

# Integrating Next Generation Technologies for Blackleg and Soft Rot Management in the USA

## A Progress Report

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EAPR Pathology and Pests Symposium

3 September 2019



# History

- In 2017 the USDA National Institute of Food and Agriculture (NIFA) awarded a \$2.5 million Specialty Crop Research Initiative grant for management of soft rot and blackleg of potato
  - Main driver was a widespread outbreak of *Dickeya dianthicola* causing widespread field losses of table and processing potatoes in the eastern US
  - Widely supported by growers, industry and other stakeholders
  - Four year funding, year three starts in October
  - Main objectives
    - Improved detection and identification using molecular tech
    - Epidemiology and spread
    - Host resistance using 'omics and screening
    - Management
    - Economic impact

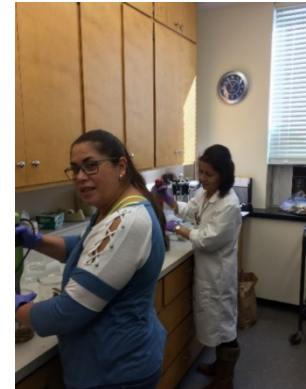


# Participants

- PI's Amy Charkowski CO, Gary Secor ND, Jay Hao ME, Chris McIntosh ID
- 10 additional scientists: Adam Heuberger CO, Bryan Swingle Cornell NY, Kasia Duellman ID, Kate Fuller MT, Keith Perry NY, Ken Frost OR, Melanie Filiatrault NY, Nicole Perna WI, Noah Rosenzweig MI, Steve Johnson ME
- 11 advisory board members from farms, commodity groups and industry: Andy Jensen NW Potato Research Consortium, Alan Westra ID Crop Improvement, Gerhard Bester Pepsico, Don Sklarkczyk Seed Farm, Don Flannery Maine Potatoes, John Nordgaard Black Gold Farms, Milt Carter CSS Farms, Pat Kole ID Potato Commission, Bob Leiby PA Potato Coop, Ron Kreuger WI, Ryan Krabill Potatoes USA

# Seed lot testing

- A post-harvest test is necessary to detect *Dickeya* in seed
- The potato scientific community has developed and validated a protocol to detect latent *Dickeya* in seed
- We recommend
  - PCR test
  - 400 tubers/seed lot
  - 25 stem end cores/sample (25 X 16 = 400)
  - Plus 25 peel samples/sample
  - Peels from stem end 1 to 2 inches long N to S
  - pelADE or Df/Dr primers for *Dickeya*
    - Sequencing of PCR product, MLSA, RT PCR to identify species
- Charkowski reference isolates
  - Multi-lab testing of DNA 98.6% accuracy
- Limited results to predict field disease based on seed lot testing results





APS Publications

*Dickeya* is highly variable and published primers do not detect all *D. dianthicola* strains.

This primer design program was tested to determine if it could quickly additional *D. dianthicola* primers.

Two additional primer sets were developed and validated.

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# Development of automated primer design workflow Uniqprimer and diagnostic primers for the broad host range plant pathogen *Dickeya dianthicola*.

Shaista Karim, R. Ryan McNally, Afnan Shazwan Nasaruddin, Alexis DeReeper, Ramil Mauleon, Amy O Charkowski, Jan E. Leach, Asa Ben-hur, and Lindsay R Triplett [✉](#)

Published Online: 15 May 2019 | <https://doi.org/10.1094/PDIS-10-18-1819-RE>

*D. dianthicola* identified in seed potatoes sent from Wisconsin to Texas differed from strains in Maine.

This strain lacks the DiaA primer site, so escapes detection if this PCR assay is used.

Genome sequences show that this strain is otherwise nearly identical to the *D. dianthicola* type strain.


