**Minutes for the WERA089 meeting**

**Denver, CO – Venue Hyatt Regency Downtown**

**March 12 & 13, 2019**

*Chair*: Kylie Swisher Grimm, USDA-ARS

*Vice Chair*: Matthew Blua, Washington State Potato Commission

*Secretary*: Kasia Duellman, UID

**Tuesday March 12, 2019**

8:00 am Call to Order (**Swisher Grimm**): Introductions; 2019 agenda, additions/approval; 2018 minutes correction/approval

Chair: Kylie Swisher Grimm

Vice-Chair: Matthew Blua

Secretary: Kasia Duellman

Attendees:

Aaron Buzza

Adam Winchester

Alex Karasev

Alice Pilgeram

Amy Charkowski

Ana Cristina Fulladolsa

Andrew Flannery

Andrew Houser

Andy Jensen

Brian Charlton

Brian Ross

Carrie Wohleb

Chakradhar Mattupalli

Chris McIntosh

Guiping Yan

Hanu Pappu

Jim Dwyer

Joe Coombs

Jonathan Whitworth

Kasia Duellman

Keith Schuetz

Ken Frost

Kent Sather

Kylie Swisher Grimm

Lynn Woodall

Mark McGuire

Mary Kreitenger

Matthew Blua

Max Feldman

Melinda Lent

Melissa Bertram

Nina Zidak

Nora Olsen

Paul Bethke

Phil Nolte

Punya Nachappa

Ravi Chitrampalam

Rich Novy

Russ Groves

Sagar Sathuvalli

Sarah Hensley

Sarah Noller

Silvia Rondon

Steve Hystad

Stewart Gray

Teresa Almeida

Vamsi Nalam

Walter DeJong

Yuan Zeng

Mary Kreitenger moved to approve minutes. Seconded.

8:20 am Administrative Advisor Report ‐ **Mark McGuire**, Univ. of Idaho

Writing committee must be formed before 2021 (by next chair, by next year’s meeting) – next deadline for funding. WERA89 report including impact statements and minutes of today’s meeting is due 60 days after today.

[Addendum: Please send your impact statements with robust list of publications and other components to add to the WERA-89 report to Kylie by April 15, 2019.]

8:30 am State Certification Reports: CA, CO, ID, MT, NE, NY, OR, WA, Others

CO: **Andrew Houser (15’)** – Colorado Seed Update

(Good snowpack!)

Since early 2000s, seed acreage has been declining, due to PVY (increased from 1975 to early 2000s); slight increase in 2019 compared to 2018

Winter test results: (Hawaii, Twin Bridge Farms) Rain delayed several days; planting depth – 4-5” – shallow to promote emergence; stakes worked better than flags; planted first week of Nov; Dec 6-20 inspections and lab testing, all leaf testing conducted on site; visual matched up well with ELISA tests; 3% recertification tolerance; must be <=5% to be sold into CO; 5-8% can be sold out of state/tag tolerance; rejections: >8%; 2018: <10% seed lots rejected and in 2019, >20% got rejected (>8%), average for 2019 6.53% (2.77% in 2017); total acreage rejected, worst ever this year (2007-2018); seed law passed in 2012, PVY was declining through 2017, but 2018 showed a spike; warmest and driest winter in 10+ years preceding 2018 – may be a link; Canela Russet – difficulty to break dormancy – average emergence is 80% (across varieties), Canela ~11% in 2014, variable among years, 2017 and 2018 about 48-57% due to their tactics; visual ID of in-season PVY spread by PCS inspectors: identify 5 healthy and 5 with PVY, test the skill of inspectors, as season progressed an additional plant was picked up (in-season spread), saw 6 by end of summer and lab picked up 7. Planted progeny tubers in Greenhouse and tested with ELISA; all ten plants had PVY, only picked up from GH growth – therefore winter test is needed; growers worry about in-season spread in HI – Andrew’s approach: mapped out plots with % PVY (red = >8%, white = eligible for recertification, etc) – lack of ‘zones’ of color indicates non-issue; comments: short time span and other data support Andrews conclusion that in-season spread in PHT is non-issue; DAP and PVY: final inspection less than 95 DAP - <4%PVY, 95-100DAP 5%, 101-105 10.1%; PVYN – limit is 1%

ME: **Jim Dwyer (5’)** – Maine certification results

(Lots of snow in Maine!)

2018 Main PHT results (Erik Hitchcock – seed certification program manager); in 2018 ME switched to all lab test for PHT (no winter grow out in FL anymore); 3 field readings during the season – early July, mid July, mid August (focus is on blackleg); reasons for change – next to new Brunswick, they switched to all lab test, has led to improved seed program, won’t plant anything >0.5% for recertification; switched to lab testing due to lack of reliability of field grow out (flooding/total loss/etc); 2017 only FY1 and FY2 went through lab only, FY3-5 went to FL; better detection in lab – specialty variety had <1% PVY in 2016-17 and >11% in 2018; others were in the ball park (grow out vs. lab); ~70% <0.55% PVY; 25% with 0.55-5.0%, 4.36 >5% virus; with lab testing, predict some varieties that are difficult to see PVY visually will be cleaned up; Q: any concerns expressed by customers re: lab test? A: No, due to New Brunswick doing it; Q: what about varietal mix? A: dealt with during field inspection; Q: Labor? A: It’s a challenge to find qualified labor for lab, two full time employees plus 6-8 part time; Q: results carried through to summer inspections? Consistency? A: Yes – took samples from FL and ran in lab and also compared with visual and they were pretty close. Q: Strain composition of PVY? A: NTN, O is declining, Stewart Gray has those numbers, Andrew plant saved all the plates for later analysis.

MT: **Nina Zidack and Steve Hystad (20’) –** Strategic changes to Postharvest Test rules and introducing direct tuber testing as a standard practice in Montana

Nina’s report:

(Highest snowfall this time in March; plenty of snow!)

Increased sample size to improve recertification decisions: reduces “width” of confidence interval, improves ability to distinguish similar prevalence levels; improves confidence in estimate of prevalence

Re-allocating PHT sample sizes to intensify sampling of earlier generations; old rules: G1, <=2acres – one sample; >2 acres, 2 samples; G2, up to 20 acres – one sample, 21-40 – 2 samples, 41-80 – 3 samples, >80 acres – 4 samples; G3-5, …

Encourage growers to send 1 of G1 and 1 G2 samples for tuber testing in the lab

How did this work?

101% increase in G1 number of samples, 71% increase in G2 samples, G3-5 samples decreased by 15%; dramatically decreased need for retesting after PHT; 2017 crop – 120 samples by PCR after PHT vs 2018 24 samples by PCR after PHT

2018 PHT summary:

56% seed lots 0% (vs 41% in 2017); 17% 0-0.5%, 9.5% @ 0.5-1%, 10% @1-2%PVY; in terms of acreage across all gens/lots: 46% of acreage 0%PVY, 22%, 12%, 12%, 8% >2%; summary of all lots;

In 2018, planted varieties that emerge about the same time together – improved picking efficiency, %visual/%ELISA was a better number because combined tuber testing results and combined with winter grow out and so increased # observations in G1 and G2, increased total test number, gives early prelim info; improving readiness for high throughput tuber testing; insurance for grow-out failure

Supplementing winter grow out in Hawaii with dormant tuber testing in November – not quitting HI any time soon but HI is never guaranteed; Q: variety mix/chem damage incidence? A: ~4 varietal issues but no chemical damage observed

Steve (nuts and bolts of how tuber testing works):

Lab testing: optimal is a combo of grow out plus lab testing

Bio-Rad thermocyclers/qPCR (2, thinking about a 3rd); 2 samples per machine per run, composites of 10, can fit 80 tests on a PCR plate;

In the past, sprout testing; over the years, tuber samples are increasing; 2017 – 24,800 tubers (800 samples/day, 2 people, 20 work days/instrument time)) vs 2018 – 51,600 tubers (using Alice’s method 6800 tubers/day, 6400 tubers/day qPCR, 10 work days/instrument time); looking at ways to increase throughput; PCR plate coated with antibody to avoid nucleic acid extraction;

Treated with Rindite to break dormancy to increase virus titer;

Immunocapture qPCR – antibody coated to PCR plate, incubate, wash, one-step qPCR, slice off stem end of tuber, ten slices per well, pressed with hydraulic press, 400 tubers per hour/person, wash and qPCR prep – 30 mins, thermocycling – 1.5 hours; will likely change protocol to collect cores from stem end plus bud end etc because PVY is not uniform in tuber;

Russet – 22 positives with HI, 17 with qPCR (not the same samples), Umatilla 31 vs 27; etc;

All varieties all generations: grow-out 0-1% PVY – 30%; 1-2%PVY – 33.5%; >2% 17% etc vs qPCR tubers: 52%, 22%, 15%;

Q: impact of where on tuber to sample? A: probably since PVY non-uniformity in tuber may vary from one variety to another

Q: what is optimal sample size? A: increased to 800 for earlier gens;

Overall: ~3600 ELISA tests, ~127 positive PVY; lab testing (ELISA tubers and qPCR sprouts) 7124 tests, 134 PVY positive

Will always be variability in grow out vs lab because separate samples, interpretation of qPCR results more complex than ELISA; immunocapture qPCR is cost effective/high throughput assay; development of new primers is necessary to increase sensitivity and specificity and comprehensive for all strains

Improve bottlenecks and increase throughput, reduce background noise, improve sample turnaround; grower cores, robotics, new primers, begin earlier to solve issues.

