

Verticillium wilt of potato caused by *Verticillium dahliae*: a soilborne pathogen that hides as an endophyte.

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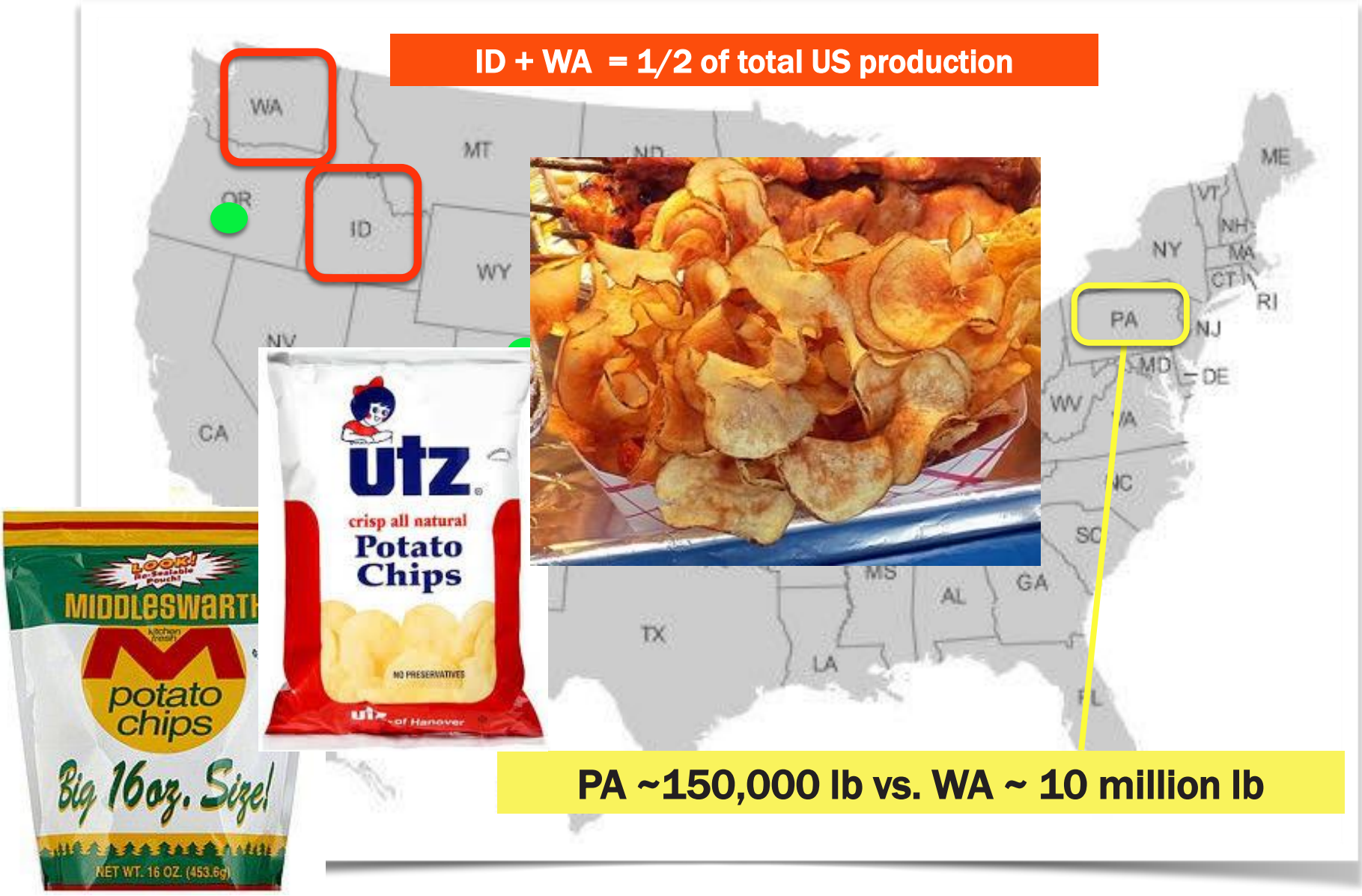
 @laura_jalon

USDA-ARS Beltsville Agricultural Research Center, Beltsville, MD. June 6, 2019.



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Potato production: U.S.

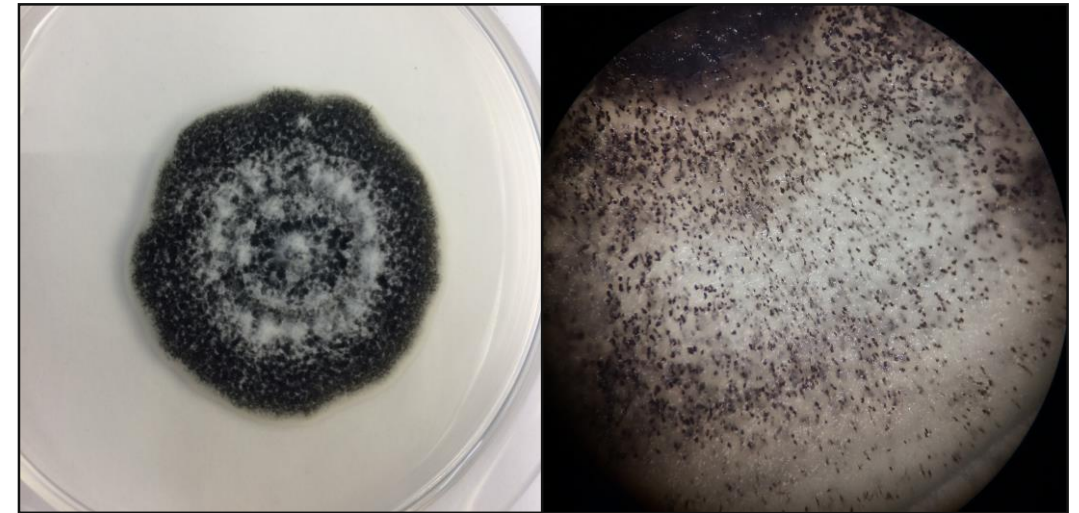


Potatoes 2016 Summary (September 2017) USDA, National Agricultural Statistics Service

Verticillium wilt of potato: the pathogen

Verticillium dahliae (*V. dahliae*)

