Minutes – WERA89
March 10, 2021 – Virtual (Zoom) Meeting
8:00 AM to 4:00 PM Mountain Standard Time

Chair: Kasia Duellman, University of Idaho (kduellman@uidaho.edu)
Vice Chair: Steve Hystad, Montana State University (steve.hystad@montana.edu)
Secretary: Max Feldman, USDA-ARS/Prosser, WA (max.feldman@usda.gov)

Wednesday March 10, 2021
8:00 AM Call to Order, Introductions, 2020 minutes approval, 2021 Agenda discussion/approval

Attendees:

Adam Winchester
Alex Crockford
Alexander Karasev
Alice Pilgeram
Amer Fayad
Amy Charkowski
Ana C Fulladolsa
Andrei Alyokhin
Andrew Houser
Andrew Jensen
Andrew westra
Anna Saum
Aymeric Goyer
Binod Pandey
Brian Charlton
Brian Ross
Brooke Babler
Bryant Davenport
carol bvindi
Carrie Wohleb
Chakradhar Mattupalli
Chris McIntosh
Colton Thurgood
David Douches
Erik Wenninger
Erin Weber
Govinda Shrestha
Gregory Elison
Hanu Pappu
Hira Kamal
Ipsita Mallik
James Woodhall
Jason Ingram
Jeffrey McMorran
Jennifer Dahan
Jennifer Rushton
Jeremy Jewell
Joe Kuhl
Johanna Sandlund
John Mizicko
Jonathan Whitworth
Joseph Coombs
Julie Pasche
Kasia Duellman
Keith Schuetz
Kelie Yoho
Kenneth Frost
Kent Sather
Kutay Ozturk
Kylie Swisher Grimm
Lindani Moyo
Lisa Tran
Lynn Woodell
Mark McGuire
Mark Pavek
Martin Lawrence
Mathuresh Singh
Matthew Blua
Max Feldman
Melanie Filiatrault
Melinda Lent
Melissa Bertram
Mike Thornton
Natalia Moroz
Nathan Gelles
Neha Gupta
Nina Zidack
Noelle Anglin
Nora Olsen
Paul Bethke
Peter Wagner
Prabu Gnanasekaran
Rachel Johnston
Renee Rioux
Richard Manasseh
Romana Iftikhar
Russell Groves
Sarah Hensley
Sarah Noller
Shane Climie
Silvia Rondon
Steve Hystad
Teresa Almeida
Tiziana Oppedisano
Vamsi Nalam
Vidyasagar Sathuvalli
Walter De Jong
Wes Bills
Ying Zhai
Yuan Zeng

- Nina Zidack moved to approve minutes; Kent Sather seconded.
- Kent Sather moved to accept agenda; Julie Pasche seconded.

8:15 AM Announcements and other business

1. Our new NIFA Rep: Amer Fayad, USDA

Dr. Fayad is National Program Leader in the USDA-NIFA Division of Plant Systems-Protection. He has been with NIFA since May 2021 and is stationed at the new USDA-NIFA headquarters in Kansas City, MO. He has a masters and Ph.D in Plant Pathology with a focus on viruses and integrated pest management. Before joining NIFA he served as the Director of the Western IPM Center, University of California Agriculture and Natural Resources in Davis, CA. Encourages attendees to keep checking the NIFA website for new RFAs. One to keep an eye on is the AFRI Tactical Sciences for Biosecurity call due on 7/22. He is the NPL of this program.

2. The Kickoff SCRI meeting, for the next round of funding, will be held tomorrow – March 11 – led by Alex Karasev
3. Other announcements

   Registration list is being logged by response and on Zoom.

8:30 AM Mark McGuire - update on the WERA89 renewal process

Renewal for WERA89 group is submitted. Currently going through the renewal process. There is a multi-state committee meeting at the end of the month, then it will go to NIFA for approval. Renewal is likely as the group is productive and enthusiastic. Approval may come in July 2021.

8:35 AM State Certification Reports (Round Table) – each state/province is invited to provide an update, with or without a Powerpoint presentation or other visual aids

Jeff McMoran (Oregon)

Same acreage as usual planted. Lower virus in tested material than usual. Roughly 13% of lots detected was type NO, 4% was NTN. There was a disappointing number of lots don't see virus a second inspection but see virus winter grow out. Some are latent for O or NO. So common wouldn't really want call varieties latent because sometimes symptoms are more obvious in the greenhouse.

Alan Westra Idaho Crop Improvement Association (Idaho)

Quietest season he can recall.

