**Minutes Summary: WERA-89 Annual Meeting**

**Embassy Suites by Hilton San Diego**

**San Diego, CA on March 8-9, 2017**

Chair: Andrew Houser

Vice Chair: Ken Frost

Secretary: Matthew Blua

In attendance: Andrew Houser, Nora Olsen, Lynn Woodhall, Carrie Wohleb, Susie Siemsen, Alice Pilgeram, Phil Townsend, Mary Kreitenger, Amy Charkowski, Ana Cristina Fuladosa, Nina Zidak, Dave Douches, Paul Bethke, Kent Sather, Alan Westra, Johnathan Whitworth, Guiping Yan, Rich Novy, Alex Karasev, Chris Benedict, Kasia Duellman Kinzer, Pablo Guzman, Mark Pavek, Russ Groves, Stewart Gray, Neil Gudmestad, Kylie Swisher, Jim Dwyer, Joe Kuhl, Silvia Rondon, Andrei Alyokhin, Chuck Brown, Chris McIntosh, Keith Schuetz, Raquel Salati, Mathuresh Singh, Andrew Nicholaus from Simplot, Debbie Inglis, Darrin Hall, Washington DeSilva, Erin Weber, Hannu Pappu, John Mizicko from Eurofins BioDiagnostics, Carolyn Keller, Teresa Almeida, Kenneth Frost, Nicole Hostert, Anna Jespersen, Mark McGuire, Alex McCandlewire, James Harris and guest.

The meeting started at 8:00 am March 8, 2016.

Welcome and introductions.

The minutes of the WERA-089 Annual Meeting of March 2-3, 2016 were corrected and then unanimously approved. Alex Karasev moved to approve, seconded by Stewart Gray.

**State Certification Reports:**

**CA - Pablo Guzman** noted California had 25 acres of G1, 150 acres of G2, 506 acres of G3, 56 acres of G4, and 28 acres of G5 seed entered into certification. There was PVY in G4 and 5 but other seed, G1-G3, were relatively clean. This year, California received seed from WI ND, NE ID CO, MN, and Canada (BC, PEI, and one or two other provinces that I didn’t catch). There was an issue with frost in 40 acres entered for certification. Pablo found mop-top in one field but is not sure if it has become established because the incidence (symptoms??) seems to be decreasing but he would like to continue to do inspections in that field. This year he found only 1 plant (0.001% PMTV infection) in which PMTV was confirmed. However, he has only been testing foliar sample and plants that are symptomatic. He has not assayed asymptomatic plants for PMTV.

**CO - Andrew Houser** discussed changes the potato certification staff in CO. Staff now includes Andrew Houser, Carolyn Keller, Greg Hess, Teresa Almeida, Sarah Shawcroft, Michelle Leckler, and Sharon Yust. He noted that acres entered into certification have been decreasing since 2012 and, in 2016, CO was at 11,300 acres entered into certification. This year, the winter post-harvest test was planted early, Oct. 31 through Nov. 2 and post-harvest readings were taken Dec. 6 – 21. For CO, most seed lots fell into the 0-3% mosaic range. Some acres were categorized in the 3.1-5% grouping and could be sold for commercial ware production in CO. A smaller number of acres were categorized as 5.1-8% mosaic, which could be sold out of state. Approximately, 8% of the seed acres were rejected outright.

In CO, the acreage rejected in the winter test increased from 1991 through 2013, and has been decreasing since 2013. Andrew noted this recent decrease could be due to the CO seed law that was put into place in 2012 (they are now finally seeing the effects). Crop oils and timing of insecticides may also be contributing as growers seem to be taking interest in better management practices.

In 2016, most seed lot rejects occurred at the winter test. Summer tolerance for mosaic is at 1.5%. In CO, winter tests are primarily visual – with some ELISA testing. In 2016-17, there were 952 plants tested for PVYNTN. About 3.5% of the plants had PVY-N or NTN (n=33), this only includes seed lots that fell between 1.0 and 5.0% mosaic. If PVY- N or NTN is over 1% in a seed lot, that lot cannot be planted back in CO, but can be sold out of state. Some additional PVY samples were sent to Stewart at Cornell, 23 plants were tested representing 17 cultivars and 21 lots/research plots. Approximately 70% PVY-WI, 17% PVY-O, 7% PVY-NTN, and the rest were mixed infections.

Comparing visual readings in HI (A sample) with lab results (B sample), HI readings were, on average, lower than lab testing samples. This suggests visual assessment may not capture the true virus prevalence, however nearly every seed lot that was rejected based on visual readings would have been rejected if the lab test was used to determine status. Alan Westra commented that the winter test serves its purpose and visual ratings are adequate for most varieties. Alex Karasev noted there may be a large cultivar effect associated with the differences observed.

In CO, acreage rejected due to blackleg was pretty constant in 2012, 2013, 2014, and 2016 at around 200 acres. In 2015, there was a spike in rejection due to black leg – approximately 750 acres – but most of the rejections in 2015 occurred in one variety.

**ID - Alan Westra** reported that acreage in ID was around 32,812 acres for 2016. This represented a drop of 3% from the previous year. There were 32,542 acres accepted with a withdraw/decline rate of 1.25% in 2016. PHT results:

       Currently recertification tolerance is 2% in Idaho, but there is a rule change pending and this will be reduced to 1% and perhaps lower in future.

       In 2016, 44% of lots had no mosaic detected, 28% had between 0 and 2% mosaic, and 27% had more than 2% mosaic and were not eligible for recertification (i.e. still eligible for commercial seed).

       In terms of acreages these represented (Clean, 5% or less, 5-10%, >10%).

Mean PVY% (or mosaic) has been increasing since 2008.  This is likely an artifact of moving the winter test to Hawaii.  In 2016 the PVY prevalence was highest in the 9 years when results are reported in terms of lots. When results are reported as acres, PVY prevalence went down a little in 2016. In general, the amount of PVY has remained constant in the program (approximately <5% as lots and acres). His general feeling is that current season spread is a problem, particularly for some specific locations.

Alan posed the question of what is an acceptable level of PVY to plant? His typical answer is that he doesn’t know, 5% makes certification in all states, 10% is the current recommended max. in ID. Do not plant greater than 10%.

Westra also noted some bacterial ring rot (BRR) testing protocols that have been adopted in Idaho and showed a flow chart to diagram their protocols. In Idaho, the number of acres rejected for BRR decreased in 2016 and no BRR was observed in summer inspections. The lab detected BRR in one lot in their screening program. Hopefully, BRR has been flushed out of the system and will not be a problem in the future. Currently, the rules in Idaho require a trace-back analysis to determine origin of the BRR (e.g., planting stock, equipment, trucks, etc.). It seems like trucks are a big issue in terms of contamination and the flush out rules are important for maintaining clean seed.

**MT - Nina Zidack** summarized her post-harvest results from the 2016 season noting that plant growth, in general, was great this year. There was some slower emergence in a few varieties, for example, they did not dip Umatilla Russet and emergence was poor in that variety. For MT, there were 53 lots with 0% mosaic, 19 lots (0-0.5% mosaic), 9 lots (0.5-1% mosaic), 10 lots (1-2% mosaic) and 8 lots (>2% mosaic). She noted there is still some concern about current season spread and aphid abundance.

Zidack summarized the performance of different varieties in the winter test, specifically how virus prevalence changes as the different generation materials pass through seed certification.

* In 2015, Alturas had high mosaic and that seems to now be getting flushed out – all Alturas g1 see was clean in 2016.
* Russet Burbank still performs strong with respect to virus/mosaic -- it does not seem to accumulate virus like some of the other varieties.
* Ranger had some virus issues in previous years, but the virus is now getting cleaned out and G1 lots in 2016 look good.
* Umatilla seems to do okay.
* Norkotah is really a problematic variety and almost no lots are very clean – even in G1 and G2 have a fair amount of mosaic. Norkotah line selection CO-2 is also problematic in terms of PVY infection, but some of the Norkotah lines perform better than the “true” Norkotah.

Zidack also discussed data that related summer leaf testing to PHT results. For G1, if you have a 0% prevalence leaf test in summer then there is an 86% chance of getting 0% prevalence in the PHT. For G2, 0% PVY prevalence in summer will have a 53% chance of having 0% (mosaic/pvy) in the PHT. For G3, with 0% prevalence in summer leaf testing there is a 33.5% chance of 0s in the PHT. There was a notable increase in PVY prevalence from 2014 to 2015, perhaps due to high in-season spread. Across all seedlots, percent of lots with 0 PVY at PHT that also had 0 pvy in summer was 71.5% (2015) and 69.4 (2016).

