graph 3). Somehow Harvanta at higher rate of 16.4 fl oz/a promoted almost 100% psyllid nymph population while its lower rate at 11 fl oz/a actually suppressed the psyllid to 50% compared to untreated control, indicating a unclear negative dose response. Meantime, all the other treatment had relatively lower than control psyllid population.

Graph 3. Treatment effect on psyllid at 8 days after F application (DAF).



Overall (Table 2), Movento and BAS4400 (14 fl oz/a) showed the best control of psyllid with total 6.5 and 7.3 count, followed by Beleaf (8.3 psyllids) and Coragen (8.3 psyllid), then 67745 at 4.125 and 5.5 oz/a (9.3 psyllid), ADA 28716 at 12 oz/a (9.5 psyllid), ADA 67754 at 3 oz/a (10.8 psyllid), Cormoran (11.3 psyllids), ADA 28716 at 6 oz/a + ADA 67745 at 3 oz/a (12.5 psyllid), and ADA 28716 at 12 oz/a + ADA 67745 at 5.5 oz/a (13.3 psyllid). It appears that ADA 67754 had a slight dose response, yet the effect was gone once ADA 67754 and ADA 28716 were mixed. There was also an obvious dose response from BAS 4400 as the lower rate (10 fl oz/a) resulted in >35 total psyllid counts which is almost 4 fold of its higher rate at 14 fl oz/a.

Table 2. Comparison of 16 Insecticidal Treatments for Potato Psyllid and Thrips in Potato.

Trt No.	Treatment Name	Rate Of Application		Number of Applications	Total Average Per 10 Leaves					
					Psyllid nymphs		Psyllid eggs		Thrips	
8	MOVENTO 240 SC	5	fl oz/a	AB	6.5	a	0	a	28.3	a
8	DYNE-AMIC	0.125	% v/v	AB						
7	BAS 44001I	14	fl oz/a	AB	7.3	a	0	a	20	a
7	DYNE-AMIC	0.125	% v/v	AB						
4	Coragen	5	fl oz/a	ABCDEF	8.3	a	0	a	21.8	a
17	Beleaf	2.8	oz/a	ABCDEF	8.3	a	0	a	17.8	a
13	ADA 67745	4.125	oz/a	ABCDEF	9.3	a	0.8	a	25.5	a
15	ADA 67745	5.5	oz/a	ABCDEF	9.3	a	0	a	22	a
9	ADA 28716	12	oz/a	ABCDEF	9.5	a	0	a	27.3	a
10	ADA 67745	3	oz/a	ABCDEF	10.8	a	0.5	a	22.5	a
5	Cormoran	12	oz/a	ABCDEF	11.3	a	0.3	a	25.3	a
11	ADA 28716	6	oz/a	ABCDEF	12.5	a	0.3	a	21.3	a
11	ADA 67745	3	oz/a	ABCDEF						
16	ADA 67745	5.5	oz/a	ABCDEF	13.3	a	0.3	a	21.5	a
16	ADA 28716	12	oz/a	ABCDEF						
12	ADA 28716	9	oz/a	ABCDEF	19.5	a	0.5	a	35.3	a
12	ADA 67745	3	oz/a	ABCDEF						
2	Harvanta	11	fl oz/a	ABCDEF	20	a	0	a	33.8	a
14	ADA 28716	9	oz/a	ABCDEF	29.5	a	0	a	32	a
14	ADA 67745	4.125	oz/a	ABCDEF						
1	Untreated Control				32.3	a	0	a	23.5	a
6	BAS 44001I	10	fl oz/a	AB	35.8	a	0	a	30.5	a
6	DYNE-AMIC	0.125	% v/v	AB						
3	Harvanta	16.4	fl oz/a	ABCDEF	62	a	0	a	32.3	a

Note: Values within each column with the same letters are not significant different based on the F test of LSD at P<0.05.

The following data is from the allied potato entomologist psyllid trial conducted by Drs. Wenniger, Reitz, Rondon, Waters and Schreiber. These results will be reported more in-depth in a separate report, but data collected from thrips on this trial are very noteworthy and the author felt it was important to include with the thrips portion of this report. There are two key finding from this trial. First, application of Brigrade repeatedly flared thrips to a level that was significantly greater than the untreated check. The sample application treatments were made in a trials that involved 10 and 20 leaves samples per plot. The flaring of thrips was only detected in the trial with 20 treatments. This leads to the second finding, that more intensive sampling of thrips resulted detection of significant treatment effects that less intensive sampling did not detect. This results suggest that the currently sampling regime of 10 leaves per plot may be insufficient for thrips sampling and perhaps other insect pests of potatoes.

Potato Psyllid Control Efficacy-NPRC intensive sampling treatment trial - 2017

Materials and Methods

During the summer of 2017, the staff at the Agriculture Development Group, Inc. conducted a research trial investigating the efficacy of Movento
Abamectin, Brigade, and Exiel for the control of potato psyllids. The experimental design for this trial was a RCB with 4 replications and plot sizes of 23ft x 25ft (i.e. two sides of four rows for total eight rows). Umatilla potato variety was planted in April, 2017, applications for this trial were made with a multi-boom sprayer calibrated to apply treatment sprays at 25 gallons per acre. Only the middle two rows of each four rows were assessed for insect pest severity ratings to minimize the potential of overlap spray at the edge rows.

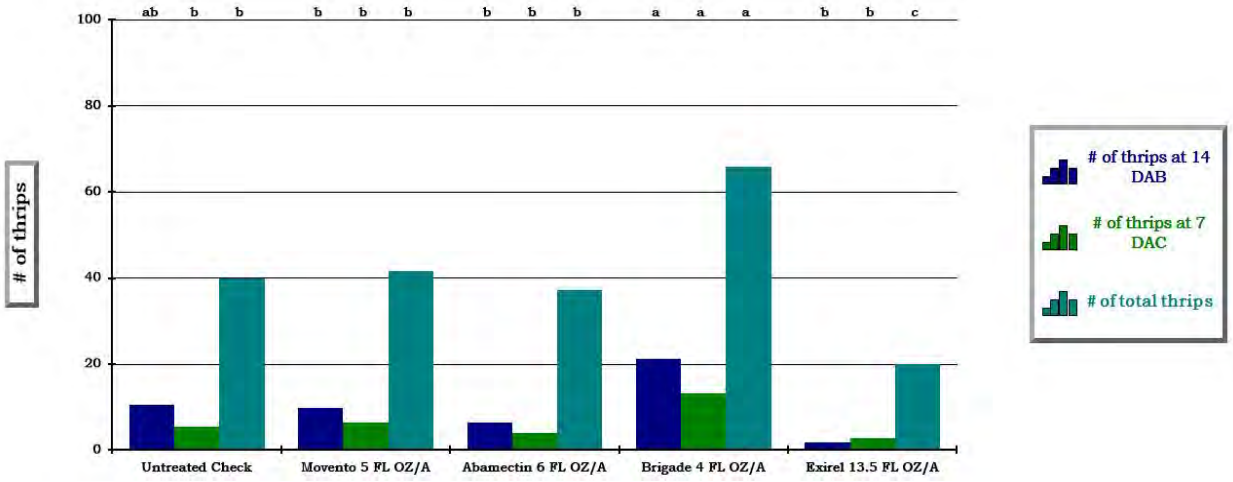
Potato psyllid, green peach aphids, and western flower thrips were observed in this trial but the psyllid pressure was very low. Three applications A, B, and C were applied on July 12, July 26 and August 9 at 14 days intervals. Evaluations for psyllids, thrips, or aphid severity were conducted at weekly basis until 14 days after C applications (DAC), by examining the number of psyllid adults, nymphs, and eggs, total winged + wingless aphid, or thrips on 20 random leaves from the middle 4 rows within each plot under microscope.

Results and Discussions

Unfortunately, the extremely low psyllid population masked the treatment effect and we only observed slightly reduced study total psyllid eggs by Movento, when compared to untreated.

Significant treatment effect was only observed on thrips at 14 days after B application (DAB), 7 DAC, where Movento and Abamectin performed as well as Exirel (Graph 1). Brigade on the other hand, promoted thrips population more than 50% compared to untreated control. Although not statistically significant, all treatments appeared to promote aphid population, especially by Abamectin and Brigade with 43% and 129% higher study total than untreated control.

Graph 1. Treatment effect on thrips at 14 or 7 days after B or C application (DAB and DAC).



Potato Psyllid Control Efficacy-NPRC standard sampling trial - 2017

Materials and Methods

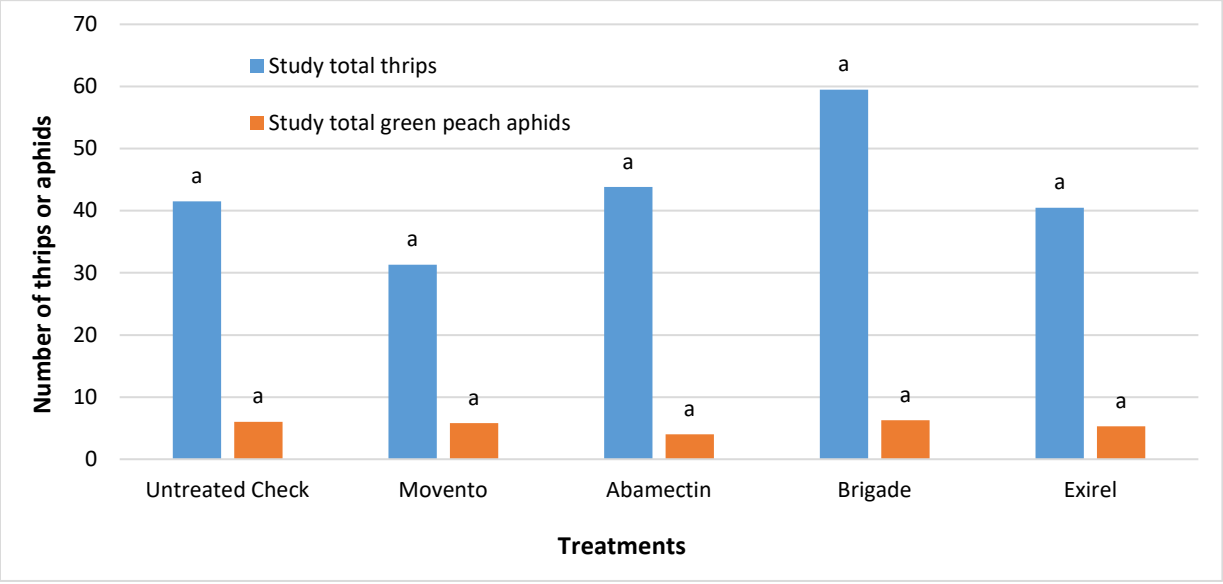
During the summer of 2017, the staff at the Agriculture Development Group, Inc. conducted a research trial investigating the efficacy of Movento, Abamectin, Brigade, and Exirel for the control of potato psyllids. The experimental design for this trial was a RCB with 4 replications and plot sizes of 11.5ft x 25ft (i.e. four potato rows). Umatilla potato variety was planted in April, 2017, applications for this trial were made with a multi-boom sprayer calibrated to apply treatment sprays at 25 gallons per acre. Only the middle two rows were assessed for insect pest severity ratings to minimize the potential of overlap spray at the edge rows.

Potato psyllid, green peach aphids, and western flower thrips were observed in this trial but the psyllid pressure was very low. Three applications A, B, and C were applied on July 12, July 26 and August 9 at 14 days intervals. Evaluations for psyllids, thrips, or aphid severity were conducted at weekly basis until 14 days after C applications (DAC), by examining the number of psyllid adults, nymphs, and eggs, total winged + wingless aphid, or thrips on 10 random leaves from the middle 2 rows within each plot under microscope.

Results and Conclusions

Overall, no significant treatment effect was observed on psyllids, thrips, or aphids. Relatively, Movento showed the best control efficacy on thrips with around 25% less study total thrips, and Abamectin showed the best control efficacy on aphids with 33% less study total aphid (Graph 1).
Unfortunately, the extremely low psyllid population masked the treatment effect.

Graph 1. Treatment effect on total counted thrips and green peach aphids.



Impact of sample size and insecticide on potato psyllid, thrips and green peach aphid.

Ranked by decreasing number of thrips.

Trt	Treatment	Rate	Appl	20 leaf sample by pest and life stage									
				Psyllid life stage by number									
No.	Name	Of Application	Code	adult		nymph		egg		thrips		green peach aphid	
4	Brigade	4 fl oz/a	ABC	0.0	a	0.0	a	0.8	a	66.0	a	8.0	a
2	Movento	5 fl oz/a	ABC	0.0	a	0.0	a	0.3	a	41.5	b	4.3	a
1	Untreated Check			0.0	a	0.0	a	0.5	a	40.0	b	3.5	a
3	Abamectin	6 fl oz/a	ABC	0.0	a	0.0	a	0.5	a	37.3	b	5.0	a
5	Exirel	13.5 fl oz/a	ABC	0.0	a	0.0	a	1.0	a	20.0	c	3.8	a
				10 leaf sample by pest and life stage									
4	Brigade	4 fl oz/a	ABC	0.3	a	0.0	a	0.0	a	59.5	a	6.3	a
3	Abamectin	6 fl oz/a	ABC	0.0	a	0.0	a	0.0	a	43.8	a	4.0	a
1	Untreated Check			0.0	a	0.0	a	0.3	a	41.5	a	6.0	a
5	Exirel	13.5 fl oz/a	ABC	0.0	a	0.0	a	0.0	a	40.5	a	5.3	a
2	Movento	5 fl oz/a	ABC	0.3	a	0.0	a	0.0	a	31.3	a	5.8	a

This table shows the Brigade flare thrips with significantly more thrips than the untreated check when the sample size was 20 leaves per plot. WSU's Dr. Tim Waters has demonstrated this effect in onions. This is the first evidence that a pyrethroid insecticide can flare aphids in potatoes. It is particularly important to note that while the same trend was seen of more thrips in the Brigade treatment as compared to the untreated check when the sample size was ten leaves per plot the difference was not significant. It is noteworthy that this is the first data showing efficacy of Exirel against thrips in potatoes.

Title: Is matrimony vine the early-June source of potato psyllids colonizing potatoes?

Personnel: David Horton, Rodney Cooper, Jenita Thinakaran (USDA-ARS, Wapato, WA)

Funding Amount: $20,000

Reporting Period: 1 April 2017 to 31 March 2018

Summary of Accomplishments:

Background objectives: Uncertainties in knowing the sources of potato psyllids colonizing potato fields of Washington have led growers to rely on calendar-based applications of insecticides to control the psyllid and prevent zebra chip disease. This project was a test of a simple model which proposes that a perennial host (matrimony vine; *Lycium*) is a critical source of psyllids arriving in potato fields in early June, and that dispersal is prompted by summer decline of matrimony vine as it enters summer dormancy in response to water stress.

Accomplishments

- Matching funds ($20,000) for this project were obtained through WSCPR.
- Potato psyllid numbers were extremely low all summer, and we were thus unable to examine psyllid movements as extensively as planned
- Phenological data and physiological data confirmed matrimony vine shows summer decline in the traits that psyllids require for continuous presence
    - *Possible consequences for industry*: These plant data are consistent with our hypothesis that psyllids depart matrimony vine in early summer due to plant decline caused by summer dormancy induced by water stress
- Even modest post-dormancy precipitation events prompted autumn flush of new tissues, likely to be attractive to psyllids
    - *Possible consequences for industry*: Autumn flush would make matrimony vine attractive to psyllids that have been displaced by potato harvest. Thus, matrimony vine likely provides overwintering shelter for harvest-displaced psyllids
- Matrimony vine appears to occur as different morphotypes in our growing region. It is unclear whether those putative morphotypes are distinct genetic types or are merely responses to local growing conditions (soil type, etc.).
    - *Possible consequences for industry*: Morphotypes appear to differ in their responses to summer stresses, and thus may differ in how important they are as sources of dispersing psyllids. This hypothesis is being tested.
- Data collected 2014-2017 indicate that infestation rates in potato in July and August can be predicted by psyllid counts on matrimony vine in spring preceding potato emergence.
    - *Possible consequences for industry:* Matrimony vine may be useful as an "early-warning-system" in allowing the industry to predict probable summer psyllid pressure.

Activities or experiments conducted:

- Potato psyllids were sampled in and around stands of matrimony vine using sticky cards to monitor psyllid dispersal. Psyllids were also counted using beat sheets and leaf samples directly in stands of matrimony vine to determine numbers present in stands.

- Plant phenological traits (chlorophyll and leaf fall) were monitored as proxies for plant summer dormancy.
- Psyllids were collected from the field and assayed molecularly to look for DNA of matrimony vine ("molecular gut contents analysis").
- Four years of psyllid counts in matrimony vine were examined to see whether counts in matrimony vine preceding emergence of potato can be used to predict timing and extent of infestation by psyllids in potato fields. Information on potato infestation was obtained from WSU-Extension (C. Wohleb, T. Waters).

Results:

Objective 1: Examine psyllid dispersal and seasonal phenology of matrimony vine.

We monitored two stands of matrimony vine (Prosser, Richland) weekly between mid-April and late-September 2017. We recorded environmental data (soil moisture, soil temperature, precipitation), plant data (leaf chlorophyll, leaves per shoot), psyllid numbers in the stands (beat trays, shoot samples), and psyllid numbers leaving stands (yellow sticky cards; Figure 1). The environmental variables and plant traits were monitored to examine phenology of matrimony vine dormancy and factors prompting dormancy. *Plant phenology and dormancy*. Leaf chlorophyll showed a steady decline between April and mid-August at both locations (Figure 2: green font and green arrows), tracking declines in soil moisture over that same interval (Figure 2: red font and red arrows). The decline in leaf chlorophyll was accompanied by declines in leaf numbers per cm of shoot at the Richland site (Figure 2: blue font and blue arrows), an indicator of leaf fall. The stand in Prosser yellowed, but retained many of its leaves through the summer (Figure 2: blue font and blue arrows). We are uncertain why leaves dropped so rapidly at the Richland site but not the Prosser site. There are obvious morphological differences between stands in shape and sizes of leaves (Figure 3) and in floral traits, and there may be more than a single species of matrimony vine in the growing region, or the population is composed of genetic variants that differ in leaf morphology and tendency to drop leaves in mid-summer. We will be exploring this question in 2018. At both sites, there was a noticeable uptick in leaf chlorophyll following late-season rainfall (blue bars in Figure2 show rainfall events); these rainfall events occurred following almost 2 months of no precipitation. The jump in chlorophyll was particularly noticeable at the Richland site (Figure 2: note leaf chlorophyll curve). At both sites, there was an autumn flush of new foliage and flowering following precipitation (Figure 4 is a photograph of autumn flush and flowering).

Figure 1. Yellow sticky card placed near matrimony vine stand, to collect departing psyllids.

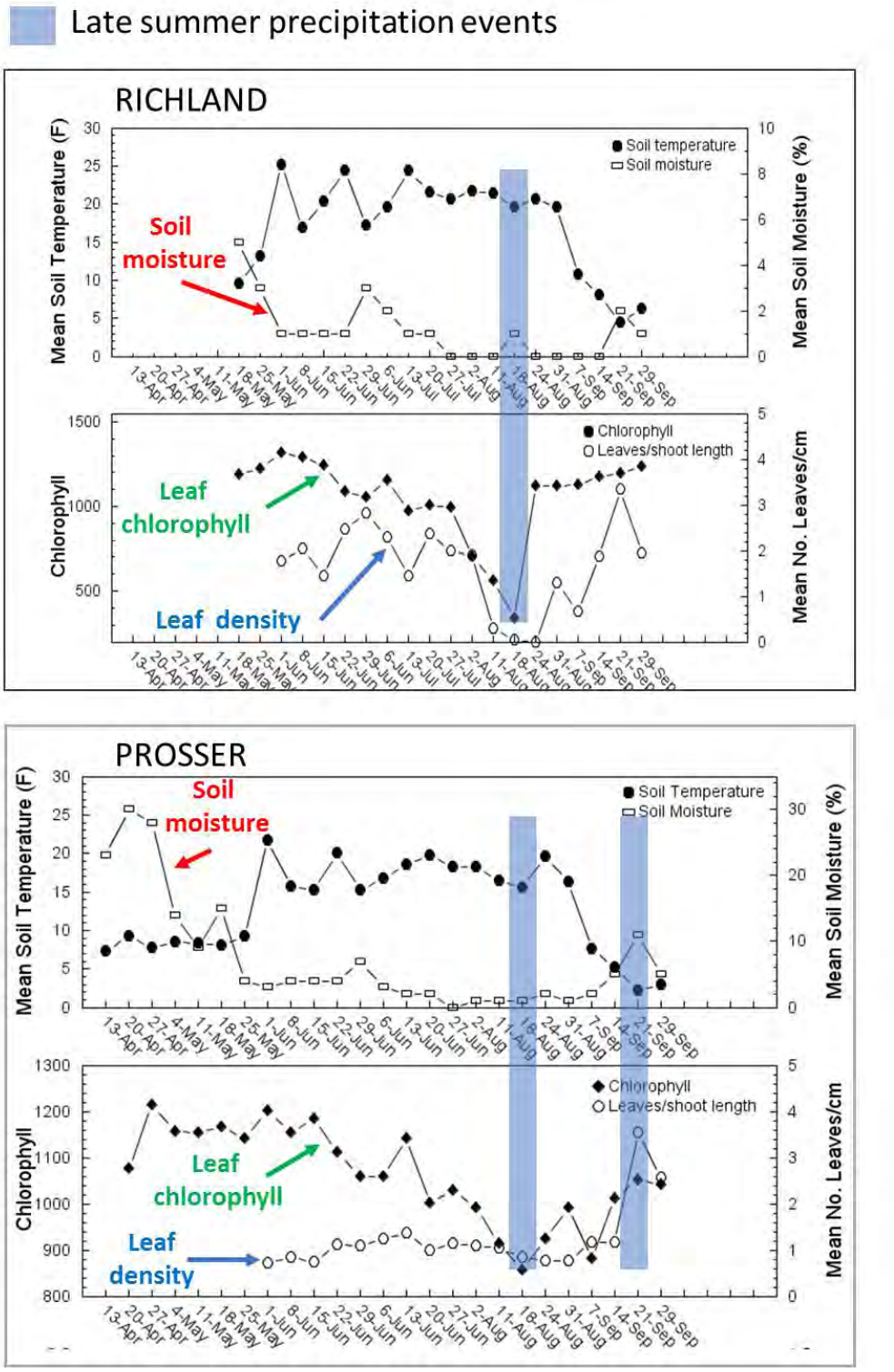


Figure 2. Environmental variables (soil moisture, soil temperature, precipitation events) and plant traits (chlorophyll, leaves per cm of shoot) through the season at two stands of matrimony vine.

Psyllid counts and dispersal. No psyllid data are shown. Psyllid numbers in 2017 were extremely and atypically low all season. We are uncertain about the reason for the low numbers, but possibly the unusually long and snowy winter in 2016-17 caused high overwintering mortality of potato psyllid. We found psyllids on traps and plants at the Prosser site only between 1 June and 20 June (corresponding to the pre-dormancy interval of matrimony vine), and again in late-September (autumn flush). We ceased seeing psyllids beginning in late-June virtually simultaneously with the onset of summer decline in leaf chlorophyll (Figure 2; note decline in leaf chlorophyll at the Prosser site beginning 22 June). No psyllids were found in any samples taken at the Richland site.

Figure 3. Different matrimony vine morphotypes (species?) from Prosser and Richland sites, with photographs showing summer complete defoliation by the Richland morph contrasted with the summer yellowing but leaf retention of the Prosser morph.

Objective 2: Matrimony vine as an early-warning tool? With our 2017 sampling data, we have now completed four-years of sampling matrimony vine during the spring flush period, and have maximum psyllid counts at the Prosser and Richland locations. We used those data to examine whether maximum spring count on matrimony vine predicts infestation levels in potato fields during the post-spring growing season. Infestation data for potato fields were obtained from the

WSU-trapping network (data courtesy of C. Wohleb and T. Waters). We examined two variables: Julian day that the first psyllids were detected in potato fields; and, percentage of potato fields within the monitoring region eventually having psyllids. Both measures were examined as a function of matrimony vine infestation. *Results.* Our results (Figure 5) indicate that spring counts in matrimony vine can be used to roughly predict arrival date of psyllids in fields and subsequent infestation levels (% of fields with psyllids). Thus, both 2017 and 2014 were low-density years in matrimony vine (especially in 2017), and in both years psyllids arrived later in fields (Figure 5: left panel) and colonized fewer fields (Figure 5: right panel) than what was observed during 2015 and 2016, both years of high psyllid counts in matrimony vine. These data suggest that potato psyllid is colonizing potato fields by dispersing from matrimony vine.

Figure 4. Autumn flush and flowering of matrimony vine, Richland site.

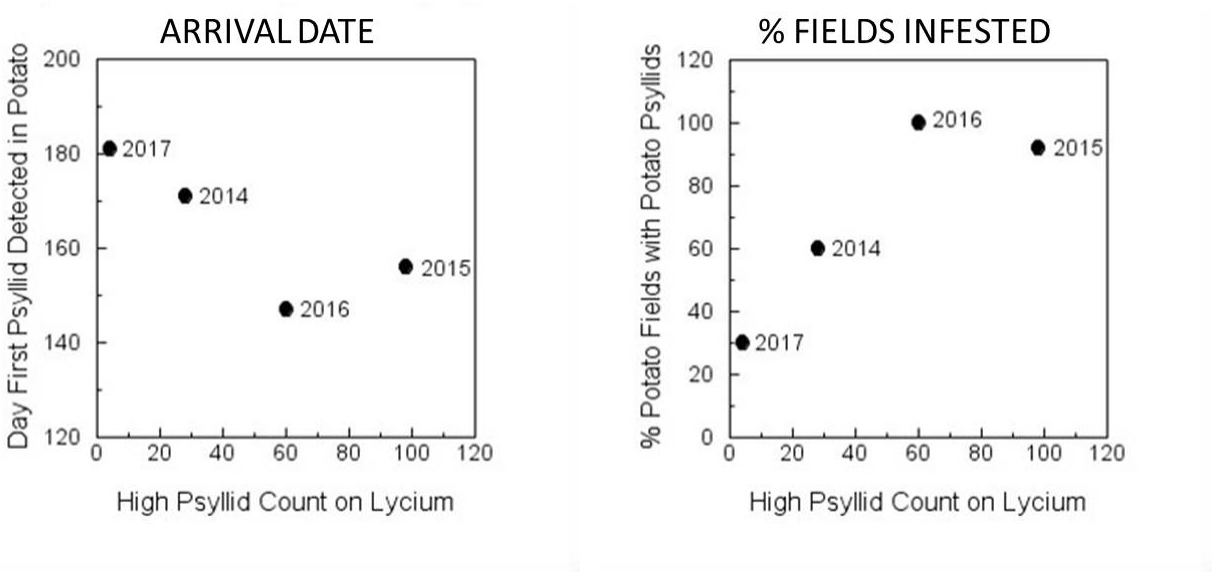


Figure 5. First arrival (Julian date) in potato fields (left panel) and final % of fields infested (right panel) shown as function of maximum psyllid counts in matrimony vine during spring preceding the summer growing season. Years 2014-2017.

Objective 3: Molecular gut contents analysis to determine dietary histories of psyllids arriving in potato fields. We are continuing to refine methods to detect and identify plant DNA in potato psyllids, to use this technology to define dietary history of field-collected psyllids. By assaying psyllids as they arrive in potato fields, we would be able to use these methods to pinpoint species of plants of importance as sources of psyllids in potato fields. Our original methods involved amplifying chloroplast DNA using universal PCR primers, cloning amplicons into bacterial vectors, and sequencing clones using Sanger-based technology. This method worked well for

small samples but is prohibitive for larger samples and for psyllids which sample large numbers of plant species. We shifted to an approach that employs high-throughput sequencing by use of a PacBio platform. This approach (a) provides a less expensive way to process larger numbers of samples, and (b) will allow us to detect multiple plant species within a single sample at reasonable levels of cost. Psyllids were collected from sticky traps near potato fields (4 psyllids examined) and from a prototype trap that collects insects into preservative (placed in a potato field in June; 8 psyllids examined). Dietary history of these psyllids was assessed using our original technique involving Sanger-based sequencing of cloned PCR products. Psyllids were also collected from matrimony vine near Kahlotus, WA in autumn 2016 following potato harvest in the region (40 psyllids); insects were stored at -80 C until they could be assayed using the PacBio approach (direct sequencing done at the WSU CORE facility in Pullman).

Gut contents results. We failed to detect a plant signal in the sticky trap insects due to deterioration of the samples on the traps. Because sticky cards are our most effective means of collecting psyllids, we are examining methods for making card samples useful to this technology (e.g., by shortening the time traps are left in the field and by using alternative DNA purification methods). Samples from traps in which psyllids accumulate in preservative had only the potato signal. Insects from matrimony vine and assayed by direct sequencing had the signal from potato (apparently due to dispersal into matrimony vine by psyllids leaving harvested potato fields), bittersweet nightshade, and matrimony vine (Figure 6). We also detected an uncommon wild solanaceous host of potato psyllid (*Nicotiana*; coyote tobacco), found at only very low densities in the growing region. This result suggests that psyllids are developing on uncommon host plants within dispersal distance of the Kahlotus stand of matrimony vine. We interpret the presence of prickly lettuce in samples (Figure 6) as evidence that psyllids which are displaced from potato fields because of harvest will feed on non-host plant taxa for maintenance as they search for solanaceous hosts. This behavior is well-known for many Psylloidea.

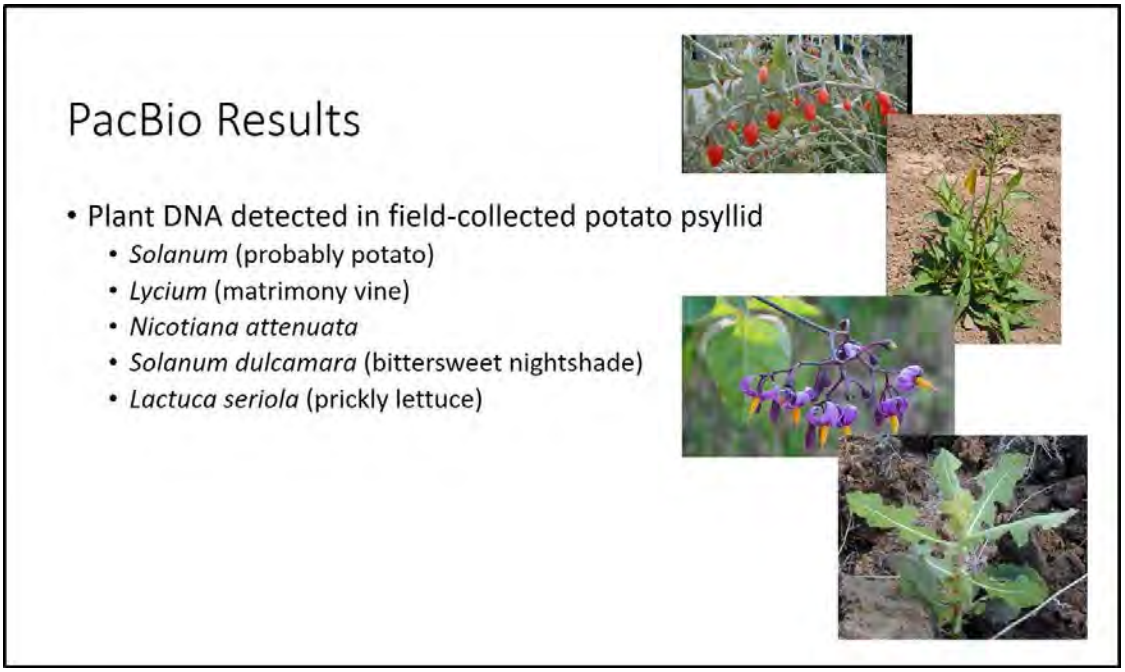


Figure 6. Identification of plant DNA detected and identified molecularly in guts of psyllids collected from matrimony vine. Psyllids were collected in autumn following potato harvest using a beating tray; Richland site (collections from 2016, stored at -80 prior to assay in 2017).

Publications:

Thinakaran, J., D.R. Horton, W.R. Cooper, A.S. Jensen, C.H. Wohleb, J. Dahan, T. Mustafa, A.V. Karasev, and J.E. Munyaneza. 2017. Association of potato psyllid (*Bactericera cockerelli*; Hemiptera: Triozidae) with *Lycium* spp. (Solanaceae) in potato growing regions of Washington, Idaho, and Oregon. *American Journal of Potato Research* 94: 490-499.

Presentations and Reports:

Cooper, W. R., D. R. Horton, and S. Garczynski. 2017. Use of molecular techniques to study psyllid ecology. 101st Annual Meeting of the Pacific Branch Entomological Society of America. 2-5 April 2017, Portland, OR. (invited talk)

Cooper, W. R., D. R. Horton, J. Thinakaran. 2017. The weed link in zebra chip epidemiology: Annual and perennial weeds as sources of the zebra chip pathogen. Annual Meeting of the Potato Association of America, 23-27 July 2017, Fargo, ND.

Cooper, W. R., D. R. Horton, J. Thinakaran, and N. Kaur. 2017. SCRI advisory committee research update, USDA-ARS, Wapato. SCRI Advisory Committee meeting, 23-October 2017, Boise, ID

Cooper, W. R., D. R. Horton, and K. D. Swisher. 2017. From molecules to landscapes: A multipronged approach for understanding potato psyllid and zebra chip disease. NPL webinar series on HLB research in the ARS. 15-November, 2017. (invited talk)

Cooper, W. R., D. R. Horton, and J. Thinakaran. 2018. Pinpointing the weed sources of potato psyllid in Washington. WA/OR Potato Conference. 24-25 January 2018. Kennewick, WA. (invited talk)

Horton, D.R., W.R. Cooper, P.J. Landolt, J. Thinakaran, E. Miliczky, N. Kaur, R. Navarre, C. Brown, K.D. Swisher, R. Novy, and J. Whitworth. 2017. Potato-related research at USDA-ARS laboratories in Washington and Idaho. *Potato Progress* 17 (#14): 1-12.

Horton, D.R., E. Miliczky, J. Thinakaran, W.R. Cooper, J.E. Munyaneza, C.H. Wohleb, T.D. Waters, and A.V. Karasev. 2017. Potato psyllid and the South American desert plant *Nolana*: an unlikely psyllid host? *Potato Progress* 17 (#16): 1-7.

Thinakaran, J. 2017. Seasonal association of potato psyllid with *Lycium* spp. in the Pacific Northwest. Annual meeting of Entomological Society of America, Denver, Colorado, 4-8th Nov 2017. (invited talk)

Thinakaran, J., D.R. Horton, W.R. Cooper and A.V. Karasev. 2017. Use of *Lycium* by potato psyllid in the Pacific Northwest: lessons from the desert Southwest? Potato Association of America Annual meeting, Fargo, ND, 23-27th July 2017.

Thinakaran, J., E. Miliczky, W.R. Cooper, D.R. Horton, J.E. Munyaneza and A.V. Karasev. 2017. Examination of ornamental *Nolana* (Solanaceae) for suitability to potato psyllid and zebra chip pathogen. Pacific Branch ESA, Portland, Oregon, 2-5th April 2017. (poster)

Annual Progress Report –2017

TITLE: Neonicotinoid Longevity in Potato Production Systems of the Pacific Northwest

PERSONNEL:
Tim Waters and Jennifer Darner, Washington State University Extension, 509 545-3511, twaters@wsu.edu , 404 W Clark Ave, Pasco, WA 99301
Mark Pavek, Washington State University Department of Horticulture, 509 335-6861, mjpavek@wsu.edu
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Vince Hebert, Washington State University Department of Entomology

REPORTING PERIOD: March 1, 2017 – January 1, 2018 (2017 Growing Season)

ACCOMPLISHMENTS:
The primary objective of this project was to determine how long at plant in-furrow, seed piece, and lay-by treatments with neonicotinoid insecticides protect potato plants from insect pests. We hypothesized that a potato cultivar with a larger canopy early in the growing season would have a lower concentration of neonicotinoid than a potato cultivar with a smaller canopy. As such, the cultivar with the lower concentration of neonicotinoid would be infested with pest insects earlier than the cultivar with the higher concentration of the neonicotinoid insecticide.
Potato cultivars differed in canopy weight early in the growing season as expected. Seed treatment with insecticide reduced canopy weight in one cultivar. Neonicotinoid concentration differed between cultivars with lay-by application of thiamethoxam and imidacloprid, but not with seed treatment of thiamethoxam for most sample periods. Thiamethoxam by lay-by application contained a significantly higher concentration of active ingredient in the plant tissue than the application by seed treatment. The psyllid slip cage portion of the experiment was performed, but the data was inconclusive. The technique provided good data early in the season, but some refinements are needed in the procedure.