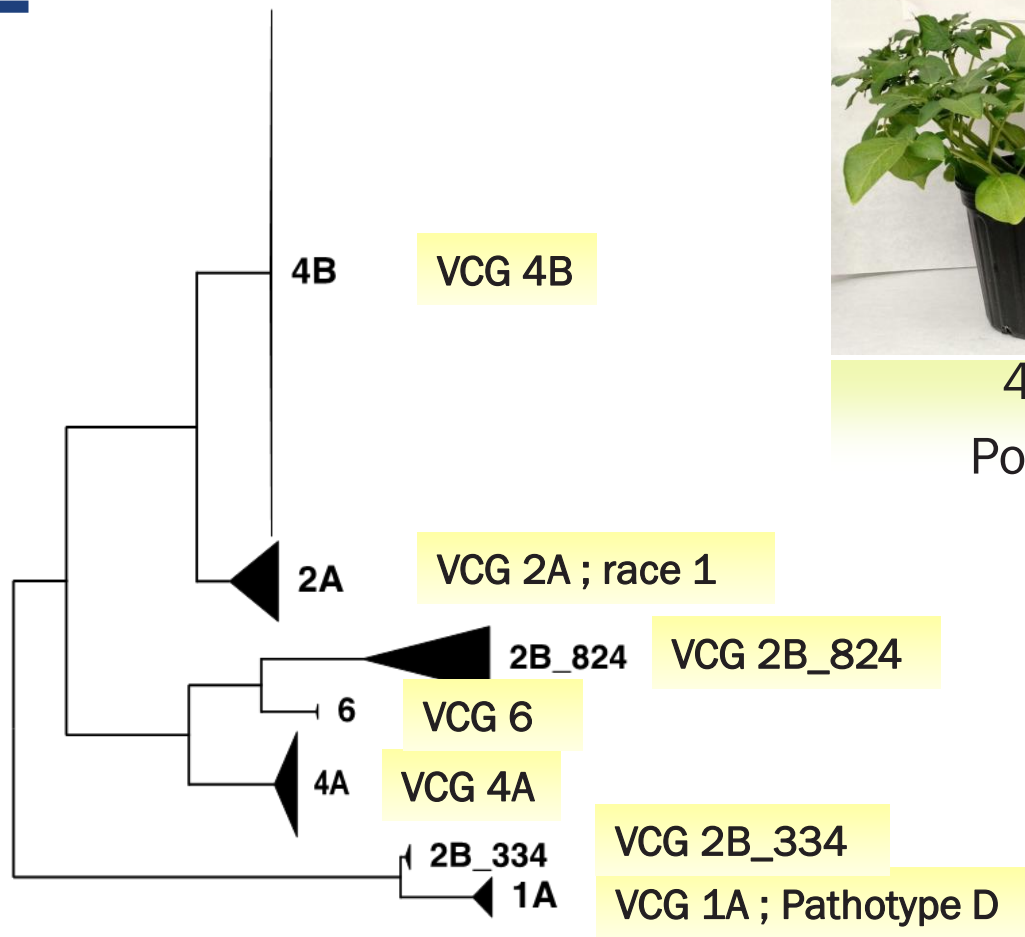
- Fungus, ascomycete
- Global distribution, especially temperate climates
- Highly clonal
- Asexual propagation: conidia, microsclerotia (survival structures)
- Susceptible host range: ~ 300 plant species (mainly dicotyledonous)
- Disseminated with soil, water, plant material, equipment, etc.



V. dahliae culture on PDA

V. dahliae microsclerotia

Variation of pathogenicity and genetic diversity of *V. dahliae*



4B 4A
Potato 'Snowden'



V 138 (orange 1A) AL42 (orange 4B) EBKH 16.3 (orange 2B) Control
D pathotype ND pathotype

Pathotype D on cotton



1A 2A 2B_334

artichoke



resistant cv. susceptible cv.

Race 1 on tomato

Pathogenic lineages of *V. dahliae* associated with agricultural systems. Neighbor-joining phylogeny constructed with 27,000 SNPs randomly distributed in the genome of *V. dahliae*. Evolutionary lineages correlate with *V. dahliae* VCGs. Branches have > 90% bootstrap support.

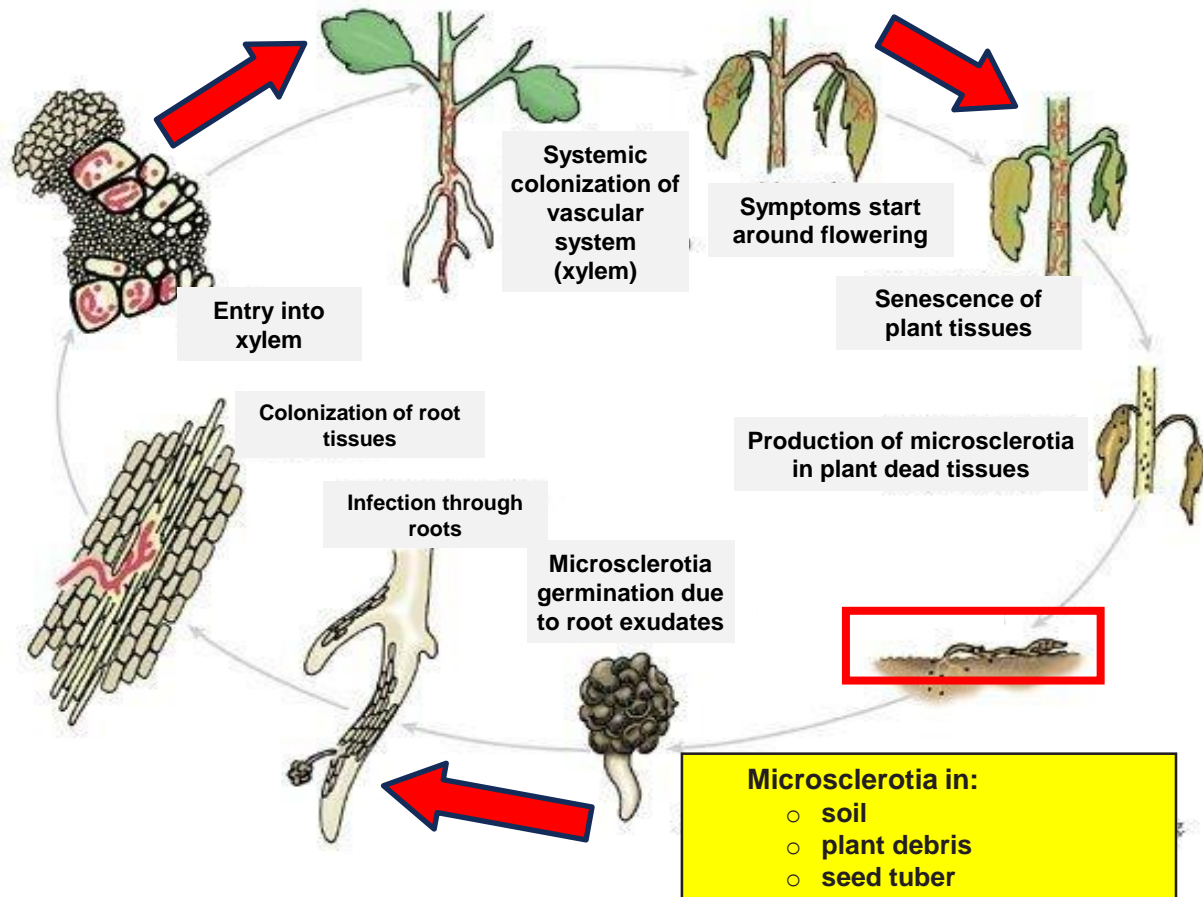
Vegetative Compatibility in filamentous fungi

- Ability of fungal isolates to anastomose their hyphae and form a new stable fungal individual
- Genetically regulated by *het* loci
- Vegetatively compatible isolates have identical alleles at *het* loci and group in the same Vegetative Compatibility Group, VCG
- **VCGs are molecular markers** from the Pre-PCR era.