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DISEASE NOTES



# First Report of *Dickeya dianthicola* Causing Blackleg on Potato in Texas

A. S. Nasaruddin , A. O. Charkowski, B. N. Babler, N. T. Perna, and J. D. Glasner

Affiliations ▾

Published Online: 13 Jun 2019 | <https://doi.org/10.1094/PDIS-01-19-0024-PDN>

# Practical seed testing results

- In cooperation with a large farming operation, our lab tested 880 samples from 55 seed lots from 20 seed farms for *Dickeya* in spring 2019
  - Cores and peels from 400 tubers divided into 16 reps of 25 tubers each/seed lot
- 98/880 (11.2%) of the samples tested positive for *Dickeya*
- Range 0-100% (0/16 – 16/16)
- 12/25 lots free of *Dickeya*
- In the field, the 100% sample has less than a 50% stand
  
- Anecdotal grower observations
- In general, any sample that has a test of 3/16 positive is considered a problem
- Seed from two states have the highest incidence, one state clean
- Positive samples seem to be more related to farm source than variety
- There is no variety specificity
- This is a very significant increase from 2018

# Seed tuber inoculation trial

Tubers vacuum infiltrated or stem end stab inoculated with *Dickeya*

Significant stand reduction in Florida due to *Dickeya* but not in ND

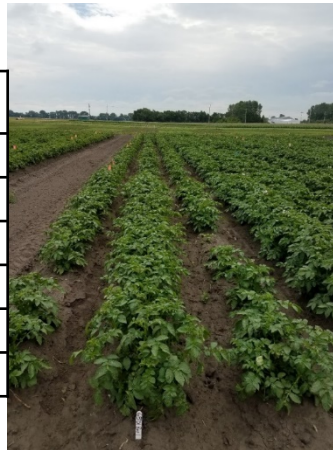


	Stand	
	FL	ND
Not inoculated	23.3 A	22.8
Infiltrate <i>Dickeya</i>	10.3 C	22.5
Infiltrate water	22.3 A	22.8
Stab <i>Dickeya</i>	13.8 B	22.5
Stab water	23.8 C	22.3
LSD $p=0.05$	2.39	NS



**But** .....in ND, *Dickeya* inoculation resulted in significant reduction in plant height up to 60 DAP. Not observed in FL

Treatment	45 DAP	51 DAP	60 DAP	65 DAP
Not inoculated	60.3 A	69.0 A	71.9 A	66.6
Infiltrate <i>Dickeya</i>	28.6 D	45.7 D	60.7 B	64.0
Infiltrate water	52.2 B	65.9 B	72.8 A	64.0
Stab <i>Dickeya</i>	38.4 C	56.3 C	64.7 B	63.8
Stab water	55.3 A	72.3 A	72.3 A	66.0
LSD $p=0.05$	5.38	4.50	5.15	ns



Sub-lethal infection by *Dickeya*??



# Tipping point trial

- Graduate student project of Kal Larson to study sub-lethal infections by *Dickeya*
- Tipping point: the concentration of *Dickeya* that changes from sub-lethal non-symptom expression to expression (stand loss)
- 2019 two replicated trials in FL and MD 2019
  - Inoculated with bacteria concentrations of  $10^0, 10^4, 10^5, 10^6, 10^7, 10^9$
  - Stand and blackleg
  - Plant height (3X)
  - Yield, grade, gravity
  - Progeny tuber testing for latent *Dickeya* infection
- Results
- Emergence NS both location
- Plant height NS both locations
- Tuber *Dickeya* by PCR. Only Significant > @  $10^9$  MD
- Stem *Dickeya*. Only significant > @  $10^5$  FL
- Yield. NS MD. Significant > @  $10^0$
- Grade. Only Significant > of 1.5-1.875 oz @  $10^9$
- It appears Concentration of *Dickeya* in seed tubers has minimal impact on growth – minor sub-lethal infection



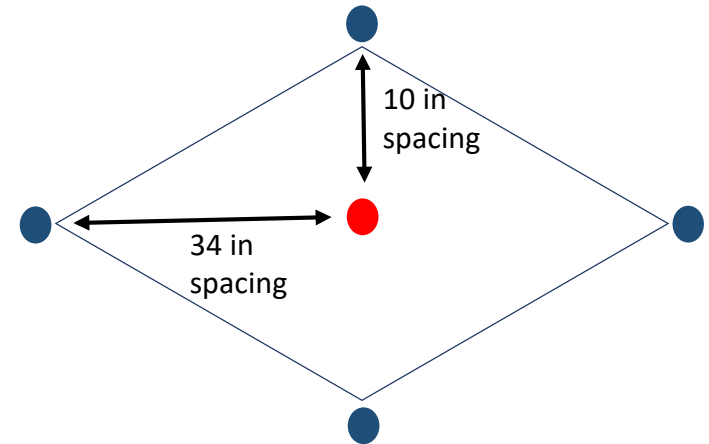
# Is *Dickeya* spread by seed cutting?