**ND – Kent Sather** reported that ND planted about 15,000 acres of seed potatoes, which was down about 3000 acres from 2015. In 2016, there was serious drown out issues from high amounts of rain. In total, ND lost ~900 acres to flood, ~900 to rejects, and 1200 acres were withdrawn to go to processing. About 12,000 acres were accepted for recertification, but not all harvested because of rain. Approximately, 8300 acres were represented in the FL winter grow out test. There are only two states left in FL, ND and ME. Sather noted that ME will be moving toward lab testing of tubers and soon ND will be the only state evaluating seed potatoes in FL.

In ND, winter growout is only required for recertification and 0.5% mosaic is certification tolerance. IN 2016, there were 6200 acres that passed for recertification. Around 58% of the acreage was at 0% mosaic, which was a little worse than in past years. There was another 1000 acres that was 0-0.5% mosaic. These increased mosaic in 2016 could be due to rain, since growers couldn’t get into their fields to vine kill or harvest (or provide various other crop management practices). IN 2016, Aphid populations were not very high, but spread seemed to be happening – maybe due to an inability to get into the fields.

ND did conduct a small PVY survey in 2016 which targeted different varieties. They were specifically looking for the most subtle symptoms in specific varieties. They then tested 36 plants for PVY and strain-typed the virus. Four were PVY-NTN and 32 were PVY-Wi. In MN, there seems to be a pretty big uptick in NTN and there is some concern/interest among growers about how to best manage. Because of the wet season and there is some concern about late blight – they may be starting to see some samples that are late blight positive in ND.

Zidack suggested that by picking mild symptoms, NTN may be underrepresented in the potato seed lots - there might actually be more than they are sampling.

Currently, ND can ship seed lots with unknown PVY prevalence for ware production but they may move to conform with the national harmonization standards in the future. Gudmestad noted that Dickeya spp. problems and late blight were way more problematic in the Midwest and that the rules in ND will probably not change soon.

**WI – Amy Charkowski** directed the program from January through August, 2016, and Amanda Gevens and Russel Groves became interim co-directors in August 2016. Amy reported that there were no problems in WI through August. Russ Groves noted that in WI, similar to ND, harvest was challenging for some growers due to rainfall and wet soil conditions. Around 9000 acres of seed potato were entered for certification in WI in 2016. In Antigo area, no late blight was observed and aphid pressure, as measured by suction traps, was low. Effectively – aphid management is prophylactic and based upon phenology models developed at the UW-Madison, so the magnitude of aphid numbers did not impact the approach to management too much. There was a little bacterial soft rot observed during harvest and an extended period of warm weather enabled harvest despite earlier wet conditions.

In 2016, there was more Dickeya testing completed in seed lots. IN 2017, all out of state lots will be tested for Dickeya and no Dickeya-positive lots will be entered for recertification. In 2016 as in previous years, the winter grow-out test was augmented by ELISA testing and focus was placed on asymptomatic varieties. In 2016, the lowest levels in virus in 5 years was observed in WI and less than 2% of seed lots were rejected.

WI now has a certified seed potato bill moving through the legislature and that is likely to be adopted – its contents were not discussed. WI seed certification just added a few people – an outreach specialist and an inspector. In the future, they will be searching for a new faculty that will take over the administration of the WI seed certification program.

There was a conversation about whether visual ratings could/should be phased out and other testing methods used to assess virus. The following comments were made:

* For at least 10 years of testing on asymptomatic varieties, ELISA leaf testing would very rarely change the certification decisions.
* Stewart Gray stated that visual post-harvest test typically works well and inspectors do a good job identifying mosaic. However, summer inspections are likely most problematic – they are good in that they can indicate the prevalence of virus that was planted, but are not a good indicator of the virus prevalence in the seed crop.
* Nina Zidack noted that every year is different in terms of symptom expression and variety plays a big role – i.e. Blazer does not express symptoms when infected with PVY and PVY infection of Clearwater Russet is often observed, but on occasion is missed.
* Russ Groves commented about the large variation of symptom expression due to variety and environment.
* Amy Charkowski suggested that summer inspections are still very good for finding very problematic lots – but lots that are borderline might be missed.

Johnathan Whitworth and Alan Westra discussed why ELISA sometimes results in false negatives and Westra is more confidant in the winter grow out visual inspection than ELISA-testing for varieties that express PVY, not Typhoid Mary varieties. Perhaps ELISA is useful for reexamining mild-expressing plants or suspect plants. Alex Karasev then noted that mosaic is caused by more than PVY and, in reality, only 85% of plants expressing mosaic are infected with PVY.

**WA – Debbie Inglis** discussed the interesting situation in WA which is one of only two states that are not conducting the winter growout test in Hawaii. They have been met with great resistance from their growers when considering moving the winter grow-out test to HI. Debbie currently has a graduate student that has looked into the social aspects of this resistance and they are currently publishing a paper on this topic. Basically, there are 5 components to adapting a new technology and growers in WA are resistant to change because they perceive a relative advantage of keeping the testing in WA – (e.g., there may be spread among seed lots in HI and they might lose advantages they have other states). They like openness and “observability” (i.e. they like to be able to observe the process). There is unknown complexity associated with moving the trial, that is perceived as being difficult. (Recent publication about adoption technology has just been published in Rural Sociology). In terms of PVY in WA, there was very little in their seed area and PVY prevalence has greatly declined since 2011. They have approximately 180 varieties, but in they are planted in very small plots. The number of varieties makes visual symptoms are challenging for inspectors scoring mosaic.

**Alex Karasev presented a few slides about HI growout testing** and the tomato PVY was discovered in HI. He basically discussed how PVY exists as a complex of strains. The PVY-O and PVY-N strains and their recombinants affect potato and the PVY-C strains affect pepper. Usually the strains are somewhat host specific, but there are some intermediate hosts that can be infected by both pepper and potato strains.

There are some concerns about having the WGO testing in HI including location issues (e.g., in field spread, local virus inoculum, aphid pressure) and phytosanitary issues (e.g., introducing a virus not currently present in HI, other infestation of local crops). They somewhat recently discovered tomatoes infected with PVY in HI. The tomatoes were field grown and exhibited symptoms of PVY. This infection was later confirmed to be PVY-H14. This strain is an O serotype and groups with the PVY-C lineage. It is only 91% identical to PVYc from pepper, 87% identical to PVY-0, and 81% identical to PVY-N. In laboratory inoculations, the PVY-C lineage induces no local symptoms on inoculated leaves and produces no systemic infection on potato. Thus, it seems potato cannot be infected by this isolate and it is most likely a pepper isolate. Alex concluded that this PVY strain found in tomato presents no danger to the winter growout in HI and it is likely an isolate of PVY local to HI (i.e., not brought to HI by potato).

**Project Reports:**

Diagnostics, Detection and Testing

**Testing for Dickeya and BRR using a New Isothermal Amplification System.** *Keith Schuetz*, Agdia. Keith reported on a new isothermal detection assay for Dickeya spp. and Clavibacter (Cms). The isothermal system is based on recombinase-polymerase amplification and is a new technology being used by AGDIA. It seems to work well on crude extracts, but one downfall is that you must have fluorimeter for detecting amplification. Currently, the Dickeya assay takes approx. 20 minutes and correlates well with the pelADE PCR. Next steps are to validate the assay on tuber soaks. Currently, the assay seems to have good specificity for the bacterial isolates they have examined. They are also trying to determine the limit of detection and optimizing the extraction procedure. The goal is to get the assay to work with water from tuber soaks. The assay for Cms detection is a 20 min or 40 min assay depending on detection method that is used. Agdia has developed two tests, one that is based on the Cms50 gene and another that is based on Cel A PCR gene targets. Both tests are comparable in sensitivity however the CelA only targets Cms isolates contains the PCS1 plasmid. AGDIA is currently trying to validate this assay. It works through amplification performed in real-time using a portable fluorimeter or in a heating block followed by amplicon detection in an “ELISA-type” strip chamber. This assay seems to be a specific assay based on the bacterial species isolates they have examined (and many bacterial isolates have been evaluated). To date, this assay does not seem to have cross-reaction with other Clavibacter species and the limit of detection was around 0.5 pg for Cms. They will and need to continue to optimize the assays and are always looking for infected materials and labs to trial the AGDIA assays.