Positive complementation between *nit* mutants from a same VCG

Verticillium wilt of potato: disease cycle



Verticillium wilt of potato: integrated disease management



Soil disinfestation: fumigation, solarization, biofumigation, ASD



Crop rotations (4-5 years) with non-hosts to separate disease cycles

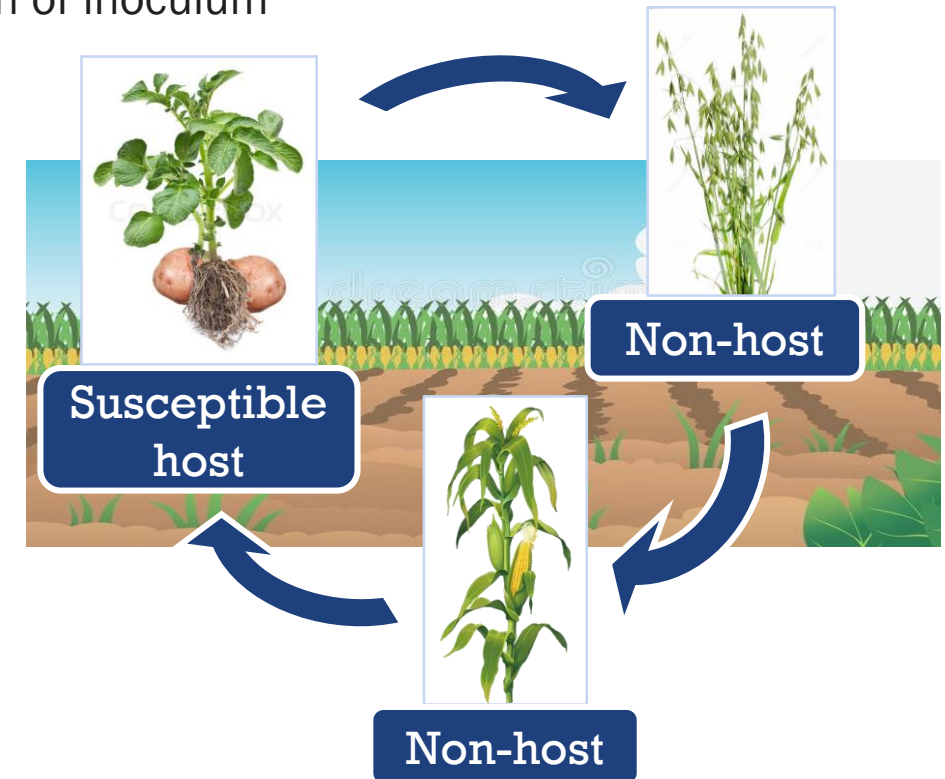


Cultural practices to improve plant and soil health

The use of crop rotations for the management of Verticillium wilt (V. wilt) of potato

- Soil fumigation is effective but expensive and restricted
- V. wilt epidemics are managed **only with crop rotations** most of the times.
- Non-host crops are selected based on absence of V. wilt symptoms

Host = V. wilt symptoms
 Production of inoculum



Non-host = no V. wilt symptoms
 No production of inoculum

Crop rotations have provided limited success in the management of V. wilt of potato

Verticillium dahliae (*V. dahliae*), the fungal pathogen causing V. wilt in potato systems,

survives for years as
microsclerotia (inoculum),



has a broad range of susceptible
hosts (few options for rotational
crops, mostly monocot species)



infects as an endophyte
rotational “non-host” crops
and weeds in the field



What diversity of *V. dahliae* is infecting asymptomatic hosts?

Hypothesis

Verticillium dahliae populations infecting as endophytes comprise non-pathogenic lineages and are genetically distinct from pathogenic populations

Main objective

To investigate the genetic diversity and pathogenicity of *V. dahliae* populations infecting asymptomatic and susceptible host plants from potato fields affected by Verticillium wilt

Diversity of *V. dahliae* infecting potato and oat cultivated under rotation



Commercial potato field in PA with long history of *V. wilt* epidemics



Potato 'Snowden'
Susceptible
N= 30



Potato 'Reba'
Mod. Resistant
N= 30



Oat 'Armor'
Asymptomatic
N= 25

N = number of isolates

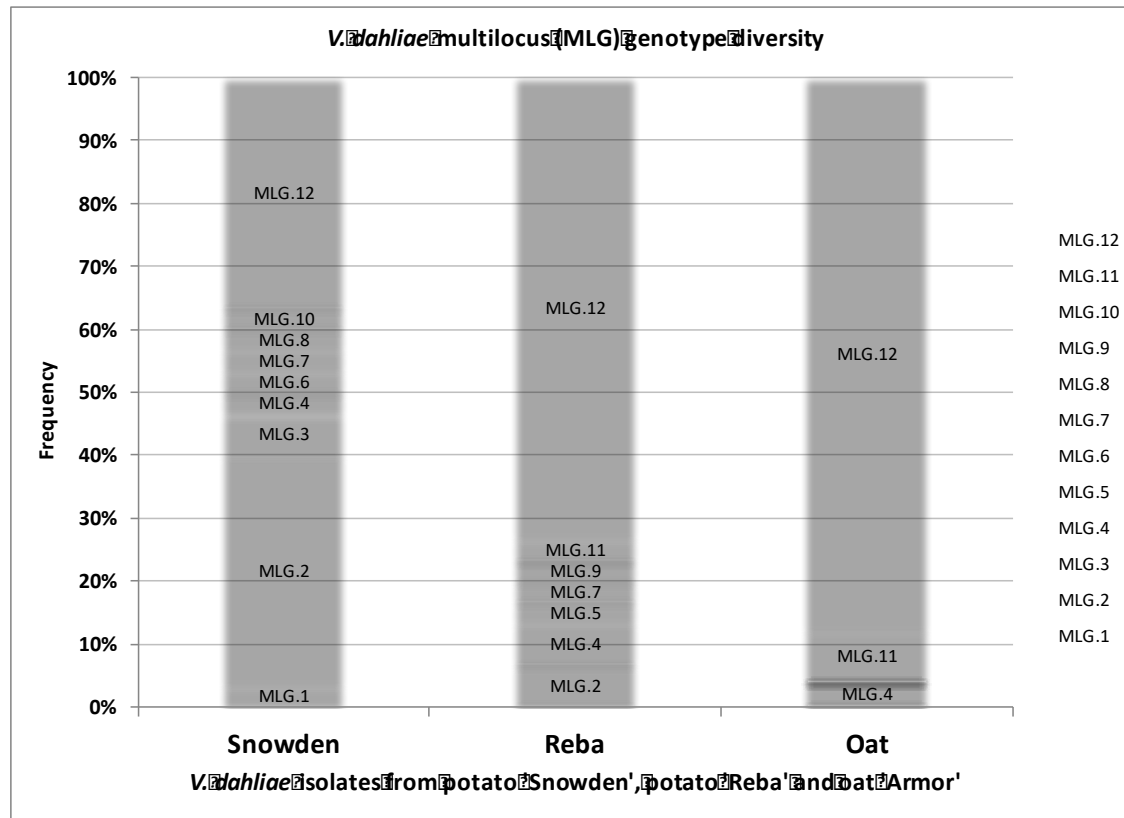
Characterization of the diversity:

- 10 microsatellites markers
- Vegetative Compatibility Groups (VCGs)
- Pathogenicity tests

Objective 1

To assess the genetic diversity and pathogenicity of *V. dahliae* infecting potato (susceptible host) and oat (asymptomatic host: traditional rotational non-host) cultivated on rotation in the same field for consecutive years

Results



Susceptibility	Susceptible	Moderately Resistant	Asymptomatic
Simpson's diversity	0.72	0.45	0.22
N of genotypes	9	7	3

VCG diversity



Potato 'Snowden'
Suscept.



Potato 'Reba'
Mod. resistant

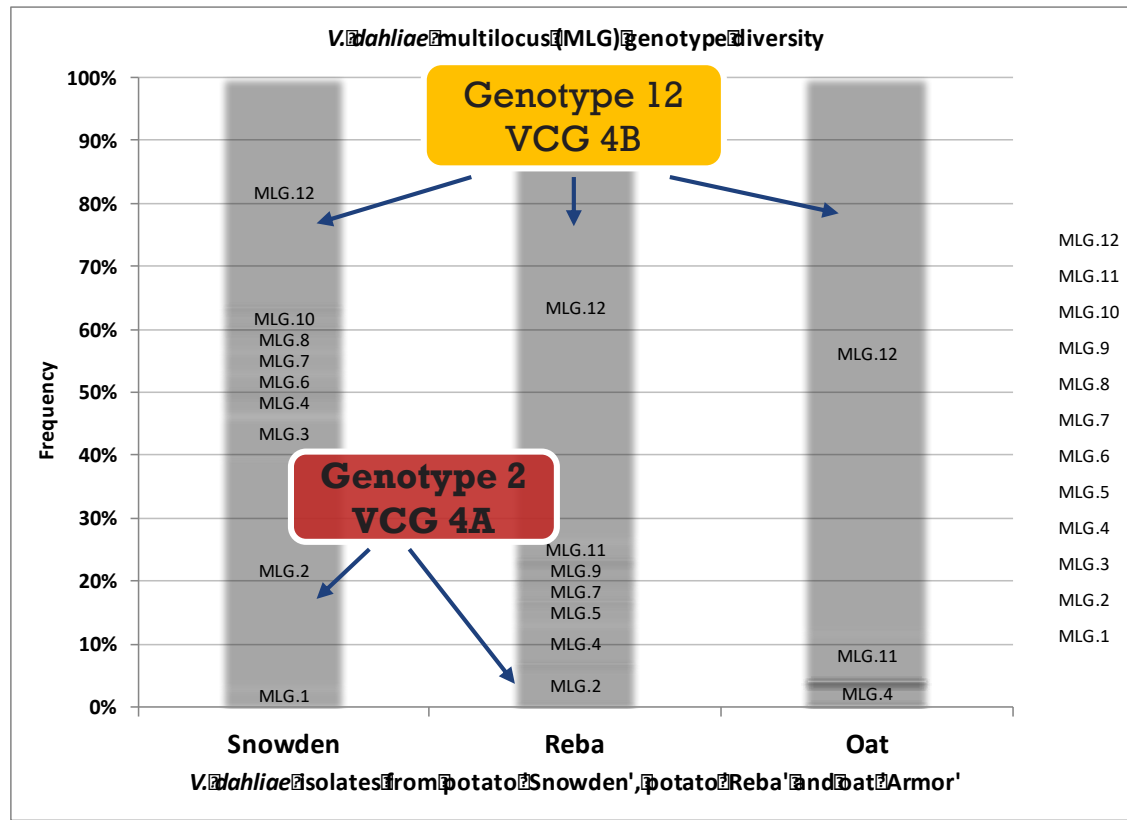


Oat 'Armor'
Asymp.