- 2017 Field trials by Steven B. Johnson, UME, showed that *Dickeya* is not readily transmitted during seed potato handling and cutting operations
- NDSU trials: *Dickeya* inoculated seed mixed with healthy seed, handled, cut and planted separately
  - 2017 Plot trial in Florida
    - No stand loss, no spread to healthy seed
  - 2018 Commercial trial in Florida
    - 200 pounds seed inoculated with *D. dianthicola* mixed with 2500 pounds healthy seed
    - No stand loss in healthy seed
    - > 90% stand loss in the inoculated seed
  - 2019 Replicated trials in FL and MD
    - *Dickeya* inoculated seed mixed with healthy seed for handling and cutting
    - No blackleg/seed decay observed either site
    - No *Dickeya* found by PCR testing of cores and peels from 240 progeny tubers (5 tubers/trt/rep) in MD
    - No difference in yield or grade



# In-field spread

- Field trials in Live Oak, FL and Oakes, ND in 2017 for in-field spread
  - Replicated, both sites irrigated
- Seed inoculated with *Dickeya* by vacuum infiltration ● surrounded by not-inoculated seed ●
- No stand loss or blackleg in-season
- Four progeny tubers collected from each surrounding plant tested post harvest by PCR for *Dickeya*
- Infection of progeny tubers was 33% in FL and 13% in ND
  - Netherlands spread of *Dickeya* to progeny tubers 27% (van der Wolf et al. 2017)
- *Dickeya* infected seed from FL and ND replanted in FL in 2018
  - 94% stand of FL tubers
  - 99% stand of ND tubers
- *Dickeya* can infect progeny tubers thus causing latent infection in both lenticels and stem-end core tissue



# Pathogen Diagnostics and Population Structure

- **Swingle, NY.** We provide molecular diagnostics services for New York State growers.
- We test pathogenesis of strains isolated from plant samples with blackleg symptoms and determine species using molecular methods and whole genome sequencing.
- **Results.** In 2016 and 2017 growing season we found blackleg disease was caused by *Dickeya dianthicola* and *Pectobacterium* spp. in roughly equal numbers
- **Results.** *Dickeya dianthicola* strains have very little genetic diversity – consistent with a recent introduction. *Pectobacterium* spp. Isolates are much more diverse – consistent with long term/endemic population
  
- Ma, X. et al. 2018. *Pectobacterium* and *Dickeya* responsible for potato blackleg disease in New York State in 2016. Plant Disease 102(9):1834-1840
- Ma, X et al. 2019. Using whole genome sequencing for pathogen identification: *Candidatus Pectobacterium maceratum*, a potato blackleg pathogen in New York State. APS Annual Meeting
  
- **Frost OR.** We isolate bacteria and extract DNA from diseased plants with symptoms of blackleg, soft rot, aerial stem rot, or lenticel rot and determine species using molecular methods.
- **Results.** In the PNW we have found *Pectobacterium carotovorum* subsp. *carotovorum* (19%), *P. carotovorum* subsp. *atrosepticum* (77%), *P. parmentieri* (1%; Not previously reported in Oregon), and *Dickeya* spp. (3%)
  
- **Hao. ME.** US population is different from European populations, and the southern states do not have the exact population as Maine