In Idaho, 30,175 acres were planted and a total of 740 seed lots were entered for testing. From this 30,101 were accepted. Thirty one (4%) were found to have mosaic. Blackspot testing identified a small proportion of infected tubers. 1st Inspection identified 8 lots (1.1%) with blackleg 824 acres (2.7%). Whereas the 2nd inspection identified 12 lots (1.8%) and 1,131 acres (3.7%). No Bacterial ringrot was found. Entered 740 lots, tested 329,200 tubers. Haven't seen any since 2016. Winter grow out went excellent. Getting results at 40 days after planting (DAP). Too difficult to harvest at 50 DAP as plants become overgrown. One metric they use is number of samples tested before Jan, 1st. Last year they were 58% complete by that date. Stand count was excellent. Out of 554 samples, average stand was 90% with only two lots exhibiting <50% density. PVY levels were lower, 45% had no PVY, 32% had less than 1%, and 21% had less that 10% PVY infection. Low disease levels in 2020.

Nina Zidack, Potato Seed Certification Program (Montana)

Field Inspection: Nina Zidack & Anna Jespersen

Database and Lab: Steve Hystad

PVY Strain Composition: Rachel Johnston

Good growing year in Oahu, took drone images of fields for the first time. In 2020, they observed 67% of seed lots with 0% PVY, 20% with 0 – 0.5%, 6% with 0.5 – 1%, 3% with 1-2 % and 4% with greater than 2%. This is derived from 10,878 acres, which is a 4.2% increase from 2019. PVY problems only in small isolated patches, not a huge problem in 2020. A total of 620 infected plants were identified (0.29%), 0.42% which were PVY. Increase from last year, 279 were from 2 growers, only for commercial sales (not seed). Stand count was good (79%). All mosaic symptom plants were confirmed in the lab. Leaves were picked from all samples and confirmed using ELISA. Montana also performs uniform testing in the summer. They test all G1 and G2 seed lots. Essentially they take 10 leaves per family unit, 200 leaves per acre.

No G1 exceeded 0.5%. Most G2 suitable for planting. G1 and G2 supply is good for all major varieties. Growers isolating seed plots, excellent results in G1 (>100 miles away from potato cropping).

Problem varieties are shifting. In the past it has been Russet Norkotah, Umatilla, Alturus. This year it was Russet Norkotah, Ranger, and Ivory. Growers using oils in early generation material have low PVY (not perfect). Twin Bridge Farms has been partner since 2009. Partnership is now dissolved; need to find a new solution to perform post-harvest testing in Hawaii.

Steve Hystad Potato Seed Certification Program (Montana)

Each year they perform dormant tuber lab testing as well as winter grow out if grower likes. Process is a qPCR test. Four cores are taken with a biopsy tool from the stem end, two eyes, and the rose end. Cores are placed into a custom pressing tray (10 tubers/well, 40 cores) and pressed with extraction buffer. The longest part of this process is the coring. Processed 120 tuber samples which was down from roughly 400 per year previously and ~20 sprout tests. They tested 88 seed lots and found 80 seed lots with at least 1 PVY positive and 8 with > 2. Decent correlation between field grow out and lab assay. At high levels of PVY, lab assay can under-estimate levels. Not all primers can detect all PVY strains. Berger primers fail on PVY N and NTN and he recommends using the PVY-Universal primers. Good to use more than 1 set as a multiplex reaction. Important takeways are ELISA, qPCR, and post-harvest grow outs are all comparable. PVY detection influenced by virus distribution, strain and titer. Immunocapture PCR (IC-qPCR) is a cost-effective, high-throughput assay ($83/400 tuber samples). They try to ID the strain on all positive samples. Currently seeing a uniform distribution of all strains across state. In Montana 85% are N-Wi, 8% are NT, 5% unknown, 1% O, 1% NO. They are trying a new Luna one-step RT Kit. Benefits are less liquid transfer, fewer consumables, and reduced reaction time 20 minutes instead of 70.

Alex Crockford Wisconsin Seed Potato Certification Program (Wisconsin)

Seeing an upward trend of seed acreage 9,294 in 2020. They used 3 methods to evaluate post-harvest testing in 2020. The first is a field grow out (visual inspection & ELISA) of 200 or 400 tuber samples planted in Homestead, FL. They evaluated 495 samples 136 with ELISA. This represents nearly all grower seed lots. They also performed a greenhouse grow out from 83 seed lots (all state farms, some researcher lots, problem varieties, duplicate samples. These were tested as ELISA only. In some cases,

direct tuber tests (IC-PCR) was performed on duplicate samples (40 samples total) on larger tuber lots (>50 acres). Performed as 400 tubers tested as 10 tuber composites. Certification decisions in WI are based upon seeing visual symptoms. Seed lot testing using ELISA was increasing (500 in 2019) until drop in 2020 (280) possibly due to the pandemic. Wisconsin's tagging tolerance threshold < 5% (Certified), Foundation seed < 0.5%. Usually 1-2% of lots do not get a tag. Results from this year are a bit below average but this changes through time.