PROCEDURES:
An experiment was conducted at the WSU research site in Pasco and maintained mimicking commercial potato production in the Columbia Basin. All plots received an at-plant in-furrow fungicide treatment, post-plant pre-emergence herbicides, an application of insecticide for Colorado potato beetle control, and foliar fungicides as needed (Table 1). The plots were arranged in a randomized complete block design with four replications. Each plot was four rows (11.33 ft) wide and twenty-five feet in length.
Treatments included neonicotinoids applied in three ways: in-furrow at planting (April 17) with imidacloprid (Admire Pro 8.7 fl oz/A), seed piece with thiamethoxam (Cruiser Maxx 0.23 fl oz/100 lbs seed) and a banded lay-by (applied May 22) application with thiamethoxam (Platinum 8 fl oz/A). It should be noted that the amount of active ingredient of thiamethoxam applied was nearly identical for the seed piece and lay-by treatments, where the seed treatment rate of 0.23 fl oz/100 lbs of seed is 2.03 oz per acre of thiamethoxam, and the lay-by application of 8.0 fl oz per acre is 2.00 oz per acre of thiamethoxam. All treatments were applied to two different cultivars. One cultivar typically produces abundant early season foliage (Norkotah), and the other that typically grows sparse, early season foliage (Alturas). Untreated check plots of both cultivars

were also planted and evaluated. We collected samples of the potato foliage seven times during the growing season (43, 49, 56, 70, 84, 98, and 112 days after planting) in order to assess how the concentration of the pesticides in the leaves changed over time. Leaf samples consisted of one gallon bags of foliage collected from at least twenty different plants per plot by collecting the newest mature leaf of the plant (4^{th} from the top). Samples were collected at the same time of day for each sampling period, placed in a container with ice packs, and sent next day shipping to Pacific Agricultural Laboratories (PAL). PAL evaluated the samples for the amount of thiamethoxam, its metabolite (clothianidin), and imidacloprid present utilizing liquid chromatography mass spectroscopy. At the designated dates as detailed above, we performed destructive plant sampling on three plants in each plot. Destructive whole plant sampling (a wet weight measurement) provided a relative assessment of the canopy size of the two cultivars at the time the concentration of the pesticide is measured. This allowed us to assess if canopy size impacts pesticide concentration.

Additionally, we determined efficacy of the insecticides by enumerating key Columbia Basin region potato insect pests. Insects were evaluated weekly from emergence to harvest using three different types of insect sampling methods: bucket samples, leaf samples, and vacuum samples with pest and beneficial insect counts performed at each assessment. In addition to evaluating the resident insect populations, slip cages with potato psyllids were utilized to assess the efficacy and residual effects of the insecticides. We infested each plot with psyllid nymphs using a slip cage on a single potato leaf. They were placed on plants on the same seven dates that the foliage tissue samplings for the insecticide concentration levels were procured. The slip cage remained on the leaf for seven days. The cages were then removed and the counts were performed to evaluate the mortality of the psyllids. This allowed us to assess how long the insecticides were effective, and to determine what concentrations were needed in the plant to be effective as insecticides. At the end of the season, the potato tubers were measured for yield and grade to determine the impact of the neonicotinoids.

Date	Pesticide	Active Ingredient	Rate	Unit
4/17/2017	Ridomil Gold	Mefenoxam	6.5	oz/A
4/17/2017	Quadris F	Azoxystrobin	8	oz/A
5/3/2017	Boundary	D-metolachor, Metribuzin	2	pt/A
5/3/2017	Eptam	S-ethyl dipropylthiocarbamate	3.5	pt/A
5/3/2017	Tricor	Metribuzin	0.35	lbs/A
6/6/2017	Coragen	Cholorantraniliprole	5	oz/A
6/10/2017	Luna	Fluopyram, Tebuconazole	11	oz/A
6/10/2017	Equus	Cholorthalonil	1.3	pt/A
6/20/2017	Bravo	Cholorthalonil	1.5	pt/A
6/29/2017	Luna	Fluopyram, Tebuconazole	11	oz/A
6/29/2017	Equus	Cholorthalonil	1.3	pt/A
7/19/2017	Zing	Zoxamide, Chlorothalonil	34	oz/A
7/18/2017	Zing	Zoxamide, Chlorothalonil	34	oz/A
7/25/2017	Revus	Mandipropamid, Difenoconazole	7	oz/A
8/1/2017	Revus	Mandipropamid, Difenoconazole	7	oz/A
8/8/2017	Zing	Zoxamide, Chlorothalonil	34	oz/A
8/19/2017	Luna	Fluopyram, Tebuconazole	11	oz/A
9/4/2017	Zing	Zoxamide, Chlorothalonil	34	oz/A
9/8/2017	Reglone	Diquat, Pyrazinediium	2	PT/A

Table 1: The maintenance pesticides applied to the plots.

RESULTS/DISCUSSION:

Potato cultivars differed in canopy weight early in the growing season as expected, and also late in the season. In the absence of insecticide treatments, Norkotah fresh canopy weight was higher than Alturas until the end of June, at which point the opposite occurred (Figure 1 and Table 2). During some sample periods, especially early season, plots treated with thiamethoxam as a seed treatment contained lower fresh canopy weights than the untreated check, but as the season progressed, that trend was no longer present (Table 2). When evaluating by specific cultivar and insecticide interactions, there were often numeric differences, but typically not statistically significant interaction affects (Table 2).

At the end of the growing season yields did not differ between plots treated with Crusier, Admire Pro, or the untreated check (Table 3). Plots treated with Admire Pro had significantly greater overall yields than plots treated with Platinum (Table 3). The overall yield did not differ between cultivars, but grades did where there were significantly more oversized Grade 1 tubers, fewer Grade 2 tubers, and fewer culls in the Nokotah than the Alturas plots.

Plant Weights	Weight in Kg						
Treatment	Date						
	30-May	5-Jun	12-Jun	26-Jun	10-Jul	24-Jul	7-Aug
Alturas	0.6801b	1.5809b	2.9238b	5.0875a	6.2778a	6.40298a	6.81135a
Norkotah	0.9789a	2.2560a	3.7333a	4.7109a	5.7154a	4.58389b	3.43961b
LSD	0.180	0.373	0.484	1.080	1.277	1.079	1.420-1.893
Untreated Check	0.8234a	1.8836a	3.5300a	5.4861a	6.2064a	5.66601a	5.57358a
Admire Pro	0.7535a	1.8595a	3.4054ab	5.0301ab	6.4460a	5.74412a	4.80971a
Cruiser Maxx	0.7645a	1.7823a	2.7644b	3.8909b	5.1873a	5.96010a	4.55911a
Platinum	0.9768a	2.1484a	3.6145a	5.1896ab	6.1466a	4.60349a	4.66473a
LSD	0.254	0.527	0.684	1.080	1.277	1.526	2.134-2.672
Alturas/Untreated Check	0.5930b	1.6208cd	2.4928c	5.6475a	6.6243a	6.74490ab	8.63111a
Norkotah/Untreated Check	1.0538a	2.1456abc	4.5673a	5.3248a	5.7885a	4.58712bcd	3.48670b
Alturas/Admire Pro	0.6403b	1.7165bcd	3.3303bc	4.9558a	6.7108a	7.02860a	6.56224ab
Norkotah/Admire Pro	0.8668ab	2.0025abc	3.4805b	5.1045a	6.1813a	4.45965cd	3.46333b
Alturas/Cruiser Maxx	0.6585b	1.1690d	2.4000c	3.9985a	5.4668a	6.56385abc	6.00997ab
Norkotah/Cruiser Maxx	0.8705ab	2.3955ab	3.1288bc	3.7833a	4.9078a	5.35635a-d	3.40853b
Alturas/Platinum	0.8288ab	1.8173a-d	3.4723b	5.7483a	6.3093a	5.27455a-d	6.29224ab
Norkotah/Platinum	1.1248a	2.4795a	3.7568ab	4.6310a	5.9840a	3.93243d	3.40046b
LSD	0.360	0.745	0.968	2.161	2.554	2.158	3.266-4.103

Table 2. Fresh plant weights for three plants per plot by various factors during the seven sample periods. Treatments with the same letters are not statistically different from one another (P=0.05 Student-Newman-Keuls test).

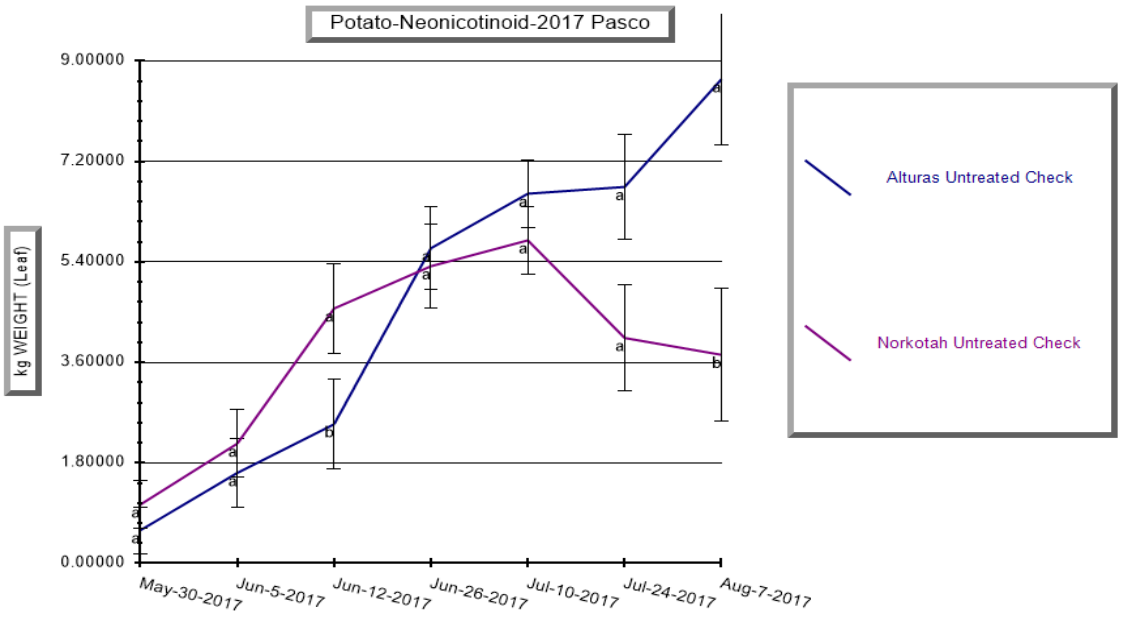

Figure 1. Fresh plant weights for three plants per plot by cultivar during the seven sample periods. Treatments with the same letters are not statistically different from one another (P=0.05 Student-Newman-Keuls test)

Yield and Grade									
Weights in Kg									
Treatment	Grade 1		Grade 2				Total		Total
	4-5.99 oz	6+ oz	<4 oz	4-5.99 oz	6+ oz	Culls	Market	Solids	Yield
Alturas	4.834a	8.223b	2.853a	0.199a	0.420a	1.333a	19.173a	17.980a	32.5a
Norkotah	3.146b	13.803a	1.608b	0.002b	0.016b	0.660b	18.780a	17.549a	31.9a
LSD	0.6324-	2.3578	0.3903	0.0696-	0.189688-	0.569431-	3.2464	0.4742-	5.51
	0.7721			0.1485	0.31696	0.59577		0.4753	
Untreated Ch	3.937a	11.688a	2.214a	0.048a	0.734a	0.734a	19.195ab	17.921a	32.6ab
Admire Pro	4.368a	12.538a	2.421a	0.120a	1.207a	1.207a	21.761a	17.881a	36.9a
Cruiser Maxx	4.024a	10.098a	2.311a	0.039a	1.014a	1.014a	18.320ab	17.516a	31.1ab
Platinum	3.394a	9.728a	0.051a	0.051a	0.946a	0.946a	16.630b	17.739a	28.2b
LSD	0.9764-	3.3344	0.5519	0.1189-	0.493944-	0.752524-	4.5911	0.6650-	7.79
	0.9769			0.2205	99999.2	0.89743		0.6745	
Alturas/Untr	4.885ab	8.723bcd	3.023a	0.118ab	0.365ab	0.843a	19.093a	18.589a	32.4a
Norkotah/Un	3.142d	14.653a	1.405c	0.009b	0.000b	0.631a	19.298a	17.276c	32.7a
Alturas/Adm	5.863a	9.185bcd	3.033a	0.480a	0.517a	1.717a	22.425a	18.360ab	38.0a
Norkotah/Ad	3.199d	15.890a	1.810c	0.000b	0.037ab	0.792a	21.098a	17.414bc	35.8a
Alturas/Cruis	4.710abc	7.668cd	2.683ab	0.154ab	0.437a	1.397a	18.720a	17.336c	31.8a
Norkotah/Cru	3.421cd	12.528ab	1.940bc	0.000b	0.000b	0.694a	17.920a	17.697abc	30.4a
Alturas/Plati	4.023bcd	7.315d	2.673ab	0.127ab	0.353ab	1.470a	16.455a	17.662abc	27.9a
Norkotah/Pla	2.843d	12.140abc	1.278c	0.009b	0.097ab	0.533a	16.805a	17.816abc	28.5a
LSD	1.2618-	4.7155	0.7806	0.2782-	0.512819-	1.210710-	6.4928	0.9486-	11.02
	1.6963			0.4167	0.6796	1.23217		0.9665	

Table 3: Potato yield and grade data. Yield and grade were performed on September 20th, 2017 and numbers are expressed as tons per acre.Treatments with the same letters are not statistically different from one another (P=0.05 Student-Newman-Keuls test).

The imidacloprid concentration was higher in Norkotah plots than in the Alturas for the second sample period, then lower on the third and fourth sample periods (Table 4). The trend that was observed was opposite of what we hypothesized, where the faster growing Norkotah cultivar contained higher concentrations of insecticide than the slower growing Alturas cultivar early in the growing season. If you compare this data with the plant tissue weights during the same time period, the tissue weights did not differ significantly until June 12 (Figure 1). Perhaps the plant began to grow rapidly prior to the sample weights being obtained, and therefore, the imidacloprid had systemically moved into the rapidly growing leaf tissue. The concentration of imidacloprid for the two subsequent samples was much lower in the Norkotah than the Alturas, like was hypothesized, but did not differ statistically. Thiamethoxam by the lay-by application contained significantly higher concentrations of active ingredient in the plant tissue than the application by seed treatment (Table 4). The Norkotah plots had a lower concentration of thiamethoxam when applied by seed treatment as compared to the Alturas plots (Table 4). The treatment of

thiamethoxam by lay-by application did not differ by cultivar for pesticide concentration (Table 4). Clothianidin is a biologically active (as an insecticide) metabolite of thiamethoxam and was, therefore, analyzed in the tissue tests. Clothianidin concentrations were lower in the faster growing Norkotah cultivar, but results were not statistically significant (Table 4). The clothianidin concentration differed in the lay-by application where there was a lower concentration of the compound in the faster growing Norkotah plots than in the Alturas, supporting the hypothesis that the faster growing, more abundant cultivar would have a lower concentration of the insecticide (Table 4). It may be expected that the concentration of thiamethoxam and clothianidin would be higher by lay-by application for a period of time since the application was made more than 30 days after the seed piece treatment of the same active ingredient. This trend of increased concentration lasted much longer than 30 days in duration, so the finding, although not surprising is significant to the potato industry in terms of managing pest insects in the potato crop. The cost of the lay-by application is something that could be considered, but most producers could apply this product at the same time that they are dammer-diking the field.

Treatment		Date						
		30-May	5-Jun	12-Jun	26-Jun	10-Jul	24-Jul	7-Aug
Alturas (Admire Pro)	Imidacloprid (mg/kg)	0.2891a	0.1350b	0.2173a	0.1365a	0.0563a	0.0360a	0.0165a
Norkotah (Admire Pro)	Imidacloprid (mg/kg)	0.2000a	0.3473a	.0623ab	0.0768ab	0.0615a	0.0368a	0.0215a
Alturas (Platinum)	thiamethoxam (mg/kg)	0.1010b	0.1162a	0.1151a	0.1230a	0.1028a	0.0435ab	0.0338a
Norkotah (Platinum)	thiamethoxam (mg/kg)	0.1352ab	0.0808ab	0.1284a	0.1415a	0.1122a	0.0753a	0.0595a
Alturas (Platinum)	clothianidin (mg/kg)	0.218bc	0.0338b	0.0616b	0.0768a	0.1297a	0.0303a	0.0220a
Norkotah (Platinum)	clothianidin (mg/kg)	0.0445a	0.0725a	0.1024a	0.1008a	0.0945a	0.0354a	0.0215a
Alturas (Cruiser Maxx)	clothianidin (mg/kg)	0.0415ab	0.0470b	0.0366bc	0.0295b	0.0217b	0.0029b	0.0000b
Norkotah (Cruiser Maxx)	clothianidin (mg/kg)	0.0433a	0.0403b	0.0254c	0.0220b	0.0122bc	0.0033b	0.0025b
Alturas (Cruiser Maxx)	thiamethoxam (mg/kg)	0.2475a	0.0888ab	0.0677b	0.0473b	0.0008b	0.0186bc	0.0064b
Norkotah (Cruiser Maxx)	thiamethoxam (mg/kg)	0.0847b	0.0479b	0.0270c	0.0299b	0.0000b	0.0074bcd	0.0079b
LSD Values	clothianidin	0.02011	0.02178	0.00689-0.03567	0.03986	0.01212-0.05135	0.00806-0.02101	0.00912
	thiamethoxam	0.04651-0.12074	0.0141-0.05486	0.01085-0.04938	0.01615-0.06767	0.01950-0.06375	0.02404-0.05476	0.01375-0.02641
	Imidaclodprid	0.02026-0.10503	0.19212	0.15689	0.10177	0.0115	0.00935	0.00862

Table 4. Residue values by treatment. Values expressed in milligrams of pesticide per kilogram of plant fresh weight. Treatments with the same letters are not statistically different from one another (P=0.05 Student-Newman-Keuls test).

The potato psyllid slip cage assays are difficult to interpret due to the high mortality rates in the untreated check plots during some assay periods. Cages were placed too high in the canopy during one sample period, leading to high mortality in the untreated check plots (June 19). Psyllids were reared in a laboratory in 2017, and then transferred to the field. This did not appear

to impact survivorship during the cooler spring days, but appeared to be problematic during warmer weather. The raw percent mortality was subjected to a correction formula to account for natural mortality attributed to the insects being caged on the plants. The raw data was subjected to the following formula:

> Schneider-Orelli Formula ((the mortality % in treated plot minus the mortality % in the control plot), divided by 100 minus the mortality % in the control plot). The result of that equation is then multiplied by 100.

For all sample periods, with the exception of the last one, cultivar did not impact psyllid survivorship (Table 5). During the last sample period, the Norkotah potato plants were beginning to senesce and as such, it was unlikely that the psyllids in those cages were actively feeding, thus explaining the higher mortality rates in Alturas plots. During the last sampling period, pesticide concentration did not differ between cultivars, so the change in mortality is not likely due to the pesticide concentration. There was a strong numeric trend for mortality rates being higher in Alturas plots. Evaluating the interaction of the two main factors, Alturas plots treated with either of the thiamethoxam applications (Cruiser Maxx and Platinum) routinely recorded higher mortality rates for psyllids. Insecticide treatment did impact psyllid survivorship, where during all but one sample period, plots treated with lay-by thiamethoxam (Platinum) had higher mortality rates for psyllids than the untreated check plots, and were more often numerically higher than the other insecticide treatments (Table 5). The trend observed in this data warrants testing these chemistries again with improvements to the psyllid slip cage techniques.

Treatment	Date						
	6-Jun	12-Jun	19-Jun	3-Jul	17-Jul	31-Jul	7-Aug
Alturas	55.92a	43.45a	31.70a	40.60a	26.80a	23.90a	41.83a
Norkotah	33.75a	19.82a	33.30a	24.60a	19.60a	21.90a	15.55b
LSD	24.53	24.23	27.91	30.05	27.46	24.15	24.99
Untreated Check	0.00c	0.00c	0.00b	0.00b	0.00b	0.00b	0.00b
Admire Pro	52.00ab	26.13bc	46.90a	29.0ab	18.40ab	37.50a	45.13a
Cruiser Maxx	45.88b	39.52ab	29.00ab	55.30a	52.50a	10.30ab	18.69ab
Platinum	81.46a	60.88a	54.00a	46.30a	22.00ab	43.80a	50.94a
LSD	34.68	34.26	39.47	42.50	38.84	34.16	35.34
Alturas/Untreated Check	0.00c	0.00c	0.00b	0.00b	0.00b	0.00b	0.00c
Norkotah/Untreated Check	0.00c	0.00c	0.00b	0.00b	0.00b	0.00b	0.00c
Alturas/Admire Pro	62.50ab	27.27bc	43.80ab	25.00ab	8.30ab	25.00ab	71.50a
Norkotah/Admire Pro	41.50bc	25.00bc	50.00ab	33.00ab	28.50ab	50.00a	18.75bc
Alturas/Cruiser Maxx	68.42ab	54.05ab	33.00ab	75.00a	55.00a	20.50ab	33.33abc
Norkotah/Cruiser Maxx	23.35bc	25.00bc	25.00ab	35.50ab	50.00ab	0.00b	4.06c
Alturas/Platinum	92.75a	92.50a	50.00ab	62.50a	44.00ab	50.00a	62.50ab
Norkotah/Platinum	70.17ab	29.27bc	58.00a	30.00ab	0.00b	37.50ab	39.38abc
LSD	49.05	48.46	55.82	60.11	54.92	48.30	49.98

Table 5. Percent mortality of psyllids in slip cages. Mortality corrected using the Schneider-Orelli Formula. Treatments with the same letters are not statistically different from one another (P=0.05 Student-Newman-Keuls test).

Pest and beneficial insects were also enumerated in the test plots. 2017 was a low in insect abundance in potato fields compared to previous seasons. Lygus counts were significantly impacted by cultivar, where early in the season, more were captured in Norkotah plots, but as the

season progressed, significantly more Lygus were captured in the Alturas plots (Figure 2). It can be assumed that this difference is a result of the overall plant health and vigor. Norkotah is a determinant cultivar, and as the crop reached its maturity, leaves began to senesce, and fewer Lygus were captured in those plots. Lygus counts were not impacted by the neonicotinoid insecticides used in this study.

Psyllid counts were extremely low during 2017 and rarely encountered in these experimental plots. Aphid counts were also relatively low. During one sample period at the beginning of July, wingless aphid counts increased in untreated check plots, but remained low in treated plots (data not shown) indicating that the neonicotinoids were still impacting aphid mortality nearly 80 days after planting.

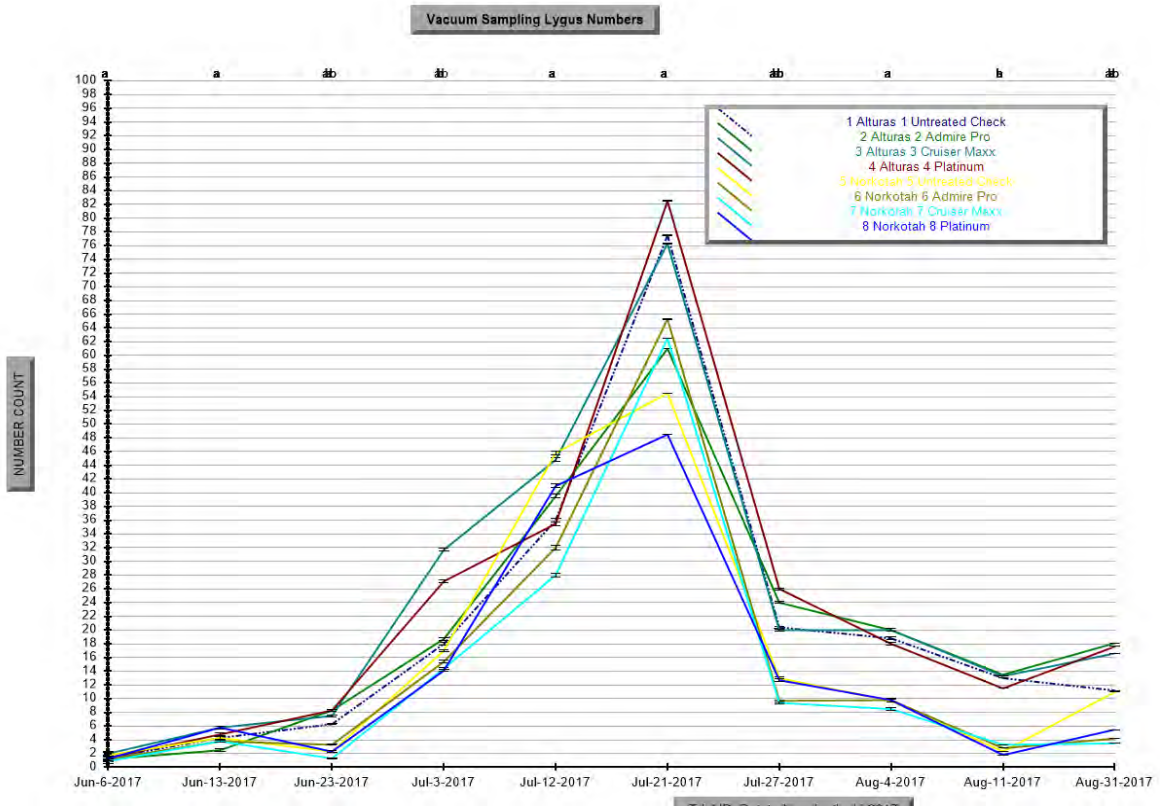

Figure 2. Lygus per vacuum sample by treatment. Treatments with the same letters are not statistically different from one another (P=0.05 Student-Newman-Keuls test).

PUBLICATIONS:

No publications have resulted from this work to date. It is anticipated that these data will contribute to the *PNW Insect Management Recommendations for Potatoes* that are annually revised by Schreiber et al. and could be published in *Potato Progress* and a peer reviewed journal after a second year of data.

PRESENTATIONS:

Waters, T.D. (January 17, 2018). Neonicotinoid Longevity in Potato Production Systems, Preliminary Report. WA POTATO COMMISSION RESEARCH REVIEW, Pasco, WA. Invited.

Annual Progress Report

Title: Quantifying effects of time of infection on zebra chip (ZC) disease development and tuber physiology both at harvest and during storage

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Reporting period: July 1, 2017 – February 15, 2018

Summary of accomplishments:

The primary objective of this study was to quantify effects of time of infection on zebra chip disease (ZC) development and tuber physiology both at harvest and after storage. Moreover, susceptibiltity of three commonly planted cultivars Red Norland, Russet Burbank and Ranger Russet to late season infections were evaluated in the greenhouse. Timing of infection was found to be an important contributing factor to the incidence and severity of ZC symptoms found in tubers; moreover, the extent to which symptoms develop over time in storage is heavily dependent upon timing of infection.

The results from the field studies suggest that a potato field that is exposed to Lso-carrying potato psyllids within ca. 1 week before vine kill should have low risk of ZC disease at harvest and after storage. Exposure at 2 weeks before vine kill may result in quite low risk of ZC at harvest, but moderate risk after 3 months in storage. For fields that are exposed to Lso-carrying potato psyllids 3 weeks or longer before vine kill, risk of ZC is moderate to high at harvest and can be quite high after ca. 3 months in storage. These results indicate that fields at risk of ZC should be processed soon after harvest, especially given that—depending on timing of infection—symptoms may be lacking at harvest, but prevalent after storage.

The greenhouse study is currently ongoing. However, our results to date indicate that the degree of susceptibility to Lso may vary over time. Although statistical differences were not detected in the first year of the study, results from our 2017-2018 trials are expected to help with highlighting significant variations in both transmission efficiency and susceptibility to infection among Red Norland, Russet Norkotah, and Russet Burbank.

Activities or experiments conducted:

Field study

Field plots were planted at the Kimberly Research & Extension Center on May 2. Cages were installed over individual plants at or just before emergence (May 31). A colony of bacteriliferous potato psyllids used for inoculations has been maintained in the greenhouse facilities at the Kimberly Research & Extension Center. Inoculations occurred on August 2, 9, 16, 23, and 30, which were 5, 4, 3, 2, and 1 week(s) before vine kill, respectively. Plants that were inoculated 5, 4, 3, or 2 weeks before vine kill were sprayed with abamectin ca. 7 and up to 14 days after inoculation to kill psyllids and prevent further inoculation of bacteria on each plant.

Vines were cut manually on September 6, and each plot was hand harvested on September 29. Tubers were stored at 55°F with 95% relative humidity (RH). Four replications of each treatment were evaluated October 6. The remaining four replications were cured for two weeks at 55°F with 95% RH; then the temperature was decreased by 0.5°F per day until reaching a final holding temperature of 45°F. These tubers were evaluated after a total of 80 days in storage.

Tubers were evaluated for ZC symptoms as follows. Each individual tuber was cut using a Keen Kut Shoe Stringer French fry cutter and a fry plank was removed for ZC symptom rating and fry quality evaluations. Each plank was given a rating of ZC symptoms of A, B, or C (A = no symptoms, B = some visual discoloration near the stem end, C = clear ZC symptoms with discoloration throughout the tuber). The remaining tissue from each tuber—separately for each of the three rating categories—was subsampled at the stem end and the composite sample was sent to the Idaho Potato Disease lab in Aberdeen, Idaho for Lso testing by PCR to confirm visual symptom ratings.

Planks were then fried in canola oil at 375°F for 3.5 minutes. Planks were again rated for visual ZC symptoms and fry quality. Fry color was determined using a model 577 Photovolt Reflection Meter (model 577, Photovolt Instruments Inc., Minneapolis, MN). Measurements were taken on the bud and stem ends of each strip. Mean fry color reflectance was taken as an average between the bud and stem end measurements. A relationship between USDA fry color and photovolt reflectance as measured by our instrument and methodology was previously established; the lower the reflectance measurement, the darker the fry color. In addition, the incidence and severity of mottling were recorded. The severity rating scale for mottling was 1= no mottling, 2 = mild mottling (light colored, non-uniform surface browning not covering the entire fried plank), 3 = moderate mottling (light colored, non-uniform surface browning covering the entire fried plank), and 4 = severe mottling (dark colored, non-uniform surface browning covering the entire fried plank). The presence or absence of sugar end also was recorded for each plank. A plank was considered to have a sugar end if a predominant color of number 3 or darker (when compared with the USDA Munsell Color Chart for French Fried Potatoes) was seen on any two sides extending ½ inch or more from the end of the fried strip.

Preliminary analyses have been conducted on all data and are presented below. Further refinements to analyses are in progress.

Greenhouse study

The greenhouse study was conducted at the University of Idaho, Aberdeen Research and Extension center, Aberdeen, ID.

Lso-infected (Lso-B), *B. cockerelli*, of the central haplotype, were reared on potato *S. tuberosum* L. (var. Russet Burbank) in a growth chamber for several generations. Colonies were maintained in 60 x 60 x 60-cm tent shaped bugdorm cages. All experimental psyllids were eventually tested to quantify Lso titer. In addition, 10 adult psyllids were removed from Lso-infected colony, prior to every inoculation event, and tested individually for the presence of Lso, using quantitative polymerase chain reaction (qPCR).

The three potato varieties Russet Burbank, Ranger Russet, and Red Norland were used in the present study. The plants were planted in the greenhouse with temperatures fluctuating between 18 (night) and 23°C (day), and a 14:10 h photoperiod (Light:Dark). The potting mix consisted of 20% peatmoss, 70% sand, and 10% of vermiculite and fertilizer. Potato plants were grown in 2-gallon pots.

Tubers were planted in December 2016 (block 1) and January 2017 (block 2). The 2017-2018 experiment, planted on November 22nd and 29th, 2017, is currently ongoing; this report only reflects 2016-2017 results.

Inoculations were conducted by releasing four Lso-positive potato psyllids into a 1-inch clip cage, for a 48-h of inoculation access period (IAP). Each time-block included two inoculation times conducted either four or one week before the simulated vine-kill. Vine-kill involved removing the vine at the very base of the aboveground stem. In each time-block, a total of 7 seed pieces were planted per variety per infestation time. Two plants per genotype were not infested and were included as non-infected controls. At the end of the inoculation access period, potato psyllids were collected off of each plant and placed into a 2 ml tube for DNA extraction and qPCR analysis. Plants were sprayed with Warrior II and Movento to assure removal of any potential nymphs that might have hatched from eggs laid during the IAP. Tubers were harvested 14 days after vine-kill.

Tubers less than 2-cm in length were excluded from the study. Each tuber (N = 1 - 4) was then sliced at the stolen attachment end and scored for visual symptoms of ZC based on a 0 to 3 scale, with ‘0’ representing asymptomatic tubers and ‘3’ representing tubers with sever discoloration symptoms. A 6-mm Harris Uni- Core^{TM} (GE Healthcare Life Sciences) tissue sampler was used to remove core samples, from the stolon attachment ends, for later Lso quantifications.

Insect samples were ground in a homogenizer for DNA extraction. Total DNA of psyllid adults (composite sample of four psyllids/plant) was extracted with CTAB method. Overall, 100% of the psyllids sampled from our insect colonies tested positive for Lso. Plants with no positive psyllids were excluded from the dataset since they all tested negative for Lso. Only plants that tested positive after inoculations were used in ZC symptom and Lso titer analyses.

For DNA extractions from potato tubers, 100 mg the tissue was placed in a 2-ml microcentrifuge tube and ground in a homogenizer. CTAB was also used in DNA extractions from tubers. Absolute quantification was performed in a CFX Connect Real Time System (BioRad). A positive control (DNA of Lso positive), a negative control (DNA of healthy tuber), and water control (no template control, NTC) were included in all qPCRs. Details on DNA extractions, Lso primers, are presented in Rashidi et al. (2017- PLoS ONE).

Results:

Results from fry evaluations were largely consistent between years; moreover, ZC ratings were similar between raw and fried samples within each rating time (i.e., at harvest and after storage; Table 1). Across both years, for ratings at harvest and after storage, no ZC symptoms were observed from non-inoculated plants or those that were inoculated 1 week before vine kill.

During 2016, plants that were inoculated 2 weeks before vine kill showed no ZC symptoms in tubers before storage, but did show slight incidence after storage. Plants inoculated 4 weeks before vine kill showed significantly more tuber symptoms than all other treatments after storage and significantly more symptoms than all but the 3-week treatment before storage (at least for the raw tuber rating). The 4-week treatment showed evidence of increased disease symptoms after storage. The 3-week treatment exhibited a response that was intermediate between those of the 4- and 2-week treatments and did not show strong evidence of increased symptomology after storage.

Results from 2017 overall were similar to those from 2016. However, we did observe slight evidence of ZC in the 2-week treatment at harvest (at least for the fried samples). Moreover, there was more clear evidence of an increase in symptoms after storage for this treatment. Further, both the 3-week and 4-week treatments showed similarly moderate levels of ZC at harvest, but higher levels after storage. For the 5-week treatment, ZC was observed at high levels at harvest and was higher still after storage.

Table 1. Comparison among inoculation treatments of mean (± SE) percentage of tubers with severe ZC symptoms.

		At harvest		After storage	
Year	Inoculation timing	Raw	Fried	Raw	Fried
2016	non-inoculated	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b
	1 week	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b
	2 weeks	0.0 ± 0.0 b	0.0 ± 0.0 a	2.1 ± 2.1 b	2.3 ± 2.3 b
	3 weeks	7.3 ± 3.5 ab	16.1 ± 11.2 a	14.0 ± 9.3 b	17.0 ± 8.8 b
	4 weeks[z]	25.2 ± 13.7 a	28.8 ± 11.0 a	48.9 ± 11.2 a	51.9 ± 9.1 a
2017	non-inoculated	0.0 ± 0.0 ab	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
	1 week	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
	2 weeks	0.0 ± 0.0 ab	2.5 ± 2.5 b	11.4 ± 8.6 b	10.6 ± 7.9 b
	3 weeks	4.6 ± 4.6 ab	4.6 ± 4.6 ab	25.7 ± 14.9 b	24.9 ± 14.4 b
	4 weeks	8.3 ± 8.3 ab	8.3 ± 8.3 ab	30.6 ± 18.5 b	30.6 ± 18.5 b
	5 weeks	38.5 ± 21.5 a	39.1 ± 21.2 a	94.1 ± 3.4 a	94.1 ± 3.4 a

Means within each column that share the same letter do not differ significantly based on post-hoc tests following Analysis of Variance.
[z]5-week treatments were planned for both years, but early senescence forced the elimination of this treatment during 2016.

Comparison of fry color among treatments largely supports the analyses on comparing ZC symptoms. Fries were or tended to be lightest for non-inoculated treatments and darker with increasing time of inoculation relative to vine kill (Table 2).

Table 2. Mean (± SE) fry color per psyllid treatment from fries tested shortly after harvest or after three months in storage, 2016-2017.

	2016		2017	
Inoculation timing	At harvest	After storage	At harvest	After storage
non-inoculated	45.3 ± 2.8 a	43.5 ± 1.9 a	38.4 ± 1.9 a	40.6 ± 0.7 a
1 week	44.7 ± 2.2 a	38.8 ± 1.2 ab	38.1 ± 1.0 a	40.7 ± 1.1 a
2 weeks	47.5 ± 1.8 a	37.5 ± 2.0 ab	37.5 ± 2.1 a	37.8 ± 2.1 ab
3 weeks	44.5 ± 2.8 a	33.1 ± 3.9 bc	33.1 ± 5.2 ab	32.9 ± 2.7 b
4 weeks	36.6 ± 3.1 b	29.8 ± 2.1 c	33.7 ± 1.4 ab	32.9 ± 2.3 b
5 weeks	—	—	24.5 ± 5.9 b	19.1 ± 1.1 c

Means within a column followed by the same letter are not significantly different based on Fisher's LSD tests.