In 2019, lab testing in conjunction with HI grow-out – backup and supplemental not 100%; sprout testing will still be offered; results from lab testing combined with grow out will be reported on Health certificates;

Q: what’s the cost? A: pretty low, ~$200/sample (lab is about $140/sample and field is about $230/sample) – cheaper than HI.

ND: **Kent Sather (15’)** – Update on ND certification

Grenora ND – early generation seed; tissue culture; isolated increase; another grower nearby within 30 miles has grown gen 2-4 but will not be growing in 2019

Cando – restricted growing area, can only be 0% for PVY, flushed out when found; nuclear and G1, will increase to G2 and possibly G3; goes to midrange growers in

Wallhala valley area – mid gen growers, somewhat isolated, not a lot of commercial potatoes, mostly certified

Oakes – commercial grower who tries to grow his own seed, G2-G5; ~80% of what he raises goes to commercial processing, doesn’t sell much maybe 10 loads, internal.

Red River Valley area – seed growers intermixed with commercial; bringing in seed built up through Cando, and if lucky can do two gens – usually only 1 because exceeds certification tolerances

Seed Acreage summary (summer acres): about the same as 2017; rejections mostly due to mosaic; from 2015 to 2016, lost a seed producer who was also a commercial grower – lost about 2000 acres

Winter test: in Florida still (growers own property in Homestead FL, Alger Farms, LLC, “old boy” connections), great growth in past three years;

Only seed lots to be recertified are required to be submitted to winter test;

Acres tending to go down, eligibility at winter test also declining, rejections tend to increase – “losing the battle”; recertification limit in ND is 0.5%, a challenge for growers to maintain;

In 2018: of 378 lots, 5500 acres eligible at 0.5% tolerance; as moving eastward and older gens, higher proportion are ineligible; 35% of seed lots had 0% PVY at winter test (vs 2016, with 58%, 2017 with 47%) – struggling!

69% acres passed for recertification, only 35% was virus-free; isolation works – continue to educate growers; PVY was seen/virus testing in field to correlate test with visual; no blackleg or Dickeya was found; 600 tubers = sample size

Q: would grower isolated down by himself in Oakes having success? A: some success even with having some commercial growers around him – 2000 acres across 5 counties so management of geography helps; one variety that he is trying (out of nuclear) – will see how that goes. Q: how much Norkotah grown in ND? A: a slight increase in Norkotah in RRV area although nothing passed winter test; early generation grower out west grows Norks and a Cando grower grows them, but they don’t do well. Limited acres. Q: Trends in why PVY increased this year? A: moisture – started with a good year but got hot and dry later in the year, so grain fields started to mature earlier – and even though aphid pops were low (for green peach aphids) we think nonspecific aphids from other crops probably caused problems combined with desire to keep spuds in the ground longer to get yield…. Early flights of aphids suspected.

NE: **Adam Winchester (15’) –** Hawaii PHTs/update on NE seed potato industry

PHT in HI, issues faced there; followed Idaho – planted 11/8-9 went okay but ran into issues over the summer due to weather – multiple hail storms, late harvest (due to snow fall), growers sent in 4-5 physiologically aged seed before dipping in GA, leading to stand issues; overall – seed acreage >6000 acres 321 lots sent to HI, only 2 rejected due to PVY; everything that had PVY in it was tested twice

2014-2017 – 96% of lots passed in HI, with exception of 2 lots for 2018 (99% passed in 2018); good PVY management due to isolation; trace amounts blackleg, curly top; varietal mix found in 5 lots 0.5% tolerance; will continue PHT because of concern with varietal mix; 64% emergence overall, lower than past three years (80-85%); varieties with issues – Umatilla, Clearwater, and …. (no emergence) poor emergence and uneven emergence

For 2019, requesting extra seed, clipping, may experiment with different GA concentrations, may look at different gassing methods (e.g. PVC pipes with wholes in middle of pallets); dipping approach may change; will request more info, including harvest date, will compare with emergence in test plots; no BRR testing – outsourced to other states, will start doing that themselves; field gen nomenclature changing from gen system to Field Year system – vote pending this summer to be in place by 2020; changing sample numbers so can send fewer samples but better representation of what’s in the field; more testing for TRV and PMTV; Nicollete and Juanita susceptible to PMTV – there more testing. Q: Nomenclature change? A: Will follow Idaho; Kent Sather is initiating discussion with ND growers; MN is already on that field year rating. Q: how testing for TRV and PMTV? A: will look more into options that are available. Q: winter test – what did you wrap pallets with? What material? A: a strong plastic wrap. R: in Colorado, they use a plastic mesh to allow air to penetrate better, may help. A: Lack of uniformity in stand may be due to infiltration of gas.

OR: **Ken Frost (10’)**

No report from Oregon

10:00 am Break

10:15 am **Seed Certification:** Potential enhancements, including sampling and high throughput testing.

*Discussion/Presentation leaders: Stewart Gray, Ken Frost*

**Chris McIntosh** **(20’)**– The optimal tolerance for PVY in certified seed potatoes

Seed certification standards have two effects:

Determines how many seed potatoes can be sold (certified)

Determines commercial yield (PVY negatively impacts yield)

Profit function for commercial grower:

Profit = yield – cost of seed

Market price of certified seed potatoes depends on price of comm potatoes, certification standard, and average cost of …

For seed grower, profit function:

Should be equal to … if seed growers operating in competitive market; solve for S to have indication of the optimal certification standard

Etc.

Results:

Cost of seed – mean of ~4%

Cost of seed plus cutting and treating – mean of ~4.6%

Recommendation (because humans like numbers ending in 0 and 5!) – 4.5%

Damage functions based largely on N-Wilga, based on Russet Burbank.

Q: is 400 tubers sufficient? A: yes, calculations appear that this is the correct number. Q: what about sample size for early gen where cap is <1%? A: Stewart Gray will provide those in a later slide

**Kylie Swisher Grimm (10’)** – Seed lot testing for PMTV and TRV in WA state

Three years: 2016, 2017, 2018

Composites, 4 tubers multiplex RT-PCR; In 2018: 30 tubers from 26 seed lots

Composites of 5

300+ seed lots per year; 2016 – 1.7% PMTV, 0.29% TRV; 2017, 2018: 4.04%, 5.5% PMTV, 0% TRV

Cores from stem and bud end; only one out of 35 PMTV-positive tubers showed symptoms

PMTV not variety specific, all years, all states showing PMTV

2018 – in-depth analysis – 26 seed lots included 3 seed lots that tested positive in initial study (not enough seed from other 14); from states with PMTV-positive lots in previous years

How concerned should we be? Trend: We are seeing an increase in PMTV; do we need to continue in-depth analysis? If so, how many?

Q: to increase sampling potential, could cut tubers in half? A: slows down Mark Pavek’s operation; Kylie’s testing is using only left-over seed from Mark. Comment: keep in mind that sampling 5 tubers or even 30 to detect virus, statistically would be levels in excess of 10%.

11:00 am **Tuber Quality:**

*Discussion/Presentation leader:* *Nora Olsen*

**Nora Olsen (20’) –** Effects of necrotic viruses on tuber yield and quality

TRV and PMTV and PVY

Proper diagnosis is critical

Impact of PMTV on quality and storability – used tubers from Gudmestad variety trial; stored; observed symptoms and fry quality at harvest and at various times after storage

2015-2016 – Alpine, Bannock, Ranger, Russet Burbank – evaluated at harvest and once or twice in storage; symptom development varies by variety and year so may have time to store before symptoms develop, virus ranged from 0-9% at harvest and 23-90% at late storage in 2015; 1-60% and 1-81% in 2016; High level of asymptomatic tubers at harvest and in storage. Low symptom development with Bannock. No apparent effect on fry color except with symptoms.

What if we store at colder vs warmer temps? How does this influence symptoms? 42 vs 48F storage temperatures:

2017: Burbank and Bannock – with Bannock no diffs whether stored colder or not; Burbank developed more symptoms when warmer. Less virus in 2018 vs pervious year – no sig diffs.

Impact of asymptomatic vs symptoms in terms of processing quality? Overall, fry color isn’t influenced unless there are visible symptoms in the tuber.

What is causing the symptoms to appear? Future-look at impact of cultural and environmental conditions. External symptoms can cause problems in storage, sloughing, etc. – packing issues;

PMTV: symptoms tend to increase in storage; Bannock low symptoms; no apparent effect on fry color except with symptoms; watch for external symptoms – skin issues;

PVY – in-season management; what is impact of the seedborne plant, looking in commercial fields – healthy vs. infected; planted 500 plants of seed lot with high level of PVY; Burbank, Ranger, Clearwater; infected with N-Wilga, NTN, or (few) O; dug individual plants (2015-2018); yield of healthy significantly higher than infected; specific gravity usually sig higher with healthy vs infected; fry color – usually no color diff,

2018 KREC Seedborne Yield:

NTN and N-Wilga – about 30% decrease in yield with Burbank, Ranger – a little more, and about 80% decrease with Clearwater. More work needed. Specific Gravity: Significantly lower in all with NTN and N-Wilga with Ranger and Clearwater.