Andrew Houser Colorado Potato Certification Service (Colorado)

Around 8,000 acres were planted and almost all was accepted (~96%). Some was rejected due to mosaic. Performed winter test grow out in a flower greenhouse instead of Hawaii largely due to the pandemic. Growers had to have their samples submitted by October 22nd and they planted the grow out in three crops started on Nov. 11th. Finished on January 23rd, 2021. Tested all samples for PVY, PVX and PLRV, no PVX or PLRV was observed. They faced many challenges. Getting uniform stands in greenhouse is difficult but sampled with 75% stand was achieved. The last sample tested was on March 4th, 2021. In total 617 samples and a total of 160,794 tubers were planted and tested. They also performed 36 sprout tests. Could see PVY symptoms but it was tough to identify which plants are which so relied upon lab test. Andrew described difficulty performing several grow outs throughout the year and the challenges of having to handle so many testing activities simultaneously and seed breakdown in the second and third crop. Required lots of labor and had inconsistent stands. Very beneficial to plant all tubers at once. Some benefits were reduced travel, additional time to verify results, and local access for growers. They learned a lot. This was the first time they have done the winter grow out locally and the first time they have lab tested each lot.

Adam Winchester Potato Certification Association of Nebraska (Nebraska)

They tested 382 seed lots in 2021: 16 FY0, 129 FY1, 110 FY2, 93 FY3, 28 FY4, 6 FY5. A total of 34 lots were taken out of certification prior to testing. Frito Lay only has field scouting done. On an acreage basis, 7,215 acres were planted in 2021: 1.6 FY0, 219 FY1, 1036 FY2, 4262 FY3, 1366 FY4,331 FY5. A total of 1,636 acres were taken out of certification largely due to chemical damage. No rejections for field inspection. They believe they have solved their emergence issues by using Rindite (not Bromoethane to break dormancy. They now track humidity, temperature, and pulp temperature in storage. The purchased a new gassing container, palletized all samples, added a chimney poll. All samples were sent to Homestead, FL for planting. Lots of Gibberella damage, blocked by grower not variety. They had great emergence (88%) but some varieties stayed small. All virus testing was performed in Florida starting on January 10th and it was completed by January 29th. They performed sprout testing for varieties that don't germinate well in Oct. – December. They did a greenhouse trial in Scotts Bluff, but germination was poor (60 – 70%). This helped identify lots with large amounts of chemical damage due to (plant growth regulator chemicals). They grew out 292 lots, 84,699 tubers and tested 76,502 leaves. PVY was detected in 15 lots, and in 13 of these cases the ELISA and visual observations matched. Two lots were detected by ELISA only. Varietal mix was identified in 5 lots. Sprout testing was performed on 58 tuber lots entered (16,425 tubers) A total of 14,128 tubers were tested (86%) and PVY was detected in 1 lot. Thirteen lots (3,550 tubers) were tested in the greenhouse. No PVY was detected but a varietal mix was identified in one lot. Slightly higher PVY than previous years. There was an increase in lots rejected due to chemical damage.

Kent Sather North Dakota Seed Potato Certification (North Dakota)

Seed grown in several parts of the state. Early generation is grown in Grenora, later FY grown at Cando and some sent to Walhalla, The Red River Valley. Oakes-Libson are commercial growers growing their own seed. In total ~15,000 acres were planted. 300 acres were rejected, with 100 being rejected to chemical treatment, 25 due to blackleg, and 200 acres due to PVY. Winter grow outs in Florida were disturbed by a tropical storm. This caused wet, and encrusted oil, decreased emergence, rain events, cold weather, weeds, etc. so the quality suffered this year. They performed immunostrip testing for PVY. In total about 7,411 acres were tested and 5,985 (81%) remained eligible. Zero PVY infection was found on 3,439 acres (46%).

Andrei Alyokhin (Maine)

Certification handled by Maine Potato Board. They no longer do a winter grow out. Switched to only doing tuber test. In 2021, ~3% were rejected (> 5% PVY), 1/3 acreage 0.56 – 5%, 63% Foundation seed (< 0.55%). Last year 7.31% entered into testing was rejected. Improved from last year, worried because, less fungicide sprayed, usually less oil sprayed.

Mathuresh Singh AAFC (Canada)

They perform 100% lab testing based upon testing sprouts and dormant tubers using PCR. They processed 427 samples which was less than previous years due to having less growers. This accounted for ~7,000 acres of potato. Approximately 66% had no PVY (0%), whereas 20% exhibited values between 0-1%, 9 % between 1-2%. The virus cap is 4% causing 2% of the crop to be rejected due to PVY. Last year was a very inconsistent growing year due to lots of drought. This caused a 20% yield reduction in most growing areas.