The results from this study suggest that plants infected with Lso within 2 weeks of vine kill will show little or no symptoms at harvest, but may develop slight to moderate incidence of symptoms after storage for ca. three months. In contrast, a preponderance of tubers from plants infected 3-5 weeks before vine kill will show ZC symptoms at harvest. Moreover, incidence of symptoms can increase over time during storage for plants infected anywhere from 2 to 5 weeks

before vine kill, with increases tending to be stronger in plants inoculated earlier. These results are similar to our previous studies showing an increase in symptoms for plants infected at 3 weeks before vine kill, but suggest that such latent symptomology does not occur as readily for plants infected within 2 weeks of vine kill. We present here a proposed risk table that summarizes these findings (Table 3).

Table 3. Likely risk of ZC symptoms in tubers in relation to timing of infection of plants.

time before vine kill	ZC risk at harvest	ZC risk after storage
1 week	low	low
2 weeks	low	low to moderate
3 weeks	low to moderate	high
4+ weeks	high	high

Risk levels are based on results from 'Russet Burbank' using the Central psyllid haplotype and Lso haplotype A.

Greenhouse study

The second year of the greenhouse study is currently in progress. This report include results from the two time-blocks which were conducted during 2016-2017 trial.

Statistical differenes in Lso titers and symptom severities have yet to be detected among the evaluated cultivars (Figures 1 and 2). In plants inoculated 4 weeks before vine-kill, Red Norland appeared to be less susceptible to Lso than the other two cultivars; however, this difference was not statistically significant ($F_{2, 18} = 0.889$, $P = 0.432$; Fig 1a). All three cultivars expressed similar degrees of symptom severity, when infected 4 weeks before vine-kill ($F_{2, 15} = 0.01$, $P = 0.989$; Fig 1b).

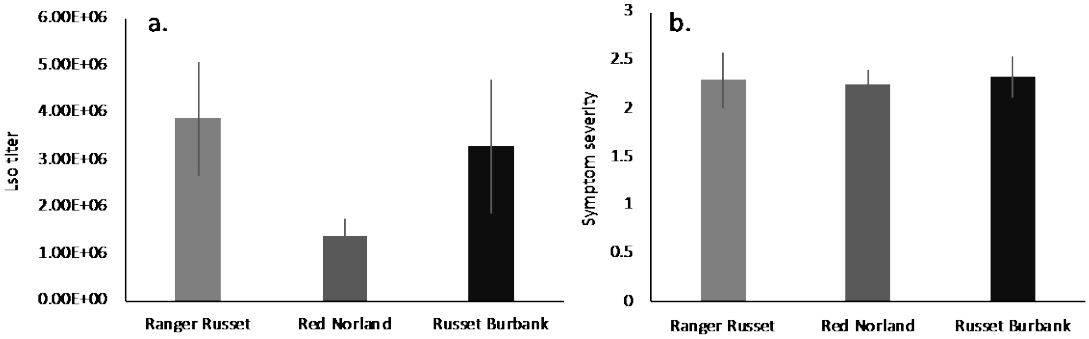

Figure 1: Mean Lso titers (a) and symptom severity scores (b) in the three evaluated cultivars, infected 4 weeks before vine-kill.

Although Lso transmission success was not statistically different (Wald X^2_2 =1.65, P = 0.438), considerable variation was observed among cultivars. While 100% transmission success was achived in Ranger Russet, the rate of successful transmission in Russet Burbank was only 37.5% (Fig. 2). Statistical differences in Lso titers ($F_{2, 12}$ = 0.593, P = 0.573; Fig 3a) and symptom severity scores ($F_{2, 9}$ = 0.564, P = 0.588; Fig. 3b) were also not detectable among cultivars, which were infected one week before vine-kill. However, in this late infestation treatment, Ranger Russet was the cultivar with relatively lower Lso titer and symptom severity when compared to Red Norland and Russet Burbank. It is important to note that only plants that tested positive for Lso were included in these analyses, thus our statistical power was limited in detecting differences. Results from the second year of our study are expected to clarifying these response patterns.

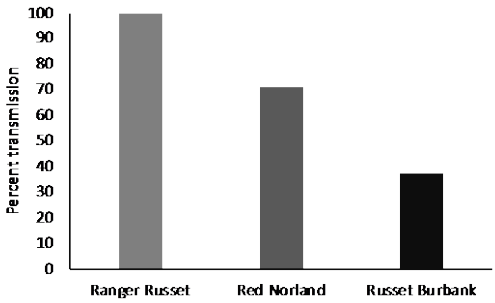

Figure 2: Percent Lso transmission success in the three evaluated cultivars, in plants infected 4 weeks before vine-kill.

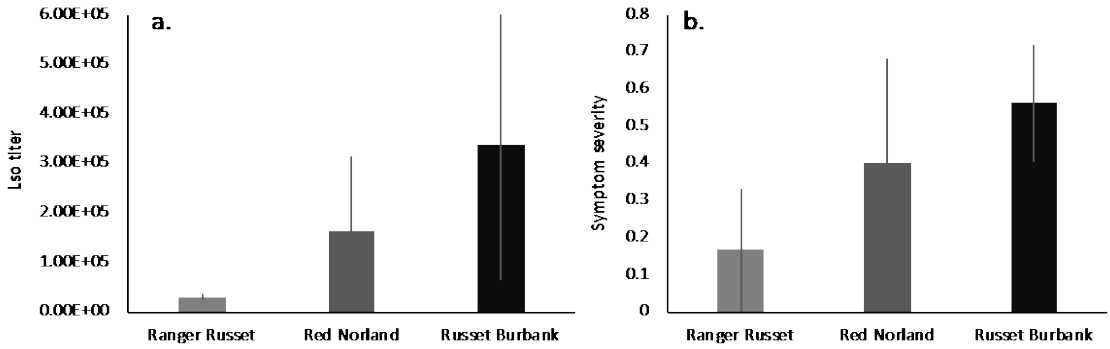

Figure 3: Mean Lso titers (a) and symptom severity scores (b) in the three evaluated cultivars, infected one week before vine-kill.

Nonetheless, the observed variability in the degree of susceptibility to Lso infection over time highlights the importance of considering this variability in screening programs aimed at identifing sources of resistance to Lso.

Publications:

No publications have resulted from this study to date. However, now that the field component of the study is complete and the greenhouse component is nearing completion, manuscripts are in preparation for scientific journal articles and for extension outlets.

Presentations & Reports:

Results were presented at the 2018 Idaho Potato Conference in Pocatello, ID, the 2018 Washington/Oregon Potato Conference in Kennewick, WA, and at the 2018 Potato Pest Advisory Committee Meeting in Kimberly, ID.

Annual Progress Report / Final Report

Title:

Strengthening soil health to suppress Verticillium wilt in potato production systems

Personnel:

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Reporting Period:

2017-2018

SUMMARY OF ACCOMPLISHMENTS:

Previous research has shown that the impacts of Verticillium wilt can be minimized by treatments focusing on improving soil health. Soil health, in this context, is the ability of the soil microbiota to fight the disease through enhanced activity and greater genetic diversity. Because the processes of creating and maintaining conditions that enable a soil to be healthy vary between geographies and industries, this project focuses on understanding the management practices and soil properties that reduce the severity of the Verticillium pathogen in the potato-supporting soils of the Pacific Northwest.

In this study, we will compare potato production fields that growers self-report as having being “severely” impacted or having a “high” amount of Verticillium with “healthy” or “low” wilt fields. Information about the management history for the past five years of these fields will be collected. Soil samples will be taken and analyzed (for soil health metrics as well as for soil microbial community composition and diversity). By making detailed comparisons of low-disease and high-disease soils from potato production systems across the Pacific Northwest, we will develop a database that should allow us to draw conclusions about which management practices and soil characteristics have the potential to facilitate soil health, and, by extension, reduce disease pressure.

Over the past months, after communication with potato growers from across Oregon, Washington, and Idaho, participation in the study was finalized and the locations of all fields to be included in the study were confirmed. Soil type information for the fields was compiled from the NRCS Web Soil Survey and enabled preliminary data analysis. With input from extension agents, academics, and growers, the five-year management history survey was created in two versions and sent to growers. Currently, this survey is in the process of being completed, and communication continues to occur between the OSU team and growers.

What we know so far is that the majority of the soils from the fields in this study are Aridisols (characterized by their salinity and low organic matter). The other two soil orders that are a part of the study are Mollisols (soils with a rich and fertile top layer) and Entisols (mineral-dominated soils). There are more diseased fields among the Aridisols than the other soil orders, and there is a higher incidence of diseased fields in Oregon than any other state.

A preliminary data analysis suggests that supposedly "better" soils, such as the Shano soil series, has been more frequently reported by the growers as having problems with Verticillium wilt and/or early die complex than the theoretically much "worse" Quincy soil. If corroborated by further investigations, this finding could potentially indicate a decoupling of Verticillium disease from soil properties that are commonly assumed to support disease suppressiveness.

I. ACTIVITIES OR EXPERIMENTS CONDUCTED:

The project started July 1, 2017. Graduate Student Larisa LaMere was hired to work on the project effective July 1, 2017. The project timeline lists two activities for this period:

Activity 1: Select 40 experimental sites by the end of the 2017 potato growing season (Jul 2017 - Sep 2017)
Activity 2: Perform survey of management history for all sites (Oct 2017 - end of March 2018)

Actions related to activity 1, Site selection:
Working closely with the Consortium represented by Dr. Jensen, Larisa contacted over 30 growers across Idaho, Washington, and Oregon. Grower response to our request for participation was generally supportive, but was noticeably stronger for growers from WA and OR than for growers from ID. From these contacts, the locations of 63 potato fields were collected and binned into two disease severity classes. To do so, fields with high disease severity (presumable susceptible to disease) and fields with low disease severity (presumable disease suppressive soils) were identified by the growers (Table 1).
To gain background information regarding general soil potentials, site location data were used to identify the NRCS Soil Series mapped for each site. Exploratory data analysis has begun, revealing that the majority of potato fields in the Pacific Northwest are on Aridisols and Entisols, with Mollisols being a minor third component. (Table 2).

Actions related to activity 2, Collect management data via grower survey:
Seeking and obtaining input from growers, extension agents and academics, a survey to collect 5 years of management data per site was developed (Figures 1a-1d) and shared with participating growers. Initial feedback from growers indicates that it will be beneficial to conduct follow up interviews. Larisa is in the process of conducting these interviews. She will also budget for in person time to clarify remaining questions when she visits the field for sample collection in spring.

Additional activity
Following a suggestion by the NPRC, a collaboration with Dr. **Ken Frost** (Hermiston Agricultural Research and Extension Center, HAREC) was initiated to assay several important soilborne pathogens of potato other than Verticillium. To this end, we will subdivide the 40 composite samples taken for soil health assessments and for each sample, share a subfraction of about 1 liter of soil material with Ken, who will also count and ID nematodes. It is our joint intention to combine the results of physical soil health testing, DNA based microbial community analysis and pathogen assays to generate inference (and resulting publications) addressing both, applied and fundamental aspects of this research.

Site Information		Geographic Information				Soil info		
Site ID	Wilt Level	Site size	Geographic location: State	Latitude	Longitude	USDA soil series name	USDA soil order	Dominant soil texture
name	description	acres	state	decimal	decimal	name	name	description
example circle	*high wilt*	*75*	*OR*	*45.960565*	*-119.352491*	*Ritzville*	*Mollisol*	*silt loam*
Circle 1	high wilt	48	ID	43.553349	-116.401191	Owsel-Purdam	Aridisol	fine-silty
Circle 2	high wilt	94	ID	43.553350	-116.401192	Owsel-Purdam	Aridisol	fine-silty
Circle 3	high wilt	78	ID	43.553351	-116.401193	Shano-Owsel Complex	Aridisol	course-silty
Circle 4	low wilt	170	ID	43.553352	-116.401194	Owsel-Purdam	Aridisol	fine-silty
Circle 5	low wilt	82	ID	43.553353	-116.401195	Shano-Owsel Complex	Aridisol	course-silty
Circle 6	low wilt	74	ID	43.553354	-116.401196	Shano-Owsel Complex	Aridisol	course-silty

Figure 1a: Survey Page 1

	Organic amendment info		Fertilizer info			Irrigation		Tillage	
	Organic amendment applied	Total amount of organic amendment applied	Total amount of fertilizer N applied	Total amount of fertilizer P_2O_5 added	Total amount of fertilizer K_2O applied	Irrigation equipment used	Total amount of irrigation	Tillage equipment	Tillage depth (write in)
	if an organic amendement (non-synthetic fertilizer) was added, what was it?	how much of the organic amendment was applied?	how much total mineral nitrogen was applied?	how much total mineral potassium was applied?	how much total mineral potassium was applied?	how was the field irrigated?	what was the total amount of irrigation during the growing season?	please describe here any activity that disturbed the soil (chisel plow tillage, drag off, fumigation application by ground rig, etc)	what was the estimated depth of the tillage?
	name	tons/acre	lbs/acre	lbs/acre	lbs/acre	description	in	description	in
	dairy manure	*7*	*120*	*100*	*200*	*center-pivot*	*21*	*chisel plow*	*5*
2013									
2014									
2015									
2016									
2017									

	Fumigant info				
	Fumigant application schedule	Amount of fumigant applied per application	Fumigant product name	Fumigant active ingredient	Fumigant application method
	how many times per year was the site fumigated?	how much fumigant was applied during each fumigation event?	what was the name of the fumigant used?	what is the first (or largest %) active ingredient of the fumigant?	how was the fumigant applied?
	#	gal/acre	name	name	method
	4	*40*	*Vapam*	*metam sodium*	*injection*

Figure 1b: Survey Page 2

	Potato Info			Disease info				Crop info		
Year	Potato cultivar planted	Potato market class	Yield	Incidence of disease	Severity of disease	Yield reduction due to early die	Other factors causing plant death	Other crop info	Other crop variety	Yield weight
Description	for the years potato was the cash crop, which variety was grown?	what was the intended market of the potato crop (fresh, fry, chip, dehy, etc.)	what was the total yield of harvested potatoes?	by your estimation, how much of the total site area was impacted by the disease?	in your opinion, how severe was the impact of early die at this site on a scale of 0 to 5?	by your estimation, about how much was the yield reduced by early die (verticillium wilt)?	if any other diseases (insects, root rot, etc) or severe weather (flood, hail, etc) caused an unusally high amount of plant death, list here.	if a cash crop other than potato was grown, what was it?	what was the variety of the other cash crop?	what was the total yield weight of other cash crop?
units	name	name	tons/acre	%	1=chlorosis, 3=wilt, 5=plant death	%		name	name	tons/acre
example	Russet Norkotah	Fry	40	25%	4	30%		onion	Welsh	16
2013										
2014										
2015										
2016										
2017										

	Cover crop info			
	Cover crop planted	Cover crop seeding rate	Termination method	Cover crop residue
	if a cover crop was planted, what was it?	how much cover crop seed was applied at planting?	how was the cover crop killed?	was the cover crop removed, incorporated, or left in place?
units	name	lbs/acre	description	description
example	winter wheat	75	tillage	incorporated
2013				
2014				
2015				
2016				
2017				

Figure 1c: Survey Page 3

	Herbicide/pesticide info			
	Herbicide/pesticide usage	Herbicide/pesticide application date	Herbicide/pesticide application purpose	Herbicide/pesticide application method
	what was the brand or generic name of the herbicide/pesticide used?	When was this product applied (for multiple applications, enter multiple dates)	why was this product applied?	how was the herbicide/pesticide applied?
	name	date	description	description
	Apron/metalaxyl	*7/9/17*	*pink rot control*	*broadcast surface*
2013				
2014				
2015				

Figure 1d: Survey Page 4

II. RESULTS:

Distribution of disease severity across geographic regions

The study involves soils from three geographically separate regions (Figure 1):
a) the Snake River Plain in Idaho
b) the Klamath Basin in southern Oregon
c) the Columbia River Basin (OR and WA)

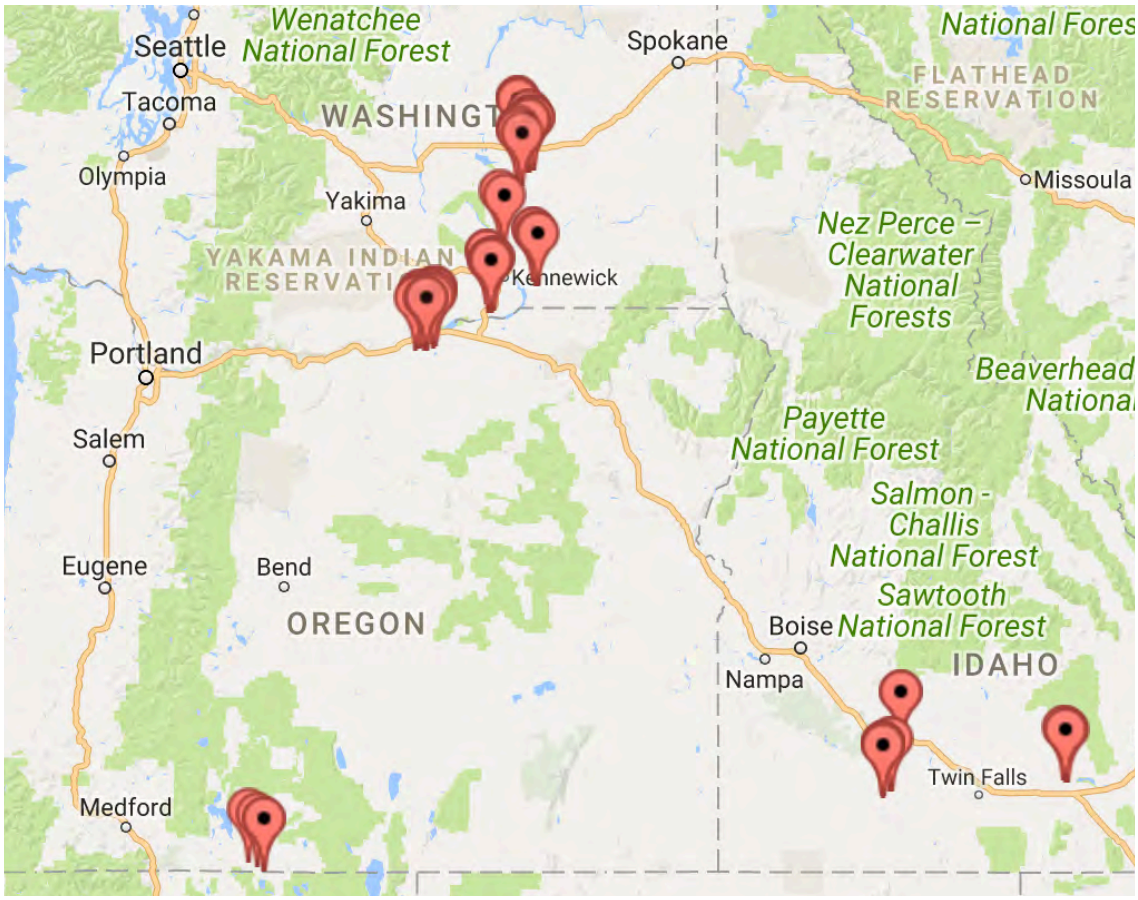


Figure 2: Distribution of participating operations across the PNW and separation into three distinct geographical areas

Growers reported more disease affected soils for inclusion in the study than healthy soils (Tables 1 and 2). There were significantly more reports of diseased soils from growers in Oregon than from Growers in Idaho and Washington. Given the non-randomized character of our data pool, we refrain at this point from any inference with regard to these statistics and take the data solely as an early indicator that regional differences in disease susceptibility may exist and should be further investigated in the course of the study.

Table 1: Distribution of potato fields submitted for participation in the study across geographical regions of the Pacific Northwest. "Severe damage" indicates fields that were considered seriously affected by Verticillium/early die complex; "Low damage" indicates fields that were reported as healthy or as having insignificant damage.

	Total	Severe damage	Low damage	Ratio severe : low
Columbia Basin	46	25	22	1.4
Snake River Plain	12	7	5	1.4
Klamath Basin	4	3	1	3.0
Total	63	35	28	1.25

Table 2: Distribution of potato fields submitted for participation in the study across the states of the Pacific Northwest. "Severe damage" indicates fields that were considered seriously affected by verticillium/early die complex; "Low damage" indicates fields that were reported as healthy are as having insignificant damage.

	Total	Severe damage	Low damage	Ratio severe : low
WA	31	15	16	0.9
ID	12	7	5	1.4
OR	20	13	7	1.9
Total	63	35	28	1.25

Soil type as a factor in disease suppression

When grouping the study sites by NRCS Soil Series and taxonomic Soil Orders (Figure 3), we noticed that potato production in the PNW occurs predominantly on Aridisols and Entisols. For a definition of these technical terms please consult Appendix A. Only 4 out of 63 study sites are on Mollisols. Two soil series, the Shano (Aridisol) and Quincy (Entisol), contribute more than half of the total population of study sites. Soil series are groups of soils with similar profiles developed from similar parent materials under comparable climatic and vegetational conditions. The soil series concept was originally introduced in 1903. Soil series were originally intended to consist of groups of soils which were thought to be the same in origin but different in texture. This principle is illustrated in the data sets describing the general properties of the Shano and the Quincy soils (Appendix B) - look particularly for differences in texture as plotted in the first row of the diagrams in Appendix B. Shano and Quincy soils occur in both the Columbia River Basin and in the Snake River Plain, and extend into all three states of the Pacific Northwest. However, the Shano is listed as Land Capability Class **2e** when irrigated), "having some limitations that reduce the choice of plants or require moderate

conservation practices" with subscript e indicating erosion and runoff as major conservation problem, while the Quincy is listed as LCC **4s** when irrigated, "having very severe limitations that restrict the choice of plants, requiring very careful management/ or both" with root zone limitations as the major conservation problem.

Hence we note that **the supposedly "better" soil, i.e. the Shano, has been more frequently reported by the growers as having problems with Verticillium wilt and/or early die complex than the theoretically much "worse" Quincy soil**. The fact that out of four Mollisols (which are supposedly even better for agricultural use than the Entisols and Aridisols), three out of four have been reported as being heavily diseased seems to extend this trend to potentially indicate that the incidence of Verticillium disease may be positively correlated with theoretical soil quality (i.e., increases the better the soil). We are aware that our database is small and not representative. However, if corroborated by further investigations, this finding could potentially indicate **a decoupling of Verticillium disease from soil properties that are commonly assumed to support disease suppressiveness**.

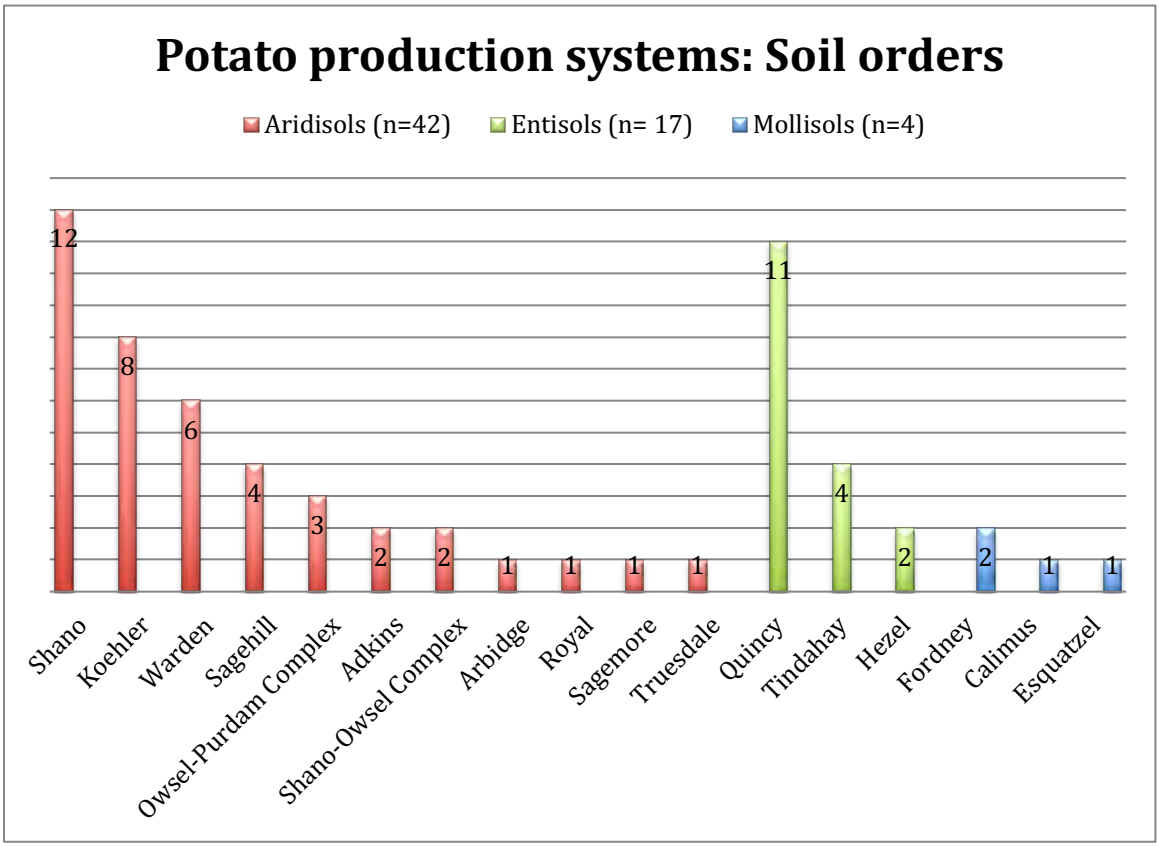
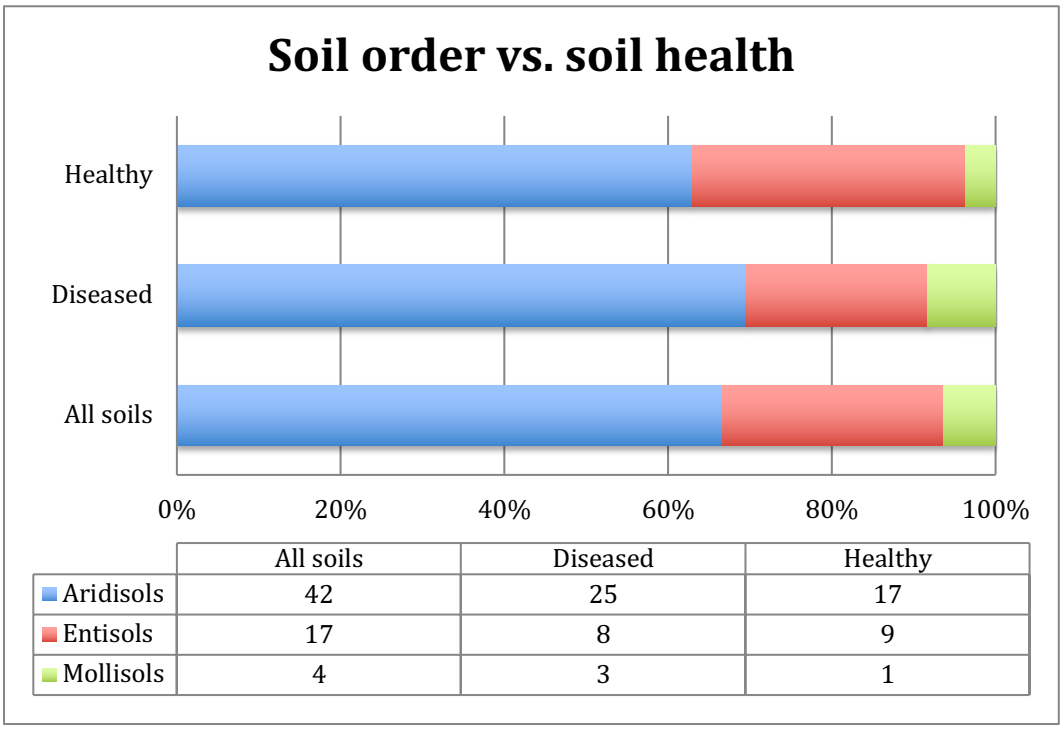


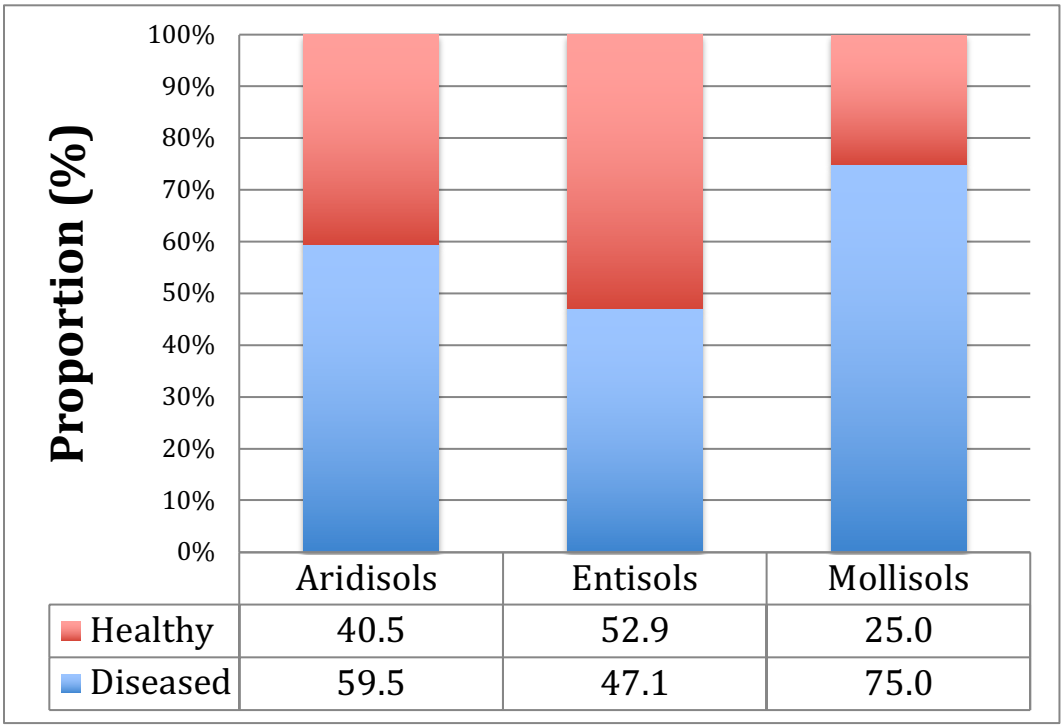
Figure 3: Taxonomic soil orders and NRCS Soil Series (X-Axis) represented in the study.

We further observed that Potato production occurs on a total of 17 soil series, however, 10 out of 17 soil orders were represented only once or twice. The distribution of healthy versus diseased soils among taxonomic soil orders is not constant (Figure 3, 4). Entisols and Mollisols contribute equal numbers of diseased and healthy soils, while diseased soils are more often reported in Aridisols.



	All soils	Diseased	Healthy
Aridisols	42	25	17
Entisols	17	8	9
Mollisols	4	3	1

Figure 4: Distribution of diseased versus healthy soils among the three soil orders



	Aridisols	Entisols	Mollisols
Healthy	40.5	52.9	25.0
Diseased	59.5	47.1	75.0

Figure 5: Entisols and Mollisols are represented in equal proportions, while diseased soils seem to have higher incidence in Aridisols

III. PUBLICATIONS:

With the project in its early stages, there have been no publications yet.

IV. PRESENTATIONS & REPORTS:

Kleber, M. 2017. Does the Soil Health movement have merit for growers in the Pacific Northwest.? Invited talk given at the 2017 Hermiston Farm Fair, Hermiston, OR, November 29, 2018.

Myrold, DD. 2018. Soil Microbiology as it impacts Soil Quality and Productivity. Invited talk given at the 2018 Washington-Oregon Potato Conference, Kennewick, WA, January 25, 2018.

V. APPENDIX

Appendix A

ENTISOLS

Entisols are mineral soils that commonly exhibit minimal pedogenic alteration. This taxonomic order was established to preserve the early Russian "azonal" concept for soils with undeveloped profiles crossing bioclimatic zones. Typically described as A-C, A-Cr, or A-R profiles, Entisols retain many physical and chemical properties of the associated parent materials. Despite minimal horizonation, however, Entisols must develop physical and chemical properties capable of supporting plants. This characteristic distinguishes Entisols from non-soil areas such as rock outcrop, water, ice, or badlands. Entisols most often occur where the influence of the soil-forming factors retards soil formation. Landforms where Entisols form are marshes and estuaries, modified urban areas, sand dunes, floodplains, and arid regions with steep or rocky slopes. These landforms are associated with wetlands, inert sandy parent materials, rapid rates of aggradation or erosion, and intensive human activity. Because of the geographic extent of these environments, Entisols are the most abundant of all soil orders covering approximately 16% of the world's ice-free land mass. Up to 60% of Entisols occur in temperate and tropical regions.

ARIDISOLS

The rationale for the Aridisol order of Soil Taxonomy **was to separate on a soil map the "sown from the unsown"; that is, the land that can be cultivated from the land that can only be grazed.** Using climate as the basis for a soil order is somewhat unique to the *Soil Taxonomy* system. Other classification systems, such as the World Reference Base, do not have an equivalent to the Aridisol order. Yet the delineation based on climate was inherited from the concept of *zonal* soils, the idea that certain kinds of soils are associated

with certain kinds of bioclimatic zones. The aridic moisture regime is based on the duration of dryness in the *soil moisture control section* during the period the soil is warm enough for plant growth. The top boundary of the soil moisture control section is the depth reached by the wetting front when 2.5 cm (1 in.) of water is applied for 24 h. The bottom boundary is the depth reached by the wetting front when 7.5 cm (3 in.) of water is applied for 48 h. Because these depths are largely a function of soil texture, finer-textured soils, such as those with fine-loamy, coarse-silty, fine-silty, or clayey textural classes, have moisture control sections that lie between approximately 10 and 30 cm. In contrast, coarse-loamy soils have moisture control sections roughly between 20 and 60 cm, and sandy textural classes have boundaries between approximately 30 and 90 cm depending on coarse fragments, which deepen these limits for all textures.

By definition, a soil has an aridic moisture regime if that soil's control section is dry more than half the year (in normal years) when the temperature at 50 cm is above 5°C. The criterion of "above 5°C" was added because cold temperatures, rather than dryness, can be the limiting factor to plant growth. Additionally, the soil moisture control section is "Moist in some or all parts for less than 90 consecutive days when the soil temperature at 50 cm is above 8°C". In other words, if a soil is moist, "not dry" (i.e., water held at tensions greater than −1500 kPa), for more than 3 months (90 consecutive days), the soil is too moist to have an aridic moisture regime.

MOLLISOLS

Mollisols are generally characterized as soils with thick, dark surface horizons (mollic epipedons) resulting from organic C incorporation. Mollisols can form under multiple environmental conditions that facilitate accumulation of organic C in the upper soil profile. Mollisols are among the most important soils for food and fiber production due to relatively high levels of native fertility coupled with climatic conditions conducive to plant growth.

The general concept of Mollisols is that of dark colored soils of semiarid to subhumid grassland ecosystems. The dark color reflects soil organic matter (SOM) enrichment in the upper portion of the profile. The formation of dark surface horizons is termed melanization, which is actually a combination of several processes involving the addition of organic matter to the soil in the form of plant residues and its subsequent transformation into humus. The high base status of Mollisols generally translates into a high level of native fertility. Both calcium and Mg, typically the dominant exchangeable cations, are required in fairly large quantities for plant growth. The pH values of Mollisols are quite variable ranging from strongly acid (5.1–5.5) to strongly alkaline (8.5–9.0). However, many Mollisols have pH values somewhere in the middle of this range and are generally considered to be favorable for plant growth without widespread use of liming agents. Their high native fertility is one of the main reasons for their extensive use in agriculture throughout the world.

Mollisols generally have not undergone intensive weathering, and therefore, their mineralogy is often dominated by minerals inherited from their parent materials. Many Mollisols have formed in recently deposited parent materials such as glacial till and loess, which has not allowed sufficient time for significant mineral weathering to occur.

Appendix B

This aggregation is based on all pedons with a current taxon name of QUINCY, and applied along 1-cm thick depth slices. Solid lines are the slice-wise median, bounded on either side by the interval defined by the slice-wise 5th and 95th percentiles. The median is the value that splits the data in half. Five percent of the data are less than the 5th percentile, and five percent of the data are greater than the 95th percentile. Values along the right hand side y-axis describe the proportion of pedon data that contribute to aggregate values at this depth. For example, a value of "90%" at 25cm means that 90% of the pedons correlated to QUINCY were used in the calculation.