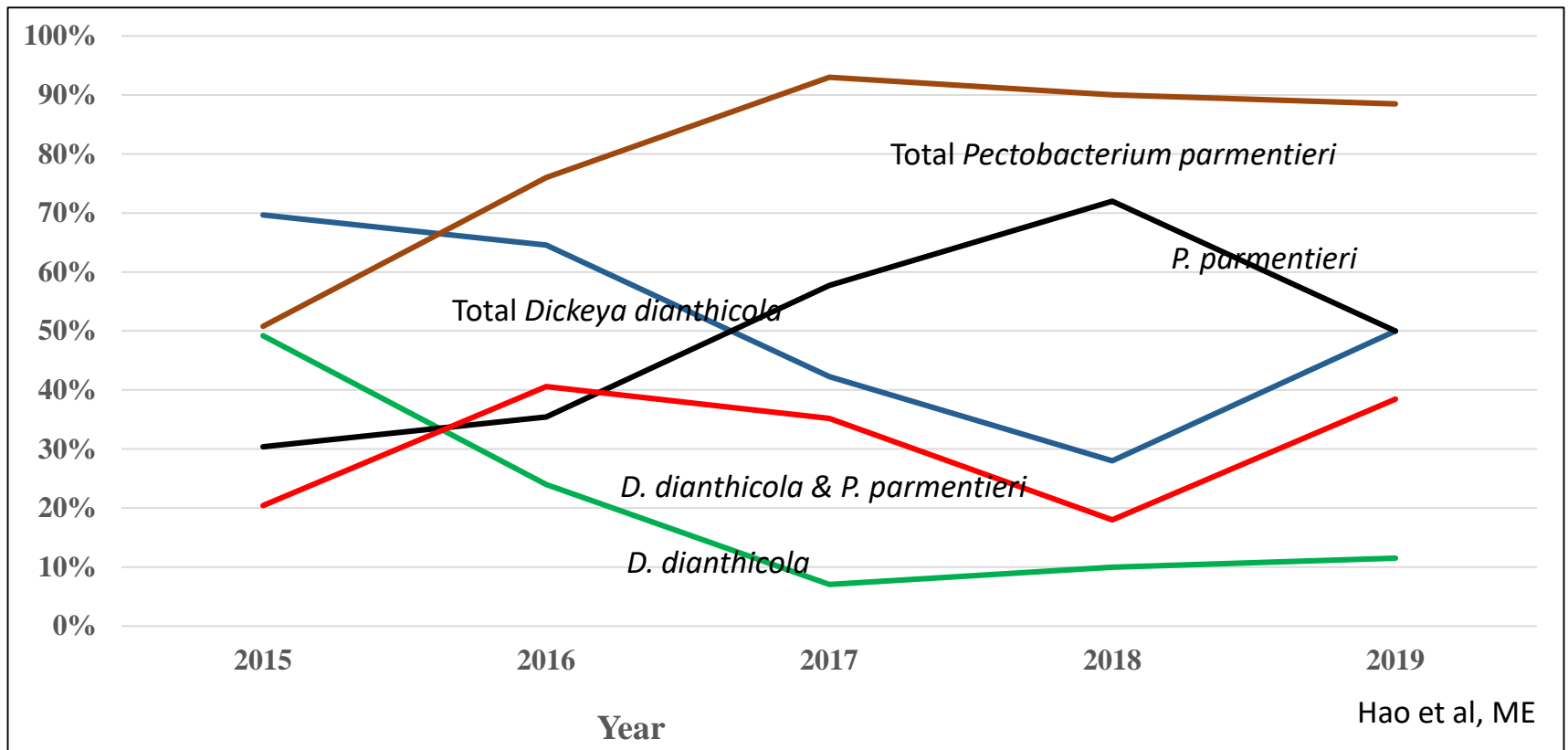
Genome Sequences

# Complete Genome Sequence of *Dickeya dianthicola* ME23, a Pathogen Causing Blackleg and Soft Rot Diseases of Potato

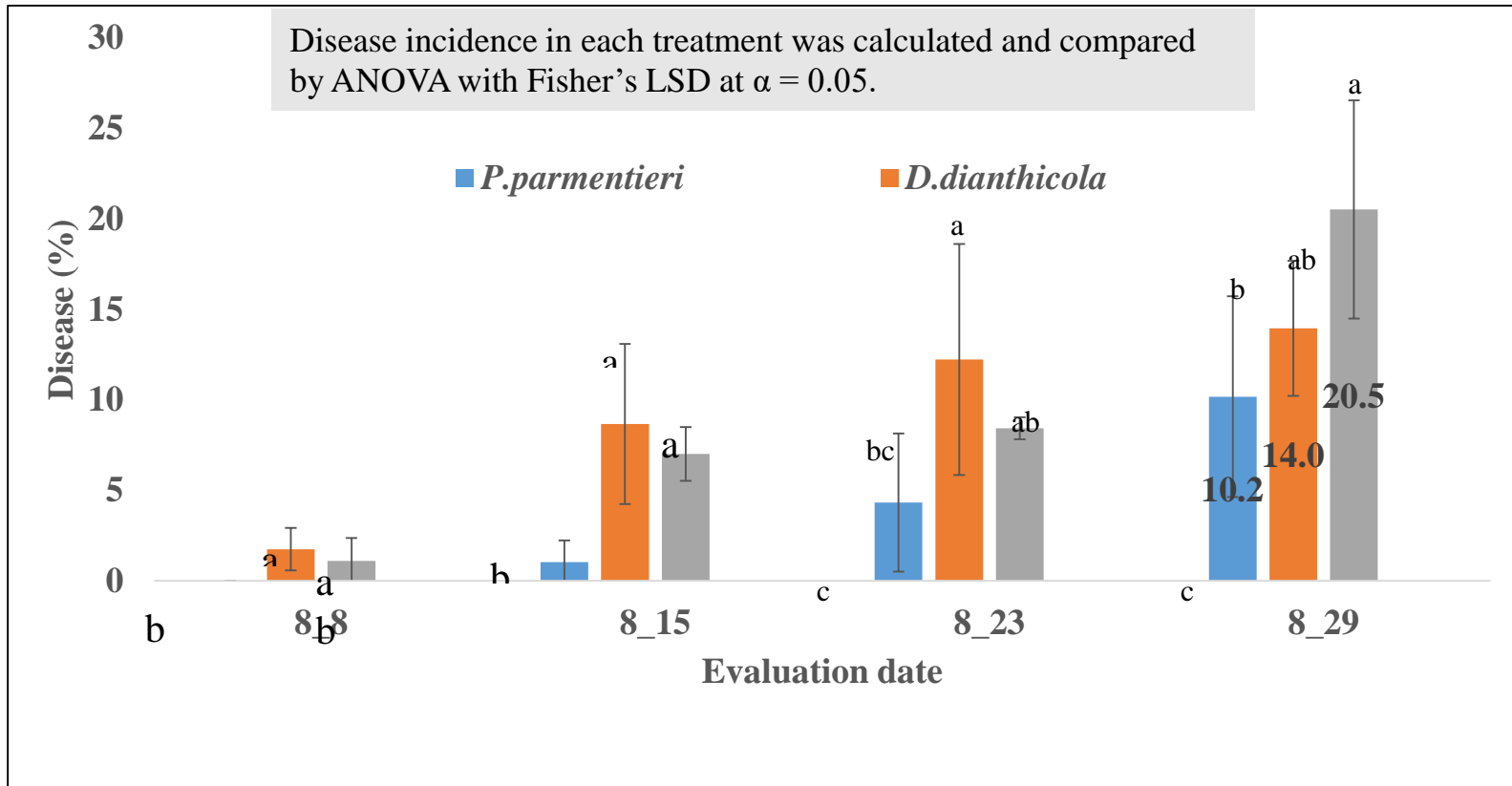
Xing Ma, Nicole T. Perna, Jeremy D. Glasner, Jianjun Hao, Steven Johnson, Afnan Shazwan Nasaruddin, Amy O. Charkowski, Shan Wu, Zhangjun Fei, Keith L. Perry, Paul Stodghill, Bryan Swingle

*D. dianthicola* genome sequence from an 2015 isolate from Maine.