10:00 AM **BREAK** (breakout rooms are available – one general room and several topic-oriented rooms; room names subject to change)

10:30 AM **Research updates**

<u>Virus-like organisms:</u>

Update on beet leafhopper vector of BLTVA and BCTV in the Columbia Basin – Silvia Rondon and Tiziana Oppedisano (Oregon State University – Hermiston R&E Center)

Rondon Lab performs insect trapping throughout the Columbia Basin on the Oregon side to track Beet leafhopper, potato psyllid, green peach aphid, potato aphid. The focus is on monitoring and control strategies for insect pests. Beet leafhopper is a major insect pest of potatoes. It goes through 5 different stages before becoming an adult and can generate 3 generations per year. Beet leafhopper feeds and reproduces on many different plants (sugar beet, potato, tomato, cucurbits, spinach, weeds) and acts as a super vector. Beet leafhoppers transmit beet curly top virus, purple top disease, and spiroplasma disease. Beet leafhopper is a major vector of Beet Leafhopper Transmitted Virescence Agent (BLTVA) which is caused by *Candidatus Phytoplasma trifolii*. Symptoms of BLTVA infection include rolling upward of top leaves, yellow, red, or purple discoloration of foliar tissue and associated moderate proliferation buds, shortened internodes, swollen nodes, aerial tubers, and early plant decline. BLTVA is caused by phyoplasma. This is a small bacterium which is a phloem-limited bacterial pathogen. It is an obligate parasite that is not cultivariable in cell-free media. It is transmitted by insects with piercing-sucking mouthparts that feed specifically on phloem tissue (leafhoppers and psyllids). Its transmission can be

described as persistant-propagative. Surveys in 2019 tested 92 plants and found 8 infected with BLTVA (8.7%). Many symptomatic plants were not BLTVA positive. Did not find any result with universal primers, or with grafting experiments. Also checked BLTVA infection rate within Beet Leafhopper populations. They tested 340 insects and found 30 infected with BLTVA (8.6%). Levels of BLTVA in the field in 2019-2020 was much lower than 2006 and 2009. They are developing at beet leafhopper phenology model based upon degree days (DD), daily maximum temperature (Tmax), daily minimum temperature (Tmin) and insect lower development threshold (Tbase). Results are variable across year and DD alone is not very accurate. Cumulative population emergence of *C. tenellus* was calculated as average of 14-year monitoring. Average temperate data of 3 weather stations was used as input data, the model was customized to include potato cropping parameters (based upon Ranger Russet and planting before 4/15). Beet leafhoppers exhibit 10% emergence during the potato vegetative stage, 50% at the tuber initiation stage and 90% at maturity. Using this data, they can predict when infection will become problematic. They also performed a study to see if carrot could act as a host for BLTVA. Despite the fact that 28.3% of beet leafhoppers acquired BLTVA during the acquisition phase 0 % of the carrots acquired BLTVA. In a similar assay it appears that BLTVA is not transmitted by lygus bugs. Future work will focus on beet leafhopper transmission efficiency of Beet Curly Top Virus in hemp. They have identified a susceptible hemp variety.

Viroids in Potatoes – Alex Karasev (University of Idaho)

Tomato chlorotic dwarf viroid (TCDVd): effect in potato cultivars

TCDVd is a pospiviroid from the families Pospiviridae and genus Pospiviroid. It contains as small, 350 nucleotide, circular single stranded RNA molecule as its genome. It is one of at least 5 related viroids capable of infecting potato. It can be transmitted mechanically or the true potato seed and is known to cause outbreaks in greenhouse tomato operations. PSTVd (Potato spindle tuber viroid) is another type of pospivirods that has been eliminated from potato in the USA, Canada, and western Europe. TCDVd found in Idaho in 2019 (APHIS May 2019, Confirmed by CPHST in Beltsville, MD). Direct tests for TCDVd found several greenhouse litchi tomato lines that were carrying the virus asymptomatically. Several potato plants in the same greenhouse bay also tested positive for TCDVd too. The source of TCDVd was likely contaminated litchi tomato seed but origin is unknown. All infected plants were destroyed. Objectives of this project are to determine the pathogenesis and time-course of TCDVd (and PSTVd) infection and relevant symptomology in solanaceous hosts (tobacco, tomato, N. benthamiana) to optimize TCDVd and PSTDVd detection. Also determine virus transmissibility, both mechanical and through plant debris in potato and other hosts, determine the virus longevity in the soil. They conducted viroid surveillance of entire greenhouse complex on campus to ensure no local source of infection. This was done by performing testing for TCDVd by RT-PCR. Plants were subjected to total nucleic acid extraction by the Dellaporta method and 2 primer sets, 1 for Pospiviriods and another specific for TCDVd. PSTVd was discriminated by sequencing band from Pospiviroid primers. Symptoms in Ranger Russet are visible 5 weeks post inoculation, and you can see large effects after 15 weeks (changing of leaf shape, potential necrotic lesions on leaves, stunting, etc.). Russet Burbank and Russet Norkotah showed similar symptomology. Some variation in resistance between Burbank, Norkotah and Ranger, with Ranger being the most resistant and Burbank being the most susceptible. Can generally detect visual symptoms after 4 weeks post inoculation and can detect TCDVd after 2 weeks post inoculation. Tuber symptoms included reduction in yield, tuber size and number of tubers. Norkotah and Ranger exhibited severe skin cracking. TCDVd can infect potato and induce foliar symptoms.