Fry color: Better with PVY statistically but essentially no visual differences unless symptoms are present and then darker color at symptom.

Issues with yield, size profile, specific gravity with seedborne PVY and fry quality with symptoms.

What about current season infection?

Growers want to know if they should change spacing, fertility, etc. and at what level of PVY?

Screenhouse inoculated plants: Alturas, Burbank, Ranger, Umatilla

At harvest, PVY-O developed tuber symptoms in all but Burbank; NTN all but Alturas; N-Wilga – none

After storage all cultivars had symptoms with PVY O and NTN and Alturas with N-Wilga.

Next year – acted differently; no O at harvest; low symptom incidence with NTN in all but none in Burbank at both timings, low symptom development with N-Wilga.

Less stress in the second year; may be playing a big role in symptom development – general recommendation: try to avoid stress.

Clearwater and Bannock -lower PVY susceptibility resulting in lower plant infection and number of infected tubers for storage.

Future-Tuber size and profile changes, impact of early death in the field

TRV:

Collaborated with Prosser/WA grown; Alpine, Bannock, Ranger, Burbank; in first year 2015, little impact on symptom development with cultivar or time in storage; in 2016 saw big diff in variety, with Alpine most affected, low with everything else. ND grown: Alpine, Bannock, Ranger, Burbank – lower symptoms lead to increase in development in storage, 2015; 2016 – no sig diffs in cultivar or time in storage; no apparent effect on fry color unless symptoms are present; Avoid Alpine Russet.

Why the big difference in symptom development between years and locations?

2017-2018: WA only. Castle, Payette, Burbank: Castle – low symptom incidence; Payette and Castle may be good choices for commercial fields.

**Paul Bethke and Erin Weber (15’)** – Impact of in-season PVY infection on harvest and post-harvest quality of chipping potatoes

Manually inoculated until before flowering; hyperspectral reflectance data were collected from asymptomatic leaves above the site of the last inoculation. The same leaves were assayed for virus with ELISA; Atlantic, Snowden, Lamoka

Atlantic – no infection in mock, 100% in inoculation with all four strains

Snowden and Lamoka– less efficient especially with NTN where only 20% of Lamoka plants and 0% of Snowden plants tested positive for PVY.

Does early season infection impact yield?

Wilga depressed yield overall by variety and as a whole.

Other strains tended to push yields down.

Specific gravity – wilga pushed it down for all 3 varieties. Specific gravity for Snowden infected with NO was also reduced.

Chip color – beautiful symptoms in foliage; no diffs in chip color, external defects, internal defects.

Percent tubers infected via peel assay: Atlantic – 0%; Snowden, PVYO – 50%, PVYNO – 8%, PVYNTN not available, PVYWilga – 50%; Lamoka – 0, 31, 2, 52, 38% peel assay via ELISA (NOT PCR).

Yield loss– Likely imposes an economic cost to growers.

|  |  |  |
| --- | --- | --- |
| **Strain** | **Atlantic yield** | **Lamoka yield** |
| O | 10% less | 20% less |
| NTN | 14% less | 10% less |
| Wi | 28% less | 17% less |

Tuber specific gravity: Likely imposes and economic cost to chip processors.

Drops in Atlantic with Wilga – 6% fewer chips/lb and 3% greater oil content

Snowden with wilga and Snowden with O – 2% fewer chips/lb and 1% greater oil content

Not really visible

Future plans – being repeated, infection status of plants looks good

Comment: Virus does not move to tubers equally among cultivars (efficiency of moving to tubers varies by cultivar)

12:00 pm Lunch, on your own

1:15 pm **Diagnostics:**

*Discussion/Presentation leaders: Nina Zidack, Alex Karasev*

**Stewart Gray (30’) –** Direct tuber testing for PVY, PMTV, TRV and beyond using PCR coupled with FTA cards

When, where, what, how, why to sample? PVY, PMTV, TRV – chronic issues in potato (vs. acute issues that have been discussed thus far)

Tuber testing desired by industry – want answers faster, don’t like risks associated with grow outs; about a decade behind Europeans; doesn’t worry about protocols so much – hundreds and hundreds on PVY diagnostics; do we need to spend lifetimes working on diagnostics for PVY? No, in my opinion. Optimizing, handling storage standardization;

When to test tubers – dormant or those that have broken dormancy (e.g sprouting)

Sampling – how to sample from the whole seed lot; on the farm – ideal; pressing into FTA cards (maybe can have farm labor press into cards); system developed by Europeans, optimizing over past 10 years; can provide details if we want; can analyze for any number of viruses, conventional or realtime PCR doesn’t matter, relatively automated using available labor to minimize upfront costs. FTA card sampling protocol:

Mash tuber core into “well” on card using an industrial press; remove debris; punch 3 holes from each well, composite of …XX tubers; buffer, shaker, kingfisher automated extraction; tubers with mixed infections or single – PMTV, TRV, and PVY; biopsy core plus sprouts from the same tuber (4 2-mm cores – one from each end and two from the middle) taken from dormancy to sprouting period; PVY in many samples with PMTV or TRV; PMTV and TRV weren’t together

Where to sample within a tuber:

Five cores out of tuber – one from each end, three from middle, two being eye, two not eye, and one from stolon; cut off skin and cut off next layer of tuber, then into flesh – therefore 3 depths = 15 samples for each tuber; sampling 8 random tubers from each of 8 fields, sampling every 2 weeks; to view internal symptoms – use ONION SLICER (rather than quarters)

Distribution in tuber is virus and perhaps cv. Specific:

PMTV: stolon end has higher titer of virus

PVY: stolon end and eyes very good

TRV: who knows; sample anywhere

How accurate is “end” sampling – rose end and stolon end; very accurate for PMTV; false negs for PVY ~50%; TRV, 20% false negatives

Conclusion: don’t sample just from the ends

Dormant vs non-dormant: will likely be a big deal; data in progress (will have >9000 data points to analyze – may be presented in 2020!)

(Stewart Gray retires this year)

May vary among cultivars, viruses, physiological age

FTA cards can be stored (long term storage); can repeatedly sample from the cards; can overload FTA cards – squishes can merge together so make sure 4 2-mm cores with biopsy tool doesn’t exceed capacity; one tuber per square, composite 10 squares (= tubers)

Need to ID fields with high virus incidence (PMTV and TRV)

Need to plan and conduct experiments so data can be directly compared

Need to agree on common procedures for sampling, testing and analysis

Need high throughput sample collection/testing

Composite samples will be the norm

Rethink tolerance limits and how they are calculated and reported

FTA cards/kingfisher extraction methods useful for range of pathogen testing

FTA cars useful for leaf tissue

[Soapbox]: Standardized field year terminology – FIELD YEAR vs. generation vs. nuclear/gen etc.

Certification agency survey – states rely on visual for many viruses

Understanding published disease estimates: 400 tuber size – if 1 out of 400 infected, reported as 0.25%; BUT 95% confidence that lot infection is below 1.57% level based on result reported from 400 tubers. E.g. 0.25% reported (1/400) may be 0.06-1.57% PLUS PVY is not uniformly distributed; Buyer should know sample size to know confidence interval because confidence interval is reduced with larger sample sizes.

PVY, PMTV, TRV cannot be visually assessed

Seed certification labs currently do not have ability to determine incidence for PMTV and TRV

Current sampling/reporting underestimating disease levels in seed

…

…

Virus disease issue affecting seed certification will only be a problem if response from industry is “Nothing”

Progress: National harmonization standards accepted by 14 states

Seed laws – year out in 3 states

Lab testing of PHT samples 100% in 3 states and some percentage in 12 states

Transition to tuber testing

National committee looking at seed certification improvements

5 0f 10 potato breeding programs engaged

Production farms engaged in tuber necrotic virus ….

**Neil Gudmestad** (via Stewart Gray): direct detection of PMTV from soil; can tell how much Spongospora subterranean (Ss) is present and whether PMTV is present (<10 copies/ul in RNA sample). If less than 100 spore propagules, couldn’t detect Spongospora. Amy’s lab is using this protocol to test for Spongospora in soil and potting mix and having good results as well.