## Dynamics of pathogens in potato blackleg samples over 5 years in Northeastern region



## Disease incidence of variety 'Lamoka' in 2018 Field trial. J Hao, UME

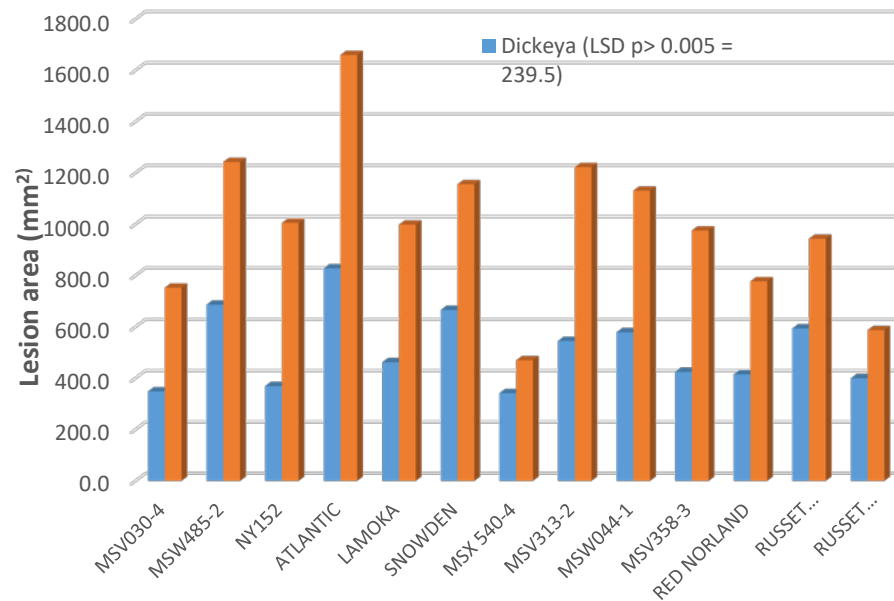


# Screening potato germplasm for resistance

- Secor, NDSU. Screen tubers of processing varieties and Potatoes USA chip selections for *Dickeya* and *Pectobacterium* susceptibility using tuber pucks – limited resistance



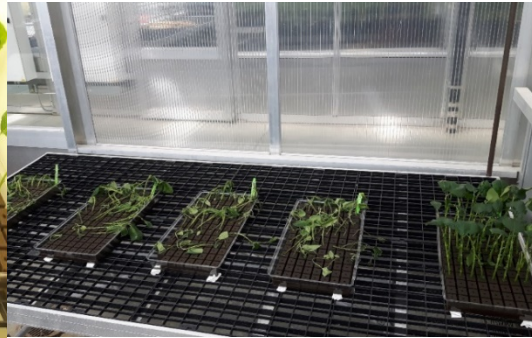
Tuber susceptibility of Potatoes USA selections to soft rot caused by *Dickeya dianthicola* (*Dickeya*) and *Pectobacterium carotovora* (*Pcc*) in 2018





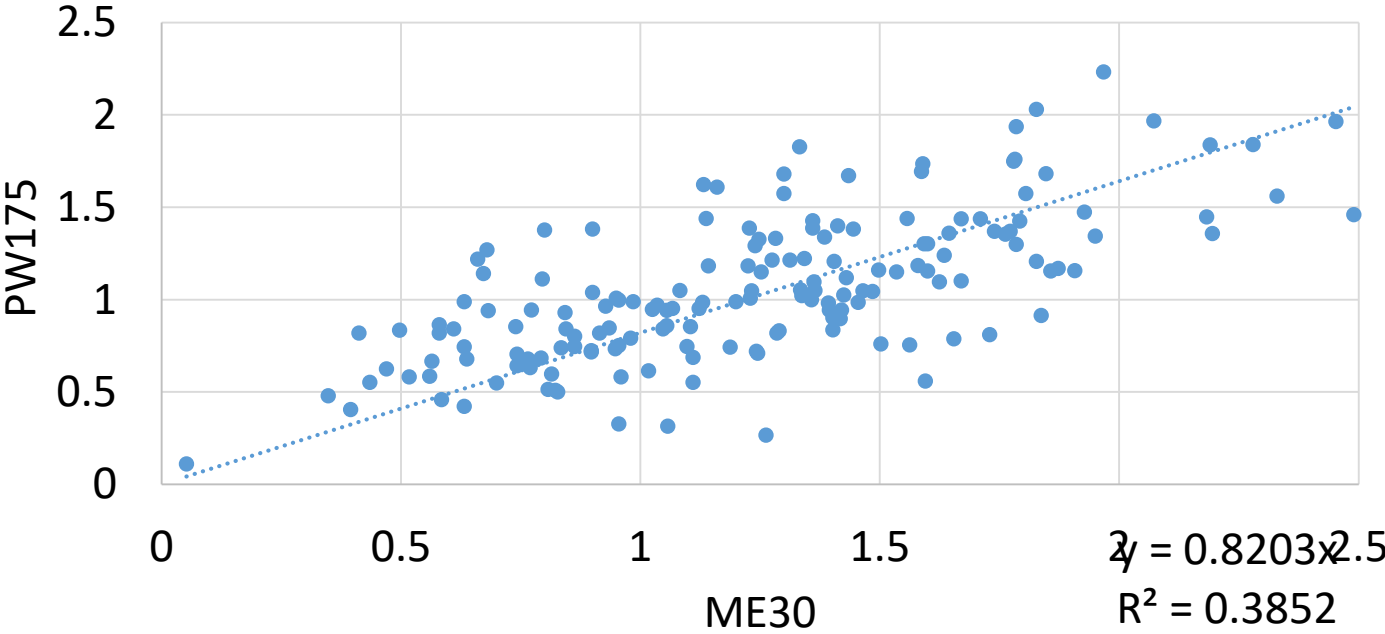
# Resistance Screening

- Edoardo Poletti is a graduate student of Susie Thompson (Secor co-advise)
- Screen NDSU breeding material for resistance to *Dickeya* using petioles and tuber pucks