Diagnostics:

Impact of tuber sampling patterns on rates of false Negative for PVY, PMTV, TRV and PVS when direct testing tubers – Jason Ingram, USDA (Cornell)

This presentation is an update on work focused on automation to perform direct testing of dormant tubers. Jason would like to recognize Sarah Noller and Sarah Hensley (Colorado Potato Certification), Gutmestad and Pasche Labs (NDSU), Chakradhar Mattupalli (CSU/WSU) and Lisa Tran ICIA for helping provide samples. Recognizes the hardest part is getting potatoes to the robot. Potato samples are collected on cards by the seed grower. One major focus has been to identify where to sample the tuber. To determine this, they took a developmental gradient at 5 points across the tuber from stem to rose end; they also investigated virus titer in skin, flesh, and depp flesh. Over 3 crop years they have tested 13 cultivars, from 6 states, 100 tubers and 15,000 nucleic acid extractions. What was found was the PMTV was more prevalent at the stem end of the tuber near the skin. PVS was highest at nodes and on each end. PVY was higher on stem end and TRV was higher on rose end. Skin generally exhibited the highest viral titer with deep flesh being the lowest. Sampling for the heel and rose end (industry standard) is probably very effective but bet results were seen when heel, rose, and 2 eyes were tested. Vine tests are not good for PMTV or TRV. Jason has found no significant differences between sampling at harvest with FTA cards, in storage with FTA cards, and spouting with FTA cards or testing sprouts with ELISA. Jason is currently growd sourcing asymptomatic tubers from stem rot plants (Dickeya or Pectobacterium). Please send him tubers!

Update on RPA-based, really rapid test for PMTV – Bryant Davenport, Ying Zhai, Keith Schuetz, and Hanu Pappu (Agdia and Washington State University)

PMTV infected plants don't always show symptoms (yellow chevron on foliar tissue, necrotic tissue in tubers). Developed a Recombinase polymerase amplification (RPA) method to detect virus. Benefits are it is 4-6X faster, it has high sensitivity, it's isothermal (not cycling), requires cheap equipment, portable, and you can use crude extracts to quantify RNA transcript. It is used for on-site screening using PMTV specific primers and can monitor presence on instrument screen in the field. Obtained sequences of PMTV from samples in CO, MD, ME, NM, WA. Four isolates were cloned, sequenced, and primers were designed that generate overlapping PCR products spanning each RNA. They performed sequence comparisions and generated a phylogeny. In total, 23 different primer pairs have been screened. Pair TGB1 is the fastest, most reliable pair (Uses P1F1R3). Specificity of PMTV RPA is excellent (no other viruses showed positives using this assay) and sensitivity is quite high (can detect virus at 10 fg/uL). Assay is quite rapid (< 20 mins) and can use crude tuber extracts for detection.

TRV/PMTV

PMTV and variety reactions in the field – Jonathan Whitworth (USDA-ARS, Aberdeen, ID)

PMTV first detected in 2001 but effective chemical treatments to control powdery scab and/or PMTV not available. Soil tests for powdery scab and PMTV are available. Foliar symptoms are rarely seen. Sometimes these symptoms resemble alfalfa mosaic virus (aka calico). Tuber symptoms are necrotic lesions (aka spraing). The symptoms of Tobacco rattle virus are similar but the vector is different (stubby root nematodes). Timeline of detection:

- 2001: Maine, Canada

- 2010: North Dakota
- 2011: Washington state
- 2012: Idaho
- 2013: Colorado, New Mexico, Oregon
- 2016: Alberta

Currently assessing threat of tuber necrotic viruses PMTV and TRV in WA state seed lot trial. In 2020, 5.67% containted PMTV and 0% contained TRV. The origin of infected seed lots was everywhere. Montana had 8, Canada/Alberta had 5, Idaho had 22, and 1 was detected in Maine. PMTV was found in Russet Burbank (5), Caribou, Clearwater (4), Columba, Ranger (3) and Umatilla (2). Some varieties are resistance/insensitive: Castle Russet and Pomerelle Russet.

They performed a field trial in 2019. The trial was in a field with a history of PMTV and TRV tuber symptoms. Fumigated field prior to planting to remove TRV vector. Tested 4 varieties (Castle, Pomerelle, Clearwater, and Russet Burbank) in 30 hill plots. At harvest visual symptoms were checked and RT-PCR was used to detect PMTV. Field was also sampled for powdery scab and PMTV in 20 x 20 grids. All four varieties exhibited virus infection but Castle did not show tuber necrotic symptoms. Disease severity index scores for each line were: Castle (0.6), Burbank (3), Clearwater (4.7) and Pomerelle (5).