**A new Macroarray from Bioreba that can detect several viruses and 1 viroid from a single sample.** *Raquel Salati*, Bioreba. Raquel gave a company overview about their services and products offered. Specifically, there are agri-strips produced by Bioreba for detection of multiple viruses as well as many PCR reagents offered for sale. She then described Bioreba’s new macroarray multiplex assay for simultaneous detection of multiple viruses from plant samples. The assay basically involves a total nucleic acid extraction, followed by PCR, and hybridization of PCR amplicons to plate. Based on binding pattern on plate, the virus species that are present in the sample can be identified. This assay has the ability to also react and identify the different PVY strains. She then described a case study of how to use the assay using potato mop-top virus as an example. Several different dilutions were evaluated for detection.

Alex Karasev noted that the PVY strain classification scheme used by Bioreba’s is old and needs to be updated. As is, the classification of PVY strains is not useful.

Johnathan Whitworth suggested that AMV and TRV should be included in the macroarray.

**What we’ve accomplished in PVY management in NB and evidence of mechanical transmission.** *Mathuresh Singh*, Agricultural Certification Services Inc, NB, Canada. Mathuresh Singh reported on long-term observational studies to examine current season spread of PVY in grower fields. In these studies, he flagged out plants in each field and then monitored the flagged plants throughout the growing season. He recorded the different management practices used in each field and tried to correlate spread with specific management tactics. He also used PCR to detect PVY in aphids visiting the fields (many ended up being non-colonizing aphid species). Growers were trying to manage aphid abundance, but it seems they were not timing their management practices effectively to reduce PVY spread. However, since 2012 In NB, mineral oil with insecticide use (i.e. 10-12 applications) has increased since 2012 and they have identified some spray programs that seem to be effective for reducing PVY-spread. In the PHT, they were able to reduce the prevalence of PVY in seed lots (as tested in the lab) and they have documented a decrease in the prevalence of PVY in New Brunswick, Canada. Since program started percentage of clean lots have increased from 22 to 72% and 92% seed lots tested between 0-1% PVY in 2016. Mathuresh also reported on mechanical transmission of PVY occurring in the field. He provided evidence of mechanical spread on wheels in the tractor row. PVY-NTN was the most-spread in this mechanical manner. In 2016, all PVY strains were started at equal prevalence, but NTN was transmitted mechanically more efficiently.

**Break for Lunch: 11:45; Meeting resumed – 1:07 pm.**

Announcement: with 49 participants, cost of the meeting will go down to $71.

1:10 pm **Administrative Advisor Report:** Mark McGuire from Univ. of Idaho gave the report. Again, the WERA-089 group was praised for being very active and submitting documents on time. Outlook on

Western Extension Research Activities = WERA

**Presentations resume at 1:12 pm.**

(Note: The remainder of the WERA program is made up of presentations from the participants from a USDA SCRI CAP project. Participants provided abstracts for each of their presentations and those abstracts are being included here as a summary of each presentation. If notable discussion occurred during or after the presentations, that will be captured as notes in between the presentations.)

**Shift in the PVY strain prevalence in the US seed potato crop is an unintended consequence of science, regulatory and farming practices.** *Stewart Gray*, USDA-ARS/Cornell University. In the past decade we have documented a rapid shift in the PVY strains; PVYO was most common prior to 2004, but now PVYNTN is increasing in incidence and distribution throughout all production areas and accounts for nearly 30% of the total incidence. The recombinant PVYNO/NWi population now dominates throughout the U.S., but PVYNWi has nearly displaced PVYNO. Other recombinant strains and genome variants have been detected, but their incidence and distribution changes over years and geographic regions. In general, the recombinant strains induce milder foliar symptoms in most widely grown North American cultivars. While tuber necrosis (PTNRD) is induced by most PVYNTN isolates, PTNRD is associated with isolates from nearly all strains of PVY. Interestingly, most North American cultivars express one or more *Ny* resistance genes manifested as a foliar hypersensitive-like response to infection by PVYO and some PVYNTN isolates. These reactions often lead to plant death or severe impairment of tuber production. Additionally, aphids do not efficiently transmit virus from these plants. The unintended *Ny* gene deployment is contributing to the disappearance of PVYO and maintenance of PVYNTN at reasonable levels, but also to the selection of PVYNO/NWi strains. Many factors are driving the transformation of PVY populations and PVY epidemiology in the U.S. potato crop, but most can be traced back to short comings in the science of developing better potatoes and in the business of producing the potato crop.

No discussion.

**Sampling, bulking, and detection of low-probability events.** *K.E. Frost*, Oregon State University. For potatoes grown for seed, certification programs are important to prevent the spread of seed-borne pathogens. Seed potato certification has relied primarily on visual inspection of the crop in the growing season and after the growing season in a post-harvest grow out assay. Pathogens that latently infect a plant or do not present foliar symptoms are challenging to detect. It would be adventitious to adopt the reverse transcriptase polymerase chain reaction (RT-PCR)-based assays for dormant tuber test. Cost is one factor that has limited the adoption of dormant tuber testing by seed certification programs. Here simulation-based models were used to examine different bulking strategies that could reduce the cost of the RT-PCR assay. Simulations were designed to reflect the seed certification post-harvest assay. Tuber populations (n = 400) were generated with varying virus prevalence, 1 to 36 virus-positive tubers distributed randomly throughout the population. Populations were aggregated according to the bulk size and prevalence was estimated. Bulk sizes varied from 2 to 50 tubers and cost was evaluated for no bulking, bulking, and two-stage bulking strategies. All bulk sizes produce biased prevalence estimates when true prevalence was 0.5% or greater. Two-stage bulk sampling estimated true prevalence. Maximum likelihood prevalence was between low and high bulk prevalence. Cost-optimal two-stage bulk sample size varied as a function of prevalence, optimal bulk size was 4 to 20 when prevalence was 9% to 0.25%, respectively. This simulation assumed perfect detection and only examined the combinatorial variation that may result due to bulking samples. Confidence in virus prevalence estimates will need to account for combinatorial variation if bulking strategies are widely adopted.

No discussion

**Investigating lab-based alternatives to field-based postharvest testing.** *Alice Pilgeram*, Anna Jespersen, Susie Siemsen, Eileen Carpenter and Nina Zidack. Montana Seed Potato Certification, Montana State University. The Montana Seed Potato Certification Program in participation with the SCRI project is investigating the scientific and economic feasibility of alternatives to the current post-harvest field grow-out to determine virus levels in seed potatoes.  The hypothesis is that data generated from current in-field post-harvest test (PHT) procedures can be obtained more quickly, more reliably and for less cost by assays conducted in the laboratory.  To test this hypothesis, the Montana Potato Lab has performed two years of parallel postharvest field grow-outs and dormant tuber testing utilizing tuber ELISA and Immunocapture RT-PCR to detect PVY. This fall, four replicate samples from four seed lots representing various levels of PVY in summer testing and visual inspections were evaluated in the postharvest test on Oahu, HI and in the lab using tuber ELISA and Immunocapture RT-PCR.  The objectives of this research were 1) determine if the tuber testing results are comparable with the summer and winter grow-out results and 2) determine if the tuber testing can be economically scaled in a typical seed potato certification lab.  Results for each of the PVY evaluations from a given potato lot will be compared for detection of virus.  Data will also be presented on different PCR techniques including reagent costs, labor costs and instrumentation requirements

Discussion: Cost for IC was on the order of $150 for a 400 tuber sample. Use SYBr instead of Taqman. The question still remains about the best place to collect the sample from the tuber – the stem or bud end.

**Workshop for Diagnostics of Potato Pathogens using High Throughput Molecular Methods.** *Susie Siemsen*, Montana Seed Potato Certification, Montana State University. The SCRI project is sponsoring my participation in the workshop “Diagnostics of Potato Pathogens using High Throughput Molecular Methods” at the NAK services in Emmeloord, Netherlands on February 13-15, 2017. The course will begin with an introduction to bacteriology, virology, nematology and laboratory tour. Diagnostic test methods in ELISA, immunofluorescence, and real-time PCR will be presented. Methods in sample preparation will be discussed. During the second day, we will isolate viruses through grinding and enrichment techniques as well as using robotics for pipetting and extracting DNA and RNA. I will attend a lecture on the quality aspects of PCR. The final day of the workshop will be used for performing PCR for viruses. The results from the PCR will be interpreted and analyzed. It is anticipated that techniques learned at this workshop can be used to refine tuber testing methods that are currently under development in our lab, and all methods will be shared with seed potato certification agencies across the United States.

Disscussion:Amy Charkoski noted that she has thought about how this type of testing model/facility could work in the US and has not come up with a good way to do it – it would need to be regional.

