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Grapevine yellows from New York to Virginia: Multilocus molecular genetic characterization of phytoplasmas affecting commercial vineyards

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Grapevine Yellows Diseases -- WORLDWIDE

Europe

Flavescence doree,
Bois Noir (Legno Nero, Black Wood)

Australia

Australian grapevine yellows
Buckland Valley grapevine yellows

North America

North American grapevine yellows
Group 16SrI
Group 16SrIII

South America

Bois Noir

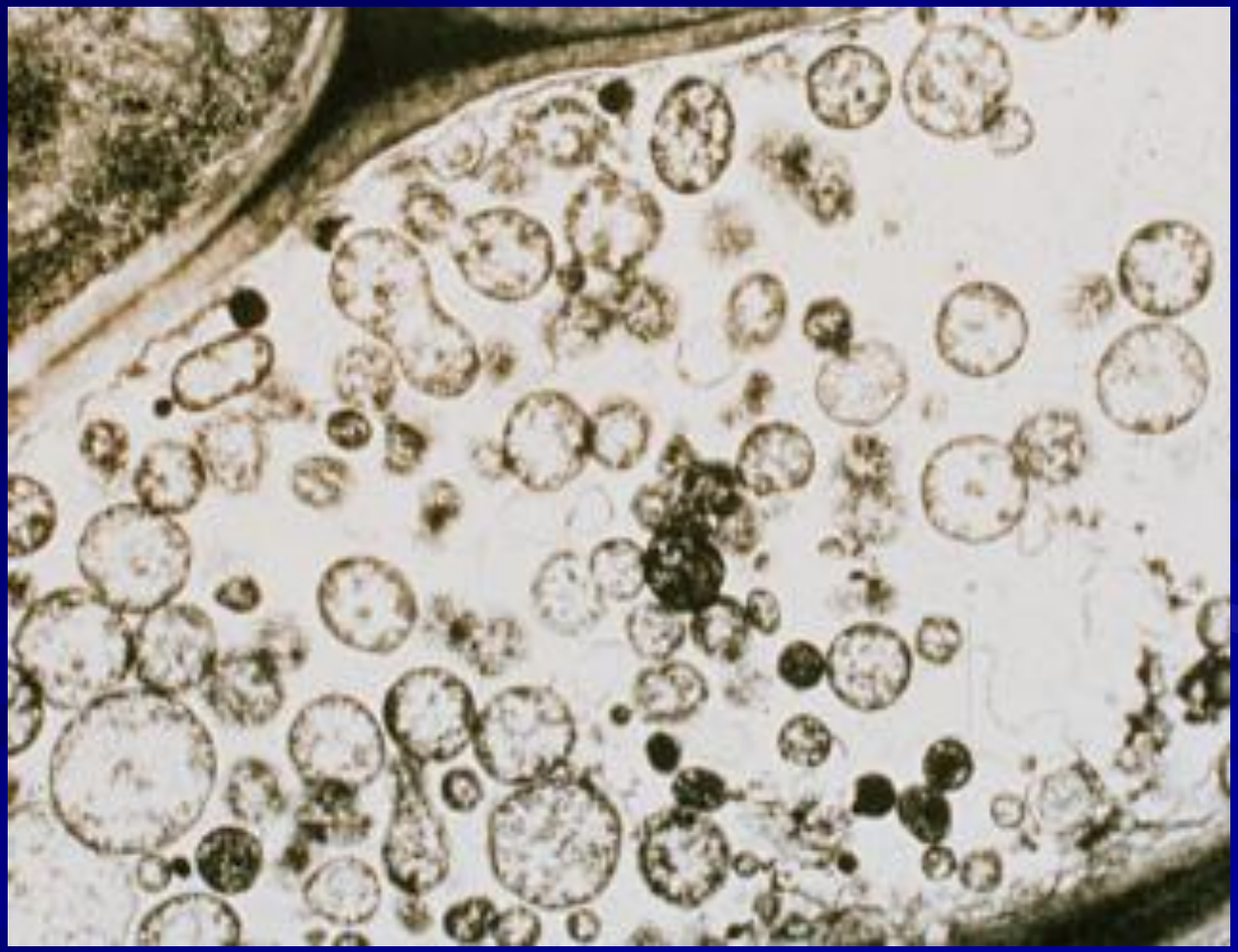
South Africa

Aster Yellows

AN EXAMPLE – Bois Noir Disease in Sicily, Italy

CAUSE(S) of Grapevine Yellows

PHYTOPLASMA – a minute bacterium that lacks a cell wall
-- cannot be isolated in pure culture
-- reside in phloem tissue of plants



SPREAD of Grapevine Yellows

LEAFHOPPERS – feed in phloem tissue

The Region Covered by this Study

- New York State

  Finger Lakes region

- Pennsylvania,

  southern Pennsylvania

- Maryland

  western Maryland, central Maryland,

  and the eastern shore

- Virginia

  western Virginia

ANALYSES OF PHYTOPLASMA GENES

16S rRNA *secY*, and ribosomal protein genes

Nucleotide sequence analyses / *In silico* translations

Taxonomic classification

Phylogenetic analyses

SNP identification / Design of NAGY strain

– specific detection & identification

Grapevine Yellows - Diseased Cultivars

CULTIVAR	No. assayed	No. phytoplasma +
Chardonnay	34	34
Cabernet-Sauvignon	8	8
Viognier	1	1
Petit Manseng	1	1
Malbec	1	1
Pinot Gris	4	4
Black Malvesia	1	1
Pinot Noir	2	2
Chambourcin	1	1
Merlot	2	2
Cabernet-Franc	2	2
Syrah	2	1
TOTALS	**59**	**59**