PCR-based markers linked to TRV resistance from Castle Russet – Sagar Sathuvalli (Oregon State University – Hermiston R&E Center)

Began with a tribute to Dr. Chuck Brown. Contributed PVY, TRV, PMTV, PLRV and Root-knot nematode resistant germplasm to Tri-State program. Release of Castle Russet was a Chuck Brown cross, developed by OSU. He will be dearly missed by all his friends and colleagues. Disease evaluations show that Castle russet does not exhibit spraing symptoms (necrotic lesions) when exposed to TRV or PMTV. Resistance was mapped in a 48 seedling population in a linkage mapping population between Castle Russet X POR08BD1-3. Resistance to TRV segregates 1:1 which indicates TRV immunity is inherited as a dominant single locus trait. SNP genotyping of this population was performed with the SolCAP SNP array v3. A major QTL was identified on Chromosome 9 and a smaller QTL was located on Chromosome 10. Data was converted to categorical before mapping (trait takes on value of 1,2,3,4; lower number less symptoms). They have developed PCR based markers based upon a SNP at position 59677060. SSR markers (36 in total) upstream and downstream of SNP PotVar0108448 were developed spanning from 57177000 to 61540751 bp. Minimap2 was used to map Castle Russet Chromosome 9 from 557177000bp to 61540751 with Atlantic to identify insertions and deletions between 20 to 100 bp and 72 pairs of indel markers were developed (ORCRSIndelmarkers 55 and 61). These markers are currently being validated on a new population and on European germplasm. Best marker so far is: ORSCINDEL490-15.

Update on TRV genetic diversity – Lindani Moyo, Gaurav Raikhy, Ipsita Mallik, Neil Gudmestad, Stewart Gray and Hanu Pappu (Washington State University, North Dakota State University)

TRV is a 2 strand RNA virus. Strand 1 encodes RNA polymerase, and a movement protein. Strand 2 is much more tolerant of deletions and encodes a viral coat protein and a protein implicate in nematode transmission. Goal is to understand genetic diversity of TRV in samples acquired from WA, ID, CO, ND, MN, IN, FL, and AK. Phylogeny of the sequences was compared using MEGA7. Basically the coat protein and gene 2b forms distinct clusters that separate virus isolates from Europe and American whereas RNA 1 there was no distinct geographical pattern (genes assessed include RdRp, 1a, 1b). Reticulate evolution

was revelated among RNA1 genes suggesting that recombination has been important in viral evolution. RdRp, RdRp-RT and 1a are conserved and under negative selection whereas gene 1b is less constrained. It is possible that gene RdRp is important for host adaptation.

12:00 PM **LUNCH** (breakout rooms are available)

12:30 PM Research Updates

PVY Biology

A pipeline for identifying PVY-interacting host proteins – Prabu Gnanasekaran, Hira Kamal and Hanu Pappu (Washington State University)

The PVY genome encodes a single polyprotein which is then post-translationally cleaved into P1-pro, HC-pro, P3, CI, VPg, NIa-pro, NIb, and coat protein (CP). In this study, to dissect the PVY-host interactome, we used PVYNTN-Nicotiana benthamiana plant as a model system. We carried out Yeast-2-Hybrid (Y2H) library screening using N. benthamiana Y2H cDNA library and pGBKT7-PVY-CP as bait. One of the proteins that was found to interact with PVY-CP was cytosolic Phosphoglucomutase (cPGM). cPGM is an enzyme that catalyzes the reversible conversion of glucose-6-phosphate to glucose-1-phosphate and is involved in controlling the partitioning of both sugar-phosphate into respiratory pathway, cell wall synthesis and sucrose synthesis pathways. This interaction between the CP and cPGM was further confirmed by Y2H approach. Yeast transformants carrying pGBKT7-CP and pGADT7-cPGM plasmids were able to grow on SD-Leu-Trp-His selection plates supplemented with 1 mM 3-amino-1,2,4-triazole, whereas the yeast transformants carrying negative control combination plasmids pGADT7 and pGBKT7, pGADT7-cPGM and pGBKT7 or pGADT7 and pGBKT7-CP were unable to grow. Similarly, yeast transformants carrying positive control plasmids pGADT7-TAg and pGBKT7-P53 grew on selection plates suggesting the interaction between TAg and P53 protein. Ongoing studies include investigation into the biology and importance of CP-PGM interaction in PVY infection of potato.

Metabolomic responses to PVY infection – Richard Manasseh and Hanu Pappu (Washington State University)

Can distinguish between NTN and N-Wilga infection in both Premier Russet and Russet Burbank using metabolomics. Samples were taken 5 days after inoculation. Potential metabolite biomarkers were identified for NTN and N-Wi infection including: dioctyl phthalate, sedoheptulose, glycerine, 1-monopalmitin, ribulose-5-phosphate, trehalose, glycerol-3-phosphate, alpha-tocopherol, 5-methoxytryptamine, and sorbitol. NTN infected tubers had higher metabolite levels than N-Wi infected tubers.