**Guiping Yan (30’) –** Current research status on molecular characterization and diagnostics of stubby root nematode species in the United States

Stubby root nematodes vector of TRV; first report in ND 2016 Yan et al Plant Disease

Sample collection and occurrence of stubby root nematodes; 10-320 stubby root nematodes/200 g soil – not very dense;

Yukon gold 2017 ND field: symptoms were apparent;

Stubby root nematode ID: morphological – time consuming; requires experienced taxonomist; morphometric measurements; molecular ID: DNA sequencing, species specific PCR, PCR-RFLP, rapid and sensitive;

Species of stubby root nematodes, all in ND, NE, ID, MN, OR, WA are P. allius; in other states, SC, FL, GA, NC: other species e.g. P. porosus, Trichodorus obtusus, P. minor;

All new sequences submitted to GenBank – 28S, 18S-1, 18S-2+ITS; sequence lengths: ITS1 region more variable among species;

Big insertions and deletions also found in ITS2 rDNA regions

D2-D3 of 28S-rDNA used in phylogenetic analysis

ITS1-rDNA used in phylogenetic analysis – monophyletic group formed

Development of molecular diagnostic assays, end-point single PCR – primers targeting ITS1 rDNA; specific

Realtime PCR – melting curve analysis; efficiency analysis (via standard curve)

Quantifying P. allius from field soil samples; taqman overestimated vs. SYBR

Multiplex (all four species); four forward primers with another reverse primer – before optimization, some bands were bright, some weak; after optimization, detected four at relatively similar band brightness; samples from each region tend to have only one species

Factors to improve assay:

Nematode body size

Small juvenile, large juvenile, small females, large females

Soil pre-treatments

Autoclave, air dry, oven-dry, non-treated – diffs in DNA concentration, yield, quality; autoclaved sign reduced yield and quality compared to others; non-treated fresh moist soil is best

Additional soil DNA purification

PCR inhibitors

Additional PCR enhancer

Replicates of soil DNA extraction

Types of standard curves

Plan – transfer realtime PCR protocols to seed certification programs and potato labs

Develop droplet digital PCR assays for detecting and quantifying P. allius and TRV on nematodes; advantages compared to realtime PCR – don’t need to generate std curve, more tolerant to cellular inhibitors

Q: how far away from detecting TRV on nematodes; A. Working on it, almost there using qPCR via Dr. Danquiong Huang; more validation needed

C:

**Keith Schuetz (20’) –** Isothermal amplification of viruses that cause tuber quality issues

AmplifyRP® operates at 39-42C; lyophilize down to pellet that contains all amplication reagents; kits that people can design their own assay – stable for at least a year; specific pathogen kits also available; for DNA or RNA; can use crude sample prep; no thermal cycling; amplification done within ~20 mins; PCR level sensitivity; molecular diagnostic experience not required; end-point or realtime detection

Viruses focusing on: PMTV, TRV, PVY

TRV will be focus today – a prototype has been developed;

Goal: geared to direct tuber testing; want to design assays for direct tuber testing; eliminate procedural challenges/critical control points (e.g. sample extraction), eliminate steps where possible; validate using instruments that labs already have, e.g. qPCR machines. PMTV currently being designed; PVY in queue for 2020.

TRV data – peel vs core: cores seemed to perform better than the peel; (TRV test has internal control that targets the COX gene? To measure inhibition)

TRV extraction timing: the longer the extract sat around the better recovery of internal control, didn’t seem to have huge impact on TRV detection.

Soaking method – works pretty well; raw soaks – not performing as well, even when spinning down; got rid of supernatant and re-suspended pellet in GEB;

Initial findings: core best tissue for TRV, more samples needed; extraction time didn’t seem to impact extraction efficiency but improved internal control; core maceration or soaking appear to work; soaking seems to have less inhibition

Where next: larger scale – more samples needed; optimize protocol; moving to high throughput using realtime PCR machines; looking for collaborators to assist in outside validation of method once optimized (let Keith know if you are interested in volunteering)

**Hanu Pappu (10’) –** Update on PMTV and TRV diversity

**(10’) –** Metabolomics and transcriptomics of PVY-potato

PMTV and TRV diversity

Two strains of PMTV – S (severe) strain, and M (mild) strain

Marker – based on CPRT amino acid sequence

Complete genome RNA 1, 2, 3) of several isolates (at least 5 from each state: ID, MD, ME, NE, WA) sequenced; genetic diversity studies

Nucleotide similarity analysis – all US isolates shared high sequence identity (>97%)

Range of sequence ID varied when individual genes were compared

CPRT was most divergent

RdRp, TGB nearly 100% identical

Similar relationship reported from Europe: CRPT most divergent

Phylogenetic analysis – genetic incongruence – all genes did not cluster together when compared to each other

More trees – will be available in upcoming publication

Genetic diversity, selection pressure, neutrality, gene flow: is one group evolving faster than another? Take home message: S strains appear to be evolving faster than M strains

Results: US – high seq ID; PMTV in US may be from single introduction

All 7 US isol appeared to be S strains

CRPT most divergent vs other genes

RdRp and TGB highly conserved

TRV:

RNA1

Tried NGS and genome assembly; ditched NGS strategy and used overlapping RT-PCR instead

RNA2

Virus tolerates deletion in RNA2 especially in nematode transmission gene; therefore RNA1 has been target for detection; RNA2 phylogenies – American isolates tend to cluster together

Evolutionary pressure varies with different genes

Low genetic diversity in US isolates

Existence of recombinants in US based on RNA1

Positive diversification in RdRp and RdRp-RT of non-potato TRV isolates (host adaptation?)

1b gene (VSR) less constrained vs other genes

TRV LAMP detection

Our results on TRV genetic diversity suggested that RdRp and 1a9MP) genes on TRV RNA1 are more conserved among different isolates

Potato Virus Y potato metabolomics studies

Can transcriptome changes be correlated with changes in metabolomics? Preliminary data – will be analyzed soon. PCA – appear to be some differential expression Premier Russet and Russet Burbank following PVY infection

Q: do we have a primer sets that can identify all strains? A: ones they’ve tried detect all isolates they have. Q: Metabolomics – experimental design two levels of variety and two levels of strains – wouldn’t it be appropriate to look at development stage, source-to-sink distribution, timing of infection, etc? Host-pathogen interaction below ground above ground, strain diffs, different time points, and growth stages, etc? A: yes.

**Alice Pilgeram (20’) –** 4-State PHT direct tuber test vs field grow out comparison (Yr 2)

Objectives:

1. Determine if results of nucleic acid-based PVY testing of dormant tubers is comparable with visual and ELISA results from field grow-out
2. Determine if tuber testing can be economically scaled for use in seed potato certification
3. …

Immunocapture (IC) – coat PCR plate with antibody just like ELISA plate; prep as you would ELISA; incubate a couple hours to overnight; wash with the same as ELISA; all virus is attached to antibody in plate; can store plate for 4-5 months or run PCR immediately. Make up cocktail just like any PCR rxn and add directly to immunocapture plate.

Adjustments: dilutions of sap from PVY+ leaves – added a standard dilution series before sending to states; a duplicate IC plate was set up for each treatment and rep; bulk samples from single rep of low, me, and high potatoes were transferred to FTA cards and send to Cornell. Individual tubers from a high rep were individually transferred to FTA cards and send to Cornell – CORRELATIONS NOT YET MADE

Primers need to be improved to reduce background

Difficult to decide what is positive – loading standards should help

In ID, IC predicted whether there was a low, med, or high level of PVY, validated by grow-out ELISA and Visual evaluations in Hawaii; for MT, IC was generally predictive of what was seen in HI; in CO – IC tended to be higher than field/visual.

Constants: same IC Plates (loaded at MSU); same machine; Variables: different RT qPCR kits from different manufacturers; Berger probe was labeled with different fluorophores, duration of tissue storage prior to processing; different varieties and genes, variation in field grow out.

Comparison of different testing:

ELISA on dormant tubers is useless – probably not high enough titer of PVY

Dormant tuber IC testing – better than Delaporta extraction; could be recovering more PVY particles – nicely protected by protein coat, whereas with extraction the protein protection is lost

Dormant tuber RNA (delaporta method) – not so great! Could be losing RNA or maybe RNA wasn’t okay; use an internal control e.g. plant housekeeping gene just to make sure RNA is okay

Field grow out – leaf ELISA – …

Starting PVY copy number – distribution in dormant tubers – more tubers of later field years have higher copy number;

-dormant tuber testing detects similar levels of PVY as field grow out

IC methods has been successfully implemented in other labs (CO, ID, WI), Cornell, Oregon, ND.

Immunocapture can be economically scaled for tuber testing in a certification lab

Comment:

**Ken Frost (15’) –** Title TDB, but on topic of diagnostics

400 tuber sampling size – confidence intervals, probability; basically, 2% incidence is not different from 0.25% or 5%; even if sample is increased, still not very good.

To call something 0.5%, may need to sample 1000 tubers.

[Stewart used program by Monsanto to calculate his numbers.]

Sensitivity: % infections correctly identified as such

Specificity: % of non-infections correctly identified as such

Ideally, true positive rate and true negative rate would be 100%; no assay is like that;

Important to know sensitivity and specificity and this data can be hard to find in all those published assays.

Positive predictive value – proportion of positive tests resulting in correct diagnosis

Negative predictive value – proportion of negative tests resulting in correct diagnosis (?)

Stewart Comment: when comparing all the different types of assays, we should stop striving for perfect correlation. Ken: but we should strive to know this info for the different assays.

False positive paradox – when rate of false positives are higher than prevalence

Bulking – combinatorial issues with bulking:

30 tuber samples 6 tests of 5 tubers each – with one positive, only one outcome; if two positives, two outcomes (both in same bulk or two bulks with one positive each) etc. as the number increases, some combinations will be more likely than others.