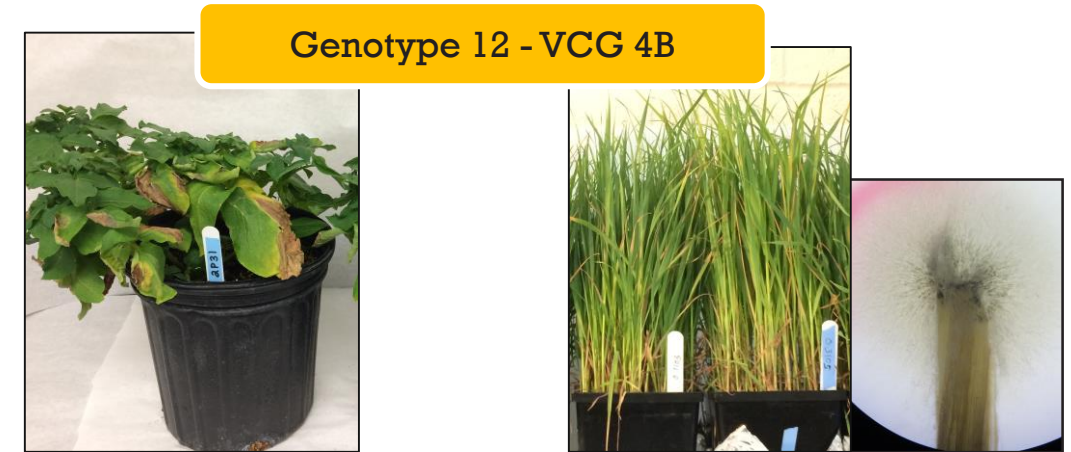
4A
4B
2A

4B

Results

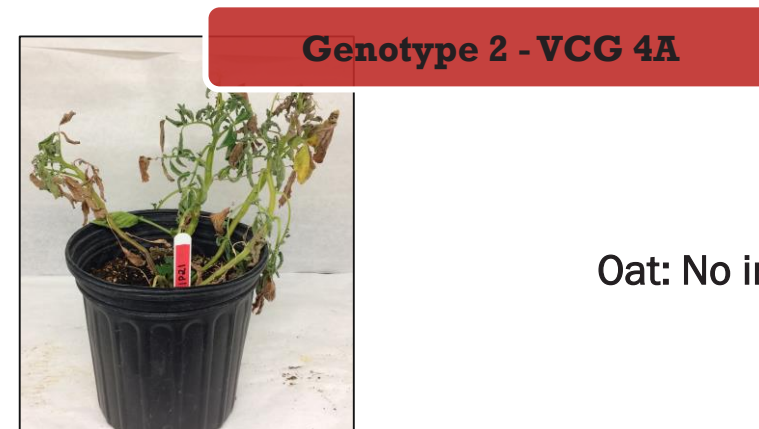


Susceptibility	Snowden	Reba	Oat
Susceptibility	Susceptible	Moderately Resistant	Asymptomatic
Simpson's diversity	0.72	0.45	0.22



Potato: Pathogen

Oat: Endophyte
No V. wilt symptoms



Potato: Pathogen
Highly aggressive

Oat: No infection

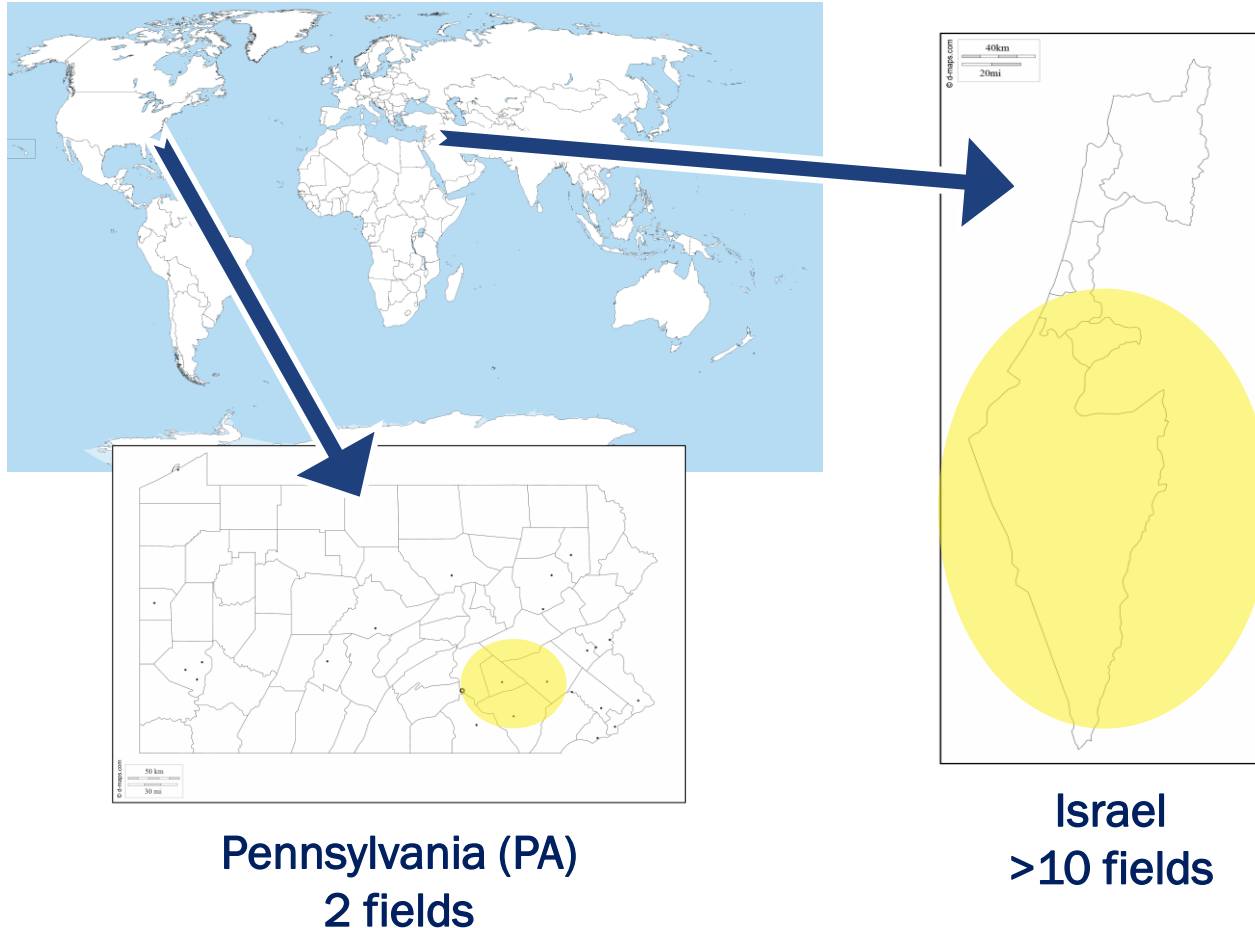
Potatoes and oats are infected differently by *V. dahliae* genotypes, probably because certain genotypes cannot infect certain hosts. Isolates infecting rotational oats as endophytes in the field are pathogens to potatoes.



1. Are these endophytic interactions happening to other hosts in the field?
Are they happening in other fields?
2. If so, what is the diversity of *V.dahliae* populations infecting as endophytes compared to populations infecting as pathogens?