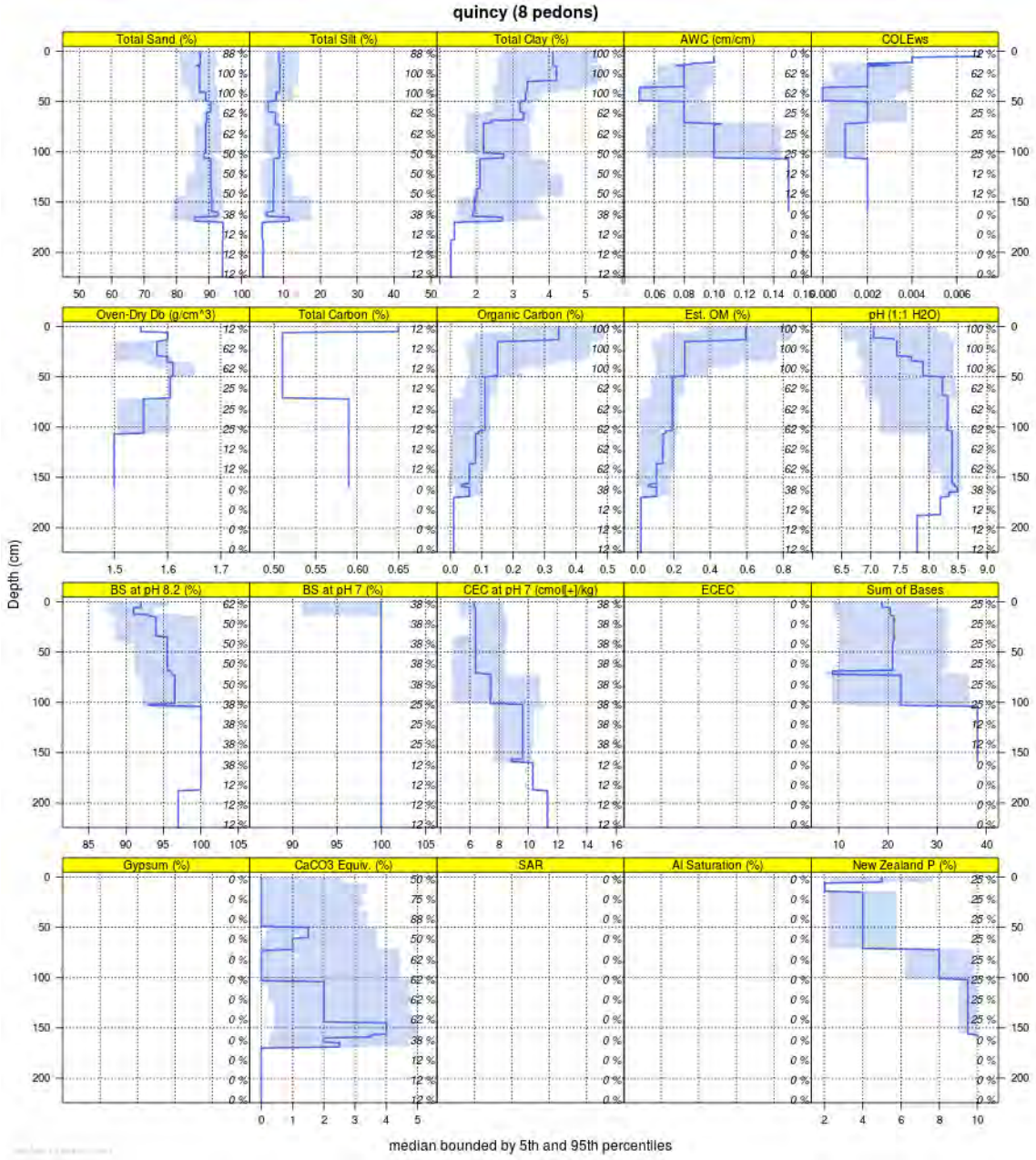


Figure 6: Aggregate lab data for the QUINCY soil series.

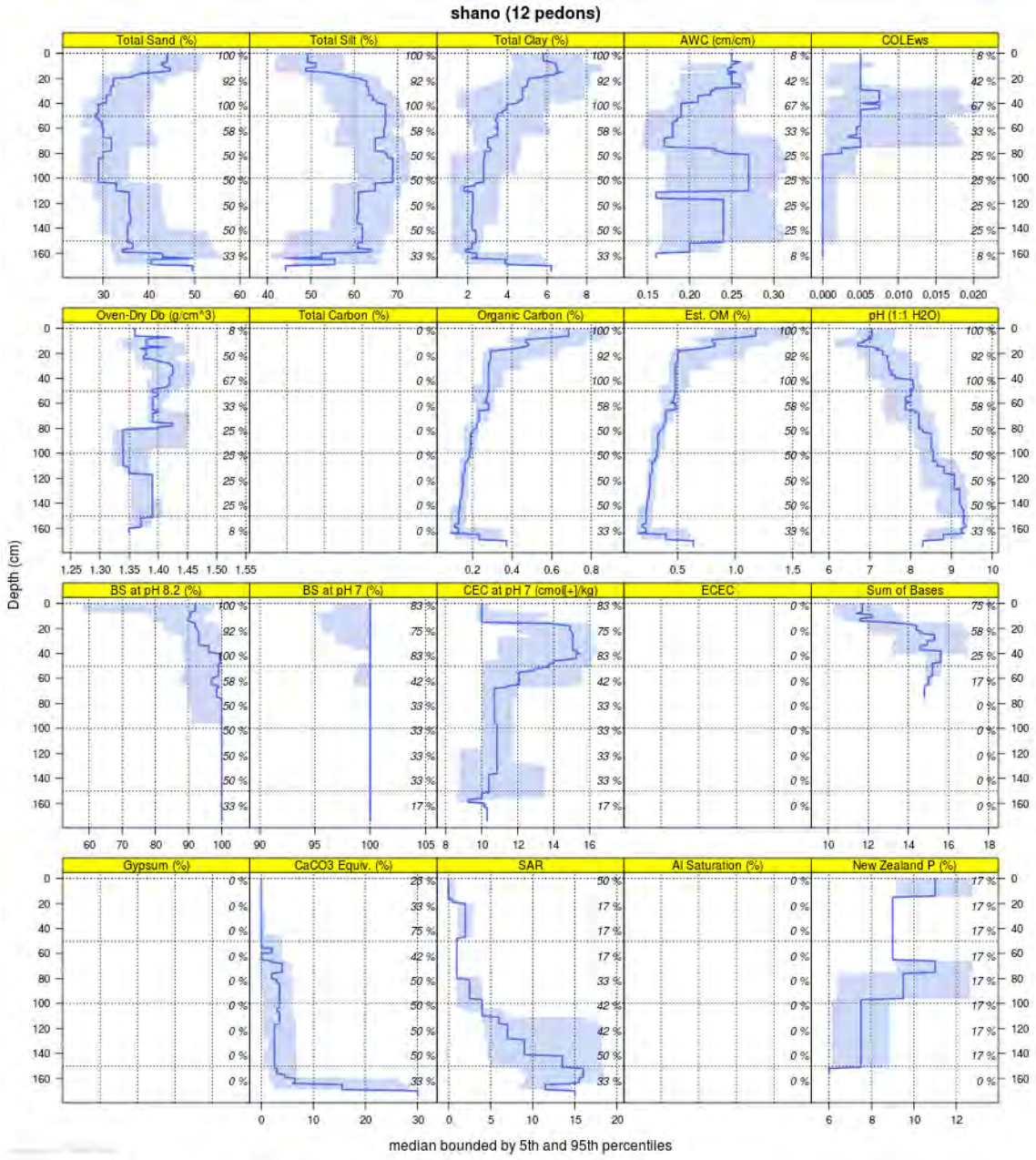


Figure 7: Aggregate lab data for the SHANO soil series

ANNUAL PROGRESS REPORT

Title: Screening for stress tolerance and development of PGR approaches to optimize yield and raw product recovery for cultivars/clones from the NWVDP

Personnel & Cooperators: Rick Knowles*, L.O. Knowles, M.J. Pavek, and G.N.M. Kumar, Dept. of Horticulture, WSU (Rich Novy and Nora Olsen, cooperators).
(rknowles@wsu.edu, 509-335-3451; lknowles@wsu.edu, 509-335-6783; mjpavek@wsu.edu, 509-335-6861; gnmkumar@wsu.edu, 509-335-3455; rich.novy@ars.usda.gov, 208-397-4181; norao@uidaho.edu, 208-423-6634). *funded program.

Reporting Period: 2017

Summary of Accomplishments - Progress to date is summarized by objective.

Objective 1 - *Screen cultivars/clones from the Northwest Variety Development Program (NWVDP) for tolerance to heat stress for retention of process quality and cold sweetening resistant phenotype*

Heat stress somehow modifies the subsequent responses of tubers to cold, inducing changes in carbohydrate metabolism that compromise process quality. Through 2016, and with the exception of Simplot's Innate cultivars, Payette Russet is the only one of many LTS-resistant clones/cultivars tested that has been shown to have robust tolerance to in-season and postharvest heat stress for retention of its cold-sweetening resistant phenotype. We determined that this trait was likely inherited from its maternal parent, EGAO9702-2 (Herman et al., 2017). Thirteen additional clones and cultivars were screened in 2017 for heat tolerance using the postharvest heat stress (PHHS) protocol we developed. This technique involves evaluating LTS of tuber samples following exposure to heat stress (HS, 21 d at 90°F), cold storage (CS, 39°F for 30 d) or their sequential combination (HS + CS). Clones tested in 2017 were selected from the late regional trials because of their superior agronomic/postharvest performance and cold-sweetening resistance. Additionally, seed of siblings and half siblings of Payette Russet were obtained from Rich Novy's program. Tubers of each clone were grown at Othello in 2017 and subjected to PHHS screening in the fall. Three of the clones, A01432-44LB, A12406-2sto, and A07548-2LB showed significant tolerance to heat stress for retention of their cold-sweetening resistant phenotypes (Fig. 1). These clones have the same maternal parent (EGAO9702-2) as Payette Russet. While EGAO9702-2 also shows tolerance to heat stress (Herman et al., 2017), inheritance of the trait is complex as A02507-3LB (sibling of Payette Russet) was both cold-sweetening and heat susceptible. A02515-2 (half sibling of Payette) was cold-sweetening resistant but heat susceptible for loss of the cold sweetening resistant trait. A03141-6 (Late Regional Trial) may also have partial tolerance to heat stress (data not shown). Screening will continue in 2018. Identification of heat resistant genotypes is a prerequisite to determining the mechanism of heat tolerance and will facilitate targeted breeding of cultivars with more durable and robust tolerance of heat stress for retention of low sugar phenotype. Graham Ellis (PhD graduate student) is working on portions of this objective.

Objective 2 - *Develop plant growth regulator (PGR) approaches to manage emergence, foliar development, tuber set, size and shape with a goal of maximizing tuber and fry (raw product) yields for selected cultivars (Payette, Alturas, Shepody, advanced clones)*

Seed treatments with GA and NAA are being evaluated for their efficacy to overcome dormancy, increase tuber set, and alter tuber size distribution and shape of selected cultivars. We

showed that GA seed treatment effectively hastens emergence, increases stem numbers, tuber set, tuber length to width (L/W) ratio, and yield of raw product (≥3-inch long fries) from 4-12-oz tubers of Payette and Alturas (Knowles et al., 2017). However, the total yield of raw product (across all tuber size classes) did not increase proportionally with L/W ratio because GA also shifted tuber size distribution away from >12-oz tubers to favor increased yield of undersize (<4 oz) tubers. Tubers ≥12 oz produce 96% 3-inch and longer fries regardless of shape. Therefore, to maximize yield of raw product, the GA-induced increase in stem numbers and associated shift in tuber size distribution needs to be minimized while retaining GA's effects on hastening emergence and increasing tuber L/W ratio. Work in 2017 showed this is possible using a combination seed treatment of GA + NAA, wherein NAA limits the GA-induced increase in stems and shift in tuber size distribution. However, the optimum relative concentrations of these growth regulators in combined treatments depends on cultivar and the desired outcome (e.g., quicker emergence and the degree of apical dominance needed for a particular tuber size distribution). Work to date has demonstrated the following:

- Fry yield per acre is dictated by an interaction between tuber shape & size distribution. Increasing the L/W ratios of 4-10-oz tubers greatly increases raw product recovery.
- 12-oz and over tubers translate to 96% fry yield regardless of shape phenotype; however, large tubers also increase defects & bruise (= penalties). This incentivizes limiting oversize in favor of 4-12 oz yield (with high L/W ratio) to maximize raw product recovery.
- In combination, NAA selectively attenuated GA's effects on increasing stems & shifting tuber size distribution, while retaining effects on quicker emergence & shape phenotype.
- GA/NAA seed treatments increased yield of raw product only when relative concentrations were adjusted to minimize loss of >10-oz yield & gain in <4-oz (undersize) yield (increases in raw product recovery ranged from 12 to 39%).
- Product (i.e. active ingredient) deposition on the seed is critical to the responses (apical dominance, tuber size distribution, U.S. No. 1 yield, shape phenotype). At a given concentration of GA/NAA, seed dip application results in more a.i. deposition on the seed than tumble spray application. In-furrow application resulted in the least amount of a.i. deposition on seed but still effectively hastened emergence and increased tuber L/W ratio.

Results from these studies were disseminated in detail in the proceedings of the WA and OR Annual Potato Conference (see Knowles et al., 2017), annual meetings of the Potato Association of America (Dean et al., 2018), and most recently at the Idaho Potato Conference (Knowles et al., 2018). Several studies will be repeated in 2018, including those described in Tables 1 & 2 and Fig. 2 using commercially registered products (ProGibb and Rejuvenate) to further define GA/NAA concentrations and application techniques (pre-plant seed spray and in-furrow applications) to maximize raw product recovery from Alturas and Payette Russet.

Objective 3 - *Evaluate effects of crop maturity on yield, tuber size distribution and storability of selected NWVDP clones/cultivars (Clearwater, Umatilla, Payette, Alturas, Ranger, Burbank)*

Key questions for the cultivars tested include: (1) how does planting date affect yield, tuber size distribution, tuber maturity, and postharvest retention of process quality and (2) what are the economic consequences (if any) for early versus late vine kill for each cultivar?

Tuber maturity was manipulated by planting at 2-week intervals (March 31, April 14, and April 28) and holding the growing and maturation (post vine-kill) periods constant at 147 and 10 days, respectively, for RB, Ranger, Clearwater and Umatilla (Fig. 3). This approach effectively shifted key developmental phases (emergence & establishment, tuberization, bulking,

maturation) into different 'windows' of the growing season, which affected tuber development, maturity, yield, tuber size distribution and storability, depending on cultivar. On average, total, U.S. 1, and marketable (U.S. 1 + undersize) tuber yields were lowest for the early planting date, highest for the mid (April 14) planting date, and intermediate for the late planting date (Table 3). The mid planting date resulted in 9% higher U.S. 1 and marketable yields ($P<0.01$) than the early planting date. Storability trials to evaluate effects of the planting date shift on retention of process quality are currently in progress. Preliminary data indicate that the effects of planting date on retention of process quality are variety dependent. For example, tubers from the mid and late plantings of Russet Burbank (cold sweetening susceptible) are maintaining better process quality than those from the early planting, despite being grown for the same number of days (147). These results are consistent with data from 2016, where tubers from the early planting of RB had accumulated 2.7- and 4-fold higher reducing sugars during the initial 30 days of storage than those from the mid and late plantings, respectively (Fig. 4). Reducing sugars in tubers from the early planting then remained higher than in tubers from the later planting dates through the remainder of storage (210 days). On the other hand, by virtue of its cold-sweetening resistance, Clearwater Russet appears less sensitive to planting date for retention of process quality.

Tuber maturity was also manipulated in a separate study (Alturas, Clearwater, Payette, Umatilla) by planting in mid-April, vine-killing 135, 149, and 163 days after planting (DAP), and then harvesting 169 DAP. The late season bulking patterns unique to each cultivar were characterized by comparing changes in yields and tuber size distributions as a function of vine kill date. Effects of vine killing/harvesting at different times on crop value for frozen processing were also assessed.

Averaged over 2 years (2016 & 17), the U.S. 1 yields of Alturas and Clearwater increased linearly by an average of 24% from 135-163 DAP with similar increases in process value (Fig. 5). For Alturas, yields of 6-oz and under tubers remained constant while 10-oz and over tubers increased. The yield of oversize (>14 oz) tubers increased the most. For Clearwater, the increases in yield from 135-163 DAP were evenly distributed among 6-10, 10-12, 12-14, and >14-oz tuber size classes. By contrast, yield and process value of Payette Russet reached a maximum at 149 DAP. The yield and process value of Umatilla was not significantly affected by vine kill date over the 2-yr study period. These trials will be repeated in 2018. Collectively, the crop maturity studies will inform beginning and end-of-season management decisions for maximizing yield, quality, and economic returns.

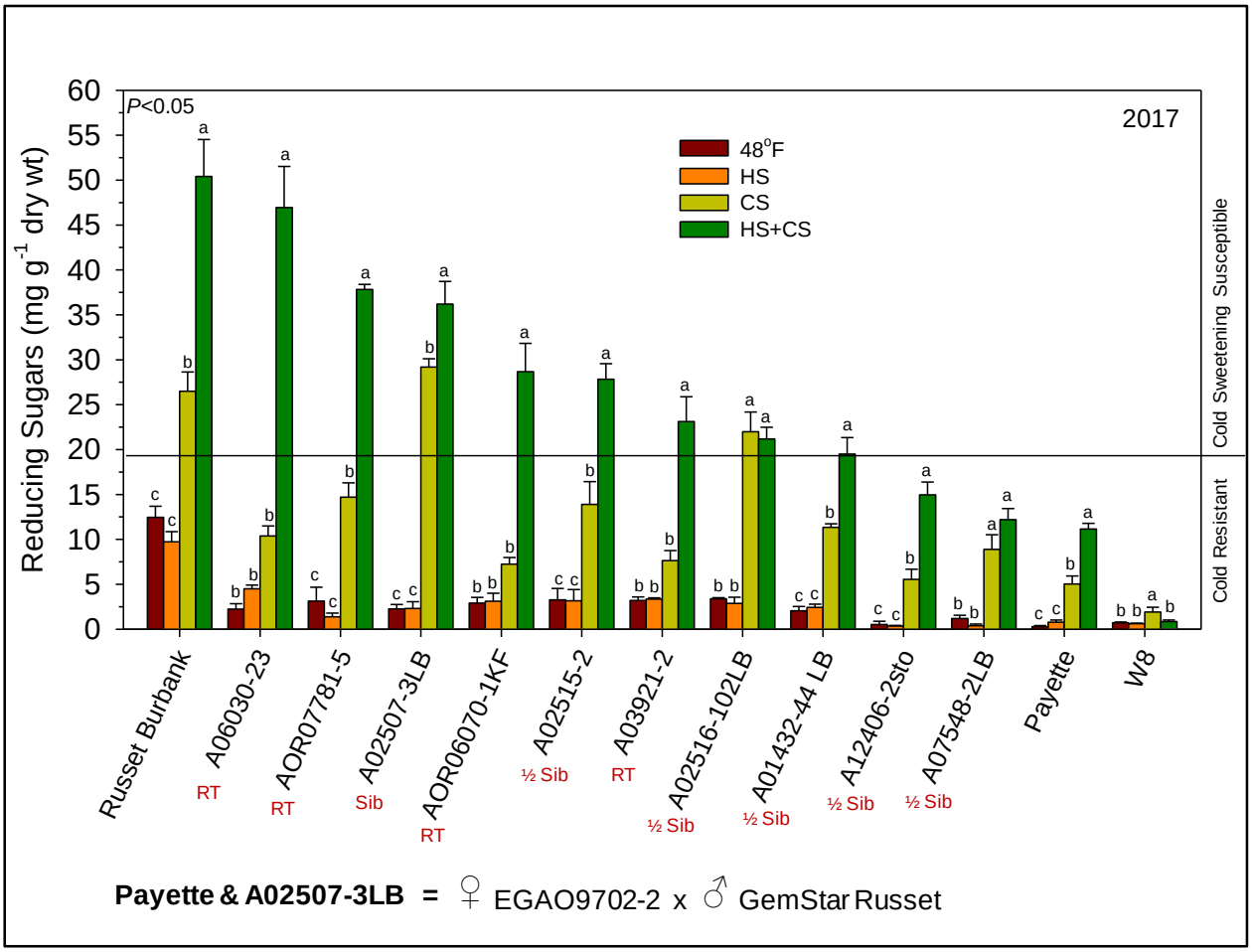


Fig. 1. Changes in tuber reducing sugar concentrations from cold-susceptible and resistant cultivars/clones as affected by heat stress (HS, storage for 21 days at 90°F), cold storage (CS, 32 d at 39°F), and the sequential combination of HS plus CS. Control tubers were stored at 48°F continuously. Tubers were subjected to the storage treatments after a brief wound-healing period directly following harvest in October. Heat stress exacerbated the cold inducible increase in reducing sugars of the cold-sweetening susceptible clones, Russet Burbank and A02507-3LB. This latter clone is a sibling of Payette Russet. Heat stress abolished the cold-sweetening resistant phenotypes of A06030-23, AOR07781-5, AOR06070-1KF, A02515-2, and A03921-2. A02515-102LB (cold-sweetening susceptible) and A01432-44LB (cold-sweetening resistant) were heat tolerant for retention of their cold-sweetening susceptible and resistant phenotypes (i.e. heat stress prior to cold storage resulted in relatively little change in reducing sugar concentrations). A12406-2sto, A07548-2LB, Payette Russet, and Innate® W8 (Russet Burbank) were tolerant of heat stress for retention of their cold-sweetening resistant phenotypes. These clones maintained USDA 2 or lighter fry color when stored at 39°F regardless of prior heat stress. Letters indicate LSD (P<0.05) for comparison of PHHS treatments within a clone. Each bar represents the average reducing sugar concentrations in 12 tubers. RT, regional trial; Sib, sibling or ½ sibling (same maternal parent) as Payette Russet.

Table 1. Effects of increasing rates of GA in combination with 120 mg L^{-1} NAA on plant emergence, stem number, yield, tuber size distribution, tuber number and size of Payette Russet potatoes grown at Othello, WA. Cut seed was treated by spraying (0.6 gal per ton of seed) with solutions of the combined growth regulators on April 10, 2017. Seed was planted April 13, 2017, vines were mowed 153 days after planting (Sept. 13, 2017), and plots were harvested on Sept. 25, 2017. Significance levels (*P*-values) for linear, quadratic, cubic, and quartic polynomial trends are given (Dev, deviations from quartic). LSD, least significant difference at $P<0.05$.

GA (mg L^{-1})	NAA (mg L^{-1})	Emerg 41 DAP (%)	Stem No.	Payette Russet Tuber Yield (T/A)									Marketable Tubers		
				Total	U.S. #1	<4 oz	4-6 oz	6-10 oz	10-12 oz	12-14 oz	>14 oz	Mkt Yld	oz/tuber	Tubers plant^{-1}	1000s/A
0	0	20.8	2.5	39.4	35.7	3.6	6.3	14.3	5.1	3.6	6.4	39.3	7.1	9.1	177.6
4.3	120	65.0	2.7	39.9	35.2	4.6	7.2	16.5	4.9	3.6	2.9	39.8	6.5	10.1	197.2
8.6	120	84.2	2.8	38.4	32.9	5.4	8.4	15.7	4.5	2.3	1.9	38.3	5.9	10.7	209.2
17.2	120	90.0	3.2	37.1	29.7	7.3	9.0	13.3	4.0	2.4	1.0	37.0	5.4	11.3	220.7
25.8	120	91.7	3.2	35.5	27.7	7.7	8.7	14.2	2.7	1.1	1.1	35.4	5.1	11.3	221.0
34.4	120	95.0	3.5	35.6	28.2	7.2	9.2	13.7	2.3	1.4	1.7	35.4	5.2	11.1	217.7
	$LSD_{0.05}$	12.5	0.4	2.6	3.1	1.2	1.5	1.7	1.2	1.2	1.8	2.6	0.5	0.7	14.3
	GA_{Lin}	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	GA_{Quad}	0.001			0.06	0.001	0.05				0.001		0.001	0.001	0.001
	GA_{Cubic}	0.001						0.05			0.07				
	GA_{Quart}							0.005							
	GA_{Dev}														

Table 2. Effects of increasing rates of GA in combination with 120 mg L^{-1} NAA on tuber length to width (L/W) ratio and recovery (yield) of ≥3-inch-long French fries from the six size classes constituting the U.S. No. 1 yield (Table 1) of Payette Russet potatoes grown at Othello, WA. Significance levels (*P*-values) for linear, quadratic, cubic, and quartic polynomial trends are given (Dev, deviations from quartic). LSD, least significant difference at $P<0.05$.

GA (mg L^{-1})	NAA (mg L^{-1})	Tuber L/W	Payette Russet French Fry Yield (T/A ≥3-inch-long fries)							Fry Yld (% U.S. No. 1 Yld)
			4-6 oz	6-8 oz	8-10 oz	10-12 oz	12-14 oz	>14 oz	Total	
0	0	1.29	0.2	0.8	3.7	3.9	3.4	6.1	18.1	50.7
4.3	120	1.50	1.8	3.3	5.4	4.3	3.5	2.8	21.0	59.6
8.6	120	1.51	2.4	5.8	6.0	3.9	2.3	1.9	22.2	67.3
17.2	120	1.59	3.3	6.5	4.9	3.5	2.3	0.9	21.5	72.3
25.8	120	1.62	3.8	6.6	5.1	2.4	1.1	1.0	19.9	71.4
34.4	120	1.68	4.3	6.6	5.8	2.1	1.3	1.6	21.6	76.5
	$LSD_{0.05}$	0.04	0.5	1.1	1.0	1.1	1.1	1.8	3.2	5.7
	GA_{Lin}	0.001	0.001	0.001	0.03	0.001	0.001	0.001		0.001
	GA_{Quad}	0.001	0.001	0.001				0.001		0.001
	GA_{Cubic}	0.001	0.002	0.003	0.001				0.02	0.02
	GA_{Quart}	0.006								
	GA_{Dev}	0.001								

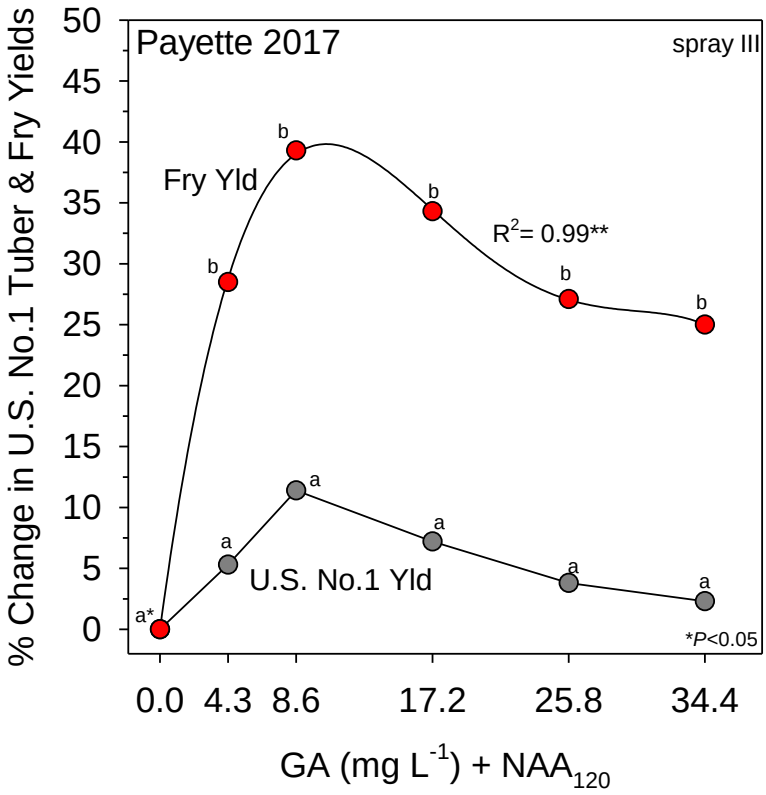


Fig. 2. Effects of increasing rate of GA in combination with 120 mg L^{-1} NAA on the percent change in U.S. No. 1 tuber yield and yield of ≥3-inch-long French fries from Payette Russet potatoes grown at Othello, WA in 2017. Cut seed was treated by spraying in furrow (22 gal per acre) during planting (April 28) with solutions containing the combined growth regulators. The photo below depicts deposition of the spray in the open furrow and on the seedpieces prior to hilling. Vines were mowed 139 days after planting (Sept. 14), and plots were harvested on Sept. 25. Letters indicate mean separation (LSD, $P<0.05$). U.S. No. 1 and fry yields are given in T/A for selected treatments.

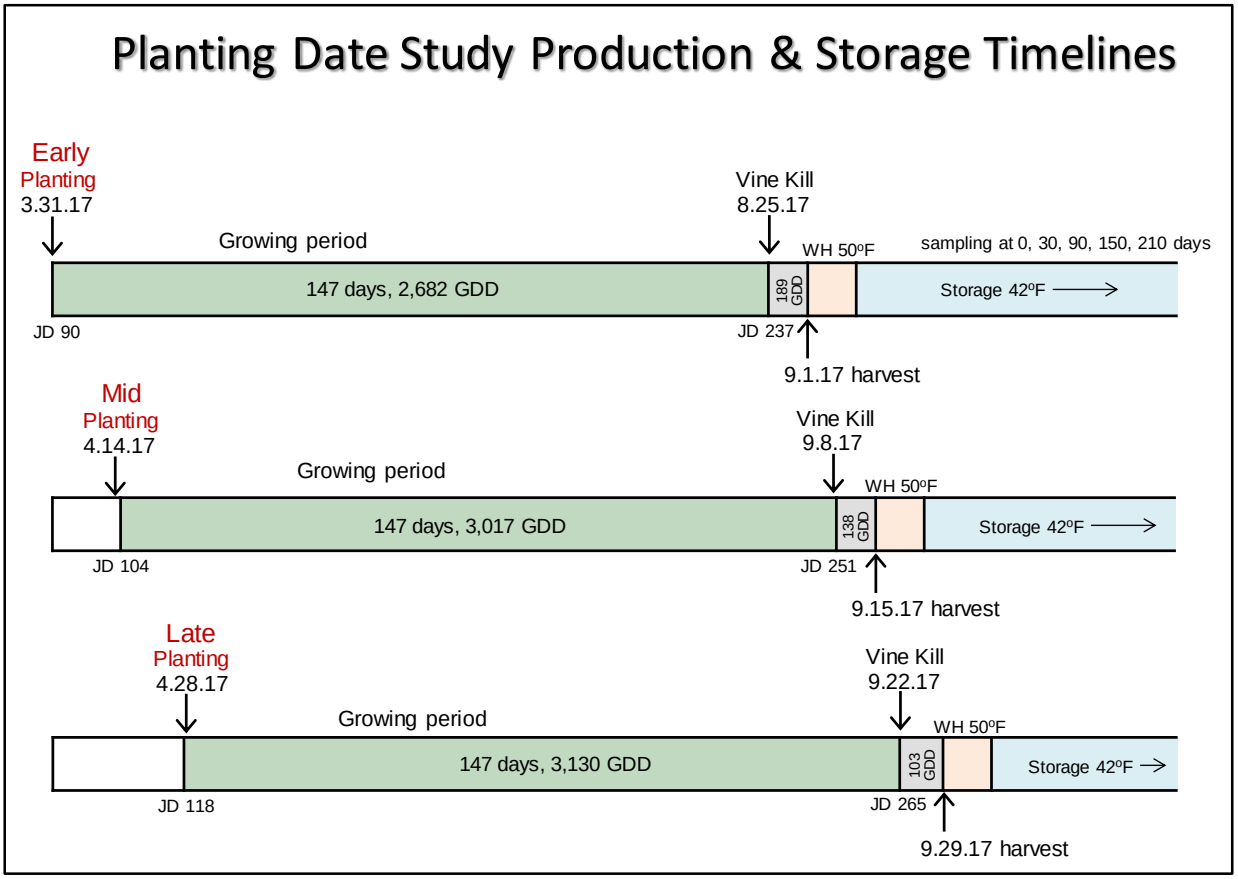


Fig. 3. Timelines for the 2017 early, mid, and late planting date trial (Russet Burbank, Ranger Russet, Clearwater Russet, Umatilla Russet). The growing period, maturation period (vine kill to harvest), and wound healing periods were held constant for all three planting date treatments. This approach forced critical stages of crop growth and development (emergence & plant establishment, tuberization, bulking, foliar senescence, and tuber maturation) into different 'windows' of the growing season. The cumulative growing degree days (45ºF base, Othello, WA) during the 147-day growing period and 7-day maturation period are shown for each planting date timeline. Plant emergence, yield, tuber set, and tuber size distribution data are given in Table 3. Changes in tuber process quality are being evaluated at 60-day intervals through 210 days of storage at 42ºF. Effects of planting date on the changes in reducing sugar concentrations of Russet Burbank tubers during storage are shown in Fig. 4 from the initial study conducted in 2016.

Table 3. Effects of planting date on emergence, stem number, yield, tuber size distribution, tuber number per plant, and average tuber size of cultivars grown at Othello, WA. Vines were mowed 8/25, 9/8 and 9/22 (147 DAP), and plots were harvested on 9/1, 9/15, and 9/29. Results of the 1-way analysis of planting date for each cultivar and the 2-way factorial analysis (cultivar x planting date) are summarized (LSD, least significant difference at $P<0.05$; LT, linear trend; dev, deviations from linearity). Data have been averaged to show the main effect of cultivar at the bottom of the table. Letters indicate mean separation (LSD, $P<0.05$).