Stewart: move industry to levels – below 1% above 1% for example; see calculator online developed by Monsanto in spreadsheet

3:30 pm Break

3:45 pm **Cultivar Development & Evaluation:**

*Discussion/Presentation leaders: Jonathan Whitworth, Kylie Swisher Grimm*

**Jonathan Whitworth (10’) –** PMTV testing in the Aberdeen breeding program: Protocols

3/13/2019:

[Stewart Gray is retiring – Jonathan acknowledges his mentorship, collaboration; etc]

Egin bench area of Idaho

Loamy sand

First implicated with mop top in 2001-2002 as PMTV+ spuds moving into Canada

Arranged to have a testing site for mop top – area has been in potatoes for a long time, with a lot of history and a willing cooperator

Criteria: history of spraing, is fumigation used to knock out stubby root nematode, every other year there is a stubby root soil test and decision to fumigate is based on numbers

Test soil for Ss and PMTV

Fumigate prior to screening (to eliminate stubby root/TRV)

Test a subset of potatoes each year for TRV and PMTV (include symptomatic and asymptomatic)

Q:If a hundred tubers are tested, how many will be infected? A: Unknown

Varieties are sensitive vs. insensitive (rather than susceptible and resistant)

68% of symptomatic had PMTV, 21% of asymptomatic had PMTV – based on work in Prosser – Crosslin/Brown/Quick/Hamlin

Some varieties that show insensitivity:

Pomerelle Russet (2014, 2015) – low sensitivity

La Belle Russet (2014) – low sensitivity

Castle Russet (2014, 2015) – lowest sensitivity/highest insensitivity

Clearwater Russet – high incidence

Comments from various attendees: More than one location with high levels is needed; infection/symptom development varies from year to year; environmental conditions influence symptom development but we don’t know what those are; if we start this screening, we have perfect situation with both viruses present – how about screening for both rather than fumigating to remove TRV.

**Rich Novy (15’) –** ARS-Idaho: Breeding for resistance to tuber necrotic viruses

3/13/2019:

Breeding for resistance to PVY

Ry genes – provide extreme R to all strains of PVY – e.g. Payette Russet

From three species: S. tubersoum andigenum, S. stoloniferum, S. chacoense,

Confer R to all strains of all strains of PVY

Monogenic and dominant

Normally in breeding, one gene (monogenic) might not be durable. Rysto has been in Europe for many decades and hasn’t broken down yet – has proven durable. Major gene resistance to PVY has been durable, unlike other major gene resistance to late blight.

Premier, Clearwater and Bannock have other Ry genes that confer some resistance….

Molecular markers for MAS for PVY Resistance

Ryadg – three markers, Rysto – one marker, Rychc – one marker; presence/absence of markers have aided in selection

Validation in conjunction with marker assays because recombination can occur

PVY screening at Kimberly ID for confirmation of R

RCB – 3 reps

PVY mechanical inoculation, aphid movement, ELISA test of daughter tubers

(also test for PLRV and PVX)

In 2007, 2009, and 2010, Payette had 0 infection while ranger and Burbank had 40-97% infection.

PA92A08-17 – a “Star” Clone in Novy’s breeding program; grandparents of Castle Russet; 59 families generated since 2000 – zero PVY from 1996-1999 (Burbank: 23-80%); potential source of Lso resistance – R towards bacterium vs. vector; Pedigree: *S. chacoense* 🡪 Colchicine doubling 🡪 *S. chacoense*-4x × Lemhi Russet 🡪 MPI 61.510/53 × 4x(tbr × chc) hybrid 🡪 PAg2A08-17; Grandparent of Castle Russet; PLRV Resistance: presumably from *S. chacoense*

Notable cv used as parents for PTNV resistance/tolerance: the Russet Market Class

Payette Russet: PVY (Rysto); MR to TRV

Castle Russet: PVY (Rysto), PMTV, TRV

Pomerelle Russet: PMTV

Gemstar Russet: PMTV

Bannock Russet: PMTV/PVY-O

La Belle Russet: PMTV

Summary: hybridizations conducted using TRV/PVY/PMTV-R parent with true potato seed generated in 2018

Seedling tubers generated in GH in 2018

TRV – 34 families – 5491 clones

PMTV – 8 fam, 1948 clones

PVY – 75 fam 16216 clones

Single hills – 1st field generation in 2018: selected 95, 182, and 62 clones for TRV, PVY, and PMTV respectively

In replicated trials, ~26 breeding clones with extreme R conferred by Rysto or andigena

**Joseph Coombs (20’)** – Genetic markers for potato mop top and tobacco rattle virus (PMTV and TRV) resistance in a tetraploid russet mapping population

Tetraploid mapping population A15001

GH tuber production – future populations need larger family sizes to produce enough tubers for phenotyping; 30% of clones did not produce enough tubers;

Global Potato Pangenome Project – Castle Russet (POR06V12-3) is one of the parents being used in pangenome project, as well as A15001

SNP genotyping of A15001 at MSU; 22K SNP Array, ~7000 markers, about 500 markers per chromosome

New Potato V4 SNP Array (available summer 2019)

Illumina V4

A15001 GWAS Analysis – GWASpoly (R package) allows putting together genetic marker info with phenotypic info to see if there are marker-trait associations; 6790 SNPs; 4 phenotypic traits: PMTV Inc and Sev and TRV Inc and Sev

The General Model detected most significant markers –

PMTV Incidence – chr 2, 35 SNPs; Chr 3, 9 SNPs

PMTV severity – no significance

TRV Incidence and severity – chr 9, all markers in similar position on chr 9; no standout annotation but others are invited to look at annotation to see if something makes sense; sig markers piling up in a region

**Alex Karasev (-20’) –** Strain-specific and other types of resistance to PVY

3/13/2019:

Strain specific and other types of PVY resistance

PVY exists as a complex of strains, making management and detection challenging

5 parental sequences/non-recombinants

Columbia Basin seed lot trials – strain ID workshop

General overview of what was seen: composition strain change – dramatic drop in O strain from 2011 and now (>60% in 2011, ~2% in 2018), N-Wilga increased, and recently NTN is on the rise as N-Wilga decreases – although still most prevalent at ~70%; prevalence of recombinants reached over 90%;

Extreme resistance conferred by Ryadg, Rsto, Rchc – broad – strain-nonspecific, durable

Hypersensitive R – Ny, Nc, Nz, etc – strain specific (maybe more than one, but not to all), sensitive to temperature; dominant; quite common in commercial cultivars

Experiments in 2015-2018: different locations, screenhouse, grow fields, greenhouse, screening various cv against diff strains of PVY

PVY-O in Ranger, Alturas – basically weak plant, almost kills it, while N-Wilga-infected plants thrive

In Clearwater, N-wilga almost kills the plant, while NTN is thriving – could explain spike in NTN

Mature Resistance: dependent on age of plant, has been studied in Europe, older plants can exhibit R to infection; can we use Mature R to manage PVY? Critical period of vector management – early season or late season, and if late season, where is the mature resistance?

Pilot experiment – 2017-2018: Yukon Gold, PVY-NTN; potato plantlets from tissue culture planted into soil and infected with strain every week after transplanting for 8 weeks; at each time point, analyzed plants for systemic infection starting 4 weeks post inoculation; assessed reaction in tubers = current season; plus 1 month after storage; collected tubers for each plant and replanted = seedborne virus. Systemic infection drops off significantly from week 4 to week 5. Most susceptible weeks 1,2,3 after transplanting; by week 4 drops a little, by week 5 almost nil.

The earlier the plant was infected, the larger the yield loss but at some point yield loss becomes virtually nil – if infection occurs mid-late season, no yield loss is expected based on this GH work.

Tuber reaction: the earlier the infection, the more serious the symptoms; late infection yields very small lesions that are not classical rings

Didn’t see translocation (doesn’t move to tubers) after later inoculation – doesn’t appear to have translocation to tubers when inoculation occurs late

Age-related R blocks systemic infection of virus and translocation to tubers

Take-home message: may be early stage of plant development which is critical and in need of protection; late in the season, plant may not need additional protection

Questions:

Study other cv and strains of virus

Connect GH to field

Can we apply this type of R to vector control?

Can this R be induced?

Comment: Days after planting/window of susceptibility hard to define in the field – a common criticism by growers

Comment: Comparing tissue culture vs field grown tubers – plants grown from field grown tubers weren’t as susceptible as the tissue culture plants in Nina’s work.

Comment: Leslie Torrent looked at Mature plant resistance – Stewart Gray will send report. NTN had much bigger effect with mature plant R vs other strains of PVY, plus there was an CV interaction.