V. dahliae lineages infecting susceptible and asymptomatic hosts in potato fields



- Potato fields affected by *V. wilt*
- **150 *V. dahliae* isolates:** 56 (asymptomatic) and 94 (symptomatic)
- **6 susceptible crops, 4 asymptomatic rotational crops, and > 20 asymptomatic weed species**
- **Genotyping-by-sequencing (GBS).**
- *V. dahliae* Lineages = VCGs

Objective 2

To characterize the diversity of *V. dahliae* populations infecting asymptomatic and symptomatic hosts recovered from potato fields affected by *V. wilt* in PA and Israel

Genotyping of *V. dahliae* isolates

Genotyping-by-Sequencing
ApeKI enzyme
HiSeq 4000
100 bp PE



Read alignment to *V. dahliae* ref. genome (bwa)

Variant calling (freebayes)

Variant filtering (vcflib)
SNPs
Read depth > 5
Mapping quality >30

SNP filtering (Tassel)

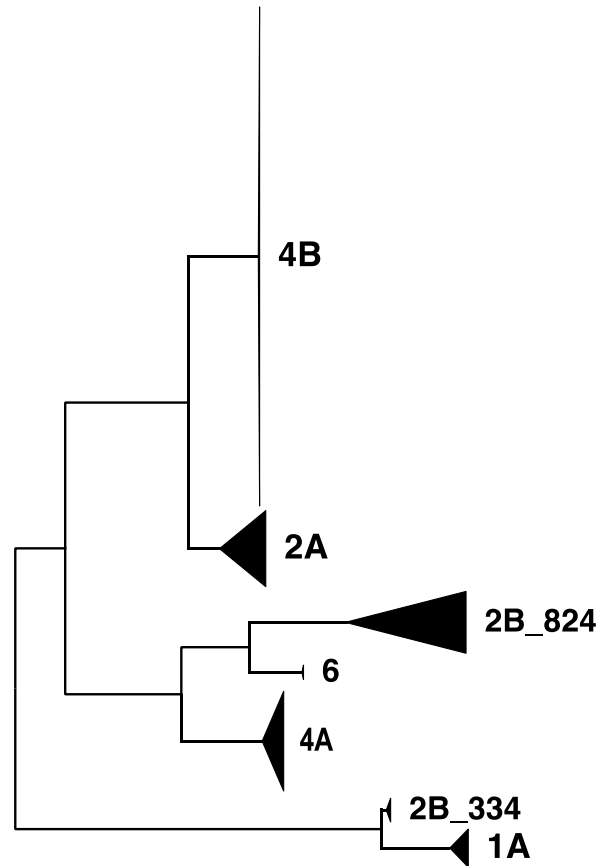
0.01 minimum allele freq
with alleles in > 75% of isolates
Isolates with allele info in >50% of sites

Thousands of SNPs

Thousands of SNPs

Thousands of SNPs

Results



Isolates from symptomatic hosts

	4B	Eggplant Pepper Potato
	2A	Eggplant, Pepper, Potato, Tomato
	2B ⁸²⁴	Chrysanthemum, Cotton, Pepper
	4A	Potato

Isolates from asymptomatic hosts

	4B	<p>Rotational crops: oat, soybean, sorghum, peanut.</p> <p>Weeds: black nightshade, silverleaf nightshade, pigweed, wild mustards, <i>Sinapsis</i> spp., <i>Diplotaxis</i> spp., <i>Calendula arvensis</i>, <i>Conyza bonariensis</i>, etc. > 20 weed species</p>
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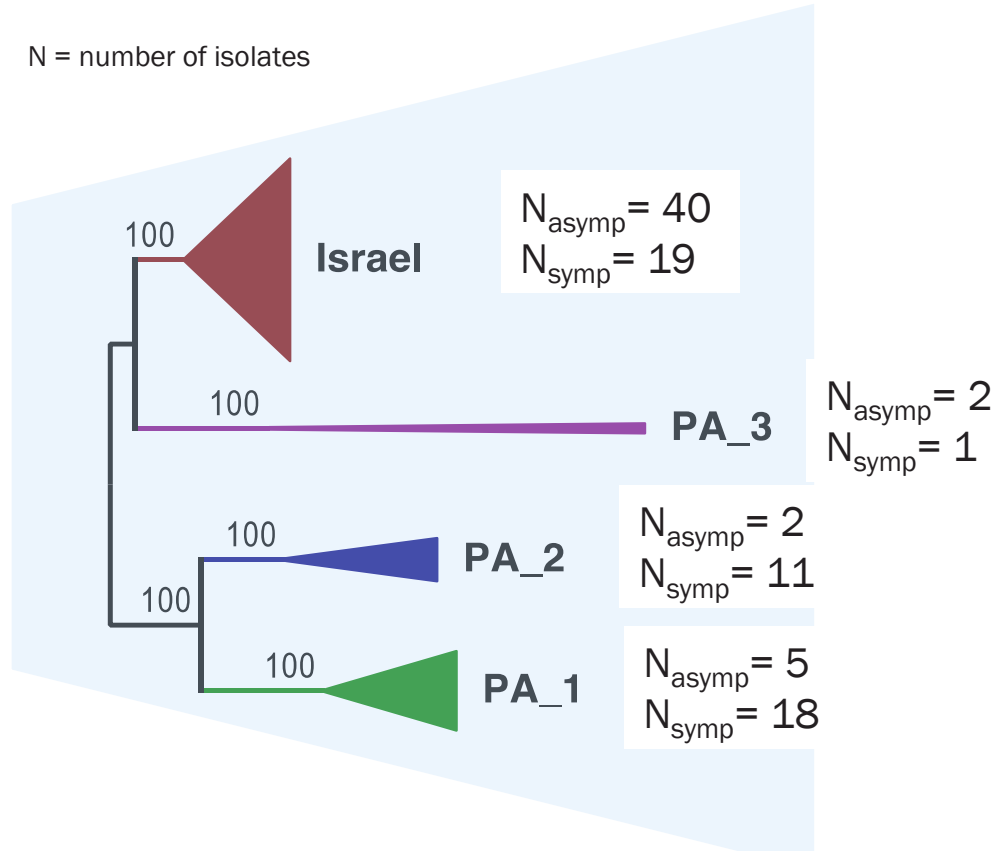
Pathogenic lineages of *V. dahliae* associated with agricultural systems. Neighbor-joining phylogeny constructed with 27,000 SNPs randomly distributed in the genome of *V. dahliae*. Evolutionary lineages correlate with *V. dahliae* VCGs. Branches have > 90% bootstrap support.

Isolates from asymptomatic hosts were all characterized as **lineage 4B**, a previously-known pathogenic lineage, together with some isolates from symptomatic hosts.