**Genetic diversity studies of PMTV and TRV.** *H.R. Pappu*, N. Gudmestad, J. Whitworth, and C. Brown. WSU, NDSU, USDA-ARS Aberdeen and USDA-ARS Prosser. Potato mop-top virus (PMTV) and Tobacco rattle virus (TRV) are increasingly becoming important in the US. To better understand the genetic diversity of these viruses, we first started with sequencing the complete genome of a PMTV isolate from Washington State (WA) followed by an isolate from MD. Phylogenetic analyses of each RNA and each gene showed divergent patterns with respect to their relatedness to the American vs European isolates. Three distinct clades were revealed for RNA-3. The biggest clade comprised of isolates from various parts of the world, including North America, South America, Europe and Asia. The WA and MD isolates fell within this biggest clade. The second clade included two isolates from East Asia, while the remaining isolates in the third clade originated from Columbia. The genome of a third isolate from Maine (PMTV-ME) was cloned and is being sequenced. The complete genomes of 15 isolates of TRV from CO, ID, MN, and ND states were cloned and sequenced. Phylogeny of these isolates with the known isolates showed clustering into American and European isolates. Phylogenetic analysis based on a comparison of nucleotide and amino acid sequences of CP and 2b protein with known isolates showed that the North American isolates cluster as a distinct group along with Asian isolates while European isolates formed a separate cluster. Significant homologies among American isolates were observed, while European isolates were found to be more diverse. The RNA-1 was found to be highly conserved compared to RNA-2.

No discussion.

**TRV and PMTV at Prosser, Contributions to a Team Effort.***C. R. Brown*, Richard Quick, Launa Hamlin – USDA-ARS Prosser and WSU-IAREC. One of the best outcomes in the management of necrotizing viruses would be new varieties with sufficient resistance to allow the cultivation in infested soils or where exposure to potato virus Y or where PVY-transmitting aphids may occur. One instance may be the breeding line POR06V12-3, which will probably be named Castle Russet. It has a high level of resistance to PMTV, and immunity to TRV and PVY (all strains). During the last year it has performed well in processors’ trials and provides a real possibility for wide acceptance in the West and Mid-West. As we learn more about biology of the host-parasite-vector relationship in the disease Corky Ringspot (Potato-TRV-Stubby root nematode), it is apparent that a large percentage of tubers which do not have tuber necrosis have nonetheless detectable virus. Additionally, the percentage of detectability was found to rise steeply when tested at harvest and at three and seven months in storage. It is a general observation that resistant clones which have very low incidences of internal necrosis also have less incidence of detectable virus. Examination of samples from seedlots intended for planting in Washington were tested for presence of TRV and PMTV. TRV and PMTV were found at an incidence level of 0.5 and 1.0 per cent, respectively. Identification of resistant germplasm, quantification of the high percentage of symptomless infection, and detection of the presence of both viruses in certified seed will guide future research priorities in management

Discussion: Alex Karasev noted that lack of symptoms does not mean the virus was not present.

**Break 2:53 pm. Resume at 3:20 min.**

**Developing Real-Time PCR Assays for Identification and Quantification of Stubby Root Nematode *Paratrichodorus allius* in Soil.** *Guiping Yan*, Danqiong Huang, and Addison Plaisance, NDSU. The stubby root nematode, *Paratrichodorus allius*, is a vector of *Tobacco rattle virus* causing potato corky ringspot disease that can cause significant economic losses to the potato industry. Identification and quantification of *P. allius* are important for effective disease management. New real-time PCR assays with TaqMan and SYBR Green chemistries were developed for direct detection and quantification of *P. allius* from soil. A pair of primers was designed from ITS-rDNA of *P. allius* for the SYBR Green real-time PCR. Another set of primers/probe was designed for the TaqMan assay. The specificity of the primers/probe was evaluated by in silico analysis and confirmed by testing 34 nematode species and communities. Standard curves were generated from serial dilutions of DNA extracted from autoclaved, *P. allius*-free soil inoculated with 20 *P. allius* nematodes, and verified by a high correlation between the numbers quantified by real-time PCR and added to the soil (R2 > 0.85). Both real-time PCR assays specifically amplified target nematodes, and the SYBR Green method was more sensitive than the TaqMan method. The real-time PCR assays were applied to naturally infested field soils, and the numbers of *P. allius* quantified by TaqMan (R2 = 0.80) and SYBR (R2 = 0.86) correlated well with those determined by the microscopic method. Compared to the estimation using microscopy, the numbers of target nematodes tended to be overestimated when using TaqMan assay but were similar when using SYBR Green assay. These two real-time PCR assays were found to have a strong correlation (R2 = 0.92).

No discussion.

**Reflectance spectroscopy, remote sensing and potato physiology and disease.** John J. Couture, Amy Charkowski, Paul Bethke, and Philip A. Townsend. Vegetation spectroscopy is the measurement of reflectance, absorption and/or transmittance of light across a continuous spectrum in live plants, and is sometimes referred to as hyperspectral remote sensing. It has the potential to detect differences in foliar nutrients, water and physiology due to differential absorption of light in narrow wavebands. Using portable field spectrometers or imaging spectrometers, we can detect diseased plants *in situ* and *in vivo*, allowing rapid assessment and potentially reducing costs of destructive sampling and associated assays. Here, we report the results of using leaf-level reflectance spectroscopy and imaging spectroscopy of potato foliage to identify and potentially map PVY-positive plants. The research incorporates two approaches. First, we use a discriminant analysis method to demonstrate that spectroscopy can be used to accurately detect (>80%) PVY-positive plants across a number of varieties. Second, we take a trait-based approach and use the same spectroscopic data to estimate important chemical and physiological traits including nitrogen concentration, leaf mass per area, lignin concentration, leaf water potential and leaf photosynthetic capacity, demonstrating statistically significant differences in all of these traits between PVY-positive and -negative plants. This allows us to assess the processes underlying our ability to detect PVY-positive plants from their foliage. Extension to imaging spectra shows good prospects for being able to map potato chemical/physiological properties associated with disease presence, but requires further work to determine the appropriate growth stage for remote imaging to be a practical approach to PVY mapping.

No discussion.

Epidemiology and Management

**Relationship of aphid abundance and diversity to local landscapes.** *Groves1, R.,* Wenninger, E., Benedict, C., Rondon, S., Dwyer, J. Alyokhin, Lagos-Kutz, K. and Frost, K. Our research focus has been aimed at objectiveIII.C.2. Our initial investigations have been aimed at establishing models that describe the flight phenology of potential Potato virus Y aphid vector species. Aphid capture data have been compiled from the NCR Aphid Suction Trap Network (<http://traps.ncipmc.org/>) from a span of 10 years (2005-2015) and 45 locations comprising over 235 species of aphids and nearly 923K individual captures in the upper Midwestern US. Additional aphid trapping data from various potato producing states are being identified to species and are planned for validation in the current models. These trap data will be standardized for each year, location, week, and cumulative growing degree-days (base50) using a random effects model. Generalized additive models will be fit to the resulting random effects and used to predict the phenology of unique aphid species in different states. Next, we are underway in trying to establish the relationship of aphid captures to local landscape element prevalence, or intensity. Over the same experimental aphid capture interval (2005-2015), aphid captures will be joined with landscape composition surrounding each suction trap location (i.e. 20 km buffer) using the USDA’s, Cropland Data Layer (CDL) (<http://nassgeodata.gmu.edu/CropScape/>). For each trap location and year, we have built crop identity data surrounding each trap using the CDL with buffer distances ranging from 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 kilometers. These data, plus landscape intensity attribute data (potato intensity, agricultural intensity, etc.) have been compiled for each trap, year location of the NCR suction trap data.

No discussion.

**Predicting End of Season PVY Levels and the Impact of PVY on Size and Grade of Russet Burbank and Russet Norkotah Potatoes.** Chris McIntosh, UID. Three years of data were collected from test plots planted in a commercial potato field near the Teton Seed Potato Management Area in southeastern Idaho. The experiments were conducted during three consecutive years from 2010 to 2012. The field trial consisted of five treatments with four replications of each target levels of PVY at emergence. Levels of PVY were measured at emergence and the end of the growing season using ELISA. Quantile regression methods were applied to the PVY level data to establish predictive models of end of season PVY. Following harvest the tubers were graded and sized according to fresh and processed market standards. At an end of season infection level of 10%, Russet Norkotah showed a significant decrease in US No. 1 and a significant increase in US No. 2 potatoes. While showing the same direction of change, at a 10% level of infection the differences were not significant for Russet Burbank. Russet Norkotah had a statistically significant decrease in total and marketable yield as well.