Comment: Virus transmitted by aphids at the time-points rather than via mechanical;

Comment: maybe inspectors have idea of which cv have mature plant resistance, so could look at inspection records; R by Gray: ME has a database online to see those varieties that always pass inspection;

Comment: regarding early vine kill – to avoid late season infection

Comment: look at model systems since in other systems, resistance doesn’t kick in until plants are older

Mature R may be related to one of the N genes

**Max Feldman (15’) –** Identifying molecular markers associated with viral pathogen resistance

3/13/2019:

Screening for corky ringspot disease (TRV) resistance

GH assays developed to study disease process using tobacco bait plants

PMTV resistance screening – identifying fields that contain both vector and pathogen; identifying markers associated with resistance/marker development

PVY resistance – taking known sources and introgressing them into their germplasm

Castle Russet – TRV, PVY, PMTV – resistant

Quantitative genetics – link between phenotype and genotype –

Corky ringspot marker development:

Castle russet X susceptible cv 🡪 score offspring for R or S

Use simple statistical test to see if R of phenotype is strongly associated with any of the markers

How to score genotypes: visual incidence, visual severity, PCR probes

R segregates as 1:1 – indicates it’s probably a dominant, single-locus trait

Using GBS – pros: decrease cost, no need for prior knowledge, platform being used by …; Cons: High % missing data; require more complex bioinformatics pipeline

Introgress existing and novel disease R into new cv; stack multiple R mechanisms

Develop new methods to more easily quickly et

Use marker assisted breeding to develop new cv

Discussion: It was noted that tolerance (asymptomatic carriers) should not be a goal since it will perpetuate a problem that can’t be seen; Feldman concurred.

5:15 pm Adjourn until 8:00 am March 5

**Wednesday March 13, 2019**

8:00 am Call to order

8:05 am Election of secretary, plans for the next year, location selection, etc

Impact statements, publications from 2018

Secretary nominations: Steve Hystad

Nomination carries. Secretary for 2020 is Steve Hystad, Montana State University

Location for 2020: Provide suggestions to Matthew Blua

Matthew Blua will move to Chair; Kasia Duellman will move to Vice-Chair.

Matthew Blua is taking suggestions for 2020 location: e.g. Boise, Portland, San Diego, et cetera

8:30 am **Disease Management:**

*Discussion/Presentation leaders: Amy Charkowski, Russ Groves*

**Silvia Rondon (15’) –** Revising the status of beet leafhoppers and phytoplasmas in the lower Basin

(was not able to present)

**Ana Fulladosa Palma and/or Yuan Zeng (20’) –** Powdery scab and PMTV - questions and updates on biology and management

**3/12 4:50PM Ana** Ss reported in 18 states; PMTV formally reported in 7 states (including Idaho); ME – 2002 1% incidence by 2018 5% incidence; went to workshop hosted by Gudmestad to learn how to detect Ss in soil; suspicion that it was present in commercial potting mix, with peat bogs most suspicious. Powdery scab symptoms observed on minitubers in growing medium. Root symptoms but not tuber symptoms can build up inoculum in soil;

Obtained two potting mix samples, tested in reps of 6 to 10; qPCR used (Gudmestad method); average of 31 sori per g soil; some were 0 some were almost 90; negative control – potting mix autoclaved 3 times; a lot of variability, probably can’t detect below 30…

Bioassay – tomato; looked for plasmodium in root hairs;

Triple checked by testing for presence of Ss in roots via qPCR

6 tomato plants of each variety – visual assessment followed by qPCR

qPCR probably not sensitive enough to detect Ss in potting mix

**Yuan** – chemical effects and host susceptibility; Omega, RIDEZ and control

Field experiment:

Neither Omega nor RIDEZ suppressed PMTV at one farm; two russets (Russet Norkotah 8 and Canela Russet) had high levels of PMTV infection without presence of powdery scab

Neither Omega nor RIDEZ showed promising effects on reducing soil inoculum level, tuber lesion development or PMTV incidence

Level of sporosori present in soil changed as results of planting different potato cv even in the field where no powdery scab symptoms were observed.

The two russets were more tolerant to tuber powdery scab lesion development in the field than the other four cultivars tested (Yellow-skinned: Satina and Soraya; Red-skinned: Ciklamen and Modoc), but not to PMTV

Environment may play significant role in development of powdery scab lesions

Greenhouse experiment:

Cultivars: Satina and Diplomat

3 inoculum levels (1, 5, 10 sporosori/gram potting mix), chemical treatments, 2 house-prepared potting mixes

Neither Omega nor RIDEZ significantly reduced soil inoculum level, tuber lesion development, or PMTV incidence

Sand/Vermiculite/Peat moss (v:v:v; 2:1:1) mixture more conducive to powdery scab and root gall formation

At optimal conditions, undetermined or low inoculum levels of sporosori resulted in severe disease development on a susceptible cv – Satina

Efficacy of transmitting PMTV was high under optimal conditions

Future/ongoing work:

Investigating non-host cover crops

Development of disease prediction model

Understanding impacts of tolerant/resistant and susceptible cultivars to Ss on rhizosphere and geocaulosphere microbiome

Identifying tolerant/resistant germplasm to both pathogens

10:15 am Break

**Russ Groves (15’) –** Risk predictions for the timing of PVY transmission and pathogen spread

Varietal variation in PVY incidence – cultivar selection; lists of ones that frequently fail certification and those that pass are available; eg Silverton Russet is highly susceptible; DRN – has a lot of zeroes, but doesn’t mean it’s resistant but tends to be self-limiting (seedborne infected plants do not grow well);

Use ARC to join cropscape data (cropland data layer) can identify what crop was grown in any parcel in any year – can look for relationships between adjoining crops (small grains – bird cherry-oat aphid, alfalfa – pea aphid) – are there crops that are driving the disease cycle; the only signal is proximity to potato was most important – related to distance to commercial potatoes – get away from sources of inoculum is the take-home message. Ken Frost and Amy Charkowski published on this.

* Clean seed
* Avoidance in space (spatial isolation)
* Early vine kill

Aphids – aphid monitoring began in Midwest due to appearance of soybean aphid and a desire to manage soybean aphid in soybean (2005-20018); 202 unique species, 54 genera membership – an incredible source of information; suction trap network.

Variation in aphid abundance, species diversity; vector environment; top five species in ME, WA, OR, IL, WI are not the same from one region to another; upper Midwest, padi and glycines are more prevalent – available via the program; CO, ME, etc; goal – to predict timing of flights

[Soybean aphid is disappearing due to neonicotiniod seed treatments]

Modeling aphid phenology: GAMM’s (2005-2013)

Fitted to degree days; across 43 suction traps across 9 states, there is a tight fit – aphids fly at predictable times; started to fit via cross validation to MN and ND data – predicted fits superimposed very well on top of actual data; tied to weather/abiotic conditions, aphids become active at predictable times

Idaho data, OR data

Risk Index Determination:

Risk score = log(count+1)\*ave infectivity

In WI, they take top five species

Perform cross validation with other locations

Generate risk predictions with the top 5 predominate species

Website: Wisconsin Vegetable Disease and Insect Forecasting Network – agweather.cals.wisc.edu/vdifn/maps

US Pest DD Map Maker – PVY, base of 39 degrees, single sine model degree days, July 20 2018 example: model suggests 2975 is when things start to happen – model shows on July 20 conditions for things to start to happen is in southern Wisconsin

For seed grower, early gen – use at plant systemic; start oils weekly early; starting early July bump up oils to 2X weekly; weekly oil starting mid-late August; at vine kill, do not oil because green/brown contrasts attract aphids – keep oiling; vast majority of aphid transmission is from non-colonizers, evening, 5-12mph wind speeds, early morning and evening flying so have a good residue at time when aphids are coming through

PVY management summary:

* Weekly paraffinic oil applications in advance of 2975 cumulative DD(base 39) have resulted in lowest overall post-harvest test readings (2009-2018)
* 2X weekly applications further reduced PVY in daughter tubers of highly susceptible dc by 35-60% initiated in advance of 2975 cum DD-39.
  + Suggests that bulk of infection/transmission occurs in late season; additive effects of selective feeding blockers/behavioral modifiers/aphicides variable and inconsistent
* Risk predictions based on upon predominant species underway

Comments: aerial applications in MT and ID may be inadequate; application coverage is critical – low volumes/low pressures/wrong nozzles/poor coverage may not work; should blast the canopy to get good coverage; aerial application does provide that turbulence and is remarkably good in other studies – evaluate efficacy of aerial application in next round of SCRI.

Pyrethroids not recommended. With low vol applications, outer canopy may get good coverage, but maybe increased contrast that attracts aphids as margins of leaves get burned;

**Jim Dwyer (15’) –** Aphid trapping studies in Maine (Alyohkin, Buzza, and M. Dwyer)

Water pans used in the past; suction traps – like Juan Alvarez’s – caught few aphids; Tower traps –sticky traps on all sides at about 6’; scouts – not as informative because can’t find non-colonizing aphids; yellow bowls – don’t work so well; yellow buckets – how much water may influence efficiency; yellow cards in field have a handling problem; green tile pant trap seemed to work very well – may depend on aphid populations; predominant species – black bean, buckthorn, foxglove, green peach, potato, willow; inconsistent species – soybean, Bird cherry oat, English grain, large raspberry; Some seasons few or none individuals of the inconsistent species detected

Timing of flights depends on aphid species

Traps differ in efficiency

Tile trays seem to be good

Tower traps – huge numbers of soybean aphids caught in some traps but not others after a big storm in 2018

Using Growing Degree Days (45 degree base) to predict initial aphid species occurrence

Buckthorn aphid – *Aphis nasturtii* – three years of data – from beginning May 15; can accurately predict when first occurrence will be – possible for predominant species but not at this time for the inconsistent species;

Comment: Russ uses 39 as a base, and Jim uses 45 as a base – because he has data from 1972. – Since Russ uses 39, the GDD number is higher (2975) while Jim’s range somewhere between roughly a couple hundred to over 400; GDD base number just changes the number so be aware

Q: Production practices similar to New Brunswick – are you using the same kind of control? A: Growers don’t want to make single application – they’d rather use tank mixes. This can cause a problem. Most are using oils, some are using pyrethroids based on what Mathuresh Singh is using; using neonicotinoids at planting (except organic growers); “better” seed growers are going for quality rather than yield – his seed ratings were fabulous and he killed early and also went weekly with oil+insecticide; some growers try to get away from oil due to perceived toxicity (may be too high concentration, or applied on stressed potatoes)

Comment: we anticipate a yield drag with oils – plant may put energy into recovering from cuticle degradation from oils; so some growers extend season by say 10 days to make up for perceived yield drag. Focus on Quality vs Quantity.