CV	Planting Date	Emerg 38 DAP (%)	Stem No.	Planting Date Study- Tuber Yield (T/A) (2017)									U.S. #1 Tubers + <4 oz		
				Total	U.S. #1	<4 oz	4-6 oz	6-10 oz	10-12 oz	12-14 oz	>14 oz	Mkt Yld	(tub/plant)	(g/tuber)	(1000s/A)
RB	3/31	2.5	2.8	44.1	37.5	8.1	10.1	13.4	4.8	4.4	10.4	41.2	8.7	7.7	169.7
	4/14	89.2	2.6	43.8	39.8	8.1	13.2	13.7	6.0	4.7	9.5	43.4	9.3	7.6	183.1
	4/28	100.0	3.0	43.5	37.2	11.8	16.2	14.2	4.4	4.0	7.4	42.5	10.5	6.7	206.0
	PD_{LT}	0.001				0.003	0.001						0.001	0.02	0.001
	PD_{dev}	0.001				0.07									
Ranger	3/31	0.0	1.8	39.1	35.4	4.7	8.7	10.4	4.1	4.9	12.2	37.5	7.0	8.9	137.9
	4/14	98.3	2.4	47.0	42.9	5.4	6.8	10.5	6.5	5.5	17.3	45.3	7.8	9.6	152.9
	4/28	100.0	2.8	46.3	41.6	7.4	9.1	8.5	4.3	5.0	19.8	44.9	8.3	8.8	162.4
	PD_{LT}	0.001	0.02	0.005	0.02	0.03					0.001	0.003	0.02		0.02
	PD_{dev}	0.001		0.05	0.04				0.02			0.04		0.03	
Clearwater	3/31	0.0	2.6	39.5	34.7	8.8	15.7	16.5	4.8	2.2	4.1	38.6	9.4	6.7	184.3
	4/14	91.7	2.8	41.7	37.1	9.8	17.2	15.6	6.0	3.7	4.1	41.5	10.2	6.7	199.3
	4/28	100.0	3.2	39.1	34.6	10.1	15.0	14.3	4.2	3.2	6.0	39.1	8.3	6.7	188.5
	PD_{LT}	0.001													
	PD_{dev}	0.001							0.09						
Umatilla	3/31	0.0	2.4	54.3	49.3	7.7	12.3	16.8	7.9	3.4	12.7	52.7	10.4	8.3	204.4
	4/14	85.0	2.9	55.6	51.4	7.6	13.0	17.2	7.2	6.1	15.1	54.8	10.6	8.4	208.6
	4/28	100.0	3.3	52.6	44.7	14.9	19.3	17.3	6.1	3.9	8.8	51.4	12.7	6.6	249.4
	PD_{LT}	0.001	0.03		0.06	0.001	0.001		0.09	0.002	0.08		0.001	0.001	0.001
	PD_{dev}	0.001			0.04	0.001	0.06				0.03		0.04	0.007	0.04
	LSD $_{0.05}$	6.4	0.8	4.8	4.7	1.0	1.5	2.5	1.2	1.5	4.2	4.5	1.0	0.8	24.3
	CV		0.06	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001
	PD_{LT}	0.001	0.002			0.001	0.001					0.09	0.001	0.002	0.001
	PD_{dev}	0.001		0.05	0.002	0.001			0.004	0.02		0.007		0.004	
	CV x PD	0.06				0.005	0.004			0.08	0.004		0.03	0.03	0.03
CV Avg	3/31	0.6c	2.4b	44.3b	39.2b	3.3b	5.2b	14.3a	5.4b	4.5ab	9.8a	42.5b	8.9c	7.9a	174c
	4/14	91b	2.7ab	47.0a	42.8a	3.4b	5.6b	14.2a	6.4a	5.0a	11.5a	46.3a	9.5b	8.1a	186b
	4/28	100a	3.1a	45.4ab	39.5b	4.9a	6.6a	13.6a	4.8b	4.0b	10.5a	44.5ab	10.0a	7.2b	202a

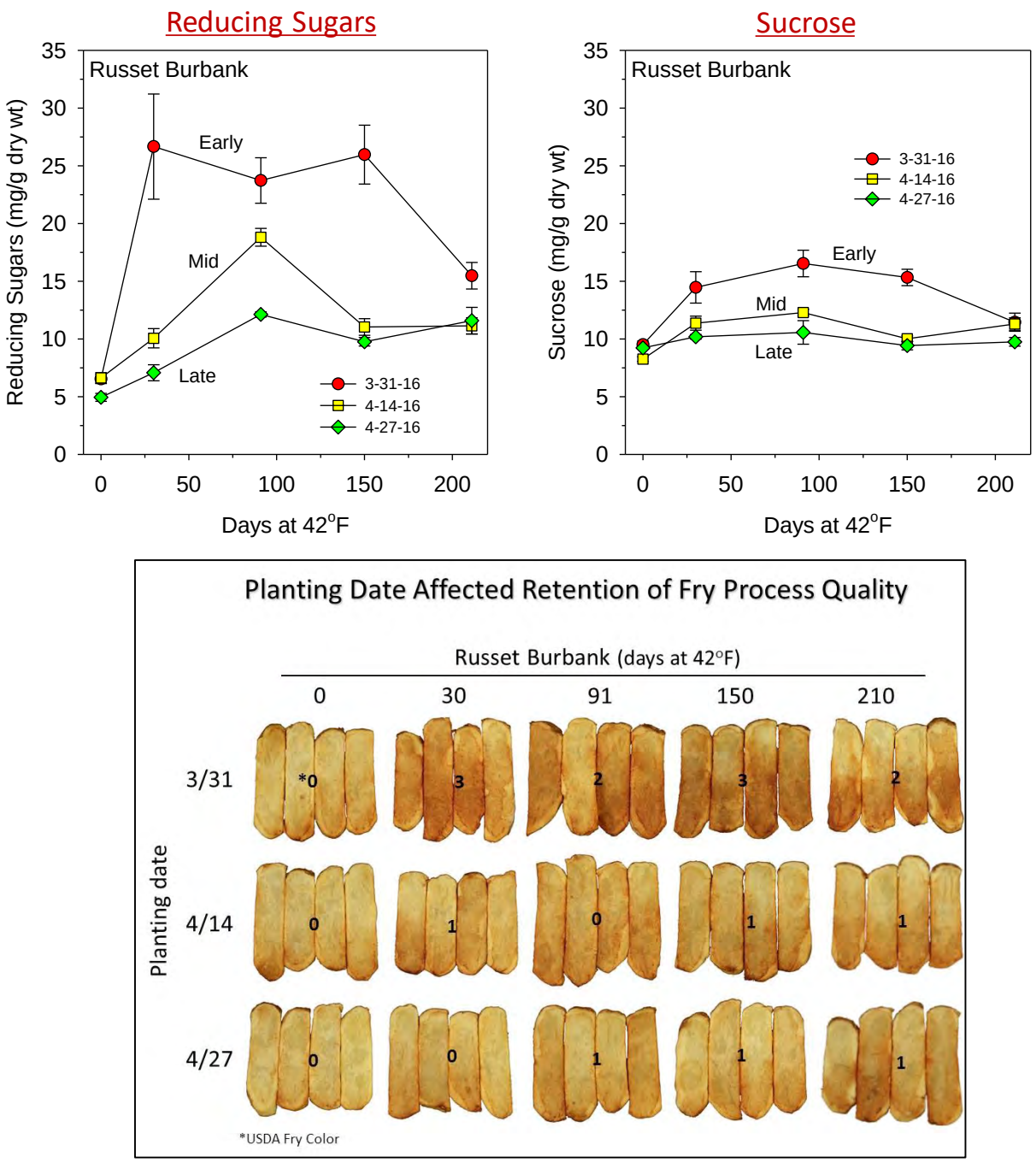


Fig. 4. Effects of planting date on changes in reducing sugar and sucrose concentrations of Russet Burbank tubers during storage at 42°F (2016/17 storage season). The 2016 Russet Burbank trial followed similar timelines as depicted for the 2017 study outlined in Fig. 3. Vines were mowed 8/25, 9/8 and 9/22 (147 DAP) and plots were harvested on 9/2, 9/16, and 9/30. Bottom photo depicts changes in fry color over the 210-day storage period. Each fry plank represents a different tuber and the 5 fry planks per treatment were chosen from a 12-tuber sample to represent the average process color. Numbers on fries indicate USDA color values of the stem ends. Fries are oriented bud end up and stem end down.

Effects of Vine Kill Date on U.S. No. 1 Yield Depend on Cultivar (2016 & 17, Othello WA)

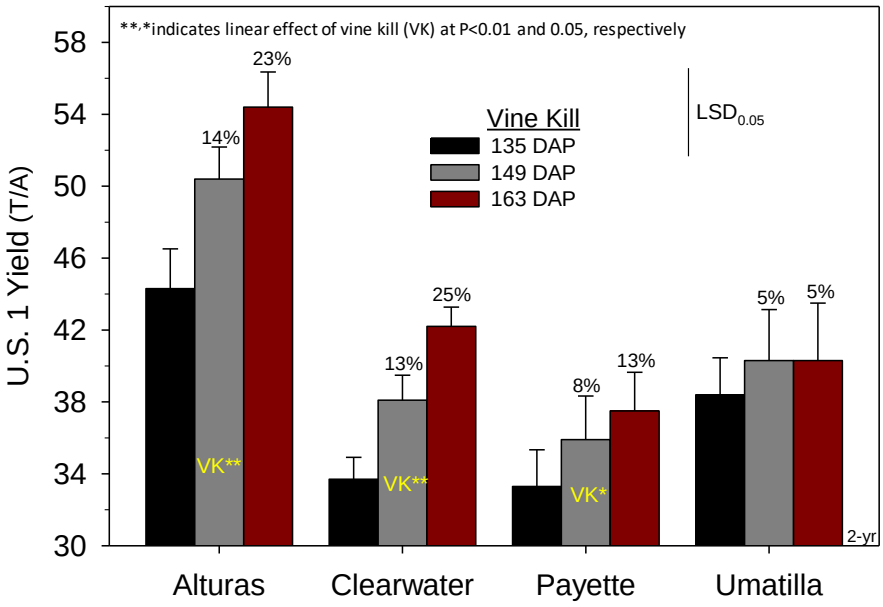
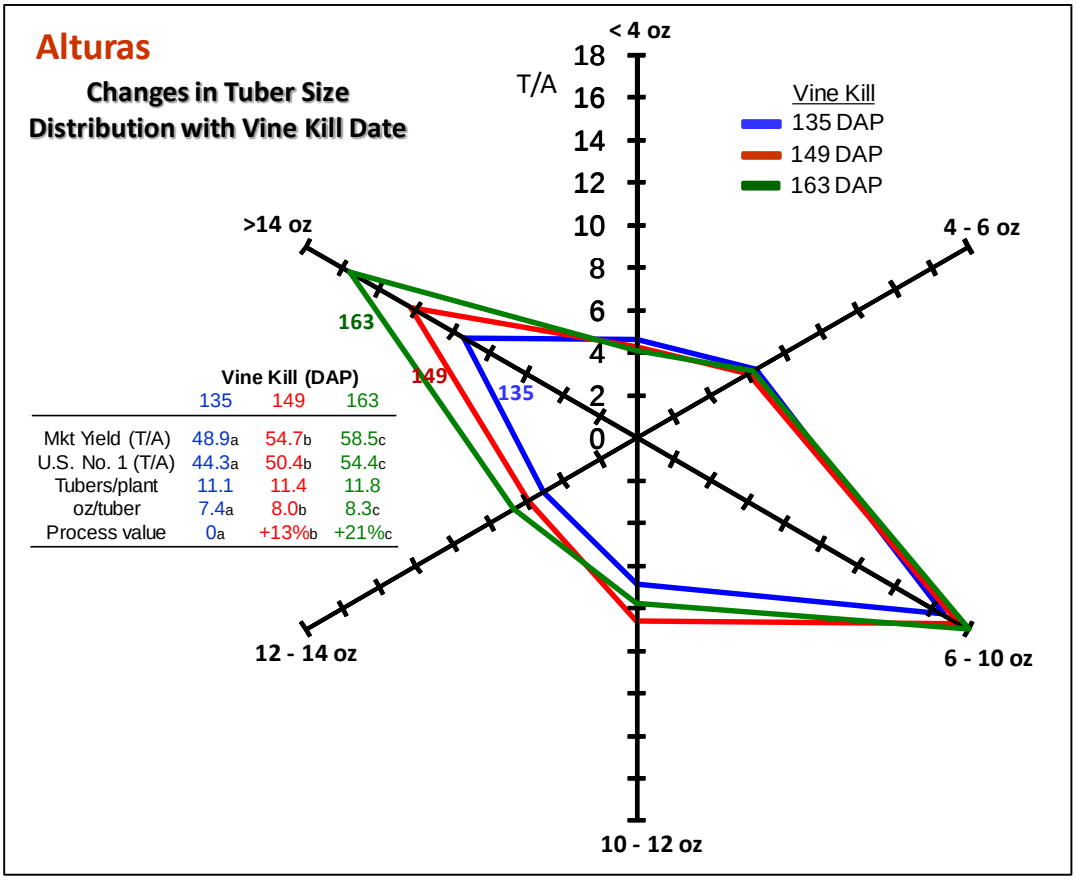
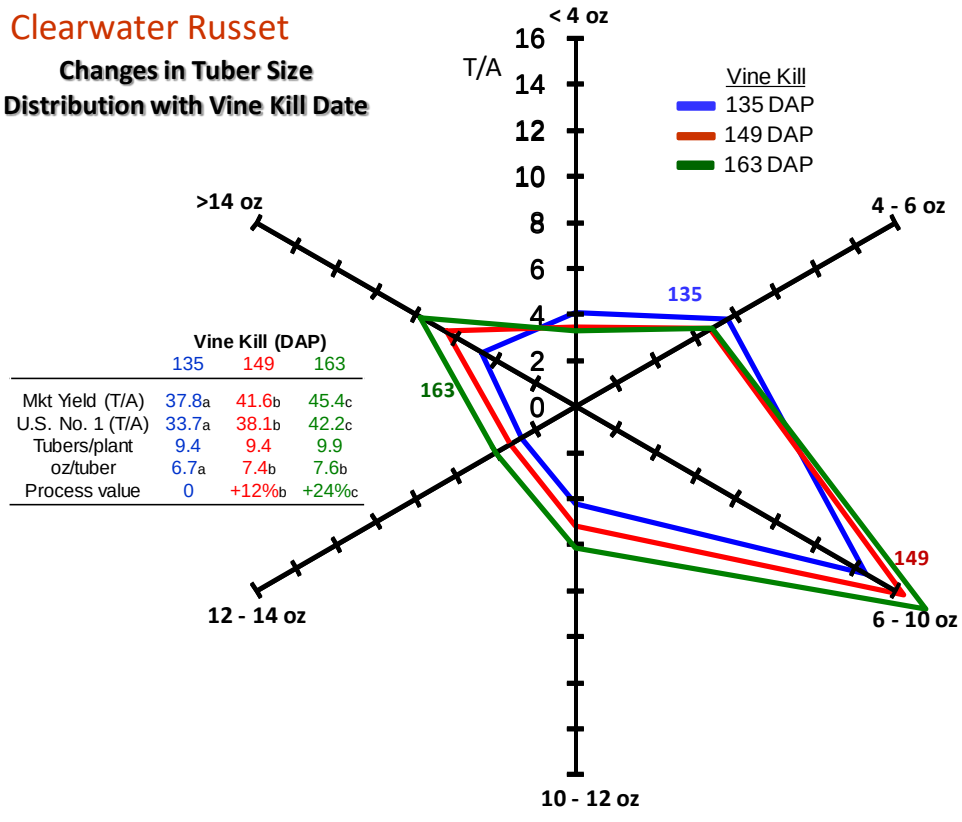


Fig. 5. Changes in U.S. No. 1 yield with vine kill date for Alturas, Clearwater, Payette, and Umatilla Russet grown at Othello, WA in 2016 and 2017 (2-yr average). Planting dates were 4/16/16 and 4/12/17, vines were mowed 135, 149, and 163 DAP. Plots were harvested 164 DAP (Sept. 27) in 2016 and 169 DAP (Sept. 28) in 2017. Effects of vine kill date on tuber size distributions and process value (relative to 135 DAP VK) are depicted for each cultivar in the polygonal diagrams below.



Clearwater Russet

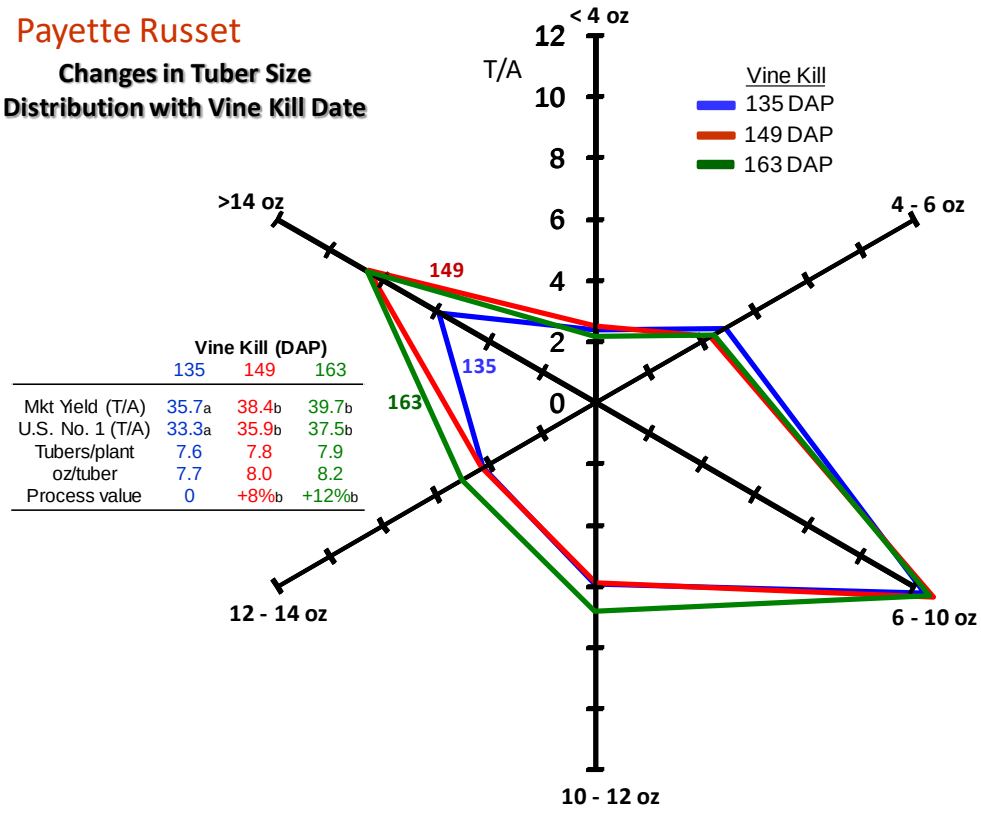
Changes in Tuber Size Distribution with Vine Kill Date



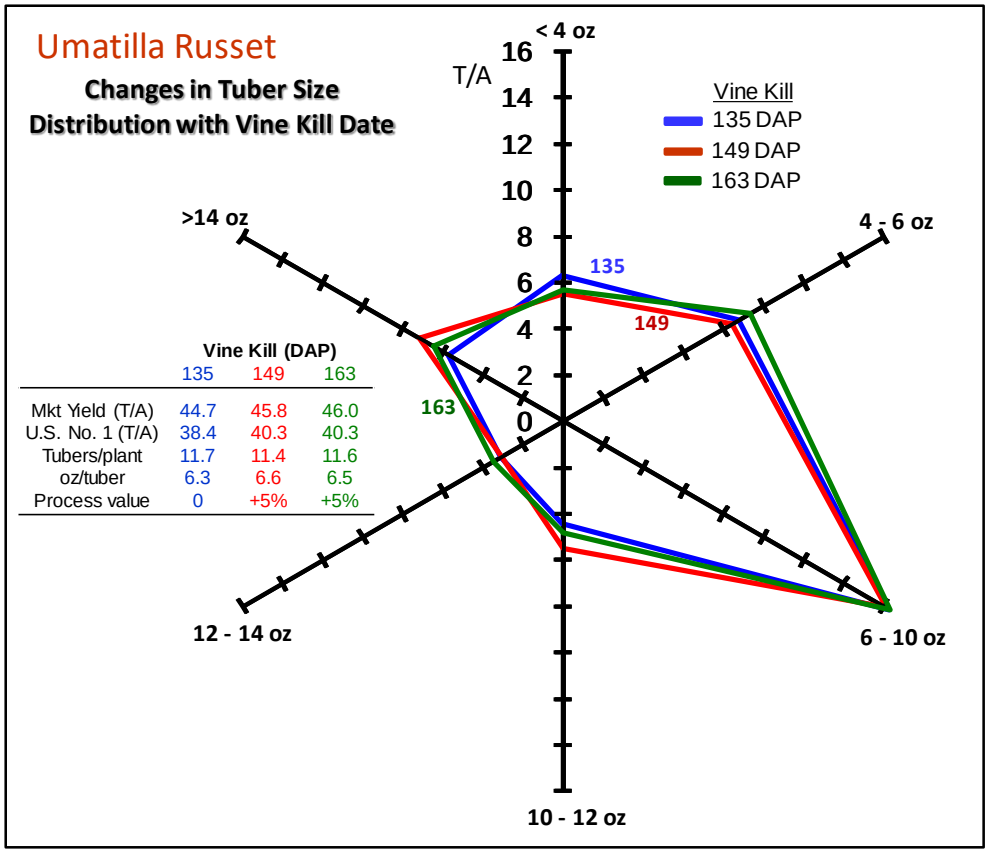
	Vine Kill (DAP)		
	135	149	163
Mkt Yield (T/A)	37.8a	41.6b	45.4c
U.S. No. 1 (T/A)	33.7a	38.1b	42.2c
Tubers/plant	9.4	9.4	9.9
oz/tuber	6.7a	7.4b	7.6b
Process value	0	+12%b	+24%c

Payette Russet

Changes in Tuber Size Distribution with Vine Kill Date



	Vine Kill (DAP)		
	135	149	163
Mkt Yield (T/A)	35.7a	38.4b	39.7b
U.S. No. 1 (T/A)	33.3a	35.9b	37.5b
Tubers/plant	7.6	7.8	7.9
oz/tuber	7.7	8.0	8.2
Process value	0	+8%b	+12%b



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TITLE: Methods of sprout and disease suppression of potatoes in storage

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Mary Jo Frazier, Support Scientist 3, UI Kimberly R & E Center
Cooperators: Andrew Hollingshead, PhD Graduate Student, University of Idaho
Mike Thornton, UI Parma R & E Center
Rick Knowles, Washington State University

REPORTING PERIOD: July 1, 2016-June 30, 2017

ACCOMPLISHMENTS

The first objective investigated early storage management temperature conditions on tuber quality response. This year's studies have helped to better understand the wound healing process and the impact to quality in three different cultivars (Russet Norkotah, Bannock Russet and Umatilla Russet). Fry color of Russet Burbank, Bannock Russet, Umatilla Russet, and Russet Norkotah was significantly darker if cured at 45°F compared to the other curing temperatures after 2 months in storage. Glucose levels were similarly impacted by the curing temperatures and demonstrates the influence of early storage management temperatures on overall quality. This study provides insight on processing quality, Fusarium dry rot development, wound healing, and weight loss as impacted by variety and curing temperature. Grower storage evaluations aid in understanding the dynamics associated with weight loss and disease development in storage. Seven varieties were grown and harvested for on-going studies evaluating for susceptibility to Pythium leak and bruise. Additional bruise and handling experiments were conducted. This includes various tuber pulp temperatures and drop heights and impacts to develop preliminary data for the seven varieties. This will help develop early storage management recommendations based upon damage and decay susceptibility.

RESULTS:

Objective 1: Investigate early storage management temperature conditions on tuber quality response.

Early storage conditions or "curing" conditions were evaluated for response to quality parameters in storage. Whole tubers (Russet Burbank, Bannock Russet, Umatilla Russet, and Russet Norkotah) were cured after harvest at 45, 55, or 65°F for two weeks then temperatures were ramped (0.5F/day) to a final holding temperature of 48°F. Weight loss, glucose and sucrose concentrations, and fry-color determinations were made at several points in the storage period. Weight loss was monitored throughout storage. Additional tubers of the varieties along with Clearwater Russet and Ranger Russet tubers were bruised by dropping through a staged drop box, inoculated with Fusarium dry-rot (mixture of 5 isolates at $1*10^5$ cfu/ml), and cured in the same conditions. Temperatures were ramped to 48°F and samples were stored for 5 months. Disease evaluation was conducted by quartering each tuber and assessing percent rot in each tuber.

Umatilla Russet had significantly higher weight loss than Russet Burbank, Bannock Russet, or Russet Norkotah (Table 1; Fig. 1). Curing at 65°F resulted in a trend ($p=0.1$) for higher weight loss compared to curing at 45 or 55°F (Table 2). There was a weak variety by temperature

interaction in weight loss at the end of storage so data is presented by variety (Fig. 1). The curing treatment had no significant effect on weight loss in Russet Burbank, Bannock Russet, or Russet Norkotah. In Umatilla Russet, the high temperature curing resulted in significantly greater weight loss. In the first week at the curing temperatures, potatoes lost 26.3% (45°F) to 22.0% (65°F) of the total weight loss in storage. This increased to a range of 40 to 43% after the first month in storage.

Cold temperature curing (45°F) immediately resulted in higher glucose (Fig. 2) in all varieties and the high glucose level persisted through approximately 224 days of storage except in Umatilla Russet which had no significant difference in glucoses concentration at the final evaluation. Depending upon variety and time in storage, 65°F curing had lower glucose concentrations compared to 55°F curing. Cold temperature curing (45°F) also resulted in darker fry color (Table 3) in all varieties. In Russet Norkotah and Umatilla Russet each curing temperature resulted in different fry color after curing. Warmer curing (65°F) generally had similar fry color to 55°F curing, except for Russet Norkotah and Umatilla Russet after the 2 week curing period only. This stresses the impact of early storage management on long-term quality of process potatoes.

In tubers bruised and inoculated with Fusarium dry rot evaluated at the end of the storage season showed higher severity (Table 4) in Clearwater Russet and Bannock Russet than in Russet Burbank, Ranger Russet, or Umatilla Russet. Russet Norkotah has significantly less rot severity than all other varieties. Norkotah had a lower incidence of dry rot than all other varieties while Bannock Russet, Clearwater Russet, Ranger Russet, and Umatilla Russet had higher incidence than Russet Burbank. Sixty-five degree curing showed significantly less tuber dry rot severity and incidence of rot 5% or more (Table 5) than 45 or 55°F curing. The effects of the curing treatment varied by variety (Table 6). Higher temperature curing resulted in lower disease severity and incidence in Russet Burbank, Clearwater Russet and Russet Norkotah but had no significant effect on Bannock Russet, Ranger Russet and Umatilla Russet. Additional research is needed to repeat this association of curing temperatures and dry rot development in relation to wound healing.

Table 1. Percent weight loss in three varieties after curing at 45, 55, and 65°F and storage for 8 months at 48°F.

Russet Burbank	**Bannock Russet**	**Russet Norkotah**	**Umatilla Russet**
6.6 % b	7.8 % b	7.0 % b	10.9 % a
Tubers were cured for 2 weeks, ramped +/- 0.5°F/day. Final holding temperature of 48°F. Different letters within row designate significance at $P<0.05$			

Table 2. Percent weight loss in three varieties after curing at 45, 55, and 65°F.

Curing Temperature	Percent Weight Loss
45°F	7.6 % ab
55°F	7.5 % b
65°F	9.1 % a
Tubers were cured for 2 weeks, ramped +/- 0.5°F/day. Final holding temperature of 48°F. Different letters within a column designate significance at *P<0.10.*	

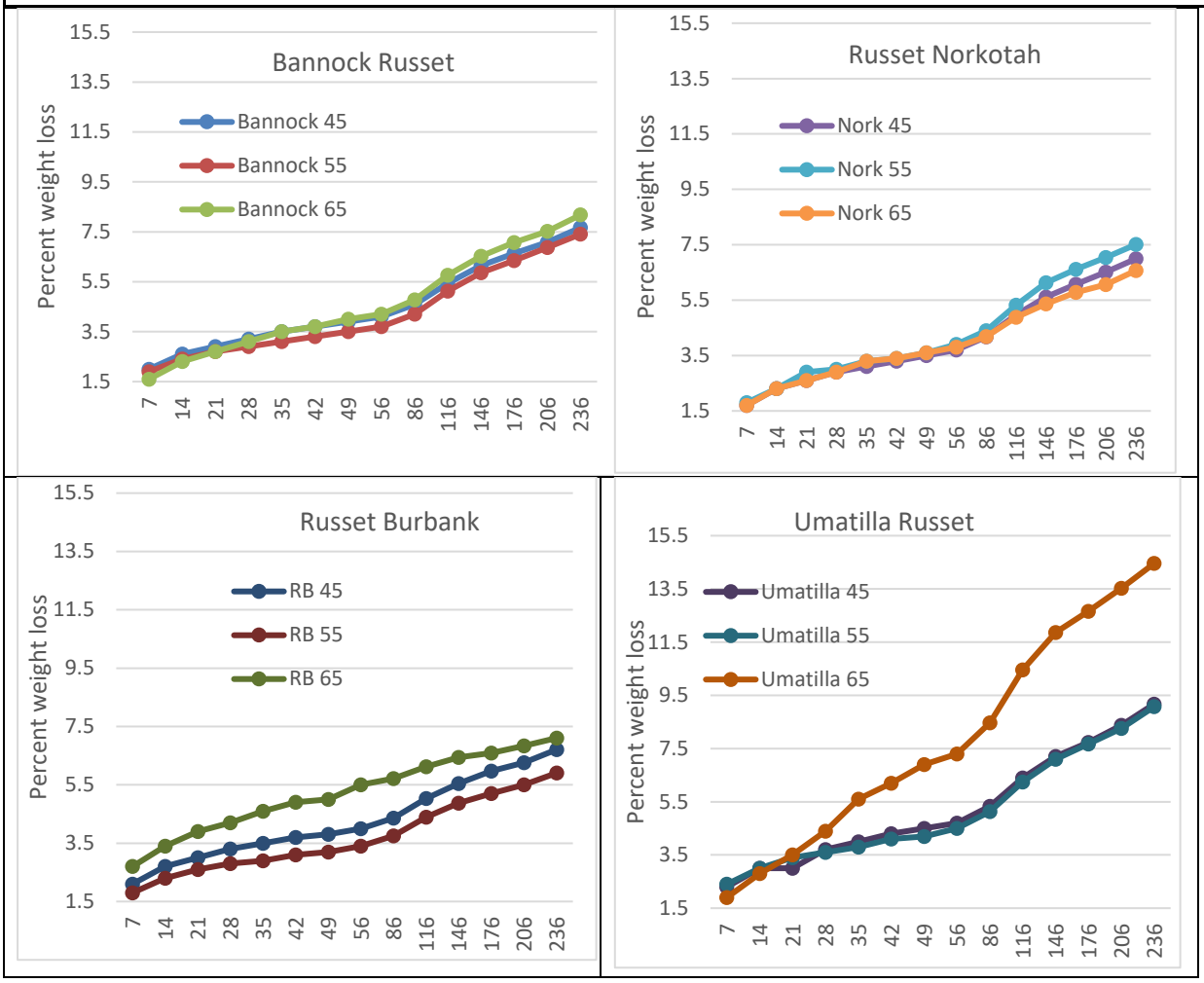

Fig. 1. Percent weight loss in samples cured for two weeks at 45, 55, or 65°F over time in storage at 48°F.

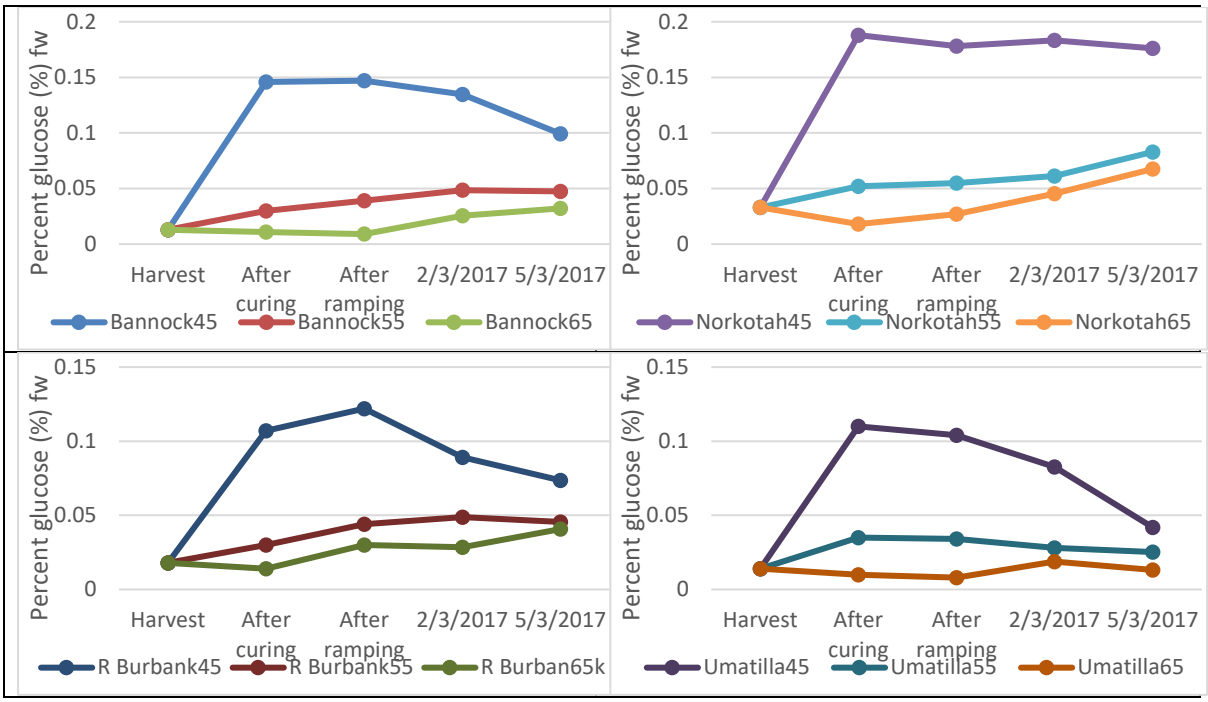

Fig. 2. Glucose concentrations of tubers cured for two weeks at 45, 55, or 65°F and stored at 48°F.

Table 3. Stem-end fry color (reflectance %) throughout 48°F storage as influenced by curing temperatures. Different letters within a column within a variety designate significance at *P<0.05.*

		Stem-end reflectance (%)				
		Days after harvest				
Variety	Curing temp	**2**	**15**	**54**	**135**	**224**
Bannock	**45F**	60	37 b	33 b	40 b	42
Bannock	**55F**		56 a	52 a	47 ab	50
Bannock	**65F**		61 a	57 a	53 a	53
Norkotah	**45F**	54	30 c	27 b	27 b	28 b
Norkotah	**55F**		48 b	45 a	40 a	38 a
Norkotah	**65F**		55 a	49 a	42 a	40 a
Burbank	**45F**	57	40 b	36 b	36	36
Burbank	**55F**		51 a	47 a	44	44
Burbank	**65F**		56 a	48 a	47	43
Umatilla	**45F**	60	42 c	42 b	46 c	50
Umatilla	**55F**		56 b	56 a	51 b	55
Umatilla	**65F**		60 a	56 a	56 a	54
* 2 Days After Harvest sample used freshly harvested tubers. Tubers were not placed in a curing treatment at this time. Means of 10 tuber samples are presented.						

Table 4. Dry rot severity and incidence after bruising, inoculating, and curing at three temperatures and 8 months of storage (48°F). Different letters within a column designate significance at *P<0.05.*

Variety	Severity %	Incidence of rot 5% or greater
R. Burbank	23 c	62 c
Ranger Russet	26 bc	75 b
Clearwater Russet	40 a	81 a
Bannock Russet	42 a	87 ab
Russet Norkotah	14 d	34 d
Umatilla Russet	28 b	76 b

Table 5. Dry rot severity and incidence after bruising, inoculating, and curing at three temperatures and 8 months of storage (48°F). Letters indicate significant differences between treatments within a column at α=0.05.

Curing temperature	Severity %	Incidence of rot 5% or greater
45°F	30 b	75 a
55°F	34 a	78 a
65°F	22 c	55 b

Table 6. Dry rot severity and incidence after curing at three temperatures and 8 months of storage (48°F). Letters indicate significant differences between treatments within each variety at α=0.05.

Variety *Curing Temp	Severity %	Incidence of rot 5% or greater
Russet Burbank 45°F	29 a	77 a
Russet Burbank 55°F	29 a	80 a
Russet Burbank 65°F	10 b	30 b
Bannock Russet 45°F	37	86
Bannock Russet 55°F	51	91
Bannock Russet 65°F	37	68
Clearwater Russet 45°F	43 a	94 a
Clearwater Russet 55°F	50 a	94 a
Clearwater Russet 65°F	25 b	71 b
Norkotah Russet 45°F	18 a	45 a
Norkotah Russet 55°F	18 a	44 a
Norkotah Russet 65°F	6 b	12 b
Ranger Russet 45°F	20	72
Ranger Russet 55°F	26	74
Ranger Russet 65°F	31	80
Umatilla Russet 45°F	30	78
Umatilla Russet 55°F	30	83
Umatilla Russet 65°F	25	67

Weight Loss in Commercial Storages

In 2016, at harvest, 30-35-lb bags of Russet Burbank, Bannock Russet, or Umatilla Russet potatoes were placed in each of three grower storages. Initial weight was recorded at harvest and end weight recorded when storage bins were unloaded. Overall weight loss was recorded for each sample bag (Fig. 3). In addition, samples were taken at harvest at various locations in the handling equipment to assess for bruise and pulp temperatures.

In the Bannock Russet storage, Pythium leak was excessive. The disease prevented the storage from being held for long-term and no weight loss bags were collected. On the two days we visited the storage during harvest we recorded pulp temperatures from 55-58°F and infrared gun temperatures of 55-60°F. Ten tuber samples were collected at several locations in the storage

loading operation. These samples were later peeled and scored for blackspot bruise and shatter bruise. The samples collected from the truck had an average of 0.7 blackspot bruises per tuber with a 30% incidence while the sample "after the even-flow" had 1.4 blackspot bruises per tuber and incidence of 70%. For shatter bruise the truck sample had 1.5 shatter bruises per tuber and an incidence of 50% while the "after even-flow" sample had 1.6 shatter bruises per tuber and an incidence of 90%. The final conveyor sample had average 1.4 blackspot and 1.9 shatter bruises per tuber with 50% blackspot and 70% shatter incidence. The samples were not replicated and there was a good deal of variability however significant bruising was occurring due to the sorting/piling operation. The results stress the susceptibility of Bannock Russet to shatter bruise and producing entry points for Pythium leak. Additional research is in progress to assess management for Bannock Russet.

In the Umatilla Russet storage, disease prevented long-term storage. Some stored samples were retrieved but only from the first day of sampling 9/27/16. Weight loss averaged 7.4% after approximately 145 days in storage. Weight loss varied in the storage and ranged from 3.5 to 14.1%. Soft rot and Pythium leak were apparent in the retrieved samples. Significant blackspot and shatter bruise was evident after two major drops in the conveyor system (data not shown). This stresses the need to minimize all drops in handling and the greater susceptibility of Umatilla Russet to bruising.

The Russet Burbank storage was held for the entire season until July 17, 2017 (301 days after harvest). Twenty bags were placed in the storage. Weight loss varied from 3.3 to 11.9% (Figure 3) with an average weight loss of 5.9% after 301 days of storage. Of the 5.9% weight loss, 1.8% was attributed to rot. Samples were placed in storage on 4 days, Sept 19, 21, 27, and 28. Sept 19 was very warm, pulp temperatures were measured from 62-70°F. Weight loss for this day was highest (7.2%). Mean weight loss on the other sampling days was less than 6% (5.7, 5.4, and 5.3 % for 9/21, 9/27, and 9/27 respectively). Rot was highest from tubers harvested 9/27 (3.1%) when tubers delivered to storage were noted as being colder and muddy. Generally, rot was very low in the storage. In addition, on September 19th, samples were pulled at several locations during the handling into storage process to assess for bruising (Figure 4). Blackspot bruising occurred at all locations and significant levels were seen from the truck.

Overall, conditions of the harvest day, storage management, and tuber conditions contributed to the weight loss potential in storage. A grower could place sample bags on the top of the pile to obtain a general idea of weight loss in the storage. Additional correlations need to be made between pulp temperatures and early temperature regimes and weight loss. In addition, the use of accumulated heat units as a tool in commercial storages to determine the end of curing needs to be established to minimize weight loss and disease development, yet in balance with processing quality.