Discussion: There was some discussion about how tuber number might have been affected by PVY prevalence. Chris McIntosh has also designed a calculator to estimate what growers “should have paid” for their seed.

**Aphid abundance and *Potato Virus Y* incidence in Oregon.** *Silvia I. Rondon*, Sudeep Bag, Kenneth Frost, Brian A. Charlton and Darrin Walenta, OSU. In 2015 and 2016, PVY incidence and aphid abundance were measured in nine potato fields in Oregon. Yellow sticky cards, yellow buckets, and tile traps were placed at four locations in each field. Aphids were collected weekly, counted and identified based on morphological characteristics. Potato leaf tissue was sampled from each location biweekly and assayed for PVY using ELISA. PVY strain composition of ELISA positive samples was determined using an RT-PCR-based strain-typing methodology. *Macrosiphum euphorbiae* and *Myzus persicae* were less abundant than all the “other aphids” combined. “Other aphids” were sent to Illinois in late November 2016 for further identification. PVY incidence was highest in Klamath, followed by Umatilla, Union, and Morrow counties. Strain typing of the ELISA positive samples revealed the PVYN:O strain to be most prevalent (56 %) followed by PVYO (10.34%), PVYNTN(5.74%) PVY NTN-NO(1.14). Disease incidence was correlated with high aphid incidence. Future studies aim to understand current in-season PVY spread.

No discussion.

**Monitoring Virus Vectors in Western Washington Seed Potato Fields.** *Benedict, C.*, B. Schacht, K. Kirkendoll, and H. Ullrich. Washington State University Extension. Seed potato production in Washington State is concentrated within Whatcom County, which is isolated from the major potato production areas of the state. There has not been an extensive study to determine virus vectors in this region. As a result, annual aphid monitoring began in 2015 in collaboration with investigators in ID, ME, OR, and WI. Two (yellow bucket and green tile) trap types were placed in ten (2015) or five (2016) seed potato fields in Whatcom County. Within each field, four sets of each trap type were placed (pre-emergence through vine kill) five meters apart and between three meters from the field edge and were serviced weekly. All captured aphids were counted and then placed into labelled vials with 70% alcohol. Aphid counts were shared with producers within 24 hours. Virus incidence was monitored through monthly foliar samples obtained from four quadrants (adjacent to traps) that consisted of ten randomly collected foliar samples per quadrant. Samples were initially tested using ELISA and if positive samples were detected were then submitted to the Karasev Lab for further confirmation and strain typing. In 2015, aphid populations followed a bi-modal distribution, with populations’ peaking in early June and in early August. Whereas, in 2016 aphid populations did not follow a clear pattern. In both 2015 and 2016, no PVY positive foliar samples were found in monitored fields.

Discussion: This area of WA is a very different system from the others where aphid monitoring is being conducted. The seed growers in this area seem to be very interested in the aphid data that has been generated by the project.

**Role of Non-Crop Hosts in Epidemiology of PVY on Maine Potato Fields.** A. Alyokhin, A. Buzza, L. Feinstein, and J. Dwyer, University of Maine. Based on published literature, Potato Virus Y is usually considered to have a very broad host range, infecting a variety of taxonomically unrelated plant species. Therefore, potential non-crop reservoirs could compromise grower attempts to manage inoculum sources within their crops. We conducted a two-year survey of vegetation surrounding seed potato fields in northern Maine. Indeed, a considerable number of samples collected from several (but not all) plant species were seropositive for PVY using standard ELISA tests. There were differences among locations and sampling dates. However, we were not able to confirm virus presence in any of the seropositive samples by PCR. Therefore, the importance of non-host vegetation for PVY epidemiology may be exaggerated due to false positive results reported in earlier published surveys.

Discussion: Should the statement that *PVY has a broad host range* be reevaluated? It seems there are many seropositives from weeds, but those seropositives could not be confirmed using RT-PCR.

**Aphid Populations and Potato Virus Y Trends in Maine.** James Dwyer, James Dill, Andrei Alyokhin, Aaron Buzza, Marc Dwyer, University of Maine. Staff at the University of Maine has been monitoring aphid populations in Maine potato fields by field scouting and trapping. Seed grower practices have been recorded, to analyze whether a correlation exists between practices and virus incidence. In cooperation with Dr. Stewart Gray, Virologist, USDA/ARS and the Maine Department of Agriculture Food and Conservation Seed Certification Program, PVY incidence and PVY strains have been monitored. Two hundred leaf samples were randomly sampled from ten seed fields and those samples analyzed in the Gray Lab for incidence and strains of PVY. The Maine Department of Agriculture Seed Certification program conducts a winter grow out test in Florida each year to assess the PVY incidence levels within Maine potato seed lots. In 2012, 10.7% of the Maine potato seed lots entered into the certification program were rejected because of the incidence of PVY. In 2016, that level had dropped to 2.0% of the seed lots rejected because of PVY incidence.

Discussion: Russ Groves asked why a 45 F base temperature was used for growing degree-day calculations? Basically, it was one of the base temperatures that had been provided.

At 5:30 pm meeting was adjourned for the day.

*Thursday March 9, 2017*

8:01 am Resume with agenda.

Effects of Virus Infection on Storage and Tuber Quality

**Impact of Virus Infection on Symptoms and Quality of Potato Tubers in Storage. *N.*** *Olsen*, L. Woodell, and M. Andros, University of Idaho, Kimberly ID; A. Karasev, University of Idaho, Moscow, ID; J. Whitworth, USDA/ARS, Aberdeen, ID; K. Frost, Oregon State University, Hermiston, OR; C. Brown, USDA/ARS, Prosser, WA; N. Gudmestad, North Dakota State University, Fargo, ND. Understanding the potential for symptom development or processing quality reductions due to virus infection from *Tobacco rattle virus* (TRV), *Potato mop-top virus* (PMTV), and *Potato virus Y* (PVY) will aid in managing the risk of storing potatoes with these diseases. Tubers (cvs. Alpine Russet, Bannock Russet, Ranger Russet and Russet Burbank) were harvested from TRV infested fields in Washington and North Dakota (ND) and PMTV infested fields in ND, and stored at 8.9°C. In addition, virus-free mini tubers (cv. Alturas, Umatilla Russet, Ranger Russet and Russet Burbank) were planted into a screen-house in OR, mechanically inoculated with three PVY strains (O, NTN and N-Wi), harvested, and stored at 8.9°C. All tubers were evaluated at harvest and periodically with time in storage for fry color/quality, virus presence, and symptom incidence and severity in 2015/16 and 2016/17 seasons. There was a relatively high level of asymptomatic TRV and PMTV tubers at harvest and in storage. The TRV and PMTV titer and internal symptoms increased with duration in storage. Bannock Russet PMTV infection was extremely low and limited tuber symptoms developed in storage. Varieties infected by PVY strains varied in symptom development. N-Wi strain showed no tuber symptoms except in stored Alturas. All varieties except Russet Burbank infected with O showed a high level of internal and external symptoms. Aside from tubers showing symptoms, limited impact on fry color and quality was observed. Studies are on-going and will provide greater understanding how quality is impacted by virus infection.

Discussion: Nora noted that tubers out of storage are currently in process for virus detection and some storage data for PVY is available but she has not had a chance to go over it.

**Effect of in-season PVY inoculation on post-harvest quality of fresh market and processing potatoes. *Erin Weber*** and Paul Bethke, UWI-Madison and USDA-ARS/UWI-Madison. PVY-infected seed has a demonstrable impact on yield, however, the impact of in-season PVY infection on post-harvest tuber quality remains unclear. To address this question, replicated field plots of five potato varieties (Russet Norkotah, Dark Red Norland, Yukon Gold, Russet Burbank and Snowden) were mock inoculated or inoculated twice midseason with four strains of PVY (O, NO, NTN, Wilga) common to Wisconsin. Leaf samples were collected from each plant three weeks after inoculation. PVY infection of each sample was assessed by ELISA, and strain identity was confirmed by immunocapture-reverse transcription-PCR. Approximately 20% of inoculated plants tested positive for PVY, although pronounced differences between varieties were apparent with Russet Norkotah having the highest rate of infection. Foliar symptoms of PVY were not observed during the later part of the growing season in any variety. In contrast, plants grown from PVY-infected mini tubers showed clear foliar symptoms as well as reduced emergence and stunted growth. PVY impact at harvest was assessed as changes in tuber size and yield. During storage, the effect of PVY on weight loss was measured monthly over 3 months for all varieties. Fry quality of Russet Burbank and chip quality of Snowden were assessed at harvest and after three months storage. Internal and external defects during storage were assessed visually. Data collected to date do not allow us to conclude that in-season PVY infection has a significant effect on any of these measures of tuber quality.