Results

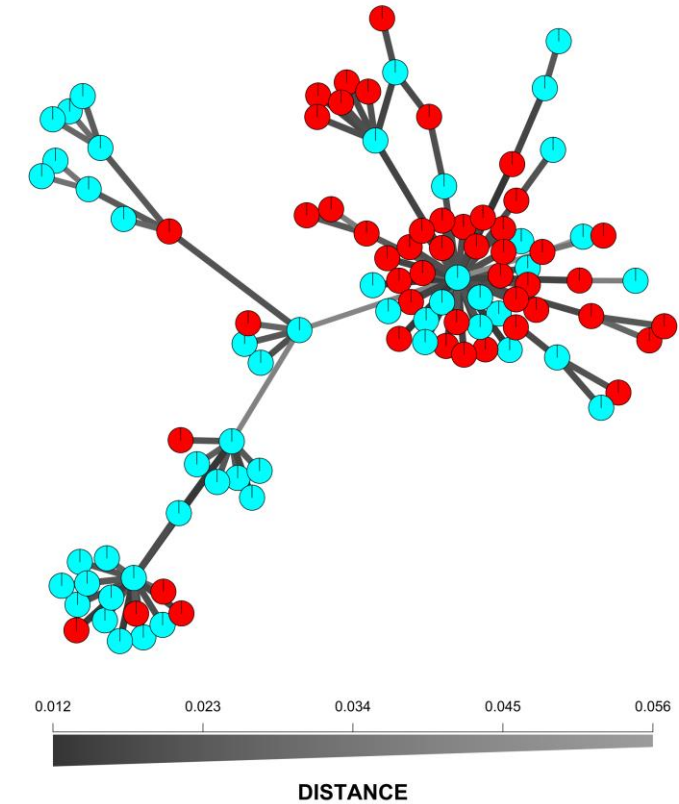
***V. dahliae* Lineage 4B**

N = number of isolates



Neighbor-joining phylogeny of *V. dahliae* lineage 4B isolates from PA and Israel using 5,335 SNPs. Branches values show percentage of bootstrap support. Isolates from asymptomatic hosts were more closely related to isolates from susceptible hosts than among them.

POPULATION
■ asymptomatic
■ symptomatic



Minimum spanning network based on genetic distance among *V. dahliae* 4B isolates from PA and Israel potato fields using 5,335 SNPs. Populations are defined based on host symptomatology. Isolates from asymptomatic hosts are genetically highly similar to isolates from susceptible hosts.

***Verticillium dahliae* 4B** isolates prevalent in potato fields as endophytes are evolutionarily related and genetically highly similar to isolates infecting susceptible hosts.



1. How are the endophytic infections of *V. dahliae* lineage 4B on rotational crops and weeds impacting the disease management on potato crops? Are *V. dahliae* 4B isolates producing inoculum in asymptomatic rotational crops and weeds in the field?
2. What are the dynamics of *V. dahliae* 4B soil inoculum when using an asymptomatic rotational crop?



Dynamics of *V. dahliae* soil inoculum in a potato-oat rotation system

Monitoring *V. dahliae* 4B and 4A soil inoculum levels in a potato-oat rotation:



Microplots infested with *V. dahliae* 4A and 4B microsclerotia

- Pathogen treatments:
 - 4A
 - 80:20 4A:4B
 - 50:50 4A:4B
 - 20:80 4A:4B
 - 4B
- Crop treatments:
 - oat monoculture
 - potato monoculture
 - potato-oat rotation
- Split-plot design
- 5 replicates, 90 total microplots
- Collection of soil samples before and after each crop
- Experiment conducted in 2017 and 2018

Objective 3

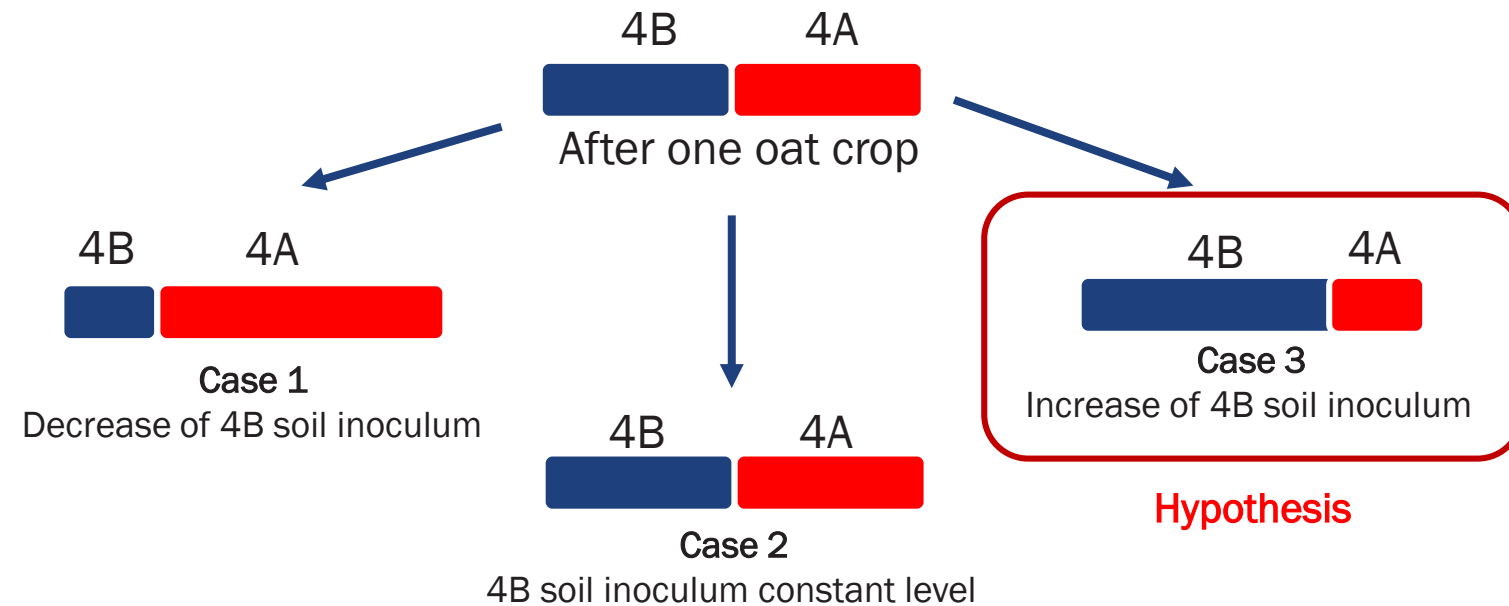
To test whether the cultivation of oat, an asymptomatic rotational crop, impacts the soil inoculum levels of *V. dahliae* lineages 4A and 4B resulting in inoculum shifts that affect disease in a subsequent potato crop.