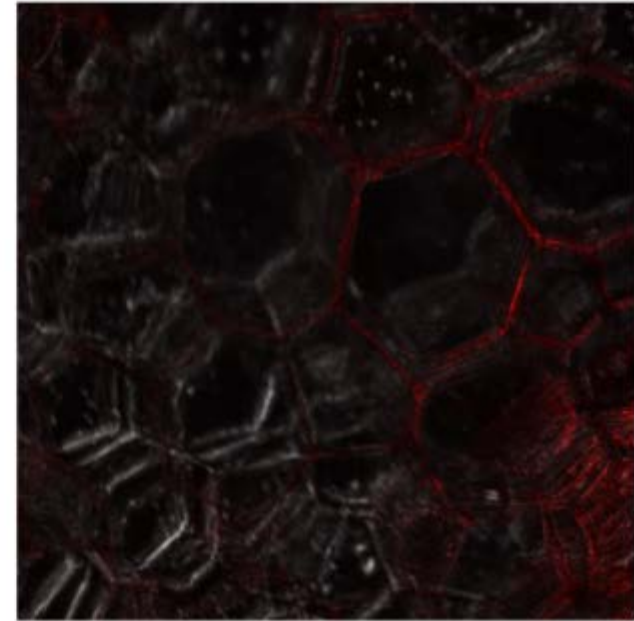
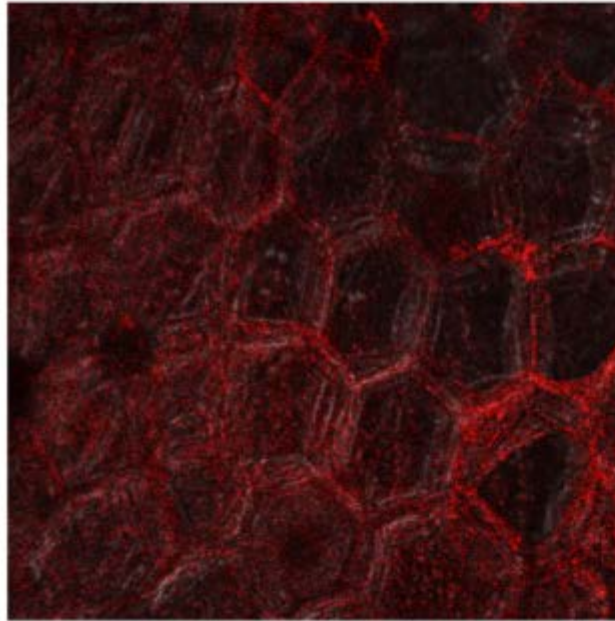
- **Bryan Swingle, Cornell NY.** In collaboration with U.S. Potato Genebank we screened wild potato species for high-level soft rot resistance.
- **Result** – We identified *Solanum microdontum* lines with high-level soft rot resistance.
- We are preparing to screen F1 hybrids of elite *S. microdontum* lines crossed with diploid *Solanum tuberosum*.

Responses of 204 potato clones to *Dickeya dianthicola* ME30 and *Pectobacterium parmentieri* PW175



One protein and two types of small molecules identified from resistant wild potatoes that appear to inhibit virulence.

These may be useful as markers for breeding for resistant potato varieties



Resistant potato cutting rooting in bacterial suspension  
mCherry-labeled *Dickeya* in resistant and susceptible plants