Q to Nina – What about LifeGard? Actigard? SAR inducers? A from Nina – no additive benefit when applied with oil because most benefit is from the oil.

Comment: Mature plant resistance – some growers think every variety has it. Per Stewart Gray – there is a big difference across cultivars and strain regarding mature plant resistance. Data sets are available from Maine – published percent of lots certified in the two different categories, can see which varieties do well across years, e.g. Kennebec, Katadon,

**Jim Dwyer, Andrei Alyohkin, Aaron Buzza, Marc Dwyer (15’) –** UMaine PVY video

12:05 PM Lunch on your own

1:30 pm PotatoesUSA PRAC update

Ryan Krabill – PotatoesUSA – manages research dept/staff research committee; staff support for group put together past couple years Potato Research Advisory Committee (PRAC) – to help provide info on what industry is looking for to better inform research proposals, national perspective; members from National Potato Council, state managers (one per time zone), research committee; industry review panel met yesterday by phone and the project was scored very favorably so a full proposal invite is probably forthcoming- deadline late April is predicted.

Erik Schroeder – Wisconsin grower – PotatoesUSA Research committee co-chair/”figurehead” of PRAC; met at NPC fly-in at D.C.; list of national priorities – necrotic viruses, and breeding efficiencies; Ryan and Blair work to help contact growers from around the country to get support letters for research proposals; 71 letters for this SCRI project plus quite a few for the diploid breeding project.

Discussion: Russ Groves asked what they see in queue for next year and the next year – are some of these larger CAP projects should we continue to think about re-upping? What’s coming out of science community re next set of submissions? A: Goal of industry in case of the soil health project for example – maybe four years from now we are doing another iteration of it, based on findings of current one. All dependent on success this year; industry may come back next year and ask for a necrotic viruses submission again if it doesn’t get funded this year. General approach: here’s a research topic and how do we use the tools available out there to best execute objectives within that particular research topic – does a piece fall under SCRI, do pieces fall under block grants, AFRI, etc?

Re: regional priorities – have you looked at different states/there’s lots of cross talk, looking at all potential funding sources e.g. APHIS had as much or more money that they’ve allocated to different research programs, State partnership programs, maybe there are some regional projects, there may be 10-007 funds available, etc.

Ryan asks us how industry can be more helpful in helping us secure funding. Paul Bethke commented we could use help getting real numbers of what a problem is costing the industry; we struggle as researchers because we don’t necessarily have access to these numbers. A: When we start talking dollars and sense, it can shut down a group of growers rather quickly because it is hard for them to talk about what is having a negative impact on their bottom line. Maybe we can weave our way through that, navigate it in a way that can elicit that information. Ryan invites us to reach out to him if we have any questions: [ryan@potatoesusa.com](mailto:ryan@potatoesusa.com)

Ken – wasn’t there talk about developing a white paper that would help identify priorities…(?) Ryan – there is a paper that broadly outlines some of the things, were going to partner with PAA – Ryan will look back at minutes. Stewart Gray recalls the meeting indicating that PAA and PRAC need to join to bring science and industry together; all ideas that we brought forth there needs to be some structure in how we attack issue of bringing industry and research together. Current format? Variation of the current format? Something else? Russ commented – likes idea of consolidating into one place; a lot of the funded problems are immediate probs now; other potential probs industry may not want to talk about now, but that may have future implications. Would these kinds of coordinated investigations be supported? E.g. Water quality issue; water shortages; etc. Trying to navigate short term, medium term, and long term “horizon” issues can be challenging. Russ Groves: As a group, scientists are looking forward, for example trying to define the concept of “sustainability.” May be “horizon” but “it is right under our feet.” Amy C: maybe a planning grant will help put together an acceptable iteration. NSF has very large grants to do environmental monitoring – nationally placed sensors etc. RG: could be recognized as emerging issues. Ryan: to topic of sustainability, it means something different to commercial growers vs. researchers; commercial growers have been trying to define it, but is likely going to be driven by consumer. RG: but industry could potentially have a say in how it is defined – can make operational definitions. Rich Novy: maybe don’t get feedback of economics/cost during meetings, but a white paper identifying industry priorities published by PotatoesUSA could be helpful in us garnering funds b/c we can cite the paper in our proposals – provides justification. Nora: NPC put together a list of priorities that is published and can be utilized by us in our proposals. RK: That is the principal meeting where people come together to talk about what those priorities are; still wrapping hands around maybe we need regional priorities and then deciding which one(s) take precedence; lots of differing opinions on the subject.

Updates on matching dollars: Potato industry at an advantage; update – it is happening. $25 million specific to SCRI program available but also have matching requirement piece – industry was blindsided by that match requirement. RK doesn’t think it is going to be impossible (e.g. per Amy C., maybe we can use fees associated with seed certification being counted as some of those matching funds); doesn’t have to be a cash match; track our in-kind contributions better to help meet the match requirement. Industry will figure out a way to get the match.

**SCRI Future Project:**

*Discussion/Presentation leaders:* *Alex Karasev, Amy Charkowski*

SCRI project plan for new grant 2019-2024

Co-Directors: Alex Karasev, Amy Charkowski, Walter DeJong, Ken Frost, Guiping Yan

Comes on heals of the one that is finishing up; new iteration

4 objectives examined individually trying to get ideas and engage those who want to be involved:

Development of sustainable system based management strategies for vector-borne, tuber necrotic viruses in potato

1. What was accomplished in the current grant (if not part of it, what related work was done previously)
2. What specifically is proposed to do in the renewal
3. Who do you plan to collaborate with on project for your specific work plan

**Objective 1**: To improve high throughput detection of PVY, PMTV, TRV and their vectors in potato tubers and soil and to train seed certification agencies in these methods

Max – hyperspectral imaging for tubers/plants; TRV infested field in Prosser/working with growers in Columbia basin; approach: imaging of entire tubers, sections of tubers, and entire plants; best combination of technologies to diagnose diseases non-invasively; Hanu generated some preliminary info on imaging on whole tubers and found decent correlation; machine learning tools to improve what can be found; Stewart: all data generated looking at correlation between visible vs. non-visual symptoms that can be correlated with infection. Formulate what you are using it for, and is this tech high-throughput enough to allow thousands and thousands of tubers to pass through and pick out infected tubers. Nina – speculates it wouldn’t be sensitive enough; FTA cards may be a robust technique for that, with higher level of sensitivity. Nina – needs for certification: 95%+ for decertification is PVY so immediate need is high-throughput testing for PVY, should survey to see how big problem is and where problems are; Stewart Gray – currently seed certification does not test for PMTV or TRV. Alex – in objective 1 we need to continue surveying for mop top and rattle; Stewart Gray says if we say we are going to survey, we will not be funded. Any survey objective cannot be explicit. Russ Groves – on discovery side, remote sensing can detect asymptomatic/pre-symptomatic; Phill Townsend shoots light into leaves and measures spectral reflectance, could be done on a tuber. Amy C: Near/medium/long term; PCR isn’t even used everywhere in seed certification; think about balance between immediate outcome and fundamental questions. Russ: we should continue the work in developing high throughput detection/why not dove tail with any kind of proximal remote sensing approach.

Vectors: Guiping: Re TRV quantifying nematodes, does nematode carry virus or not? May be can apply method to disease risk assessment. Alex: what about mop top detection in the virus – Amy C: Method learned from Neil re Ss testing, challenge is interpreting results – can tell growers what they’ve got but we don’t have a threshold for Ss in soil – ties into resource management as we frame this problem. How to use limited resources (farmer’s budget) to get biggest bang.

Stewart: We need an overarching goal of each objective rather than get lost in minutiae. Frame it in the sense that we have ten-thousands of seed lots that need to be tested, if we can fly a drone over a field and see 0 disease issues, we could choose not to put any more resources into that seed lot for testing; instead put resources into seed lots with detectable disease – spend resources wisely. Same thing for soil sampling – can determine whether we have nematode, Ss, or PMTV in Ss – but can we use this remote sensing tool as a resource management tool.

Amy – lesson from blackleg project – enough diffs in lab’s abilities to detect Dickeya

Baseline requirements – same language, same protocols, same methods, ring tests, etc, must be understandable for everyone.