Discussion: For 2017, Paul would like to discuss using mini tubers instead of regular seed. Quality was only assessed on a random sample of six tubers and water loss was measured on a 5 kg sample of tubers.

Breeding and Cultivar Evaluations

**Screening North American varieties against multiple strains and isolates of PVY.** *J. Whitworth*, S. Gray, J. Ingram, D. Hall, USDA-ARS and Cornell University. Field demonstration plots and greenhouse screening plots are being done to characterize the reactions of North American varieties to PVY. Demonstration plots involving 40+ varieties positive for three PVY strains were used for training of certification inspectors, seed and commercial potato growers and processors in Othello, Washington in June of 2016. Greenhouse screening trials are being done using ten isolates representing five PVY strains. This work is being done in New York and Idaho and when complete will cover 70% of the varieties planted in the U.S. Greenhouse studies show that the N:O and NWi recombinant strains are typically more mild in symptom expression than NTN, NE-11, and O strains. Variety susceptibility to necrotic lesions in tubers is also being evaluated. Preliminary results show that visual identification of PVY symptoms in the field is difficult and the industry would benefit from seed certification that uses ELISA lab testing for PVY detection.

No discussion.

**Sensitivity of potato cultivars to PMTV- and TRV-induced tuber necrosis under North Dakota conditions.** Shashi Yellareddygari and *Neil C. Gudmestad*, NDSU. Tobacco Rattle Virus (TRV) and Potato Mop Top Virus (PMTV) are two soil borne viruses affecting potato in most of the important production areas of the U.S. Both TRV and PMTV cause tuber necrosis in potato tubers making them unmarketable. TRV is vectored by a number of stubby root nematode species complex in the genus *Paratrichodorous/Trichodorous* and PMTV is vectored by the powdery scab pathogen, *Spongospora subterranea*. There are no effective chemical or cultural control options for PMTV-induced tuber necrosis. Although soil fumigation with 1,3 dichloropropene or applications of oxamyl can be effective in reducing stubby root nematode numbers, both of these chemical options are compromised by a lack of available product. Genetic resistance to TRV and PMTV, or the lack of sensitivity in the expression of tuber necrosis symptoms, is the only means to effectively and sustainably manage the tuber necrosis diseases caused by these two viruses. Sixty three potato cultivars, representing each market class of potato, were evaluated for sensitivity in expressing PMTV and TRV tuber necrosis in two separate field experiments in 2015 and 2016. There was no relationship among the cultivars tested in their sensitivity to PMTV and TRV-induced tuber necrosis. Regardless of market class, the majority of potato cultivars evaluated were classified as not sensitive to the development of PMTV-induced tuber necrosis. Conversely, the majority of cultivars evaluated were found to be sensitive to TRV-induced tuber necrosis. Ten potato cultivars were classified as being not sensitive to tuber necrosis caused by PMTV and TRV.

Discussion: The question - Did you look at correlations with powdery scab development? – was posed. Neil suggested that there was a correlation with tuber powdery scab and PMTV infection, but not root infection (i.e. galling) and PMTV infection. Stewart noted that red la soda had severe symptoms but was classified as an insensitive variety.

**Strain-specific resistance to PVY in potato and its effect on prevalence of PVY strains in the field.** *A.V. Karasev*, C.N. Funke, K.E. Frost, N. Olsen, UID and OSU. In 2015 and 2016, we evaluated the ability of three strains of *Potato virus Y* (PVY), PVYO, PVYNTN, and PVYN-Wi, to systemically infect four potato cultivars, Russet Burbank, Ranger Russet, Umatilla Russet, and Alturas, under semi-field conditions in Hermiston, OR. The experiment was arranged as a randomized block design with four replications and planted in a protected screen-house. In total, 40 plants of each cultivar were inoculated with either PVYO, PVYNTN, and PVYN-Wi or not inoculated. After mechanical inoculation with PVY strains in late May, each of the 640 plants was tested for the development of systemic infection 1 and 3 months later. In both seasons, three of the tested cultivars, Ranger, Umatilla, and Alturas, exhibited a pronounced strain-specific resistance to PVYO only, while being susceptible to the PVYNTN and PVYN-Wi infection. The predominant exclusion of the PVYO strain was clearly visible in the screen-house within five weeks of inoculation, reducing the number of PVYO-infected plants by at least 25%. This effect on the PVY strain prevalence resembled the changes observed in the potato production area of the Columbia Basin where between 2011 and 2016 the proportion of PVYO-positives in the total PVY infected samples dropped from above 60% to below 6%.

Discussion: Why is there no PVY-N resistance? (I did not catch an answer)

**Interactions of three PVY strains with five fresh market potato varieties.** *D.A. Inglis*, B. Gundersen, A. Beissinger, and A.V. Karasev, WSU and UID. PVYO, PVYNTN, PVYN-Wi, and buffer each were used to inoculate nuclear seed of All Blue (AB), Chieftain (Ch), French Fingerling (FF), Yukon Gold (YG), and Russet Burbank (RB) on 28 Mar 2016 at WSU Mount Vernon. Pots were arranged in a RCB design in four replications (240 plants total) on greenhouse benches. Symptoms appeared on Apr 14 and plants were rated weekly. Confirmatory testing for PVY on 26 Apr was by ELISA. Vines were cut on 26 May, tubers rated on 6 Jul, then placed at 4oC to measure weight loss on 19 Jan 2017. PVY symptoms across cultivars were evident on 51/60 plants (85%) for PVYO, 59/60 (98%) for PVYNTN or PVYN-Wi, and 0/60 (0%) for buffer; 85% of symptomatic plants tested PVY+. Mosaic displayed on all cultivars. Veinal necrosis was minimal on PVYO x Ch; PVYNTN x FF or RB; and, PVYN-Wi x RB. Leaf drop did not occur on PVYO or PVYN-Wi x FF; was highest for PVYO x RB or YG, and PVYNTN x YG; and, was limited or did not occur with PVYN-Wi. The leaf drop finding reaffirms that missed detections of PVYNTN and PVYN-Wi in WWA may be due to its over-reliance as a key PVY indicator. There were significant (*P*<0.0001) strain x cultivar interactions for yield. Percent (by number) of PTNRD or cracked tubers was highest for PVYNTN- and PVYO-infected YG (59.8%, 9.3%, respectively), and across FF treatments. Fewer cracked tubers occurred with PVYN-Wi, and cracking did not always associate with weight loss.

Discussion: Is this an increase of virus titer over generations? There was a comment on garden center experience – Nina has also surveyed garden centers for potato pathogens and has found PVY at a level of ~ 20% and has also detected Cms in some cases.

**Potato and Potato Virus: The Effect of Cultivar, Seed Type, and Defense-Inducing Agents.**  Nina Zidack, Elisa Boyd, Eileen Carpenter, Michelle Flenniken,Montana Seed Potato Certification. (no abstract). There is a perception that there is a higher rate of PVY infection in plantlets, microtubers, greenhouse grown minitubers when compared to field grown seed. Also, there seems to be lower current-season spread in field produced tubers. Nina presented data on experiments designed to measure if there were differences in virus accumulation by younger planting materials – those planting materials with less environmental exposure. A related question is to determine if there is a “generational-type” resistance and how we could induce this type of resistance in younger generation planting materials. When inoculated, plants grown from minitubers or g3 tubers had a lower PVY incidence and some evidence of age or generational resistance was presented. Nina also discussed an experiment to see if SAR inducers would change the susceptibility of younger generational planting materials. In Burbank, there appears to be an effect of SAR inducers, the SAR induced plants had lower PVY incidences post-inoculation than the non-induced controls. These experiments will be repeated in the future, including more varieties. They will also be looking at differential expression of the varieties through transcriptome analysis.

 **Developing populations to map virus resistance in potato**. *Dave Douches*. Michigan State University. The purpose of our SCRI/VIRUS objective is to conduct genetic mapping studies to understand the genetic makeup of PVY, PMTV and TRV. We have developed mapping populations that are segregating for the virus resistances. A new PVY resistance gene was identified and mapped. A segregating population (H25) was developed at Cornell University and germinated, propagated in tissue culture. The material was sent to Cornell for phenotyping while MSU did the SNP analysis. Cornell did the QTL analysis. A15001 is a mapping population for PMTV and TRV. The seed was germinated, propagated in tissue culture then two rounds of greenhouse propagation took place in the greenhouse. DNA was also isolated for SNP analysis which was completed. Tubers will be used for PMTV and TRV phenotyping in 2017.