PVY Management

Testing new biologics against PVY – Richard Manasseh and Hanu Pappu (Washington State University)

Ninja (aka SP2700) a biochemical produced from soil bacterial fermentation (SePROP Corporation, NC) was tested. It is advertised to activate plant innate defense system and demonstrates anti-viral properties. Reported to control Tobacco mosaic virus on tobacco as a local lesion host and Tomato bushy stunt virus on pinto bean (local lesion host). Nicotiana benthamiana is a susceptible systematic host. Ninja is used extensively in plant viral disease management outside the USA. Experiment was to

inoculate leaves and sample after 5 days, then test younger leaves for systematic infection after 14 days using ELISA. Treatments included:

1) PVY
2) Buffer
3) Ninja + surfactant + PVY inoculum
4) Ninja powder + surfactant + PVY
5) Surfactant + PVY
6) Surfactant + PVY solution

Results show that Ninja decrease primary infection rate relative to experimental treatments #5 and #6.

The second question was to see if Ninja can cure/reverse PVY infection. Treatments included:

1) PVY
2) Mock
3) Buffer + inoculation
4) PVY inoculation
5) After 24 hours PVY treated with
    a. Buffer
    b. Ninja
    c. Surfactant

Results show that Ninja prep cannot reverse infection. Did a time course of curative treatment:

1) Buffer only
2) PVY inoculation only
3) PVY inoculation followed by Ninja at 7, 14, 21 days post inoculation
4) Same as above but with surfactant

Fewer symptoms were found on Ninja treatment leaves, but virus load was still high as measured by ELISA. Possible Ninja may help deactivate PVY. These trials are also now being performed on potato.

Update on recent field trials of mineral oil, insecticides and biological control agent for PVY management in New Brunswick, Canada – Mathuresh Singh (Agricultural Certification Services Inc., New Brunswick, Canada)

PVY abundance has declined over the last decade from 13% in 2009 to less than 1% in 2020. Currently 60% of available seed is totally clean. They did an experiment to assess if field management can really reduce PVY during a 3-year period between 2018 and 2020. They performed a replicated (4) and randomized trial to assess 200 plants/replicate in 8 row plots. 100 tubers were tested from rows 3-6. A total of 8 different spray regimes were tested (Traditional oil-insecticide mix, alternative insecticides, and biologicals). Insecticide and oil plots showed low PVY spread, some better than the industry standard (Silencer). This is good because Silencer may be banned. In this case Deltamethrin (Decis) is perhaps the best alternative. Biological additive (LifeGard®) was effective at controlling PVY. Lots of variation observed between years. The big picture summary is as follows:

No spray: Poor PVY control, Good yield

Chemical insecticide OR mineral oil: Poor PVY control, Good yield

Chemical insecticide AND mineral oil: Good PVY control, Good yield

Biological agent: Good PVY control, Poor yield

Alternative treatments are cost-competitive. Range of input costs (spray chemicals) per acre for whole season program vary between $75 to $215. The best PVY control costed between $145 to $210 per acre. Also calculated standardized cost (cost to get 1% PVY reduction). Best is Decis + mineral oil program = $26.42/acre/1% PVY decrease. Typical Silencer treatment costs 75% more, whereas biologicals are only 30-45% more. Found that leaves sprayed with insecticide (Silencer) and oil retained pyrethroid agent at higher levels for longer periods of time than insecticide alone. Insecticide alone treatment exhibited loss of pyrethroid agent below detection level quickly after spray. Theory is that insecticide is soluble in oil and can help penetrate biological tissue. Take home messages are: PVY pressure has been low recently. This is largely due ot reduced inoculum pressure (1) planted inoculum: Planting clean seed and managing neighouring fields is most cost effective. Rogueing volunteers and seed-borne PVY. Also due to reduced inoculum pressure (2) aphids. Spray with insecticide and oil (or biological) early as aphid pressure is a good predictor of post-harvest PVY presence. Advice: Spray early before full emergence, particularly on most sensitive plants. Use cost-effective sprays but don't skimp. Weekly spraying 2-3 Liters/acre of mineral oil and supplement frequently (each week) with tank-mixed insecticides (Silencer, Decis, Beleaf) or biological sensitizers (LifeGuard WG) as recommended.

1:30 PM Other topics

High resolution 3D characterization using Cryo-EM – Martin Lawrence (Montana State University)

Dr. Lawerence is looking for collaborators interested in virus structure (at atomic level, molecular basis of virus interactions. He is a structural biologist. He presented on 3 techniques of Cyrogentic Transmission Electron Microscopy (cryo-EM).

a) Single Particle Analysis (SPA) – Icosahedral viruses
b) Helical Reconstruction (HR) – Helical viruses
c) Cryo Electron Tomography (CET) – Virus Host Interactions

X-ray crystallography has also been used for non-structual viral proteins.