**Objective 2.** To improve virus-vector management through development of epidemiological models and through research-based recommendations for potato production.

Brian – interested with respect to powdery scab, vector is problem regardless of PMTV. E.g. in southern OR, he planted the worst powdery scab seed and rarely sees scabby tuber at research station; this year, exploded in a field with no known history of potato.

Silvia – crop rotations; does using Sudan grass around potato fields really reduces number of aphids moving into crop? Nina – in terms of border crops – used quite a bit in Montana, including Payette russet – advantages of using potato next to potato. Would like to see potato borders incorporated into a research project to see if they are useful. Stewart – with respect to PMTV and TRV, use golden nematode in New York as a model, relies on the use of R and S cultivars of potato, where R potatoes aren’t necessarily desired as a food but would be part of a management strategy – use those varieties as a management tool, whether as border crop (for PVY) or trap crop (for PMTV/TRV).

Alex – piggybacking on that idea – determining whether Castle russet can convert stubby root nematode to non-virulent. RCBD with Burbank and castle russet to monitor viruliferous nature of nematode in stubby root nematode.

Stewart – bring it as a total strategy, not “this lab will do this, that lab will do that”;

Sagar - how can using border crops influence seed quality

Guiping – new cover crops, mustards, mixtures, etc for nematode control

Jonathan – mineral oils in west, we don’t know how good they work under irrigation; tools to trace virus, as well as aerial application.

Russ: combination of inputs that lessen disease is great, but if resources accessible to you, in cooperation with growers, can use post-hoc data – ask questions like why does field A continue to have issues but field B does not; if producers share info, we might be able to parse out what practices work.

Alex: Suppression of vectors – primarily soilborne – nematode, Ss; Amy and Neil are the ones looking at this and will probably continue to do so in second iteration.

**Objective 3.** To develop molecular markers diagnostics for resistance genes against PVY, PMTV, TRV, and Ss; to clone at least one PVY resistance gene: and to use proteomic and metabolomics studies to understand virus impacts on physiology of stored potatoes – Walter DeJong will take lead

Sagar – genome sequencing/developing more markers and to clone gene for PVY resistance within four years. Castle has same Rysto as Payette.

Primary need – screen for resistance to vector; Andy says Kewamu Tinaka at WSU is already doing this – lab based assay – talk to him to see if it fits. Neil Gudmestad/ND field provides good disease pressure and may be useful for screening for resistance. Alex – in current grant, we did have special area focused on testing different cv for sensitivity to tuber symptoms. Is this finished or do we expect to continue this? Stewart – doesn’t think proposing to continue it won’t go far; do exactly as said earlier – need reliable phenotyping system – person at WSU for Ss may be useful; Amy C has also presented some GH data; look at nematode resistance/TRV transmission by nematode, phenotype populations provided by breeders to enable marker identification – cannot be field-based; reliable phenotyping system and existing populations, can still take a period of time – does it fit within time frame. Walter – we already have a good idea of TRV and PMTV resistance genes. Max – a number of lines with resistance but we don’t know how many different mechanisms they originate from; cross known R with susceptible varieties and identify the number of independent mechanisms; Rich – since Amy has reliable screening tool, then we can proceed; the bottleneck has been a lack of reliable screening. Amy – connect with Tinaka; field is too unreliable. GH is very easy to get symptoms. Rich – even if we can adopt that at Aberdeen, would be good. Amy C. – just keep GH under 65F and soil too wet. Sagar – good pipeline in terms of getting markers with Castle, within next year or two should have decent markers from Castle russet for TRV and PMTV.

Alex – sounds like there is a lot of collaboration among groups so may be easy for us to reach this objective

**Objective 4.** How to identify economic or incentive barriers to effective disease management and to use this info to aid industry in adoption of improved management strategies and harmonized regulations

Chris McIntosh – will take the lead (?)

Alex to Nina – how to adopt all recommendations that we may generate – new detection methods, how to change certification rules, how to include requirement for soil vectors/PMTV/TRV. Nina – getting everybody work together – the same methods. Using the same methods/harmonizing will improve communication between agencies. Nina – basic objective: the exercise we did that year where every sample collected by MT was also tested by her lab and by Alex’s lab – a huge ring test. Russ – when we talked about this several months ago, social component – may need a sociologist; at UW we have life science communications people whose specialization is to characterize impediments to adopting new tech; a slide he didn’t show that Ken Frost made, out of WI seed certification program data, they looked at grower as a source of variation (coded farms) – there is a grower component to a lot of the problems; a retrospective look at databases will identify successful vs. non successful to help ferret out differences. Ken Frost – rotation strategies etc but how about looking at good vs bad growers to see what practices work. Chris McIntosh – soil health grant: choice games for growers to look at various barriers to adoption; could be fairly straightforward to set up for this; to assess attitudes/likelihood to adopt a tech; Amy C: cost, how people make decisions, the incentive piece/big picture is a huge problem too – by the way certification is set up, you get punished for taking risks, and no reward for succeeding at making positive change. Who studies incentive/change? Chris M: sociologists or economists.

Stewart – one thing to look at may be situation with citrus greening in FL - $350million spent on trying to solve it but haven’t yet solved it but there is that sociologist component – we can look at that and pull out some info from that and propose to do some of those things. Russ – this is in the wheelhouse of expertise of people who do just this type of work so why not bring on board one or two people with this specific skill who can bring this scholarship to this proposal.

Stewart - Nina brought up a good point. As an administrator rather than a scientist, he looks at this at a hundred thousand foot level – really important only have 6 weeks to pull this together and only a few people involved in pulling this together – it is really helpful to get people to write the specifics of what they are planning to do. Five years ago, about a dozen people submitted and it had to be distilled from over 100+ pages to the proposal. Ballooned to over 30 PI’s – so they said just give us a budget and the whole thing got budgeted. After it was funded, then many of the PIs provided what they were going to do that wasn’t even part of the proposal. If you get accepted into project, you get accepted for doing a specific function; that function may change within 5 years, but you are going to get paid to perform a task. Do the task that you are assigned otherwise it is a real nightmare for those managing the project. Need to stay on task. Reporting is getting much more serious. Year by year. Match requirement/more paper work; Just because you get on the project in Year 1 doesn’t mean you’ll be on it in Year 2, 3, 4, or 5. Managers need to keep track of where money goes, and they need to communicate with Andy Jensen/ Ryan Krabill/etc/industry to continue to assess needs. Hard job. Show 1. You can do it. 2. That it’s important to the industry.

Other funding opportunities

2:30 pm Break

3:00 pm **Disease Management Recommendations:** Aphid management, vector-borne disease management, soil-borne disease management, disease management in storage. *Discussion/Presentation leaders:* Russ Groves

Please send your edits to the Document that Russ Groves sent out by email before the meeting.

Go to site to see Virus management plan at website: <https://blogs.cornell.edu/potatovirus/>

Mary K has asked us to make comments and send back so Russ can assimilate them into a working

draft so that updates can be provided. Would benefit by having much more detail that goes along with this. At website go under each of the viruses – quick facts, biology of pathogen, descriptions, management. Scroll through webpage and see how it is structured. It can be viewed as an executive summary. Amy C. suggested to ask someone like a graphic artist and consider making slide sets.

Ultimately this can be a publication that each University can put on as their own Extension publication.

Please take a look at the document and go through it with edits; responses will be collected and assimilated. Make sure that it is relevant for you as you understand the content/info that is being provided.

Stewart – Mary has done the bulk of the work of putting this website together, so when you see her, send her a note saying how much you appreciate it. Mary did not write the stuff – all content was written by many people who were experts on the different viruses so don’t feel slighted if your particular edit was not included in final draft because with so many of us providing edits, it is not likely every single change will be incorporated. Goal: Dynamic document that will change; can be added to it if you have data sets that you want to put out there; for example, cv reactions to the different viruses; if published in journals can be referenced; etc; can provide links to other websites, videos, etc; want it to be a comprehensive site that anyone can go to in a language that the lay person can understand; provide pictures that can be added to the gallery; etc.

Russ will send us the “Track Changes” version so we can see edits.

4:00 pm **SCRI current project:**

*Discussion/Presentation leader: Stewart Gray, Amy Charkowski*

Objectives for 2019

Final reporting

All money for current SCRI must be spent by August 30, 2019 – no exceptions, drop dead deadline

Final report due 90 days after this grant ends; Stewart will retire before that so he asks for your final report by end of September – 30 days

Show that you did a lot of “stuff” and have really helped the industry. Website is the public face – so if you want to add stuff, that’s great, but number of publications matter – even if not quite published yet, please give comprehensive list, including manuscripts in preparation.

For next round: Provide explicit details in what you are planning to do or in what you would like to do. Preproposal has been reviewed by the industry (growers, not necessarily potato growers) and they have decided to invite it for submission as a full proposal. The full proposal is evaluated by a scientific panel – they are looking for science, rated based on science so PLEASE when writing up your part make sure your science is sound. Indicate why what you are doing is relevant, better, why it will help industry and science. If the scientists don’t like the science, it will not be funded.

5:00 pm Adjourn

Respectfully Submitted,

Kasia M. Duellman