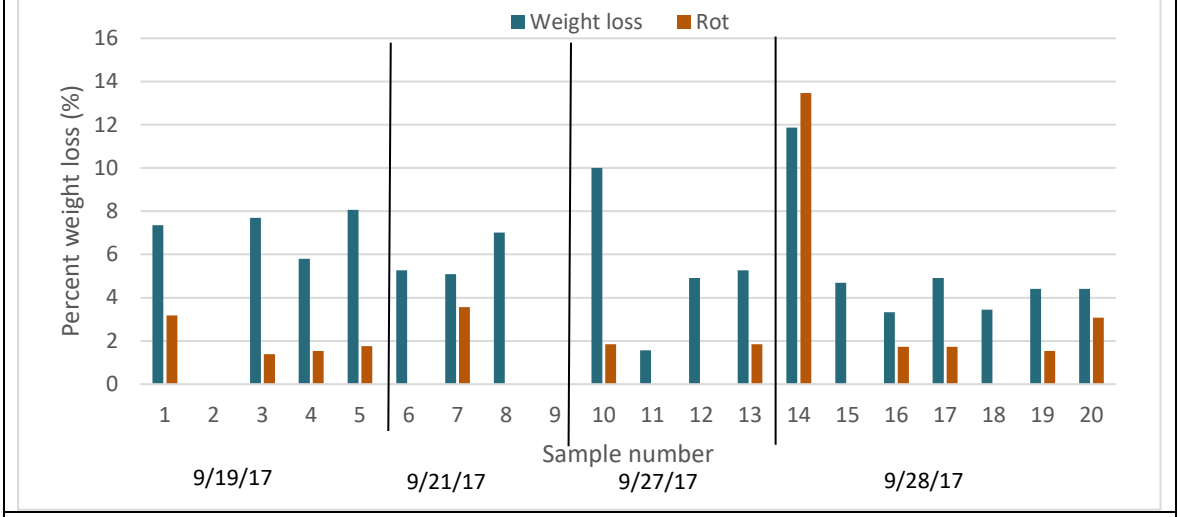

Fig. 3. Weight loss (%) in a Russet Burbank commercial storages during the 2016-17 season. Stored for 301 days.

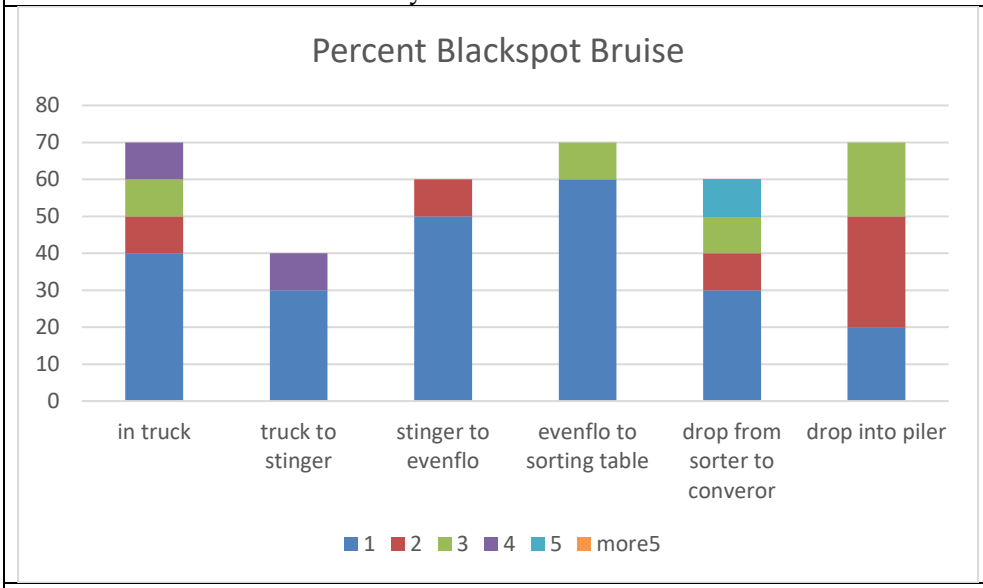

Fig. 4 Percent blackspot bruise from the Russet Burbank storage sampled 9/19/17

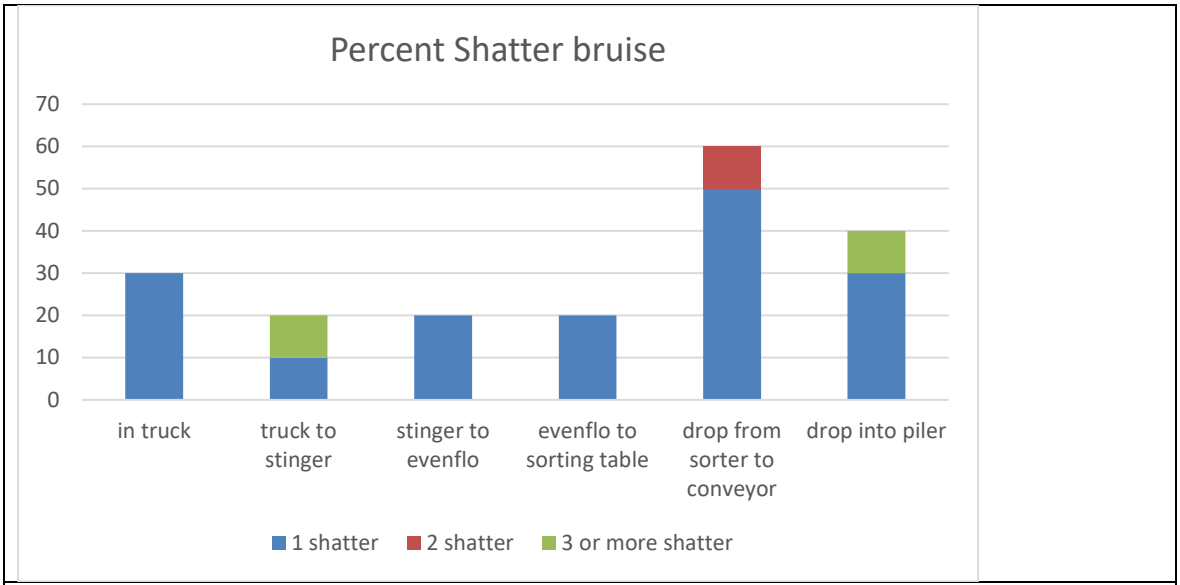

Fig. 4 Percent shatter bruise from the Russet Burbank storage sampled 9/19/17

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Olsen, N. and M. Thornton. 2016. Battered and Bruised: Managing bruising during harvest. Potato Grower Magazine 45(9):30-1. September 2016.

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Olsen, N., N. Gudmestad, J. Miller and Y. Wang. 2017. Storage Management Workshop. Idaho Potato Conference. Pocatello, ID, January 18, 2017.
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Annual Progress Report

TITLE: Long-Term Impacts of Manure Application on Production of Potato and Other Crops

PERSONNEL: April Leytem, Research Soil Scientist, Kimberly

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ACCOMPLISHMENTS:
We have completed the fifth year of this eight-year dairy manure application study. Dairy manure was applied at rates of 20, 40, and 60 ton/acre (wet weight basis) either annually or biennially from 2012 to 2017 on irrigated research plots located on the USDA-ARS research station in Kimberly, Idaho. As potatoes were not grown in the fifth year of this study, only grain yields will be discussed in this progress report.

Soil nutrient accumulations

In the fifth year of this eight year study, soil nutrients continued to accumulate with increasing manure rates and/or manure application frequency (Table 1). In year 5 (March 2017), soil Olsen P levels increased over the code 590 threshold of 40 ppm for both annual and biennial applications of dairy manure at rates of 40 and 60 wet ton/acre, suggesting these application rates and frequency are greater than P removal potential of the barley, sugar beets, wheat, and potatoes that had been grown in this field since 2013. In addition, there were elevated levels of P in the second foot depth for the annual 40 and 60 wet ton/acre as well as the biennial 60 wet ton/acre treatments, suggesting that P saturation is occurring in the first foot of soil with P leaching through the soil profile (data not shown). Elevated soil K and EC are also prevalent with high manure application rates and may become a concern for salt sensitive crops. There were also increases in total N, nitrate, and soil organic matter with increasing rate of manure application at both the annual and biennial application frequencies. Relationships between soil nutrients and grain yield components are currently being evaluated.

Barley yields and quality

The 2017 barley grain yields and select yield components are listed in Table 2. Grain yield was greater for all treatments compared to the control, however there was no difference among treatments. Lodging ratings were higher than the fertilizer and control treatments for all manure treatments, with the 20 ton/acre biennial treatment being lower than the other manure treatments. Grain protein was greater in all manure treatments compared to the control but there were few differences among manure treatments. Both plant height and number of heads per square foot were greater for all treatments compared to the control with no difference between treatments (data not shown). We are currently evaluating the impact of soil nutrient accumulations on yield components to further understand how and why manure application affect these parameters.

Wheat yields and quality

The 2017 wheat grain yields and select yield components are listed in Table 2. Grain yield was greater for all treatments compared to the control, however there was no difference among treatments. Lodging ratings were higher than the fertilizer and control treatments for the biennial 60 ton/acre as well as the annual 40 and 60 ton/acre treatments with no difference among manure treatments. Grain protein increased with increasing manure application rates at both biennial and annual application timings, and were higher for all the manure treatments compared to the fertilizer and control. There were more heads per 3' row for all treatments compared to the control but no difference in number of kernels per head (data not shown). All treatments except for the 60 ton/acre had higher plant height than the control (data not shown). We are currently evaluating the impact of soil nutrient accumulations on yield components to further understand how and why manure application affect these parameters.

Table 1. Spring 2017 preplant soil nutrient and salts response to repeated dairy manure applications in Kimberly, Idaho at the 0-12 inch soil depth.

Manure rate (dry ton/acre)	Application Frequency	Soil Total N (%)	Soil Organic Matter (%)	Soil Nitrate-N (ppm)	Soil (Olsen) P (ppm)	Soil (Olsen) K (ppm)	Soil EC (ds/m)
Control	NA	0.09 e	1.3 e	5.6 e	9 e	130 f	0.53 e
Fertilizer	NA	0.09 de	1.5 de	6.9 e	16 e	126 f	0.62 e
20	Biennial (Applied in 2012 and 2014)	0.11 cd	1.6 cd	10.7 d	27 de	254 e	0.89 d
40		0.12 c	1.8 c	17.1 c	45 d	398 d	1.29 c
60		0.14 b	2.2 b	20.5 b	84 c	633 c	1.66 b
20	Annual (Applied in 2012, 2013, and 2014)	0.12 c	1.7 cd	12.4 d	39 d	380 d	0.98 d
40		0.16 a	2.4 b	20.8	107 b	766 b	1.44 c
60		0.18 a	2.7 a	25.4 a	154 a	1101 a	1.89 a
p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

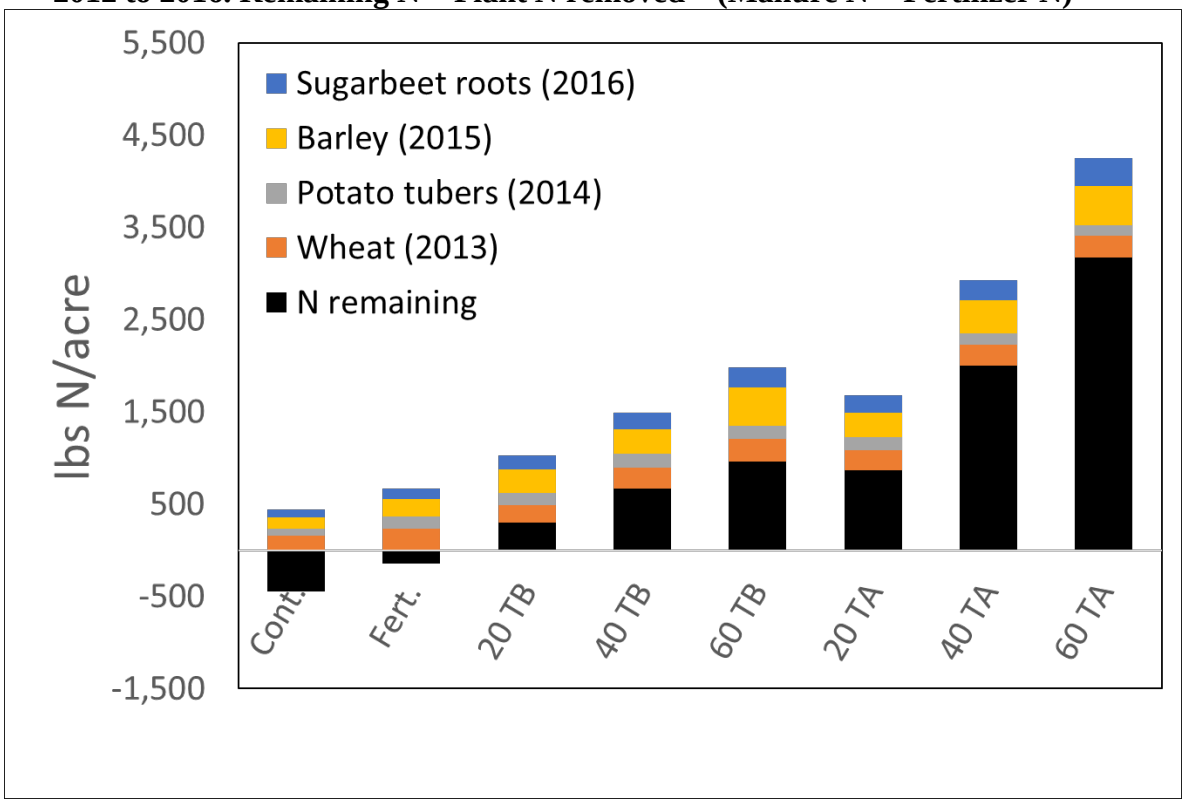
Table 2. Wheat and barley responses to manure study treatments in 2017, year 5 of an eight year long term dairy-manure application study in Kimberly, Idaho.

Manure rate (dry ton/acre)	Application Frequency	Jefferson Hard red spring wheat			Moravian-69 Spring malt barley		
		Yield (bu/acre)	Protein (%)	Lodging rating (1-9)	Yield (bu/acre)	Protein (%)	Lodging rating (1-9)
Control	NA	61 b	10.0 e	0.8 c	64 b	9.1 d	1.0 c
Fertilizer	NA	116 a	12.6 d	1.8 bc	131 a	9.4 cd	1.3 c
20	Biennial (Applied in 2012 and 2014)	112 a	13.6 c	3.0 abc	132 a	9.8 abc	3.3 b
40		106 a	14.4 abc	4.5 ab	133 a	9.6 bc	6.3 a
60		112 a	14.9 ab	5.0 a	128 a	10.0 ab	7.3 a
20	Annual (Applied in 2012, 2013, and 2014)	109 a	13.9 bc	4.0 ab	137 a	9.9 abc	6.8 a
40		109 a	14.9 ab	5.0 a	137 a	9.6 bc	7.0 a
60		103 a	15.1 a	5.3 a	126 a	10.2 a	7.5 a
p-value		<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001

Plant nitrogen removal and uptake over 4 years by crop

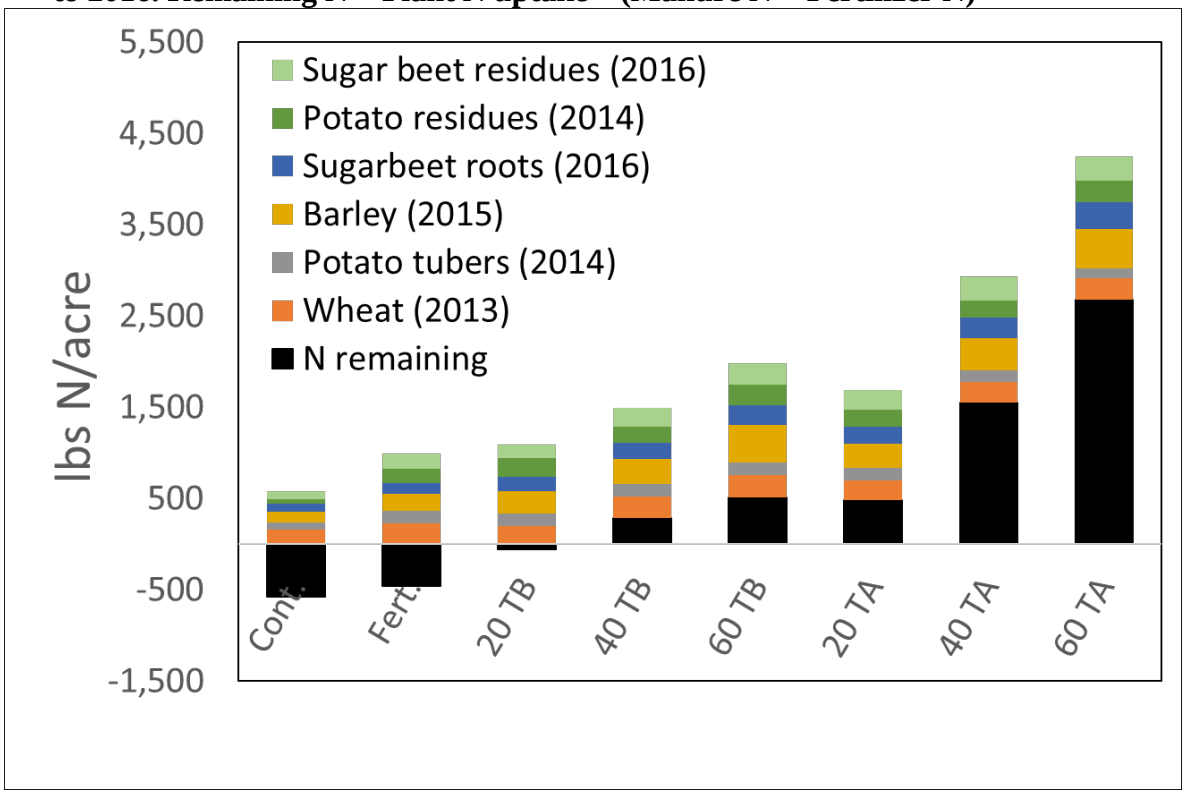
Plant N removal (material harvested and removed from field) was calculated over the four-year rotation and is presented in Figure 1. Both sugarbeets and barley showed a response in plant N uptake, with N uptake increasing with manure application rate. Nitrogen uptake by wheat and potatoes did not show this same trend. Both the control and fertilizer treatments had negative "N remaining" which is defined as the amount of N removed vs. what was applied with manure and fertilizer. In these instances, N is being mined out of the soil. All manure applications treatments had more N remaining than taken up by the plants with increasing N remaining with increasing manure application rate. This excess N could contribute to nitrate leaching and groundwater quality issues in the future.

Figure 1. Plant nitrogen removal response to repeated dairy manure applications from 2012 to 2016. Remaining N = Plant N removed – (Manure N + Fertilizer N)



The plant nitrogen uptake data (all N taken up by plants but not necessarily removed from field) are shown in figure 2. When evaluated on an uptake basis, there is less of a surplus of nitrogen left in the field compared to what was applied. Significant amounts of nitrogen are taken up by both sugarbeet tops and potato vines. If this biomass is removed from the field it substantially reduces the nitrogen remaining in the field that could be subject to loss via leaching. Finding ways to utilize these crop residues and remove them from production fields would help to reduce the environmental impact of these treatments.

Figure 2. Plant nitrogen uptake response to repeated dairy manure applications from 2012 to 2016. Remaining N = Plant N uptake – (Manure N + Fertilizer N)



PROJECTIONS:

Results from these studies will allow us to develop clear recommendations for Idaho potato growers on how to get the most out of their manure application without compromising crop production or soil quality.

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McKinney, C., R.S. Dungan, A.B. Leytem, and A.D. Moore. 2018. Occurrence and abundance of antibiotic resistance genes in agricultural soil receiving dairy manure. FEMS Microbial Ecol. *In press.*

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Annual Report
to
Northwest Potato Research Consortium

BKK918, BKK036 & BKK037are independent projects but connected with each other toward the same long-term goal. Thus, the results of three projects are presented as one report.

TITLES:

BKK918 [fiscal 2016 - 2017]: Characterizing starch and its digestibility of Tri-State potato varieties for future cultivar development

PI: Lin
Co-PI (funding): Thornton
Co-PI (non-funding): Bohlscheid

BKK036 [fiscal 2017 - 2018]: Development of a sensory texture profile of French fries for identifying its correlation with starch properties

PI: Lin
Co-PI (funding): Thornton & Hopfer
Co-PI (non-funding): Bohlscheid, & Cantley

BKK037 [fiscal 2017 - 2018]: Identifying potato macromolecular and microstructural features associated with sensory qualities of French fries

PI: Lin
Co-PI (non-funding): Thornton, Olsen, Novy, & Bohlscheid

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Catherine Cantley, Senior Manager, Sensory and Product Development, J.R. Simplot Company, 16733-E Simplot Blvd, Caldwell, ID, 208-780-4262, Catherine, cantley@simplot.com

REPORTING PERIOD:

July 1, 2017 – January 30, 2018

SUMMARY OF ACCOMPLISHMENTS:

A major problem in the potato industry is the quality stability and sustainability. To solving the problem, a reliable assessment tool is needed for variety developer, growers, and processors. Our short-term research goal is to *identify measurable structural features associated with sensory attributes of French fries*. We have collected two years data of starch characteristics of selected varieties grown at Parma Research and Extension Center at the University of Idaho in 2016 and 2017; a few tubers were also collected in 2015 and the data were included in this report. Our data confirm that **specific gravity is not an accurate measurement to assess potato quality associated with sensory attributes.** Dry matter and starch content calculated from specific gravity (referred to as "estimated content" in this report) do not agree with the content measured by physical and enzymatic analysis (referred to as "measured content" in this report). Also, the difference between estimated and measured content varies across varieties.

We identified that **"measured" starch content and starch granule swelling capacity strongly correlates with variety acceptance by QSR**. Among selected varieties, those accepted by QSR had a higher quantity of starch and larger granule swelling capacity than other varieties. Starch granule swelling capacity is determined by starch granule integrity. Thus, we also examined **starch granule size, thermal property, and pasting viscosity that are also correlated with the acceptance by QSR.** However, **none of them alone has a statistically high possibility to indicate the potential acceptance by QSR at this point.**

In addition, our previous research shows that the capacity of starch granule swelling is influenced by non-starch macromolecules. Our data demonstrated that **some varieties – such as Burbank, Umatilla, and Clearwater – have "strong" cells that can hold swollen starch granules without rupturing during cooking**. The variation in cell wall composition and properties plays a role in starch granule swelling behavior and potentially influences potato food quality.

Some varieties are less stable than others in two years. For example, Ranger obtained in 2015 and 2016 were significant differences in their starch properties. Burbank had high specific gravity, measured dry matter, measured starch content, and high pasting viscosity in 2016, but had low numbers of all of the measurements in 2017. Umatilla, Clearwater, Pomorelle, Premier, and A06021-1T are relatively consistent in 2016 and 2017.

ACTIVITIES OR EXPERIMENTS CONDUCTED:

In fiscal 2016 – 2017, we established methods and examined starch properties of selected varieties. The experiments including specific gravity, measured dry matter, measured starch content, starch isolation, starch granule size distribution, starch granule morphology, apparent amylose content, starch gelatinization temperature, starch pasting viscosity, starch swelling factor and swelling power, potato flour macro element content, and potato pectin content.

In the fiscal 2017 – 2018, we grew selected varieties for the second year and repeated all of the experiments described above. In addition, we developed methods to measure the individual specific gravity of each tuber. For pasting properties, in addition to starch pasting viscosity, we also measured the rheology (yield stress and storage module) of mashed potato tissues at different temperatures. We have been isolating cell wall materials for characterizing its fine structure. We also examined cell morphology before and during heating. We plan on generating starch fine structure data that will assist us to examine the mechanism of swelling capacity.

We will conduct the sensory study in March 2018. We have developed methods to examine cell morphology and texture attributes of French fries. These two techniques will be performed onsite while conducting the sensory study in Caldwell (ID) to obtain data from panelists, texture analyzer, and microscope of the freshly prepared French fries at the same condition. All of the tubers that will be used in the sensory study have been analyzed, a new batch of Burbank will be used to replace the batch grown in Parma that had low specific gravity, low starch content, and low starch pasting viscosity.

RESULTS:

We are processing data and working with a statistician to analyze our data. In this report, we demonstrated several figures to support our statement above. Some data generated from 2015 (before research projects were awarded) were also included, but the sample size in 2015 was smaller than it was in 2016 and 2017. All of the tubers were from the same location except the additional batch of Burbank that will be used for the sensory study; the biochemical data of this new batch are not completed yet. Data with comprehensive statistical analysis will be presented in the next report.

1. Potato tuber characteristics

1.1 Specific gravity, dry matter, and starch content

We examined the specific gravity and dry matter of the selected varieties in two or three years. Some varieties were removed from the study in 2017 because we started to focus on those varieties selected for the sensory study.

Specific gravity range was 1.057 - 1.093, 1.053 - 1.094, and 1.053-1.103 for the all studied varieties in 2015, 2016, and 2017, respectively (Figure 1). In general, Burbank, Clearwater, Umatilla, Ranger, Payette, A03921-2, and Premier had specific gravity near or above 1.090. However, we obtained a quite different level of specific gravity of the same varieties in different years. For example, Burbank's specific gravity was only 1.07 in 2015 and 2017. Umatilla, A03921-2, and Premier had a relatively stable specific gravity in our trials. Figure 1

demonstrates the specific gravity of each variety measured in two or three years.

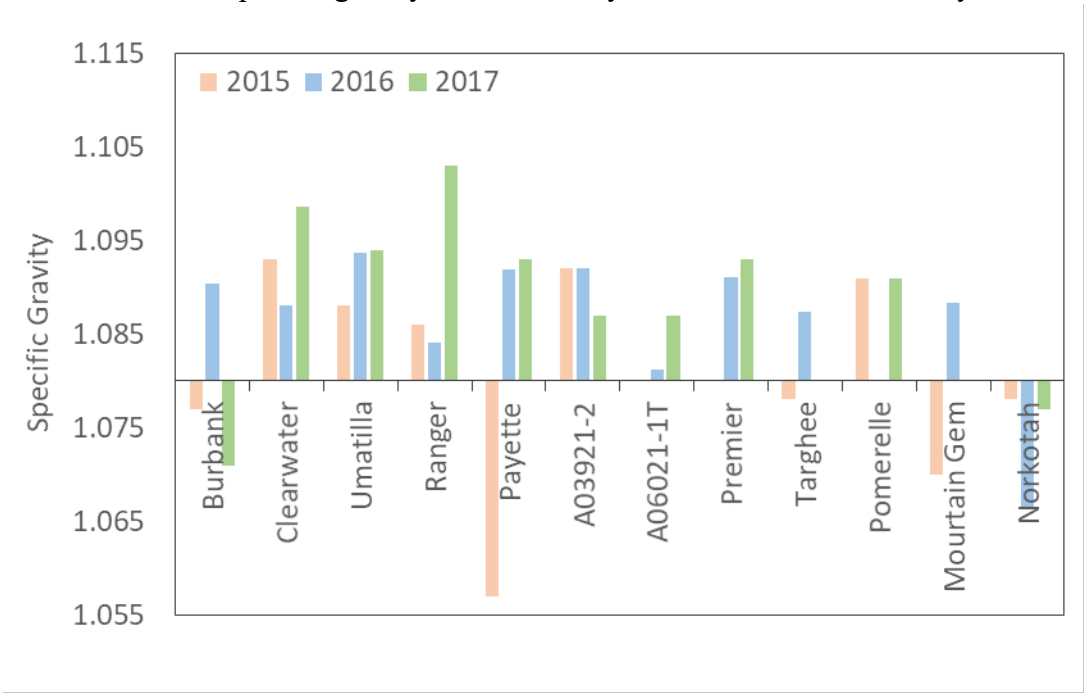


Figure 1. The specific gravity of selected varieties in 2015, 2016, and 2017. Some varieties had two years data (2015 &2016, or 2016 &2017), some had three years measurement. All of the tubers were grown in Parma, ID.

We calculated dry matter ("estimated dry matter") using specific gravity. However, we found the data did not agree with the measured dry matter. Thus, the data of estimated dry matter were neither reported nor discussed in this report. We removed water from tubers and measured the dry matter. Burbank, Clearwater, Umatilla, and Targhee had relatively high dry matter. However, this observation was not consistent in another year. Overall, Clearwater had the high dry matter in three years, Mountain Gem and Norkotah had the lowest dry matter. Figure 2 demonstrates the measured dry matter of selected varieties in two or three years.

We analyzed starch content using a combination of enzymatic and chemical analyses. In general, the trend of the measured starch amount was similar to the trend of measured dry matter, but there are inconsistency between two years. Clearwater had the highest starch amount, and Mountain Gem and Norkotah had low starch content (Figure 3).

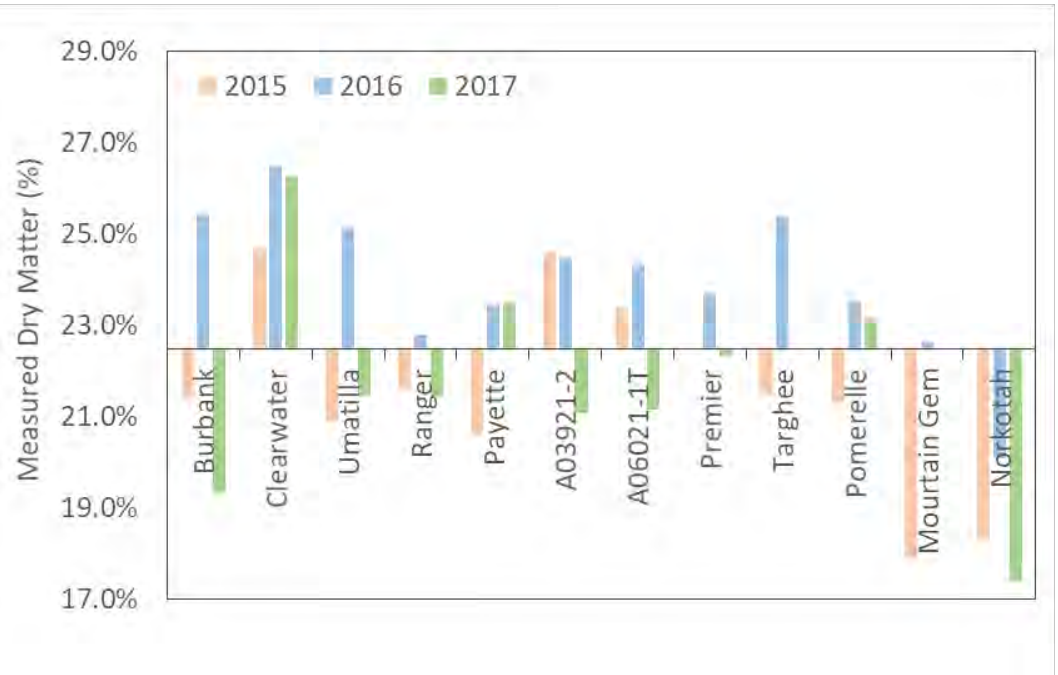


Figure 2. The measured dry matter of selected varieties in 2015, 2016, and 2017. Some varieties had two years data (2015 &2016, or 2016 &2017), some had three years measurement. All of the tubers were grown in Parma, ID.

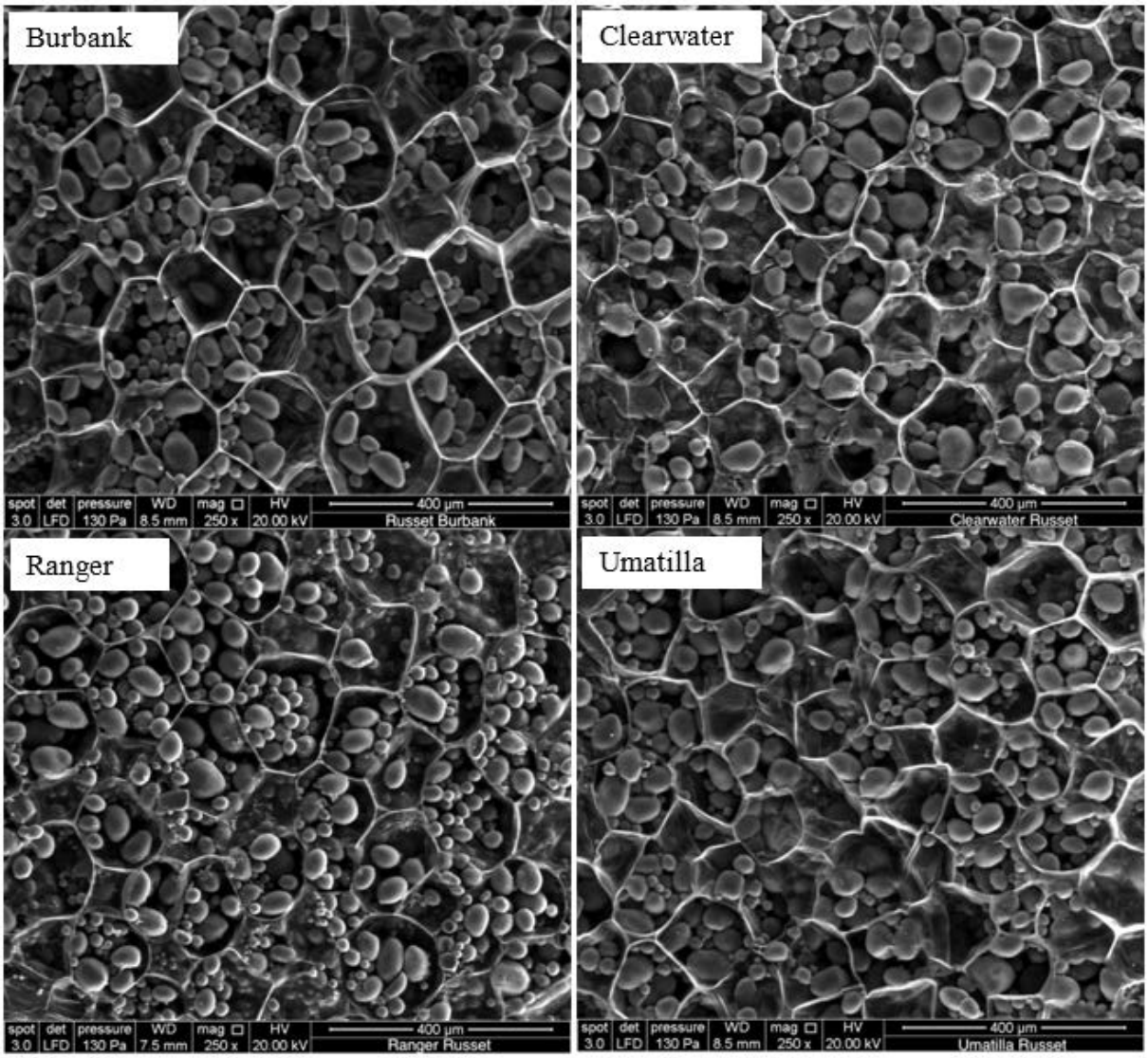
Figure 3. The starch content of selected varieties in 2015, 2016, and 2017. Some varieties had two years data (2015 &2016, or 2016 &2017), some had three years measurement. All of the tubers were grown in Parma, ID. The starch amount was determined by a combination of enzymatic and chemical analysis. The percentage is based on the dry weight of tubers.

2. Starch characteristics

2.1 Morphology of starch granules and cells

We examined the morphology of starch granules in the fresh potato tubers using the scanning electron microscopy (SEM) (Figure 4). The size and number of starch granules vary at different locations in potato tuber (Figure 5). Thus, all of the figures demonstrated in Figure 4 were sampled from the same location in tubers.

Clearwater and A06021-1T had large and uniform granule size, and Pomerelle had the smallest size. The size of other varieties is similar. Some varieties have a very diverse size of starch granules, such as Mountain Gem. Most of the varieties have the typical oval shaped starch granules, Clearwater and Norkotah both have irregular shapes for their large granules and spherical for their small granules.