There are several advantages of using CET. It requires no heavy metal staining and work can be done in vitreous ice to maintain native protein structure. Huge benefits for determining structures of helical viruses, as this used to require somewhat heroic efforts. Some technological advances in microscopes, cameras, and software have made the resolution revolution possible. He has performed a lot of work on viruses from Yellowstone including Sulfolobus Turreted Icosahedral Virus (STIV), and Acidianus Tailed Spindle-shaped Virus. Can get at virus-host interactions of STIV bound to pili on S. solfactaricus using CET. Can generate 3D volume (Z-stack) but on higher resolution of subject that enables virus-host interactions to be observed/characterized at the molecular level. Can look at timepoints in viral life cycle, to study genome entry, replication, assembly egress, and transport. Interested in making progress on helical plant viruses to 1) correlate virus structure with function and 2) characterize virus-host interactions.

Transmission of *Dickeya* by insects – Andrei Alyokhin, University of Maine

Dickeya dianthicola is a gram negative, non-spore forming anaerobic soil bacterium. Pest of potatoes associated with "black leg" disease and slow wilt. Can cause loss during storage. Dickeya is more virulent thatn pectobacterial and requires a lower infective dose. Replicates at temperatures > 28 degrees C. and plant dies ~12 days after inoculation. Dickeya can infect peach aphids and makes them sick. More deadly towards wingless aphids. Aphids are considered to be a possible vector of Dickeya. Two potential insect vectors Colorado potato beetle (CPB) and Green peach aphid (GPA) were tested to see if they could transmit Dickeya. CPB is a oligophagous pest of nightshades, they are mobile, and rapid evolve insecticide resistance. GPA is highly polyphagous and vectors PVY and PLRV. GPA has a complex lifecycle with both winged (alate) and wingless (apterous) phases. Goal was to answer 2 questions. Question 1: Do insects discriminate between infected and non-infected plants? CPB was tested in a wind tunnel. It was found that no discrimination was observed or that odor is not a sufficient cue to drive feeding preference. Foliage treated with 2,3-butanediol elicted a significantly lower response rate, moreso than even 2,3-butanediol itself.

GPA was assessed using a Y-Tube olfactory aphid choice assay. Individual aphids preferred infected issue whereas uninfected tissues had more aphids/leaf.

Question 2: are insects capable of transmitting Dickeya between plants? This was assessed by infecting a healthy plant with Dickeya strain ME30 and incubating it for 5 days at > 28 degrees C. Insects were then introduced to the infected plant and allowed to feed for 2 days. Insects were then transferred to a clearn plant and incubated for 5 days, followed by a PCR test and bacterial culture. This was performed for both GPA and CPB. No transmission was detected, indicating that neither CPB nor GPA are vectors for Dickeya. See publication in APS PhytoFrontiers.

1:55 PM POLL – to determine breakout room topics for the upcoming brainstorming session

2:00 PM **BREAK** (breakout rooms are available)

2:30 PM Formal Breakout sessions/Brainstorming sessions

- Enter a Breakout Room that interests you and brainstorm with colleagues to identify research and collaboration opportunities
- Attendees within a Breakout Room will assign their own moderator or spokesperson
- Once the session is over, everyone will return to the main session

3:00 PM Conclusions, Ideas for future work and collaborations

- Moderators (or spokespersons) from the breakout sessions will provide a brief update of what was discussed/concluded
- Action items will be identified, if applicable

Brief Updates of what was discussed/concluded in Breakout Sessions:

Diagnostics and pathogen detection

Conversation focused on direct tuber testing, particularly Jason Ingam's work. Discussion about multiplex and being able to test for many viruses per sample. Discussion about generating powdery scab

inoculum in greenhouse. Amy Charkowski and Yuan Zeng have had good success. Keys are lots of water, and cool temperature (below 18 degrees C). Potting mix is a good medium. Cold tolerant tomato is a good host for powdery scab. Nobody has seen foliar symptoms of PMTV.

Breeding

Discussion centered on value of developing PVY resistant material. The importance of soil-borne disease is important (PMTV) as insect vectored diseases can be controlled using insecticides and oils. Ideal to use marker assisted selection. The major bottleneck to identifying QTL/markers is the phenotyping of structured genetic populations.

Vector-pathogen relations

Assessment of management practices to control powdery scab and PMTV was discussed as was vector attraction to host and non-host plants. Vectors in some cases may be manipulated by the pathogen. There is a need in Washington state to update action thresholds on insect and other pathogens. Need to better understand the interaction between powdery scab and microbiome particularly to do with suppressive soils.

Host-virus interaction

Discussion focused on defense against pathogens, molecular mechanisms of PTI, viruses in the apoplast, technology to visualize PVY expression in the plant (mcCherry construct), PVY in aphids, CM with colored markers to get better visualization of virus in the plant.

Post-harvest test group

Winter grow outs are indispensable. Need to have a good field site to do winter grow outs. Post-tests, usually merging both visual and dormant tuber/lab tests. Particularly to focus on false negatives.

3:50 PM 2020 Publications & Annual Report, Election of Secretary

Julie Pasche is nominated to serve as Secretary. Kent Sather moves to elect Julie Pasche, Seconded by Nina Zidack. Unanimously approved.

4:00 PM **Adjourn**