Discussion: Novy asked what is chromosome 00? Douches replied that it is simply markers that do not map to the current draft genome of potato. A question was raised as to whether we should genotype before phenotyping so as not to waste time phenotyping? Answer – yes.

**10:00 am Morning break and meeting resumed at 10:20 am.**

**Aberdeen Program Update: Breeding for Resistance to Potato Tuber Necrotic Viruses.** *R.G. Novy* and J. Whitworth, USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID 83210. A primary objective of the ARS-Aberdeen Potato Breeding Program, in collaboration with colleagues of the Pacific Northwest (Tri-State) Potato Variety Development Program, is the development of potato germplasm and varieties with resistance to tuber necrotic viruses. Payette Russet, released in 2015, is a processing variety with extreme resistance to PVY conferred by the resistance gene *Rysto*. Challenge inoculations of Payette Russet with multiple strains of PVY have not resulted in infection. Payette Russet also has moderate resistance to corky ringspot disease caused by TRV. The variety Pomerelle Russet, also released in 2015, exhibits resistance to PMTV and moderate resistance to TRV based on field screening data provided by ARS colleagues at Prosser, WA . Additional detail regarding these two russet varieties, as well as our current efforts in breeding for resistance to tuber necrotic viruses*,* will be presented.

Discussion: Is Alex Karasev looking for a strain of PVY that will break the Payette Russet resistance? Yes Alex is always looking for new strains!

**Mapping a PVY resistance gene in *Solanum chacoense.*** Amy Charkowski and Ana Fulladosa, Colorado State and UWI-Madison. The *Potato virus Y* (PVY) extreme resistance gene *Rychc* has been identified in the wild relative of potato *Solanum chacoense*, but has not been introduced into North American cultivars. We identified a PVY resistant, diploid clone of *S. chacoense* (CHC 39-7) in accession 275138 and crossed it with a susceptible, diploid clone of *S. tuberosum* (US-W4). The resulting F1 clone, XD3, was selfed and the segregating population was phenotyped. SNP genotyping data, correlated with phenotypic results, suggested that CHC 39-7 carried the PVY resistance gene, *Rychc*, and that it was located at the end of chromosome IX. The genome of CHC 39-7 was sequenced and fine mapping of *Rychc* was carried out through the development of PCR-based molecular markers along the end of the chromosome. Four markers (two TaqMan markers and two SCAR markers) co-segregated with resistance in the XD3 population. Recombination data for each marker suggested that *Rychc* is located further towards the end of chromosome IX. The region containing the gene has been narrowed down to 720 kbp, containing approximately 120 genes. Validation of the molecular markers on a second population derived from CHC 39-7 is in progress.

Discussion Stewart asked if the markers would be directly transferable from diploid to tetraploid potato. Douches and Fuladosa both thought yes, they should be transferable.

**QTL analysis of tuber and foliar symptoms caused by PVYNTN in a segregating tetraploid population** Walter De Jong1, Stewart Gray2, Dave Douches3, and *Washington DaSilva1*, 1Cornell University, 2USDA-ARS/Cornell University, 3MSU and 4Cornell University. Potato tuber necrotic ringspot disease (PTNRD) is a tuber deformity associated with infection by the tuber necrotic strain of potato virus Y (PVYNTN). It negatively impacts tuber quality, reduces marketability, and poses a serious threat to both seed and commercial potato production worldwide. Recent surveys have shown that PVYNTN now predominates in Europe and is on the rise in the USA. The cultivars ‘Waneta’ and ‘Pike’ express severe and no PTNRD symptoms, respectively, when infected with PVYNTN isolate NY090029. To map loci that influence tuber and foliar symptoms in potatoes infected with PVYNTN, 244 F1 progeny of a cross between Waneta and Pike were genotyped with a potato SNP chip. Plants were grown in the greenhouse and inoculated with isolate NY090029 at the 8-10 leaf stage. Foliar symptom type and severity were monitored for 10 weeks; at maturity, tubers were harvested and evaluated for PTNRD expression. The progeny segregated for type of foliar symptoms and PTNRD expression with 93%, 73%, and 14% of the lines expressing foliar mosaic, veinal-necrosis, and Pike-like foliar necrosis symptoms, respectively. Tubers harvested from 61% of the clones expressed different levels of PTNRD. QTL analyses revealed major-effect QTLs on chromosomes IV and V, IV, and V for mosaic, PTNRD, and foliar necrosis symptoms, respectively.

No discussion.

**Response to PVY in a segregating population *Kuhl, J.C.***, A.V. Karasev, S. Struble, C.N. Funke, and M. Chikh-Ali, UID. Vegetative propagation of potato renders virus management particularly problematic leading to the requirement of seed certification programs and the propagation of sterile plantlets for starting new seed. In many potato growing regions *Potato virus Y* (PVY) continues to be a persistent problem year after year, leading to decreased yields and quality issues related to potato tuber necrotic ringspot disease (PTNRD). Potato cultivars experience a wide range of responses to PVY isolates ranging from susceptible to extreme resistance. The activation of necrotic lesions in response to PVY may activate resistance or allow the continued spread of the virus. Potato cultivar response to PVY is generally strain specific, however few instances so far have recorded specific responses to recombinant isolates. We have documented the development of necrotic local lesions in progeny from a Yukon Gem by Russet Nortkotah cross. Progeny segregate in a simple 1:1 ratio for the present absence of necrotic lesions. However, resistance to virus spread segregates in a more complex manner. Initial marker data from this population will be presented.

**\*\*\*Developing a modified hydroponic system for furthering investigations of potato powdery scab.** Amanda Gevens, Stephen Jordan, Haley Higgins, Department of Plant Pathology, University of Wisconsin-Madison. Powdery scab caused by the Plasmodiophorid pathogen *Spongospora subterranean* f. sp. *subterranean* has increased in occurrence in North American potato production since its recognition in 1911. Due to the pathogen’s increased presence in soils dedicated to potato rotations and its capacity to vector the tuber necrotic potato mop-top virus its management by cultural and chemical means is critically important. *Spongospora* species are notoriously challenging to work with in the laboratory; the pathogens are not culturable and require careful manipulation of infected plant materials for generating controlled infection. Powdery scab host resistance investigations have typically relied upon naturally infested field soils resulting in useful, field-relevant data. However, to date, controlled experimentation with single colony isolates outside of the complexity of field soils has not been possible. We are developing a modified hydroponic potato growth system and inoculation process to enable the empirical study of *S. subterranean* interactions with potato roots and tubers. To date, we built a hydroponic potato system with capacity to accommodate 4 individualized replicates for each of 4 experimental treatments or plant varieties. Adjustments from a standard hydroponic system were made to accommodate an enlarged root and tuber zone with easy plant removal access for non-destructive analyses. Published inoculum generation protocols are currently being conducted and compared. Our preliminary results indicated that published protocols do not consistently produce viable zoosporic inoculum necessary for plant inoculations. Refinement of strategy to consistently generate infective inoculum is ongoing. We anticipate completion of this design and inoculum optimization phase in Spring of 2017. Disease evaluation of 4 cultivars identified as highly susceptible, moderately susceptible, moderately resistant, and highly resistant from field disease assessments will be carried out in the new hydroponic system during Summer and Fall of 2017.

*\*\*\*Amanda Gevens could not attend the meeting but sent a slide bank with narrative. We did not listen to the entire slide bank, but Stewart Gray just made a few comments about this aspect of the project. Basically, Amanda has run into some issues with generating infection in this hydroponic system and producing viable inoculum. If you have ideas about how to resolve these issues or how to improve the system, please contact her directly.*

**Meeting turned back over to WERA-089 chair – Andrew Houser.**

**Election:** There were two nominations for Secretary: Kasia Duellman Kinzer (Univ. of Idaho) and Kylie Swisher (USDA-ARS, Prosser). A brief election was held by paper ballot and Kylie Swisher was elected to serve as secretary (2018) by a majority. Ken Frost will move from Vice Chair to Chair. Matthew Blua will move from Secretary to Vice Chair.

**WERA-89 Meeting in 2018:** Most of the people in attendance agree that early March is still the best time and potential dates for 2018 are:

March 7-8, 2018

March 14-15, 2018

Suggestions for location:

San Antonio, TX

New Orleans, LA

Tucson, AZ

San Diego, CA

**The meeting adjourned at 11:47am time March 9, 2017.**

**Accomplishments:**

**Impact Statements:**

**Old impacts:**

We have a better understanding of the different PVY strains circulating in North American potato cultivars thanks to work that has been conducted by team members.