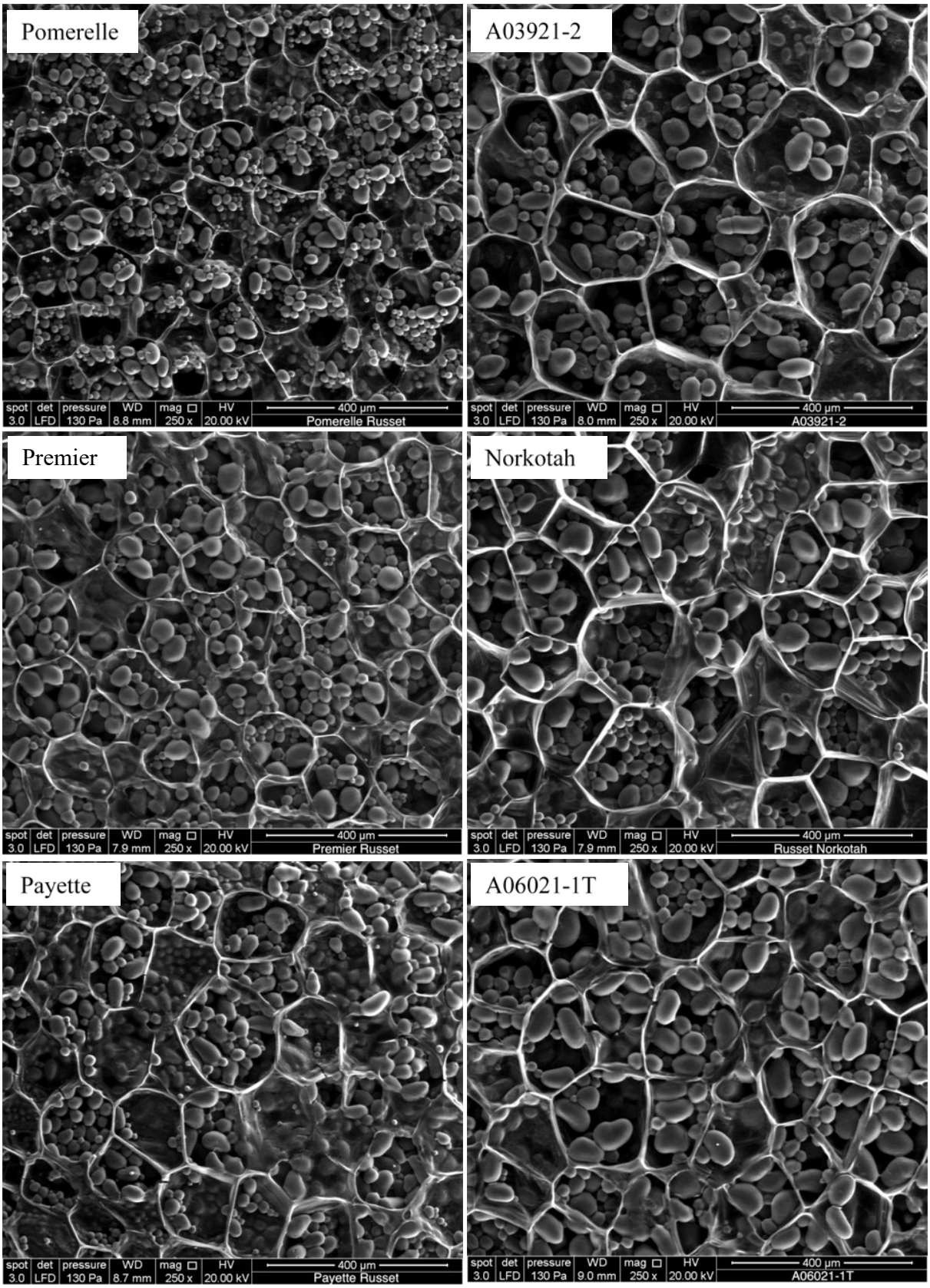


Figure 4. Scanning electron micrograph of selected varieties at 250x of magnification.

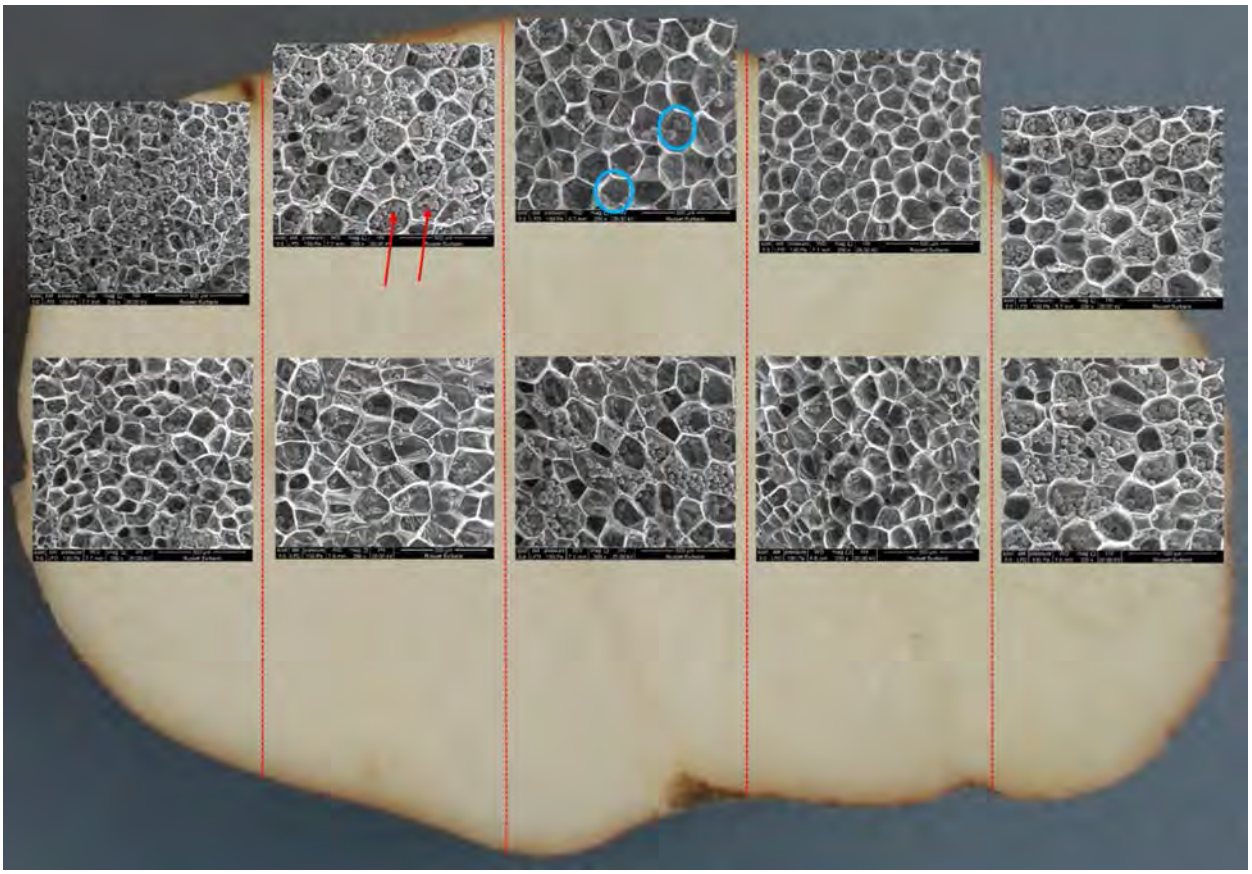


Figure 5. The size and number of starch granules vary at different locations in a potato tuber. The small oval-shaped granules (as indicated by the red arrows) are starch; the honeycomb structure (as indicated by the blue circles) is comprised of cells. The core area of a tuber has fewer starch granules per cell (the three center pictures in the second row) than the outer areas (vascular areas). All the pictures above are at the same magnification.

2.2 Starch granule size distribution

Our SEM images demonstrated that the size of starch granules was distributed in a broad range. We used a particle size analyzer to examine the granule size distribution (Figure 6). We separated granules to several fractions – extreme small, small, medium, and large. In 2016, Clearwater had the lowest proportion of extreme small granules (3.15%, < 20 μm) compared with others varieties. Burbank had less small granules (34.58%), the highest proportion of medium granules (35.75%, 36-53 μm), and large granules (24.87%, > 53 μm) than other varieties. Burbank obtained in 2017 was not analyzed yet. Tarhee and Mountain Gem were excluded from the study in 2017.

*Figure 6. The volume percentage of **extremely small** starch granules (with a diameter smaller than 20 µm) in tubers obtained in 2016 and 2017.*

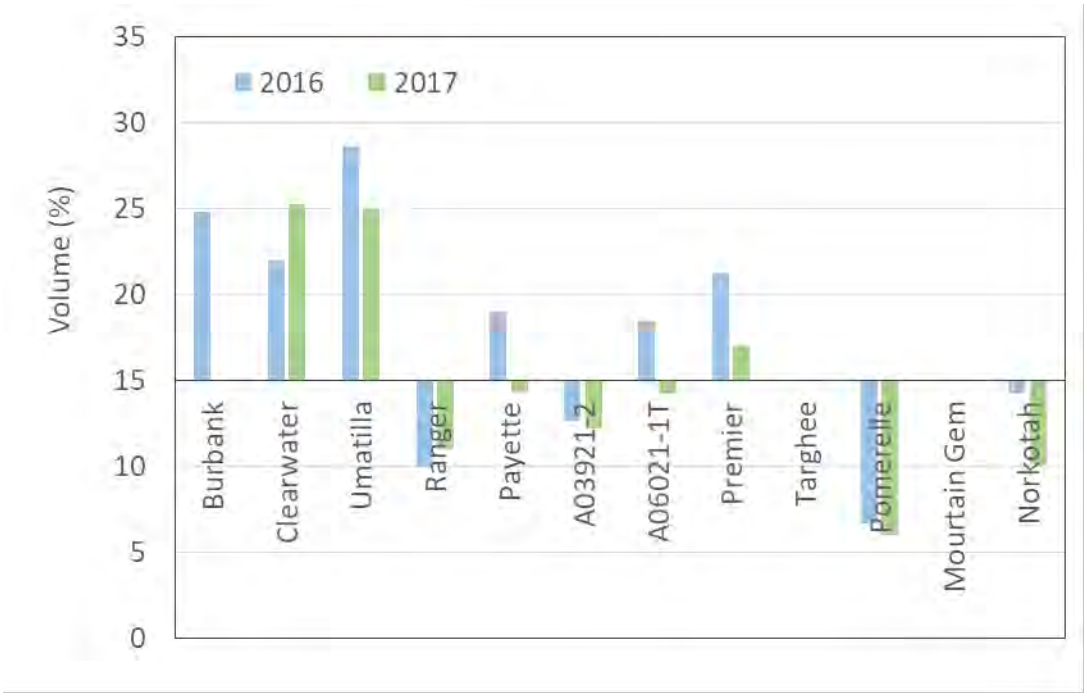


*Figure 7. The volume percentage of **large** starch granules (with a diameter bigger than 53µm) in tubers obtained in 2016 and 2017. Burbank obtained in 2017 was not analyzed yet. The percentage was based on the volume equivalent to a sphere.*

2.3 Starch granule thermal property

We examined the thermal property of starch granules, including onset, peak, conclusion temperatures and the change of enthalpy. Thermal property is associated with starch granule integrity and crystalline structure. It demonstrates the needed temperature and energy to initiate the gelatinization and generates pasting viscosity. The onset temperature of selected varieties is presented in Figure 8. Burbank and Clearwater started to gelatinize at a relatively low temperature, similar to Targhee.

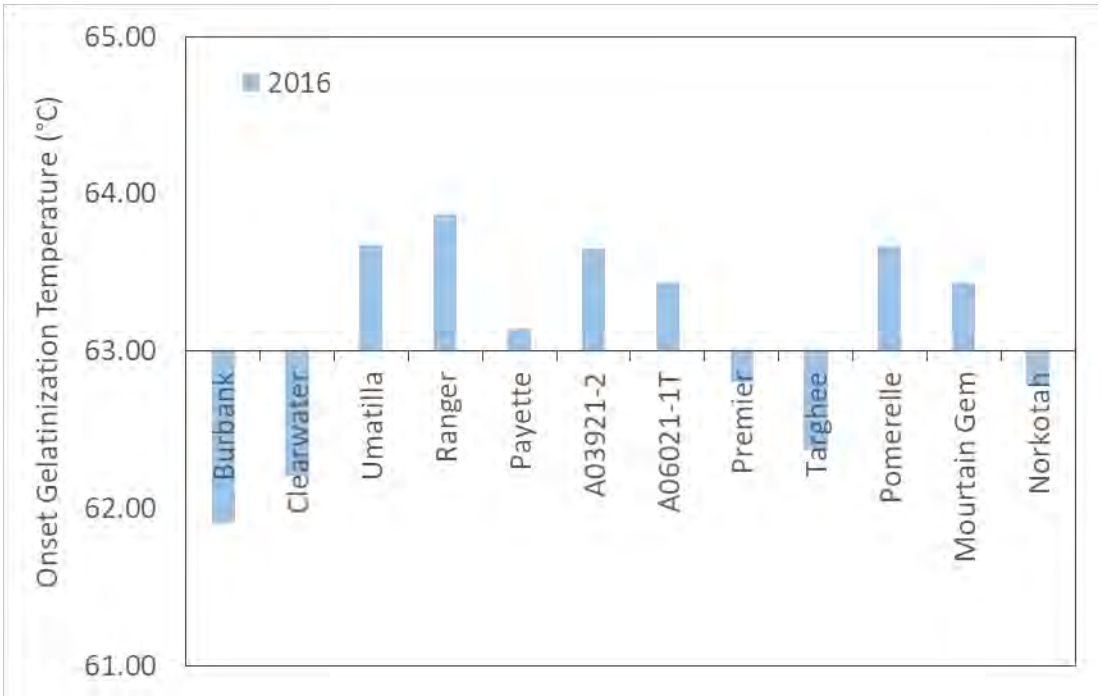


Figure 8. The onset gelatinization temperature of starch granules isolated from tubers grown in 2016.

2.4 Starch pasting viscosity

We examined the starch pasting viscosity, including peak, trough, breakdown, final, and setback viscosity. We also recorded peak time and pasting temperature. In this report, peak viscosity, which is the most relevant parameter to warm product texture, is presented in Figure 9. Overall, Burbank (2016 only), Clearwater, Umatilla, Payette, and A06021-1T had a higher peak viscosity than others, such as Ranger, Premier, Pomerelle, and Norkotah (Figure 9). In addition to the pasting property of purified starch, we are analyzing the rheology properties (stress yield and storage module) of cooked potato tissues.

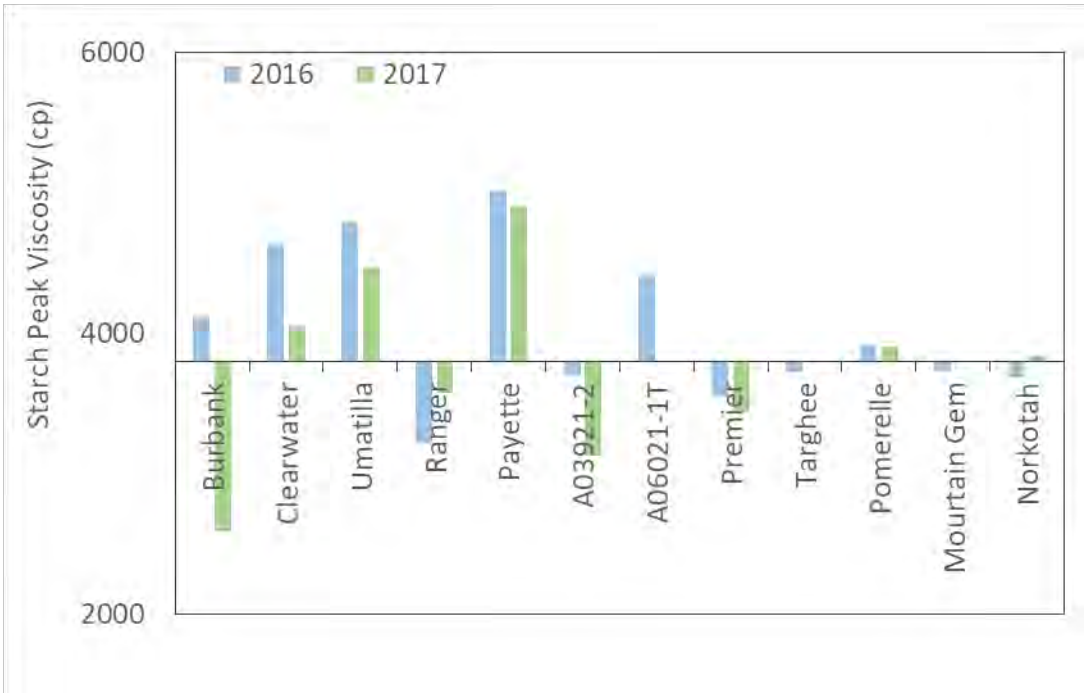


Figure 9. The peak viscosity of starch isolated from tubers grown in 2016 and 2017. Targhee and Mountain Gem were excluded from the study in 2017.

2.5 Starch granule swelling capacity

We examined the volume of swelled granules at five different temperatures. We selected data obtained from three temperatures, which had the most significant difference between each variety, to present in this report. Overall, Burbank, Clearwater, Umatilla, Ranger, and Payette had a bigger swelling capacity than others, such as Premier and Pomerelle (Figure 10). Among all of the starch characteristics examined in our studies, the swelling capacity had the strongest correlation with the acceptance by QSR. However, it cannot be used alone as an assessment. For example, Norkotah also demonstrated a good swelling, but Norkotah is known for fresh used, instead of processing (Please see the next section for the explanation).

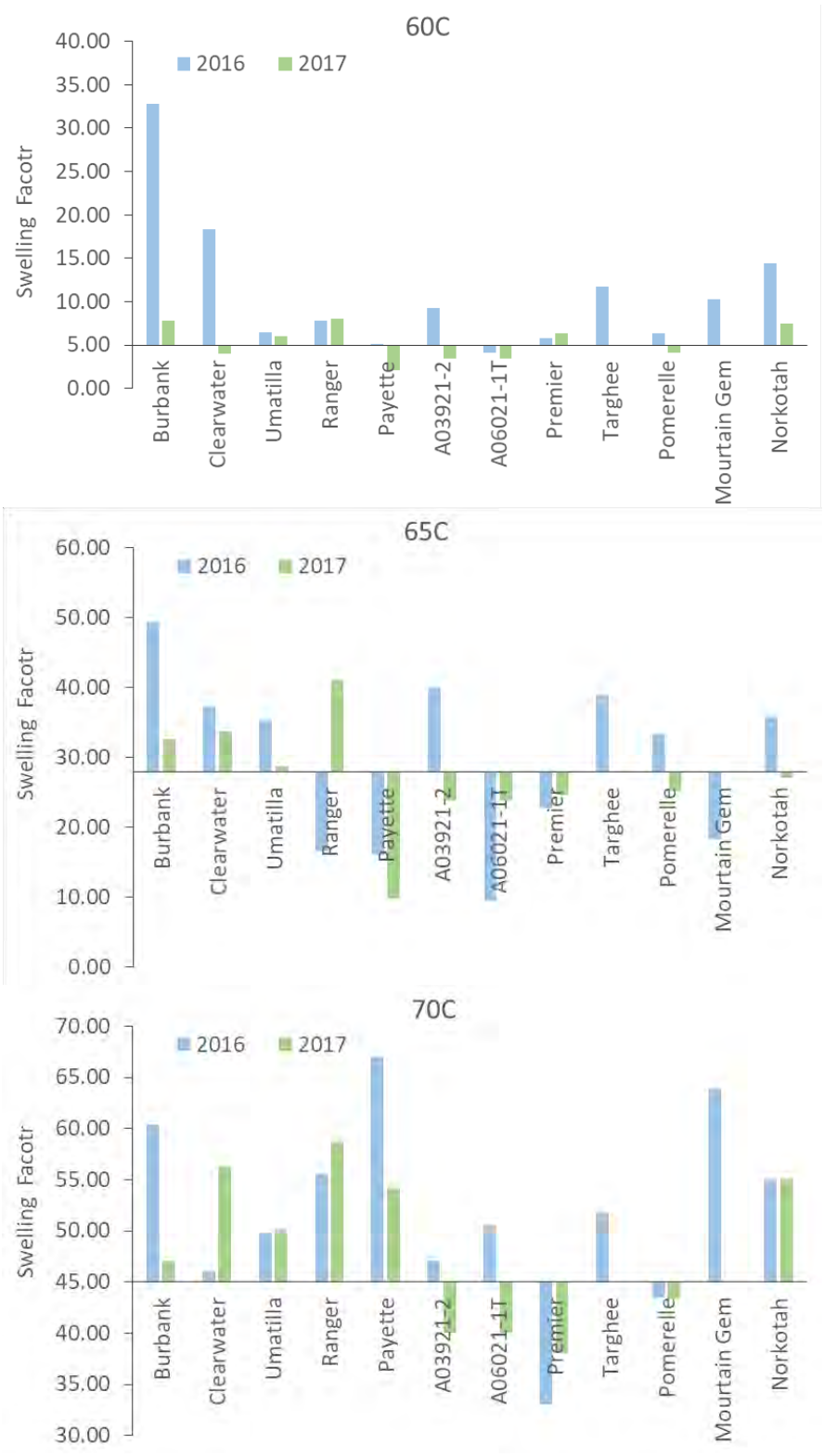


Figure 10. The volume of swollen granules isolated from tubers grown in 2016 and 2017 examined at various temperature.

2.6 Morphology of gelatinized starch and cells

We examined the morphology of starch granules and cells in potato tubers during gelatinization. Our pictures (Figure 11) made of Burbank (2016) demonstrated granule swelling inside of cells, and cells maintained their original shape and size until starch was fully gelatinized. When examining other varieties, we revealed that cells of some varieties (i.e., Norkotah and Pomerelle) could not hold gelatinized starch; their cells broke, and gelatinized starch leaked (Figure 12). This is a novel finding and explains why starch characteristics have a strong correlation with the variety acceptance by QSR, but none of them alone can be used as a sole assessment tool. The interaction between starch and non-starch macromolecules (i.e., cell wall materials) must be considered.

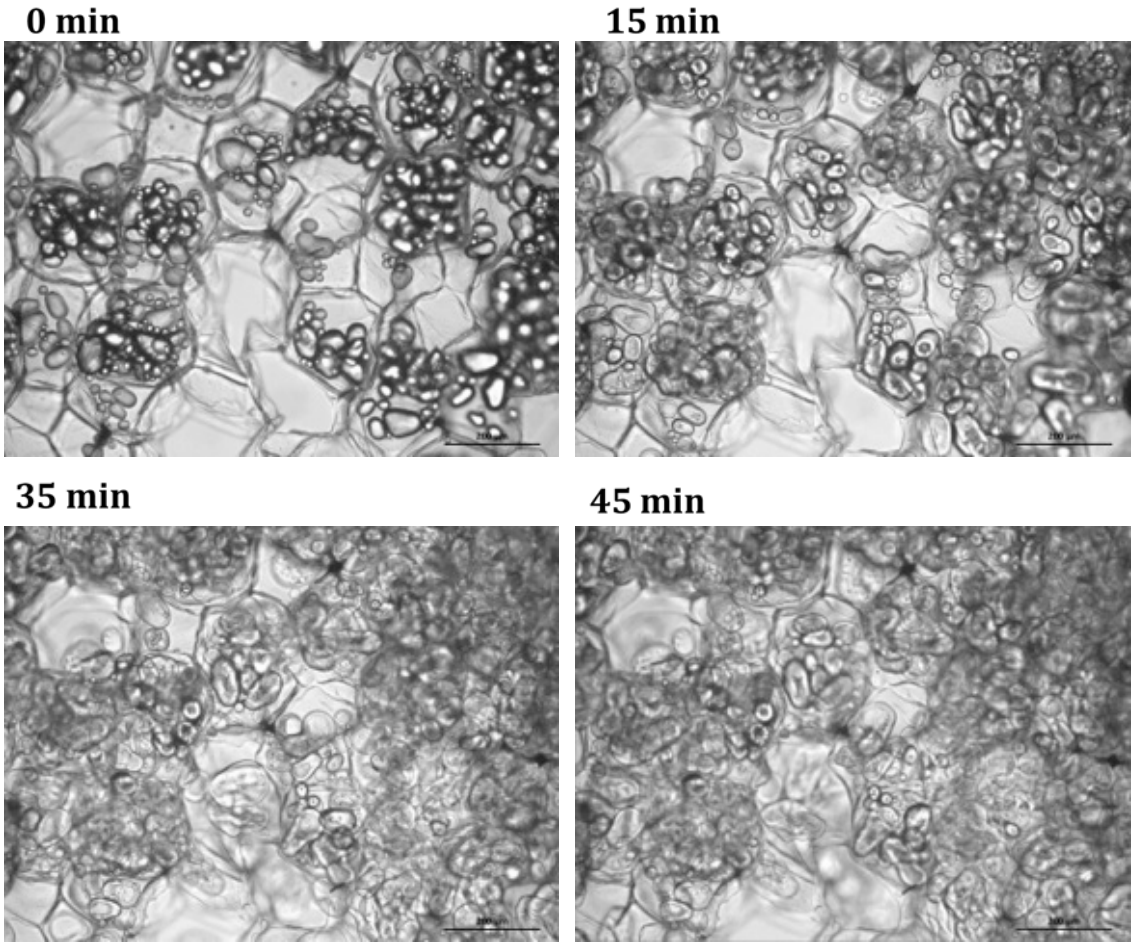


Figure 11. Morphology of gelatinized starch and cells during heating potato tuber tissues.

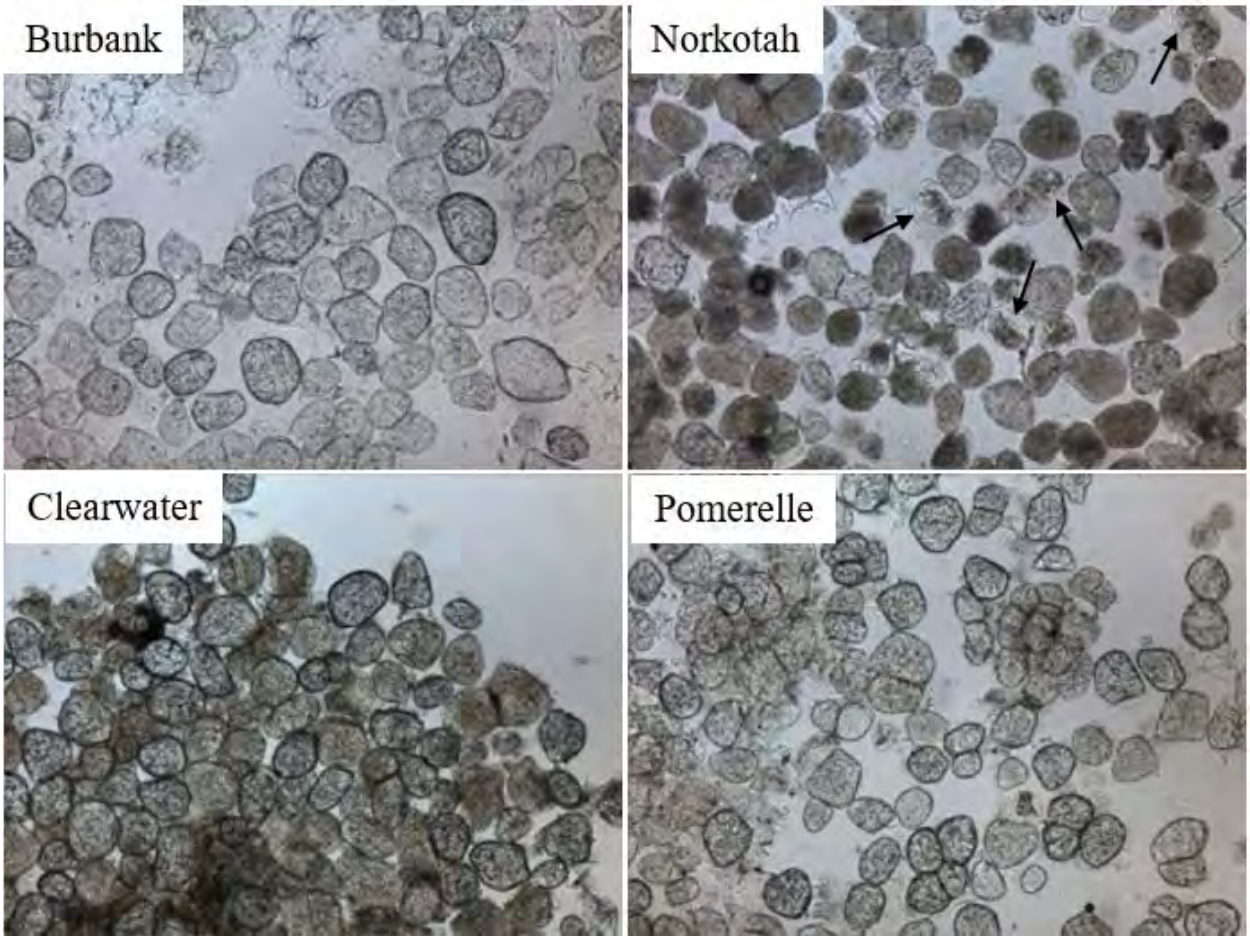


Figure 12. Morphology of gelatinized starch in the cells from cooked potato tubers. Arrows indicated rupturing cells and leaked starch molecules

PUBLICATIONS:

We plan on publishing part of the data after completing the sensory studies in 2018.

PRESENTATIONS & REPORTS:

Lin. A. Starch characteristics associated with the industry acceptance of potato varieties for making French fries. J.R. Simplot (Caldwell, ID), October 24, 2017

Lin, A. *Proposal presentation.* Northwest Potato Research Consortium Proposal Review Meeting, Best Western Vista Inn (Boise, ID), October 24, 2017

Shao, Y. Characteristics of potato starches from PNW varieties and their influence on French fries texture. Innovation Show Case. University of Idaho (Moscow, ID). April 13, 2017.

Lin, A. *Potato Research Update to Basic American Foods.* University of Idaho (Moscow, ID), April 4, 2017

TITLE: Determination of Factors that Regulate Tuber Glycoalkaloid Content.

PERSONNEL: Roy Navarre, Jeff Stark, Sagar Sathuvalli, Mark Pavek.

USDA-ARS, Prosser, WA; Washington State University, Pullman WA; Oregon State University; and University of Idaho, Parma, ID

Cooperators: Chuck Brown, Joanne Holden, Susie Thompson, Walter De Jong, Isabel Vales, Creighton Miller, Rick Boydston.

REPORTING PERIOD:2017

INTRODUCTION:

The major relevance of glycoalkaloids (GLKs) for the potato industry is their potential to be toxic to humans if present in high concentrations. Potatoes and many other *Solanaceae* contain glycoalkaloids that have a variety of effects depending on the concentration. Generally, the first indication that GLKs are higher than normal is a slight burning or tightening of the throat and bitter taste that passes quickly. However, bitterness alone does not mean glycoalkaloids are present, as other compounds can also contribute to bitterness. Symptoms become progressively more severe as the concentration of GLKs increase, including cramping, vomiting, nausea and other ill effects including death in extremely rare cases (Hopkins, 1995; McMillan and Thompson, 1979). Most of the effects of GAs are due to their disruption of cell membranes and cholinesterase inhibition. They occur in higher concentrations in fruits, leaves, and sprouts than in tubers, with GA concentrations of 18 grams/kg FW reported in sprouts (Valkonen *et al.*, 1996).

There are no regulations about glycoalkaloid concentrations in the United States, but breeders voluntarily adhere to a maximum amount of 20 mg/100 grams fresh weight in new cultivars. Many of the types of potatoes consumed in the Andes contain far higher amounts than this, and historically some potatoes were placed in streams to leach out the bitter compounds before consumption.

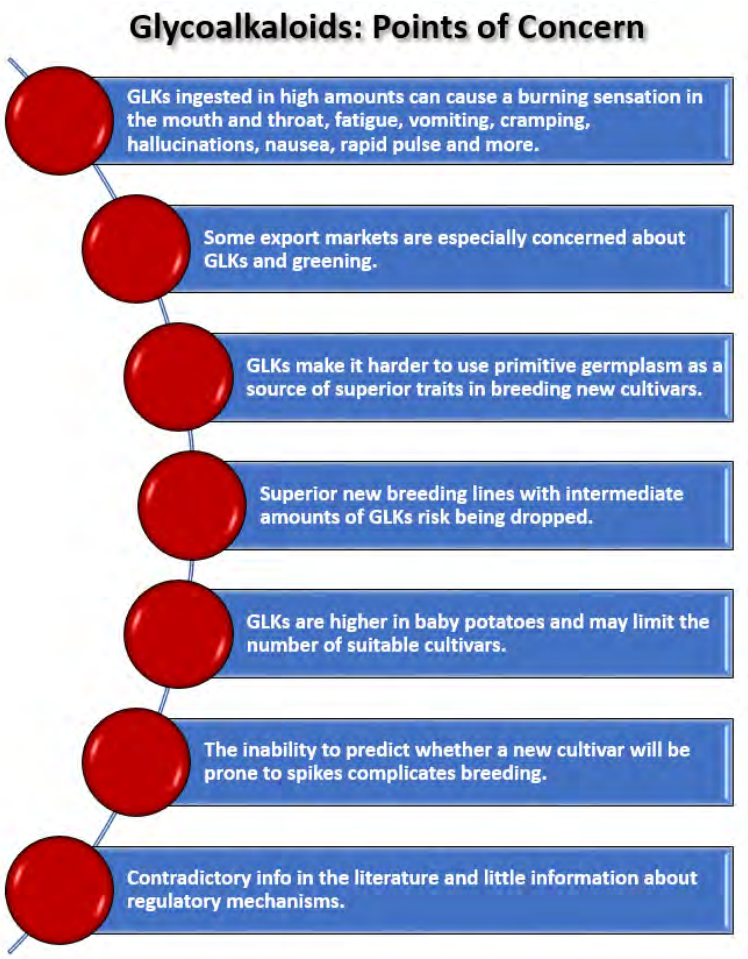
Most new cultivars developed in North America contain less than 10 mg/100 grams FW, but there appears to be increasing discomfort about potatoes in the 10-20 mg/100 g FW range. The 10-20 mg range is historically accepted to encompass safe amounts of glycoalkaloids (GLKs). In increasingly risk adverse modern society, the worry about breeding lines in the 10-20 mg/100g FW is that their GLKs *might possibly* increase above 20 mg/100g FW in response to unpredictable environmental triggers.

This concern is understandable. For example, the Pacific Northwest has competitive advantages for trade with Asia, the fastest growing export market for PNW potatoes. This market has an especially low tolerance for greening of potatoes. Light-induced greening is also thought to increase the amount of GLKs present in potatoes. Thus greening can result in shipments being rejected. An article in Potato Pro.com Sept, 2017 about an incident with green potatoes in Taiwan reported *"Taiwan's health authorities will conduct stringent checks of potato suppliers after reports that French fries served at fast food restaurants were found with green patches." Cheng urged that green potatoes shall not be sold from the upstream suppliers.*

According to Cheng, food vendors which were found to either sell or use potatoes containing solanine can be fined around NT$30,000 (US$993.5) to NT$3 million based on the Act Governing Food Safety and sanitation. The scoop of green-tinted potatoes was first posted online by a user of PTT, the largest terminal-based bulletin board system (BBS) in Taiwan. He wrote that his tongue felt weird when he was eating potato wedges at McDonald's and he found there was a green tint in the food."

Given that these actions resulted from a single consumer reporting "his tongue felt weird" one can see why there is concern about the potential for spikes resulting from light exposure, or other less understood environmental factors. Spikes in GLK concentrations has happened in the past with multiple cultivars, including mainstays like Russet Burbank, which have been reported on rare occasions to have dramatic spikes in GLKs up to 80 mg/100 g FW. The chipping cultivar Lenape was withdrawn from production in North America after a range of 16-65 mg/100 g FW was found in a 1970 survey, in which potatoes from Parma, ID contained 65 mg/100 g FW. The Parma levels may have been associated with a killing frost two weeks before harvest. Levels of 4-35 mg have been reported for Kennebec and 2-21 mg for Katahdin. In a Canadian study, GLK levels in Burbank tripled after 6-8 hours exposure to sunlight near freezing temperatures. GLKs in the Swedish cultivar Magnum Bonum were found to vary between 6-67 mg/100 g FW.

Worry about the potential for spikes exists because spikes are not necessarily predictable with the exception greening. The environmental, physiological and genetic regulation of GLK levels in tubers are not well understood and the lack of such knowledge is why predictability is lacking. Consequently, a fear for new breeding lines is that their GLK amounts established as acceptable over years of evaluation in trials, could nevertheless inexplicably spike to an unacceptable amount when grown on an even larger scale due to unpredictable causes. This concern would be reduced if we could better predict the effect of environment on any given cultivar. Increased understanding of the biochemical and molecular mechanisms leading to increased GLK synthesis in tubers would increase our ability to predict how a given cultivar will respond to a



range of environments.

Concerns about spikes can cause breeding programs to try to breed for lines as low as possible, a strategy that has drawbacks. If 10-20 mg is precluded as an acceptable range, this can slow new cultivar development. Such a line may have numerous outstanding traits such as disease resistance, yield and processing quality, but risks being dropped because of intermediate GLK levels. Moreover, requiring unnecessarily low amounts of GLKs in any new line can restrict the ability of breeders to use primitive germplasm as a source of highly desirable traits not found in the domesticated potato gene pool. Modern cultivars only use about 1% of the genetic diversity available in primitive potato germplasm, so it is highly likely such germplasm contains untapped genes that would confer superior performance. However, progeny derived from primitive germplasm can have higher amounts of GLKs than the typical modern cultivar, therefore excluding 10-20 mg as an acceptable range will make it more difficult to tap into the potato genepool to enhance performance. But also important is that GLKs at the proper concentration can positively influence potato flavor. Moreover, the role of GLKs in the plant appears to be to enhance pest and pathogen resistance, so decreasing GLKs to unnecessarily low levels may increase the requirement for pesticides and fumigants.