Dynamics of *V. dahliae* soil inoculum in a potato-oat rotation system

Monitoring *V. dahliae* 4B and 4A soil inoculum levels in a potato-oat rotation:



Example of microplot infested with 50:50 ratio of 4A and 4B inoculum:



Hypothesis

The cultivation of oat, which get asymptotically infected by *V. dahliae* 4B, will result in significant increases of *V. dahliae* 4B inoculum in soil after the crop because the pathogen is producing microsclerotia in the asymptomatic plants

Dynamics of *V. dahliae* soil inoculum in a potato-oat rotation system

Development of a PCR-based protocol to monitor *V. dahliae* 4A and 4B in soil

De-novo Whole Genome Seq

PacBio + HiSeq 4000
T003, S-228 (4A)
U073, S-39 (4B)

De-novo genome assembly

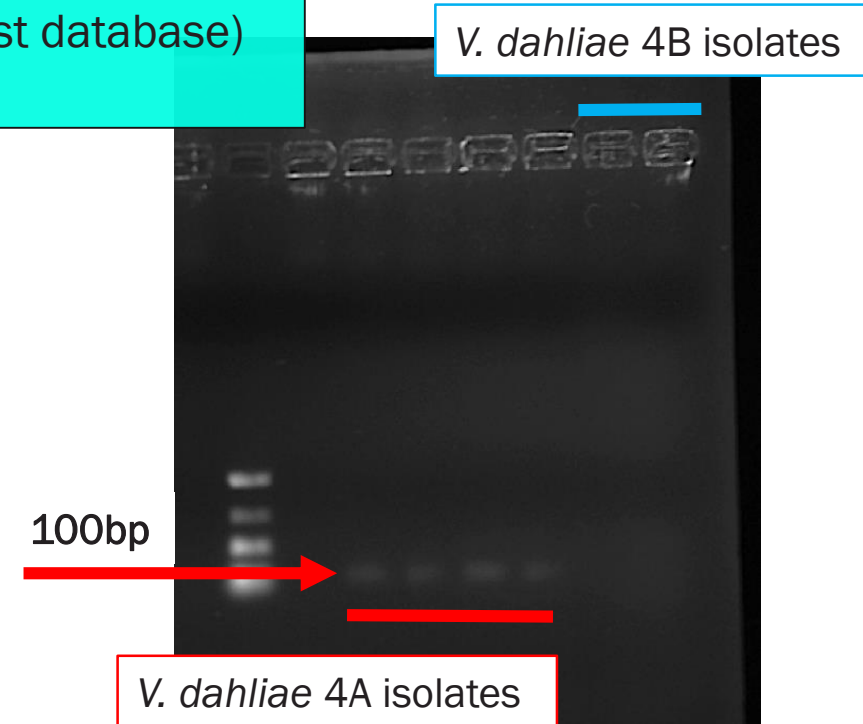
(Canu + pilon + quast)
Long reads -> scaffolds
Short reads -> nucleotide correction

Identify LS regions for designing 4A and 4B markers

- Comparative genomics of *V. dahliae* lineages genomes (1A, 2B_824, 2A, 4A, 4B)
- Repetitive elements identification (RepeatMasker)
- De-novo gene prediction (glimmer)
- Mapping characteristics (bwa, samtools)

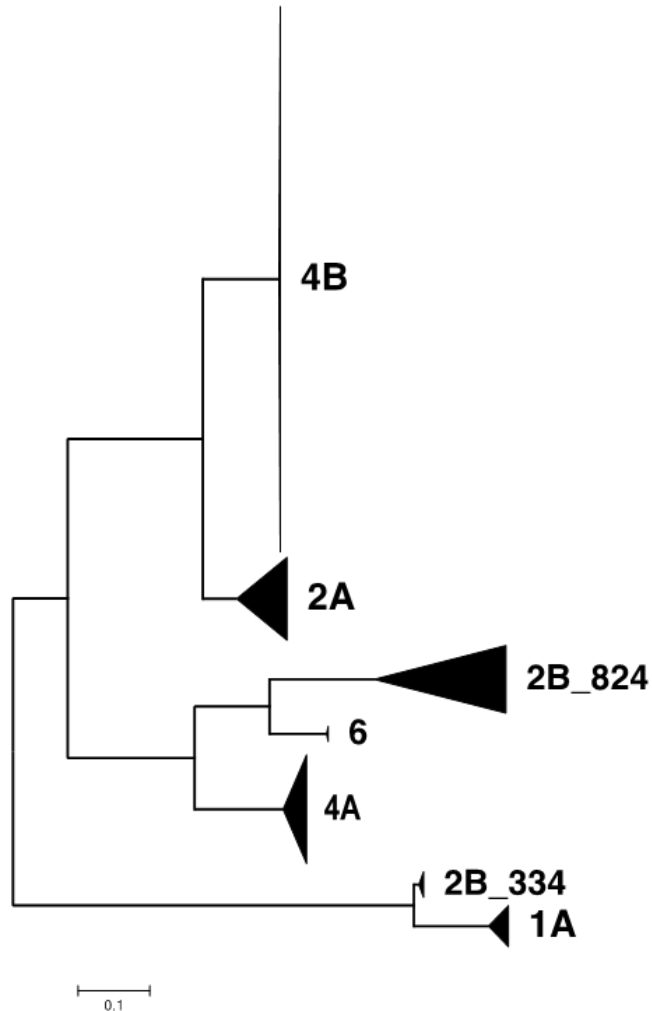
Marker Development

Primer design (Primer Blast)
Coding sequences, CDS
Specificity (Blast database)
qPCR/ddPCR



Agarose gel showing amplification of *V. dahliae* 4A PCR marker

Development of a PCR-based protocol to monitor *V. dahliae* 4A and 4B in soil



Marker Development
Testing primers on DNA from representatives of *V. dahliae* Lineages

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Conclusions and Implications

- In the field, potatoes are **rotated with crops** that are **asymptomatically infected** by *V. dahliae* lineage 4B, a lineage that is pathogenic to a broad range of crops including potatoes.
- Common **weeds** present in potato fields are also **infected by *V. dahliae* lineage 4B**.
- *V. dahliae* 4B isolates seem to have an **endophyte-pathogen dual role** depending on the host they infect.
- How does a potato-oat rotation impact the levels of *V. dahliae* 4A and 4B soil inoculum?

Acknowledgements



PennState
College of Agricultural Sciences

- Glenna Malcolm, Beth Gugino, Maria Jimenez-Gasco, Gary Moorman (Penn State)
- Michael Milgroom (Cornell U.)
- Leah Tsrer and Omer Frenkel (ARO, Israel)
- Bob Leiby (PA Potato Coop)
- PA and Israel potato growers
- Genna Tesdall, Callista Millers, Morgan Gannon, Jenny Mazonne, Amanda Mainello, Michele Mansfield and Clara Miller (Penn State)
- Sara E. Getson (Michigan State U.)
- Penn State Plant Pathology research farm
- Penn State, Plant Pathology and Environmental Microbiology

Funded by



United States Department of Agriculture
National Institute of Food and Agriculture



Project number GNE17-144



United States – Israel
BINATIONAL AGRICULTURAL RESEARCH AND DEVELOPMENT FUND
BARD – קמח



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Questions?

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