There has been significant progress in the development and release of PVY resistant cultivars. This is the result of making PVY resistance breeding a priority. This will have a significant positive impact on the potato industry; as PVY resistant cultivars are more widely grown there will be fewer rejections and less downgrading of seed due to PVY infection, fewer yield losses due to PVY infections, and fewer quality defects caused by necrotic strains of PVY.

Efforts to characterize symptoms of many different potato cultivars infected with different viruses (PVY, PMTV, TRV) and virus strains (PVY-O, PVY-NTN, PVY-N/Wi) have helped personnel with seed certification agencies correctly identify virus-infected plants when they are doing inspections.

**New Impacts:**

**Dave Douches**
Use of the potato SNP arrays has allowed us to create high quality potato populations to conduct genetic mapping of virus resistance traits. The high density of the genetic markers that are generated will allow for mapping in tetraploid populations by the breeders. Seed tubers are generated of these populations so that PMTV and TRV can be phenotyped.

**Russel Groves**

Aphid capture data have been compiled from the North Central Regional, Aphid Suction Trap Network (<http://traps.ncipmc.org/>) from a span of 10 years (2005-2015) and 45 locations comprising over 180 species of aphids and nearly 1M individual captures in the upper Midwestern US. Additional aphid trapping data from various potato producing states are being identified to species and are planned for validation in the current models. These trap data will be standardized against cumulative growing degree-days (base50) using random effects models, and these will be used to predict the dispersal phenology of unique aphid species in different states.

**Neil Gumestad**

Sixty potato cultivars representing every market class were tested for their sensitivity to PMTV- and TRV-induced tuber necrosis in field trials conducted in 2015 and 2016. Expression of tuber necrosis by each virus was variable among cultivars with a number of them identified as being insensitive to the tuber necrosis phase caused by each virus. The commercial potato industry has cultivars available within each market class that can be used to escape economic loss by utilizing potato varieties that do not express tuber necrosis.

**Debbie Inglis**

1. Shallow, suberized canoe-shaped cracks on tubers of fresh-market potato varieties can be a symptom of Potato virus Y, especially if the infections arise from seedborne virus. 2. Over three trials at WSU Mount Vernon NWREC (field and greenhouse), ‘All Blue’, ‘Chieftain’, ‘French Fingerling’, ‘Russet Burbank’, and ‘Yukon Gold’ were susceptible to PVY strains O, NTN, and N-Wi, exhibited strong mosaic symptoms, and varying levels of cracked tubers. 3. Potato growers need to employ season-long PVY management when planting these varieties, and avoid purchasing seed tubers from lots exhibiting the cracking symptom.

**Christopher McIntosh**

This past year work on a beta-test version of the PVY seed calculator was completed. The calculator represents a “dashboard” style reference for the economic impact of PVY. By selecting an estimated infection level in seed, the user can obtain a mean and 95% confidence interval on the forecasted economic damaged caused by the virus.

**Mathuresh Singh**

It has been demonstrated that mechanical PVY transmission (not vectored by aphids) can readily occur in the field due to farming operations. Inoculum tubers known to be infected with PVY of three strains (PVYO, PVYN:O, PVYNTN) were planted in a tractor-trackway row and a control row away from tractor traffic. The virus spread was 3-5 times higher in tractor row than non-tractor row with PVYNTN spreading far more than the other strains (ca. 70% of new infections).

**Jonathan Whitworth**
Documenting PVY symptoms of potato varieties in the greenhouse and field has shown that recombinant strains have mild symptoms, making visual inspections difficult.  Results are being shared with growers and the industry.  A PVY demonstration trial was done in Washington in 2016 and additional trials will be done in WA, WI, and ME in 2018.

**2017 WERA-89 – Publications**

Benedict, C., McMoran, D., Inglis, D., and Karasev, A.V. (2015) Tuber symptoms associated with recombinant strains of Potato virus Y in specialty potatoes under northwestern Washington growing conditions. American Journal of Potato Research 92: 593-602. Fig. 1 of this paper has been selected for the cover of the October issue of American Journal of Potato Research.

Cating, R.A., Funke, C.N., Kaur, N., Hamm, P.B., and K.E. Frost. (2015) A multiplex reverse transcription (RT) high-fidelity PCR protocol for the detection of six viruses that cause potato tuber necrosis. The *American Journal of Potato Research* 92 :850-864.

Chikh-Ali, M., Bosque-Perez, N., \*\*Vander Pol, D., Sembel, D., and Karasev, A.V. (2016) Occurrence and molecular characterization of recombinant Potato virus YNTN (PVYNTN) isolates from Sulawesi, Indonesia. *Plant Disease* 100: 269-275.

Chikh-Ali, M., \*Alruwaili, H., \*\*Vander Pol, D., and Karasev, A.V. (2016) Molecular characterization of recombinant strains of Potato virus Y from Saudi Arabia. *Plant Disease* 100: 292-297.

DeBlasio, S.L., Johnson, R., Mahoney, J., Karasev, A.V., Gray, S.M., MacCoss, M.J., and Cilia, M. (2015) Insights into the polerovirus-plant interactome revealed by co-immunoprecipitation and mass spectrometry. *Molecular Plant-Microbe Interactions* 28: 467-481.

DeBlasio, S.L., Johnson, R., Sweeney, M.M., Karasev, A.V., Gray, S.M., MacCoss, M.J., and Cilia, M. (2015) The Potato leafroll virus structural proteins manipulate overlapping, yet distinct protein interaction networks during infection. *Proteomics* 15: 2098-2112.

# Domfeh, O., Bittara, F., and Gudmestad, N.C. 2015. Sensitivity of potato cultivars to *Potato Mop Top virus*-induced tuber necrosis. Plant Dis. 99:788-796.

# Domfeh, O., Thompson, A.L. and Gudmestad, N.C. 2015. Sensitivity to tuber necrosis caused by *Potato Mop Top virus* in advanced potato (*Solanum tuberosum* L.) breeding selections. Amer. J. Potato Res. 92:636-647.

Domfeh, O. and Gudmestad, N.C. 2016. Moisture management as a potential disease control strategy for *Potato Mop Top virus*-induced tuber necrosis. Plant Dis. 100:418-423.

Fulladolsa, A.C., F.M. Navarro, R. Kota, K. Severson, J.P. Palta, and A.O. Charkowski. (2015) Application of marker assisted selection for Potato virus Y resistance in the University of Wisconsin Potato Breeding Program. *Am. J. Pot. Res.* 92:444-450.

# Mallik, I., Anderson, N.R., and Gudmestad, N.C. 2012. Detection and differentiation of *Potato virus Y* strains from potato using immunocapture multiplex RT-PCR. Am. J. PotatoRes. 89:184-191.

# Mallik, I. and Gudmestad, N.C. 2015. First report of *Potato Mop Top virus* causing potato tuber necrosis in Colorado and New Mexico. Plant Dis. 99:164.

Mondal, S.; E. J. Wenninger; P. J. S. Hutchinson; J. L. Whitworth; D. Shrestha; S. D. Eigenbrode, and N. A. Bosque-Perez. (2016) Comparison of transmission efficiency of various isolates of Potato virus Y among three aphid vectors. *Entomologia Experimentalis et Applicata* 158: 258-268.

Rowley, J.S., Gray, S.M., and Karasev, A.V. (2015) Screening potato cultivars for new sources of resistance to Potato virus Y. *American Journal of Potato Research* 92: 38-48. – Fig. 8 of this paper has been selected for the cover of the February issue of American Journal of Potato Research.

Wohleb, C.H., T.D. Waters, E.M. D’Auria, and D.W. Crowder. (2015) WSU Potato Pest Alerts – Providing Regional Pest Information and IPM-based Recommendations to Aid Management Decisions. Abstracts of the Papers Presented at the 99th Annual Meeting of the Potato Association of America. *Am. J. of Potato Res.,* 93(2)*.*

Wohleb, C.H. (2015) Development and impact of a pest alert system for potato growers in the Columbia Basin of Washington. 8th International IPM Symposium.

# Domfeh, O., Bittara, F., and Gudmestad, N.C. 2015. Sensitivity of potato cultivars to *Potato Mop Top virus*-induced tuber necrosis. Plant Dis. 99:788-796.

# Domfeh, O., Thompson, A.L. and Gudmestad, N.C. 2015. Sensitivity to tuber necrosis caused by *Potato Mop Top virus* in advanced potato (*Solanum tuberosum* L.) breeding selections. Amer. J. Potato Res. 92:636-647.

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