# Understanding the defense response of potato plants to *Dickeya* sp.

- Melanie Filiatrault Lab – USDA and Cornell University
- Graduate student project Yingyu Liu
- Global transcriptomic analysis to understand the molecular interactions between *Dickeya* and susceptible or tolerant diploid potatoes (from Amy Charkowski)
- Data mining revealed several enriched terms and gene expression patterns when comparing the expression profiles between tolerant and susceptible potatoes, indicating possible roles in disease resistance
- One class of genes represent promising candidates that encode for putative, previously uncharacterized, antimicrobial peptides (AMPs) are hypothetically involved in resistance to soft rot pathogens
- Also identified other genes of interest for breeding for blackleg tolerance in potatoes because their homologs are involved in resistance to other pathogens in other plant hosts
- Our study of potato global gene expression patterns provides critical information to facilitate the development of novel disease management strategies and accelerate disease-resistance potato breeding processes
- Poster Presentation: Liu, et. al. Searching for host resistance: studying early potato stem response to *Dickeya* inoculation via RNA-seq. 2019. IS-Molecular Plant Microbe Interactions Conference. Glasgow, Scotland. (abstract)

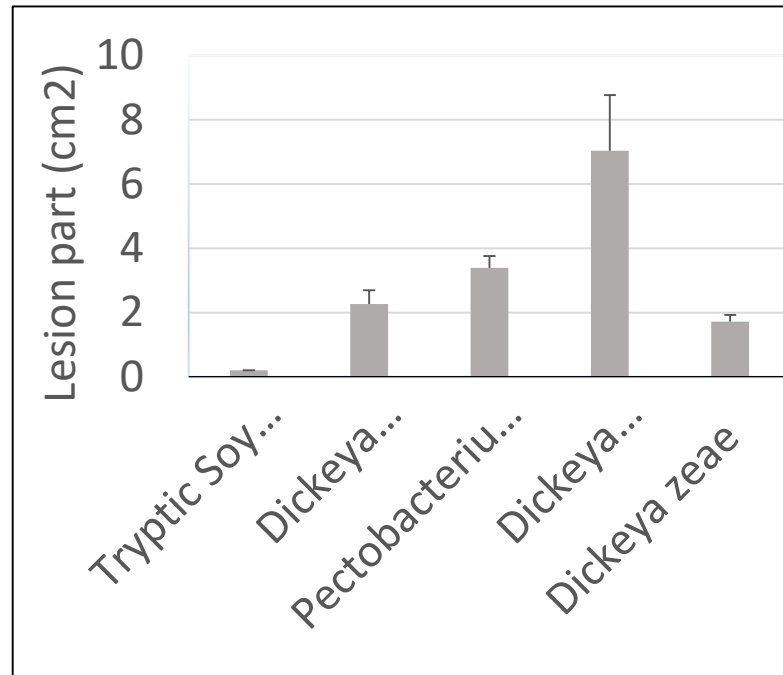
# Potential sources of Dickeya

- Interesting story
- Ordered 20 Dahlia bulbs from a well known European mail order source
- 4/20 tested positive for Dickeya
- Culture and sequence shows *D. chrysanthemi*
- Pathogenic to potato
- Also reported as a source of Dickeya in Australia, South Africa and NY (Bryan Swingle)



# Surface Water

*D. aquatica* (most frequent), *D. zeae*, and *D. dianthicola* found and all are pathogenic to potato



# Economic Impact of Dickeya

- 2019 Replicated field trials conducted in ME, MI, OR, ND
- Dickeya infected seed mixed and planted with non-infected seed at different ratios
  - 0, 5, 10, 15, 20, 25 %
- Data
  - Stand
  - Blackleg
  - Yield and grade
  - Economic analysis (Chris McIntosh and Kate Fuller)



# Communication of soft rot/blackleg/Dickeya management progress to industry

- Advisory Board
- Investigators and Board meet annually
  - 2019 meeting be open to the public
  - 7-8 November Intercontinental Hotel MSP Airport
- Very important to keep our industry informed of MANAGEMENT
  
- Grower seminars
- Michigan State uTube

Links to videos are here:

<http://potatobacteria.org/wordpress/faqs-and-extension-bulletins/>



# End Thoughts

- *Dickeya* most frequently found in eastern half of the US
- We do have a good standard assay for detecting *Dickeya* in seed lots
  - Stem end cores + peel + PCR
- *Dickeya* is not readily spread by seed handling and cutting
- *Dickeya* can spread in the field to adjacent tubers FL trial 33%
- *Dickeya* infection may remain latent in seed potato tubers and not result in disease expression after planting
  - Sub-lethal infections
- Several labs are screening germplasm for resistance to *Dickeya* using various methods
- There may be several sources of *Dickeya* - ornamentals, flower bulbs, weeds, water – that need to be identified
- Still wondering where initial infection comes from
- *Pectobacterium* populations are changing