Millions are invested in the TriState program R&D by the commissions, state and federal institutions, processors and seed growers. A clone found with intermediate amounts of GLKs may be dropped. In the case of advanced breeding lines this may represent a six figure dollar loss because the line will have been in development over ten years and trialed nationally.

GLKs also impact the growing West Coast market for “baby potatoes”, whose appeal extends to non-traditional potato consumers and that present opportunities to diversify PNW potato production. Baby potatoes have higher amounts of GLKs than at maturity and this can present a bottleneck for bringing new consumer-oriented baby potato cultivars to the market. In addition to such potatoes having higher GLK levels than at maturity, baby potatoes are typically eaten with their skin on and minimally processed, which may contribute to having a higher risk for glycoalkaloid content than processed potatoes.

Although GLKs have been much studied over the years, especially in the 1960s-1980s, a case can be made that neither researchers nor industry know as much about glycoalkaloids as once thought and that new developments change how GLKs should be perceived. It is not difficult to find contradictory published scientific literature about GLKs. Injury can elevate amounts as much or more than greening. A combination of cold, wet soils and clouds may stimulate an increase in GLKs. Magnesium is reported to increase GLKs, whereas mixed results have been found with nitrogen amounts. Inconsistent findings are also reported with vine killing. Based on limited, older data, one paper suggested spikes in glycoalkaloids may be more common in cultivars with GLK averages greater than 10 mg/100 g FW, but like much of the older GLK literature, the data is not conclusive.

RESULTS:

Our major focus this past year was on identifying the potential of different cultivars to experience spikes in GLK concentrations. This objective has two purposes: **1)** To evaluate whether different cultivars behave similarly across locations in terms of GLK content. In particular, we were interested in the stability of GLK content across locations in Castle Russet, a newly released TriState line with intermediate amounts of GLKs, but good processing

characteristics and disease resistance, including to PVY, TRV and PMTV. **2)** If we do observe large differences in GLK content based on where the potatoes are grown, then try to identify the regulatory mechanisms responsible at the molecular level.

With the help of our cooperators, the same 13 cultivars or breeding lines were grown in Idaho, Oregon, Washington, New York, North Dakota and Texas. Seed was all from the same source.

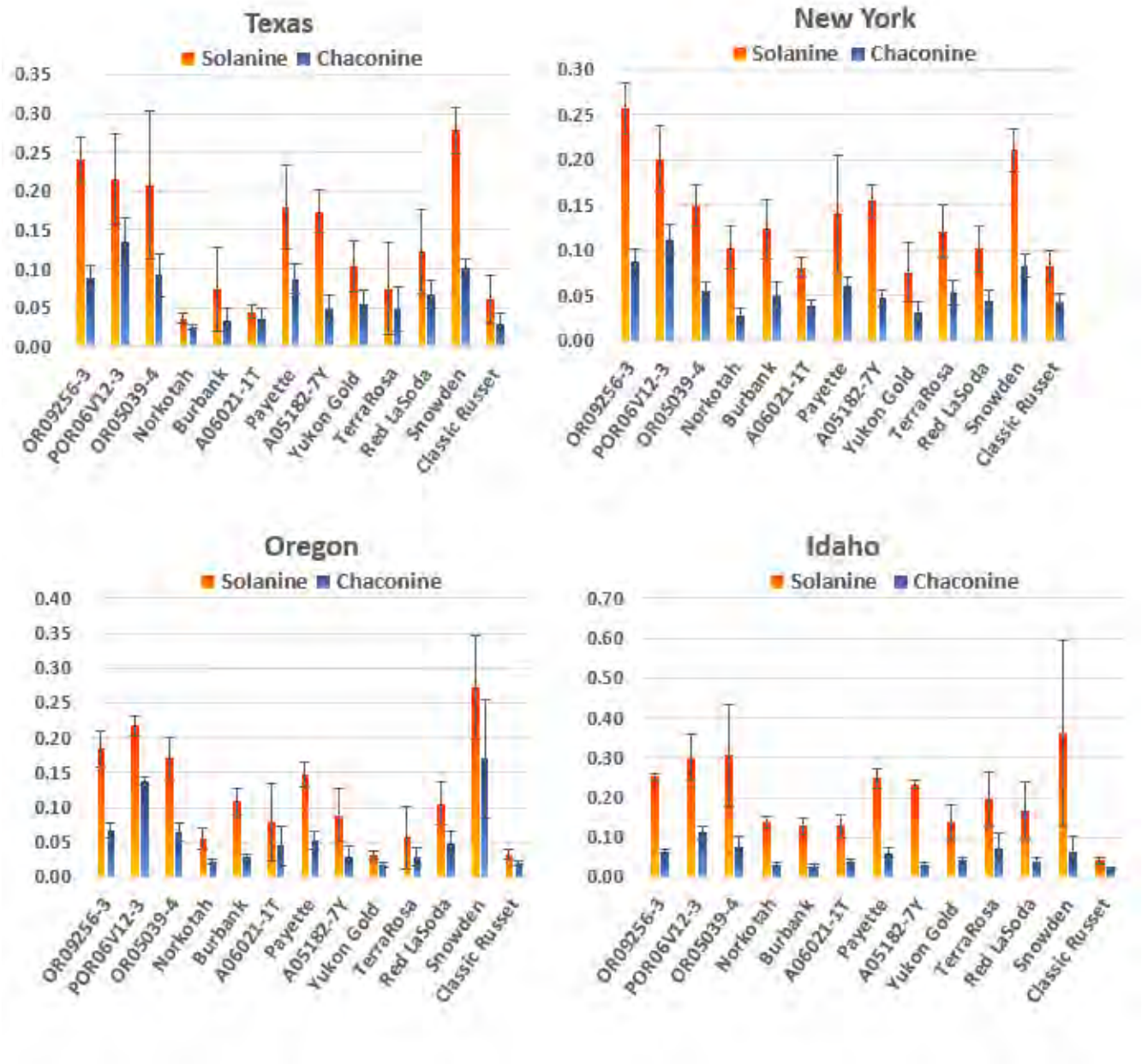


Figure 1 (see next page for description)

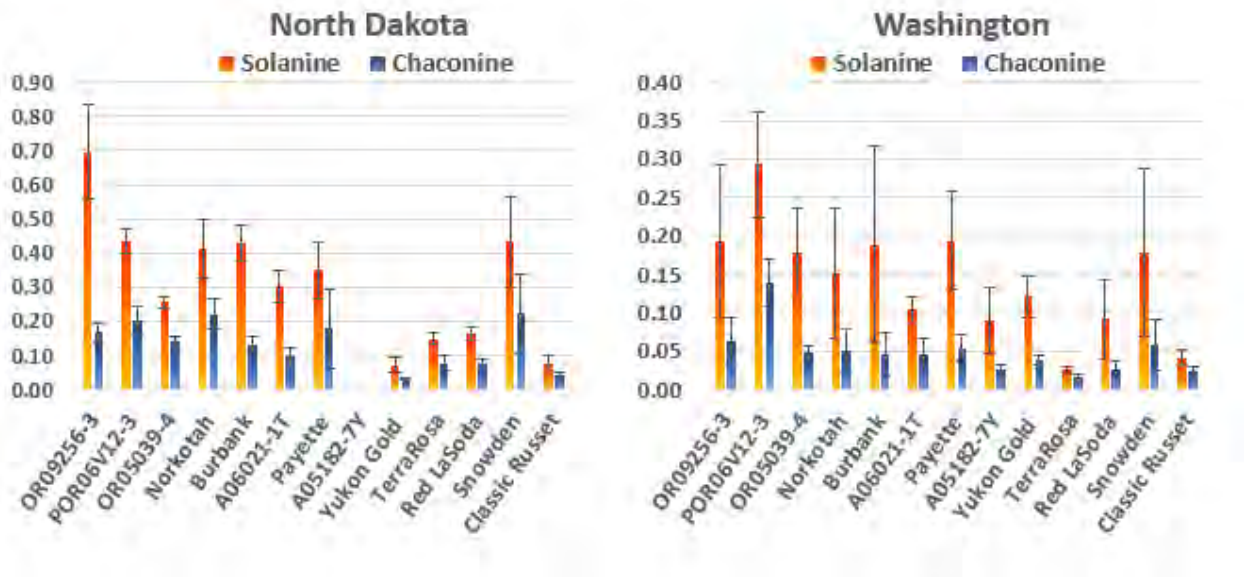


Figure 1 continued. Solanine and chaconine concentrations in 13 cultivars grown in six States. Concentrations are expressed in mg glycoalkaloids/gram dry weight. Glycoalkaloids were quantitated using a triple quadruple mass spectrometer.

Potatoes were freeze-dried and glycoalkaloids extracted and quantitated by LCMS. As seen in **Figure 1**, solanine is more abundant than chaconine in all cultivars and constituted from 67% (Classic Russet) to 83% (Norkotah) of the total amount of GLKs present (**Figure 2**). Solanine is reported to be better tolerated than chaconine and less bitter, so this ratio can be considered desirable compared to a potato that has a higher amount of chaconine than solanine. The GLK data in this report should be considered preliminary and will be reported on a mg/100 gram fresh weight basis later based on the actual fresh weight of each potato. However, one could estimate the amount on a mg/100 g FW basis from this data by assuming each cultivar is 80% water. In that case 20 mg/100 g FW would correspond to 1 on the scale used in **Figure 1.** All cultivars contained less than this amount of GLKs. The variation in GLK content among the different locations was modest. North Dakota had the highest amount of GLKs for several cultivars among the locations, such as OR09256-3 (**Figure 1**). Although, in almost all instances the amounts were still below 10 mg/100 g FW assuming the potatoes were 80% water. Figure 3 shows the amount of solanine in each cultivar among the six different

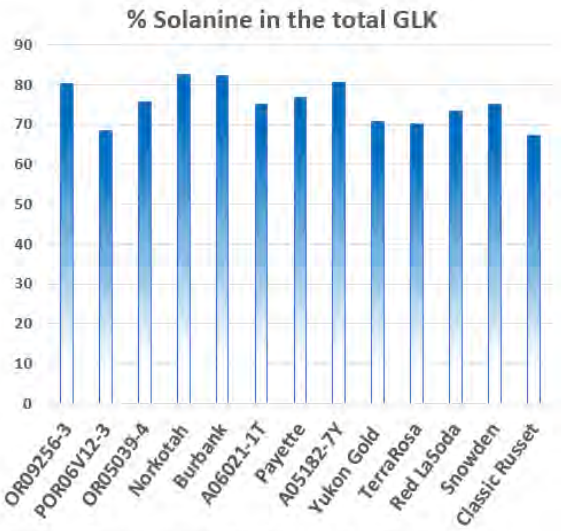


Figure 2. Percent contribution of solanine to the total amount of glycoalkaloids present.

locations and makes it easier to see the amount of variation between locations.

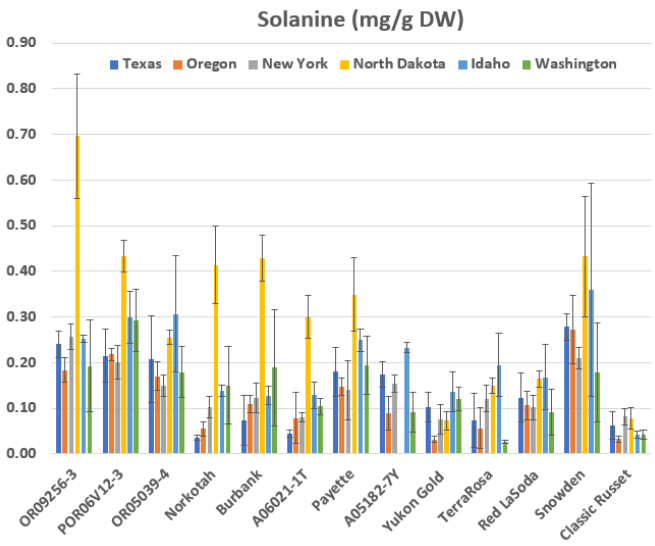


Figure 3. Amount of solanine in 13 cultivars grown in six States.

The GLK amounts in POR06V12-3 (now named Castle Russet) was of particular interest because in earlier trials it often tested in the 10-20 mg/100 g FW range and there was interest in what its GLK amounts would be when grown on a wider scale. As seen in **Figure 1** and **4**, the GLK amounts in V12 (Castle Russet) remained well below the threshold of 20 mg/100 g FW. The three lines with the highest amoungs were OR09256-3, Castle Russet and Snowden, whereas Classic Russet had the lowest amounts (**Figure 5 and 6**).

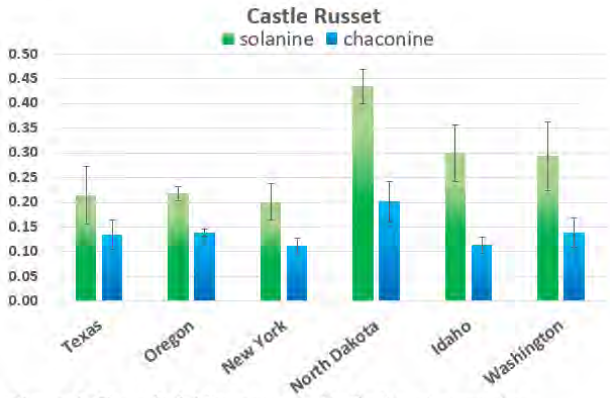


Figure 4. Glycoalkaloid amounts in Castle Russet grown in six States.

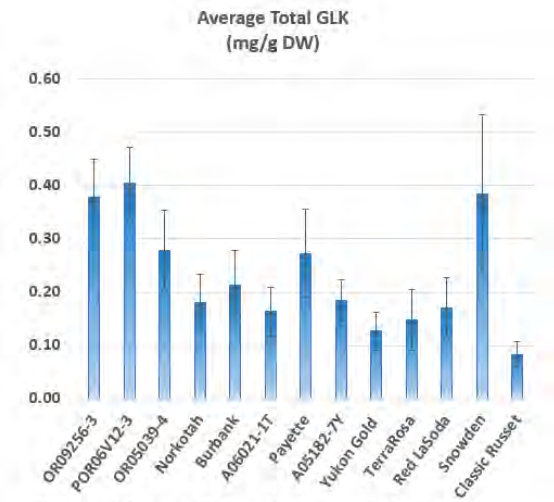


Figure 5. Average total glycoalkaloids for each cultivar among the six locations.

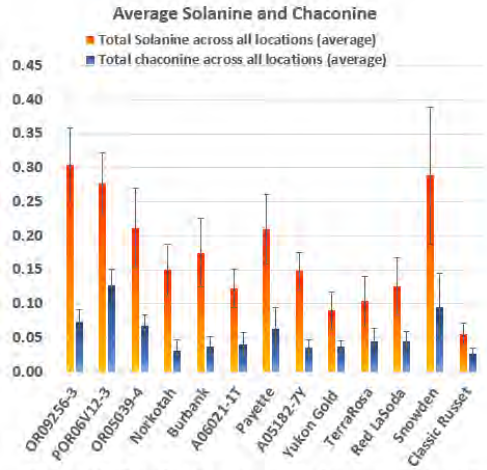


Figure 6. Total solanine and chaconine for 13 cultivars averaged across locations. Amounts are mg/g DW.

One important question about GLK content is whether some lines are more prone to spikes than others. Some older literature has suggested that lines in the 10-20 mg/100 g FW range might be more prone to spikes, and this is a potential concern about new breeding lines with intermediate levels of GLKs. The trial in the six different States will be repeated a second year, but year one of the trial does not support the hypothesis that intermediate lines are more prone to variation. The variation in GLK levels can be considered multiple ways. If we look at the fold variation between the high and low amounts of GLKs in each cultivar across all six locations (**Figure 7**) ranged from about 2-11 fold, with Norkotah having the highest spread because of one location. But even the highest amount in Norkotah was well below the 20 mg/100 g FW threshold. So considered this way, Norkotah was more prone to spikes than Castle Russet.

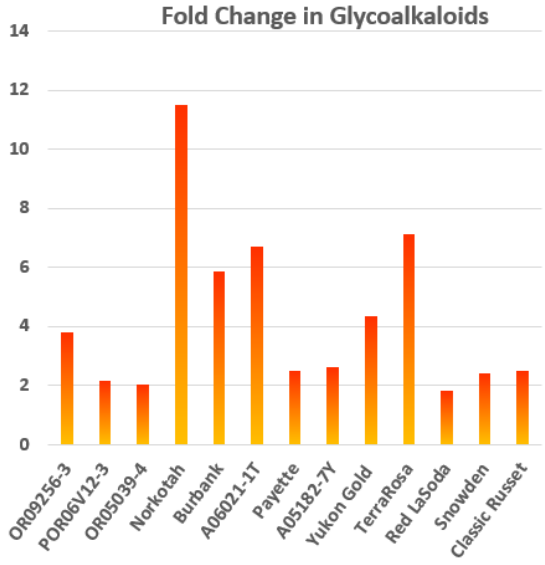


Figure 7. For each cultivar, the range between high and low GLK concentrations was determined across all six locations. The y-axis reports the fold difference between high and low amounts.

Another way to consider to what extent each line was prone to variation in GLK content is to calculate the standard deviation among all samples of each cultivar as a percent of the total GLK in that cultivar (**Figure 8**). When evaluated this way Snowden and Terra Rosa had the most variation. Snowden is thought to be prone to spikes, in which case evaluating the data as in Figure 8 may more accurately capture this capacity than Figure 7. Classic Russet also shows greater variation when evaluated this way, which may reflect that even if a cultivar has very low amounts of GLKs, it may still have as much variation as cultivars with higher concentrations of GLK when the variation is expressed relative to the amount of GLKs present. Castle Russet had amongst the lowest variation in **Figure 8.**

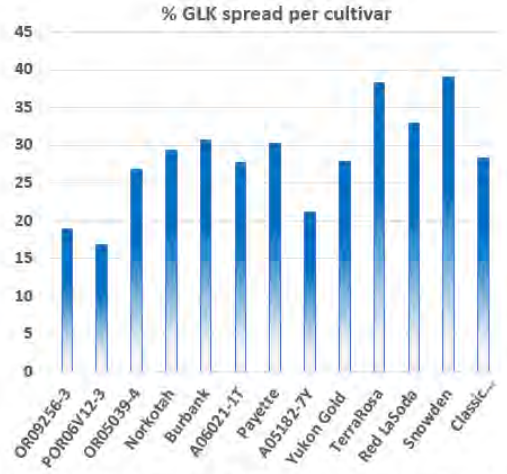


Figure 8. The standard deviation of all samples of a given cultivar are expressed as the percent of the total GLK in that cultivar.

PENDING DATA:

Additional Samples.

Several hundred potato samples have been processed and freeze-dried, and are now awaiting GLK analysis. These include germplasm from NRSP-6 grown in Wisconsin, harvested and freeze dried, and will be analyzed shortly. Likewise over 60 baby potato lines have been freeze-dried and will be analyzed for GLKs, along with baby potatoes vine killed by different methods to evaluate the potential effect on GLK levels.

Molecular Analysis.

Primers are in the process of being designed and tested to analyze gene expression at various points in the GLK pathway. These will be part of the effort to attempt to determine the regulatory mechanisms responsible for spikes in GLK content.

DISCUSSION:

A second year of data from the multi-site field trial is needed before drawing conclusions. However, year one showed that all 13 lines evaluated had at least a ~2-fold range between high and low amounts of GLKs. Quadruplicate samples, each consisting of at least 3 bulked tubers, were analyzed for each line. In no instance did any sample exceed the 20 mg/100 g FW threshold. Nor did the data suggest that Castle Russet was more prone to variation than cultivars with lower amounts of GLKs.

Given data generated over the last decade that shows a substantial amount of GLKs are lost during commercial processing, combined with numerous recent studies showing multiple health-promoting effects of GLKs, including anti-cancer properties, it would seem that the historically accepted range of less than 20 mg/100 g FW of GLKs should continue to be the accepted standard. Eliminating GLKs from potatoes or developing breeding lines with exceptionally low amounts, may not be an optimal strategy given the drawbacks to such an approach discussed in the first part of this report, combined with the abundant recent literature showing health promoting effects of GLKs.

We were hoping to find some potatoes that had well above the 20 mg/100 g FW threshold, and use these for analysis of the regulatory mechanisms that determine whether a cultivar is prone to spikes. Although Norkotah had over an 11 fold range between high and low, the amounts were still far below the accepted threshold, therefore this is not an ideal sample to explore the molecular basis of spiking. The second year field trial may yield more extreme variation, otherwise alternatives will be explored.

PRESENTATIONS: A workshop on glycoalkaloids was presented at the Idaho Potato School in January 2018.

Progress Report

TITLE: Development of genomic resources and enhancement of breeding efficiency for important potato pests

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REPORTING PERIOD: 2017-2018
OBJECTIVES:

1. Validation of new sources of resistance to Columbia Root Knot Nematode and Verticillium Wilt
2. Development of sequencing resources (whole genome and transcriptome) for *Solanum bulbocastanum*
3. Identification of genes and genomic regions that confer resistance to PVY through development and utilization of potato genome resources.
4. Development of sequencing resources to differentiate different races of *Meloidogyne chitwoodii*

1. Validation of new sources of resistance to Columbia Root Knot Nematode and Verticillium Wilt

In order to expand the genetic base of resistance to CRKN, we screened 40 plant accessions from nine wild potato species for their resistance to *M. chitwoodi*. Greenhouse screening identified fifteen clones from *S. hougasii*, one clone from *S. bulbocastanum*, and one clone from *S. stenophyllidium* with moderate to high levels of resistance against three isolates of *M. chitwoodi* (Table 1.). Geographical mapping of resistance sources identified in our study revealed that all the new resistant sources from wild potato species are concentrated in one region in west-central Mexico (Jalisco and Michoacán). To identify novel sources of resistance to Verticillium Wilt (*Verticillium dahliae)*, we inoculated a panel of 80 clones from nine wild potato species from North and South America with *V. dahliae* in the greenhouse. We identified two clones from *S. andreanum*

and one clone from *S. bulbocastanum* that had resistance that was equal to or greater than Ranger Russet, the resistant check (Table 2). These new sources for CRKN and VW will be introgressed into elite potato populations to allow the development of potato cultivars with durable resistance to CRKN and VW.

Table 1. Geometric means of reproduction factors (Rf = number of eggs extracted/initial number of eggs) and HSD tests for wild clones and checks, against three *M. chitwoodi* isolates.

Clone	WAMCRoza		WAMC1		WAMC27	
PI161726hou-3mc	0.317$^{(bc)}$	PH	0.000$^{(d)}$	NH	0.123$^{(bcd)}$	PH
PI239423hou-1mc	0.063$^{(bcde)}$	NH	0.000$^{(d)}$	NH	0.290$^{(bc)}$	PH
PI239423hou-2mc	0.186$^{(bc)}$	PH	0.039$^{(bcd)}$	NH	0.563$^{(b)}$	PH
PI239423hou-8mc	0.000$^{(e)}$	NH	0.000$^{(d)}$	NH	0.271$^{(bc)}$	PH
PI239423hou-10mc	0.000$^{(e)}$	NH	0.001$^{(d)}$	NH	0.390$^{(b)}$	PH
PI239424hou-2mc	0.000$^{(e)}$	NH	0.000$^{(d)}$	NH	0.009$^{(de)}$	NH
PI239424hou-3mc	0.001$^{(e)}$	NH	0.006$^{(cd)}$	NH	0.162$^{(bcd)}$	PH
PI239424hou-6mc	0.001$^{(e)}$	NH	0.003$^{(cd)}$	NH	0.020$^{(cde)}$	NH
PI239424hou-9mc	0.000$^{(e)}$	NH	0.000$^{(d)}$	NH	0.331$^{(bc)}$	PH
PI255518blb-4mc	0.330$^{(bc)}$	PH	0.000$^{(d)}$	NH	0.010$^{(de)}$	NH
PI283107hou-5mc	0.000$^{(e)}$	NH	0.000$^{(d)}$	NH	0.062$^{(bcde)}$	NH
PI283107hou-6mc	0.109$^{(bcd)}$	PH	0.186$^{(b)}$	PH	0.337$^{(bc)}$	PH
PI283107hou-9mc	0.001$^{(e)}$	NH	0.001$^{(d)}$	NH	0.087$^{(bcde)}$	NH
PI545815sph-9mc	0.033$^{(cde)}$	NH	ND		0.372$^{(bc)}$	PH
PI558402hou-2mc	0.186$^{(bc)}$	PH	0.004$^{(cd)}$	NH	0.155$^{(bcd)}$	PH
PI558402hou-4mc	0.159$^{(bcd)}$	PH	0.017$^{(cd)}$	NH	0.108$^{(bcd)}$	PH
PI558422hou-2mc	0.622$^{(b)}$	PH	0.027$^{(bcd)}$	NH	0.121$^{(bcd)}$	PH
Rutgers	20.286$^{(a)}$	H	10.254$^{(a)}$	H	6.013$^{(a)}$	H
Vernema	0.012$^{(de)}$	NH	0.000$^{(d)}$	NH	0.101$^{(bcd)}$	PH
Red Core	0.144$^{(bcd)}$	PH	0.062$^{(bc)}$	NH	0.000$^{(e)}$	NH

NH –Non-Host (Rf: 0 to 0.1)
PH – Poor Host (Rf: 0.1 to 1)
H – Host (>1)

Table 2. Replicated evaluation of resistance the higher the value, the greater resistance to verticillium wilt

Accession	Resistance
PI498148adr-1vd	14.875[a]
PI498148adr-2vd	14.500 [a]
PI498011blb-1vd	14.125 [ab]
Ranger Russet	13.125 [abc]
PI283107hou-1vd	11.625 [bcd]
PI275181iop-1vd	11.000 [cd]
PI275182iop-1vd	9.250 [d]
PI275182iop-4vd	9.125 [d]

2. **Development of sequencing resources (whole genome and transcriptome) for *Solanum bulbocastanum***
We are updating the draft genome assembly of *S. bulbocastanum* accession SB22, with the ultimate goal of producing a chromosome-level representation of the genome. Long-read DNA sequence data was obtained from the Pacific Biosciences Sequel instrument at the OSU Center for Genome Research and Biocomputing. With these data, we were able to re-assemble a much less fragmented version of the genome, with fewer, much longer strings of DNA. The mean size of the DNA strings in the assembly increased from 9,854 in version 1 to 2,300,000 in version 2. The number of annotated protein-coding genes in the updated assembly is 32,943. Chromosome scaffolding is currently underway using a method called Chromosome Conformation Capture. The SB22 genome resource enables study of genetic control of important agronomic traits including disease and pest resistance.

3. **Identification of genes and genomic regions that confer resistance to PVY through development and utilization of potato genome resources.**
Background: *Potato virus Y* (PVY) is one of the most economically important pathogen of potato and exists as biologically distinct strains. The virus-derived small interfering RNAs (vsiRNAs) from potato cv. Russet Burbank individually infected with PVY-N, PVY-NTN and PVY-O strains were recently characterized. Plant defense RNA-silencing mechanisms deployed against viruses produce vsiRNAs to degrade homologous viral transcripts. Based on sequence complementarity, the vsiRNAs can potentially degrade

host RNA transcripts raising the prospect of vsiRNAs as pathogenicity determinants in virus-host interactions. This study investigated the global effects of PVY vsiRNAs on the host potato transcriptome.
Methods: The strain-specific vsiRNAs of PVY, expressed in high copy number, were analyzed *in silico* for their proclivity to target potato coding and non-coding RNAs using psRobot and psRNATarget algorithms. Functional annotation of target coding transcripts was carried out to predict physiological effects of the vsiRNAs on the potato cv. Russet Burbank. The downregulation of selected target coding transcripts was further validated using qRT-PCR.
Results: The vsiRNAs derived from biologically distinct strains of PVY displayed diversity in terms of absolute number, copy number and hotspots for siRNAs on their respective genomes. The vsiRNAs populations were derived with a high frequency from 6K1, P1 and Hc-Pro for PVY-N, P1, Hc-Pro and P3 for PVY-NTN, and P1, 3' UTR and NIa for PVY-O genomic regions. The number of vsiRNAs that displayed interaction with potato coding transcripts and number of putative coding target transcripts were comparable between PVY-N and PVY-O, and were relatively higher for PVY-NTN. The most abundant target non-coding RNA transcripts for the strain specific PVY-derived vsiRNAs were found to be MIR821, 28S rRNA,18S rRNA, snoR71, tRNA-Met and U5. Functional annotation and qRT-PCR validation suggested that the vsiRNAs target genes involved in plant hormone signaling, genetic information processing, plant-pathogen interactions, plant defense and stress response processes in potato.
Conclusions: The findings suggested that the PVY-derived vsiRNAs could act as a pathogenicity determinant and as a counter-defense strategy to host RNA silencing in PVY-potato interactions. The broad range of host genes targeted by PVY vsiRNAs in infected potato suggests a diverse role for vsiRNAs that includes suppression of host stress responses and developmental processes. The interactome scenario is the first report on the interaction between one of the most important *Potyvirus* genome-derived siRNAs and the potato transcripts.

4. **Development of sequencing resources to differentiate different races of *Meloidogyne chitwoodii***

Accomplishments: We successfully finished sequencing of CRKN race 1, CRKN race 1 Roza pathotype and CRKN race 2 using Illumina HiSeq 3000 and Illumina MiSeq. Initial assembly has been finished with Illumina HiSeq 3000 and the data is available for the researchers to use. We are currently working on comparing the three genomes to identify regions of dissimilarity for future marker development.

The overall objective of this proposal is to design PCR-based markers to identify the CRKN pathotypes. PCR-based markers typically means that a thermocycler and imaging systems are necessary to run the reaction and to visualize the results. We wanted to enhance the functionality of the CRKN identification assay for future use in field settings. Therefore, we have worked on loop-mediated isothermal amplification (LAMP) assays for identification of CRKNs. LAMP assays take advantage of the *Bst* DNA polymerase with strand displacement activity to amplify large amounts of DNA in a short amount of time (<1 hour) at single temperature, eliminating the need for the thermocycler (Notomi et al. 2000). LAMP assays can be colorimetric, removing the need for expensive imaging systems. The Gleason lab recently has developed a loop-mediated isothermal

amplification (LAMP) assay that can specifically detect the CRKN isolated from soil samples (Plant Disease, submitted). The LAMP primers were stable for all the CRKN pathotypes tested. Thus, it can distinguish the CRKN from other species of root-knot nematodes found in Washington State. With this proof and concept completed, we can now use information regarding sequence polymorphisms between isolates to develop new LAMP primers and potentially generate pathotype specific LAMP assays.

Activities conducted: For the LAMP assay, primers were designed based on DNA sequence differences in the intergenic spacer 2 (IGS2) region between 5S rDNA and 18S rDNA of *Meloidogyne chitwoodi*. When the LAMP products were separated by agarose gel electrophoresis, they produced ladder-like banding patterns (Figure 1). To compare the sensitivity of LAMP assay with conventional PCR, a series of 10-fold dilutions of *M. chitwoodi* race 1 genomic DNA was used as the reaction template for both a LAMP assay and PCR. The LAMP assay was 100 times more sensitive compared to conventional PCR in amplifying products from purified DNA. The LAMP primers we designed could amplify products from three *M. chitwoodi* pathotypes: race 1, race 2, and Roza. No products were obtained in the LAMP assays using DNA from the tropical root-knot nematodes (*M. incognita, M. javanica)* and the Northern root-knot nematode *M. hapla* (Figure 1).

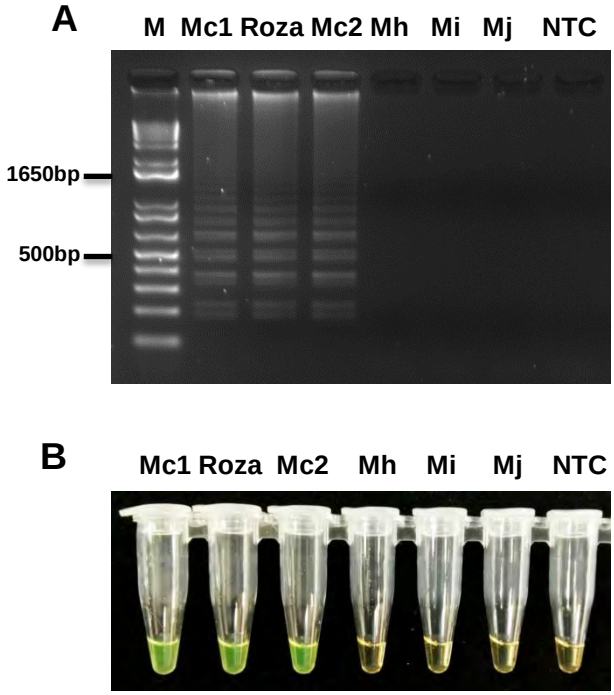

Figure 1. LAMP assay for the CRKN. LAMP assays were performed using genomic DNA from Mc1 (*M. chitwoodi* race 1), Roza (*M. chitwoodi* pathotype Roza), Mc2 (*M. chitwoodi* race 2), Mh (*M. hapla* VW9), Mi (*M. incognita* VW6), Mj (*M. javanica* VW4), and NTC (non-template control). Positive LAMP results were visualized by **A,** banding patterns on an agarose gel electrophoresis, and **B,** SYBR Green I staining of the LAMP reactions. There were positive reactions for all three *M. chitwoodi* isolates tested. There were no positive reactions for *M. hapla, M. incognita,* and *M. javanica* samples.

Publications:

1. Moyo, L., S.V. Ramesh, M. Kappagantu, N. Mitter, V. Sathuvalli, H. Pappu (2017). The effects of potato virus Y-derived small interfering RNAs of three biologically distinct strains on potato (*Solanum tuberosum*) transcriptome. Virology Journal 14:129

ANNUAL PROGRESS REPORT SUBMITTED TO THE NORTHWEST POTATO RESEARCH CONSORTIUM

Title: Education Efforts Specific to the Potato Sustainability Initiative Survey

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REPORTING PERIOD: July. 1, 2017 - Feb 15, 2018

Summary of accomplishments: *This project was initiated in the summer of 2016 and is currently in progress for the second funded year.*

A manual with accompanying resources was developed and posted at www.uidaho.edu/potatoes on May 4, 2017. Lynn Woodell attended two Potato Sustainability Initiative (PSI) audits that summer to evaluate the effectiveness of the manual in aiding grower efficiency in preparing for the audit, understanding and answering audit questions and providing documentation of their stewardship practices. Woodell presented a poster on the topic at the Potato Association of America Meeting in July 2017. Olsen was actively engaged with the PSI governance committee in altering questions for the 2017 survey which was released Nov. 2, 2017. A workshop regarding the survey and audit was held at the Idaho Potato Conference in January 2018. Significant changes were made to 2017 survey questions resulting in the need for extensive edits to the manual. This manual is being reviewed for publication and posting at www.uidaho.edu/cals/potatoes in February.

Activities or experiments conducted:

A Potato Sustainability Initiative (PSI) Survey and Audit Organizational Manual for the 2016 Survey and 2017 Audit with accompanying resources was developed to aid growers in navigating the survey and audit. It included fill-in documents, examples of standard operating procedures, and resources to help answer interview questions. The manual was reviewed by the PSI committee and select auditors. Both word and pdf versions along with resources were posted at www.uidaho.edu/potatoes on May 4, 2017. Several articles were published regarding the survey and audit and alerting potential users the manual was ready for download. The survey was updated in November 2017 and a revised manual was produced and currently in review.

Audits performed on two grower operations were observed to evaluate the effectiveness of resources provided in the manual and changes were made as needed. A poster was presented at the Potato Association of America Meeting in Fargo, ND in July 2017 describing the PSI survey/audit and our educational efforts.

Nora Olsen participated as the academic member in the Potato Sustainability Initiative (PSI) Governance Team twice monthly meetings as well as the annual meeting in Lethbridge, Alberta June 21-23, 2017. The 2016 survey was altered by the PSI Governance Team and the 2017

Survey was released on November 2, 2017. These alterations resulted in extensive revisions to the existing manual and accompanying resources by the members of this funded proposal. The manual is under review and will be posted soon. Newsletters and alerts will be sent at time of publication.

A workshop regarding the survey and audit was held at the Idaho Potato Conference in January 2018. This workshop introduced the audit, objectives and examples of the manual and provided a "mock" audit with an ISDA auditor.

Results: Feedback from the growers and industry has emphasized the contribution from the manual and education programming as a standard means for growers to use for the audit. The usefulness and application of the manual and education have provided a consolidated resource for the Northwest potato growers to navigate the survey and audit.

Publications:

Potato Sustainability Initiative (PSI) Survey and Audit Organizational Manual for the 2017 Survey and 2018 Audit (In review) and will be posted at www.uidaho.edu/cals/potatoes.

Potato Progress (May 4), Potato Pulse (May 4), WSU Potato Pest Alerts (May 19), and PNW PestAlert (May 24). A trade magazine article was published in the May issue of Potato Grower Magazine. Additional newsletters will be written to provide details regarding the new survey and audit.

Presentations & Reports:

Woodell, L., N. Olsen, T. Waters, and C. Wohleb. *Sustainability is not Just a Buzzword*. Poster presentation, 101th Annual Potato Association of America meeting, Fargo, ND, July 25, 2017.

Woodell, L. and N. Olsen. 2018. Sustainability Audit Workshop. Idaho Potato Conference. January 18, 2018.