Grapevine Yellows - PHYTOPLASMAS

CULTIVAR	16S rDNA RFLP Group I	Group III
Chardonnay	3	25
Cabernet-Sauvignon	1	7
Viognier	0	1
Petit Manseng	0	1
Malbec	0	1
Pinot Gris	2	1
Black Malvesia	--	--
Pinot Noir	2	0
Chambourcin	--	--
Merlot	2	0
Cabernet-Franc	2	0
Syrah	1	0
TOTALS	**13**	**36**

Grapevine Yellows in North America

Maryland **Chardonnay** **August 2011**

Pennsylvania Pinot Gris August 26, 2011

Eastern Shore Maryland Chardonnay
August 17, 2011

Pennsylvania Pinot Gris August 26, 2011

Western Maryland Chardonnay August 2011

Western Maryland Chardonnay August 2011

Pennsylvania Pinot Gris August 26, 2011

Pennsylvania Pinot Gris August 26, 2011

Pennsylvania F Chardonnay August 26, 2011

Pennsylvania H Pinot Gris

Pennsylvania F **Pinot Noir**

Pennsylvania F **Chambourcin**

Pennsylvania F Syrah August 26, 2011

Maryland Black Malvesia August 2011

NAGY PHYTOPLASMAS: 2 different species

Group I

= Group 16SrI ‘*Candidatus* Phytoplasma asteris’-related

Group III

= Group 16SrIII, ‘*Candidatus* Phytoplasma pruni’-related

IMPLICATIONS –

the 2 NAGY phytoplasmas likely have different insect vectors

and

possibly different alternate plant hosts

NAGY Group III Phytoplasma

is Related to

the Phytoplasma Causing X-Disease of Stone Fruits

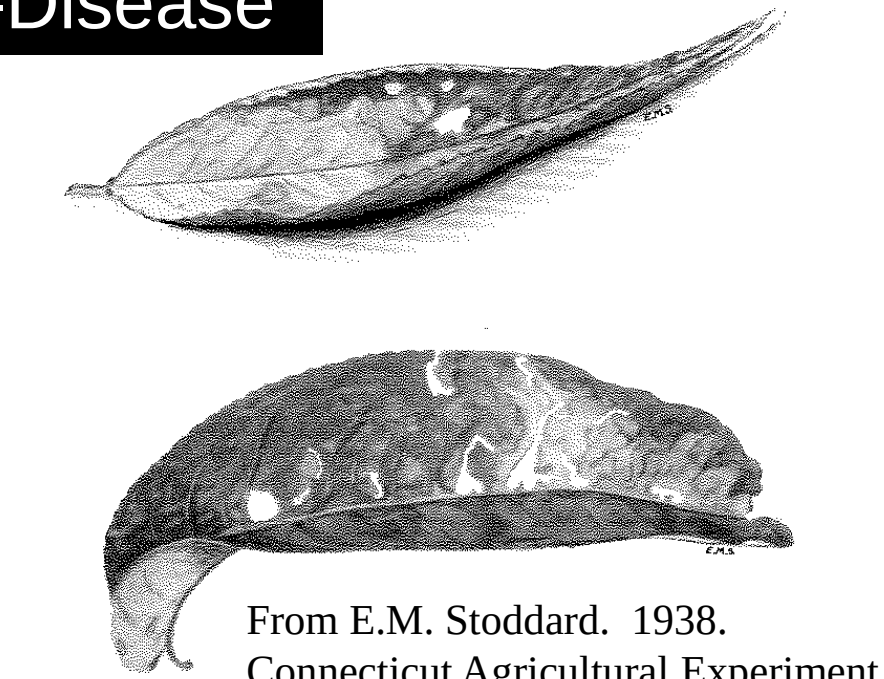
All 13 signature sequences

of '*Candidatus* Phytoplasma pruni'

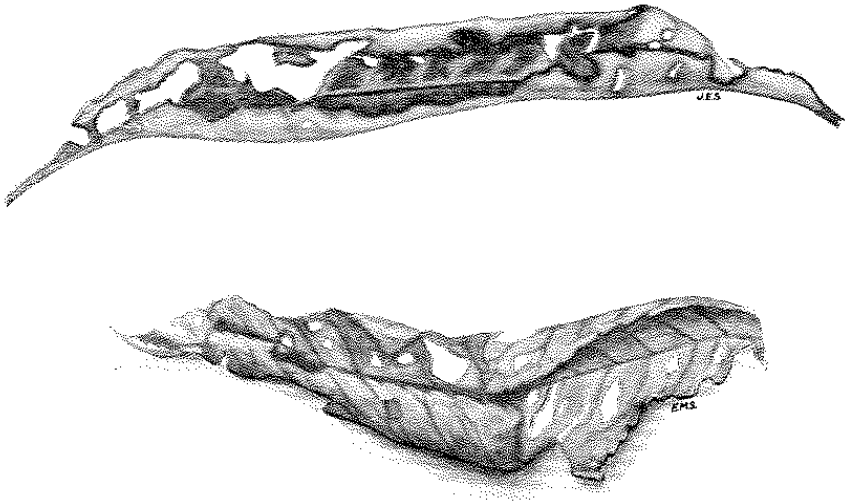
are present in the Group III Strains Associated with

North American Grapevine Yellows

Peach X-Disease



From E.M. Stoddard. 1938. Connecticut Agricultural Experiment Station, New Haven. Circular 122.



Characteristic Leaf Symptoms of X Disease.

Do these phytoplasmas cross infect their agronomic hosts ?

Do the NAGY and X-disease phytoplasmas

cross infect grape and peach in nature ?

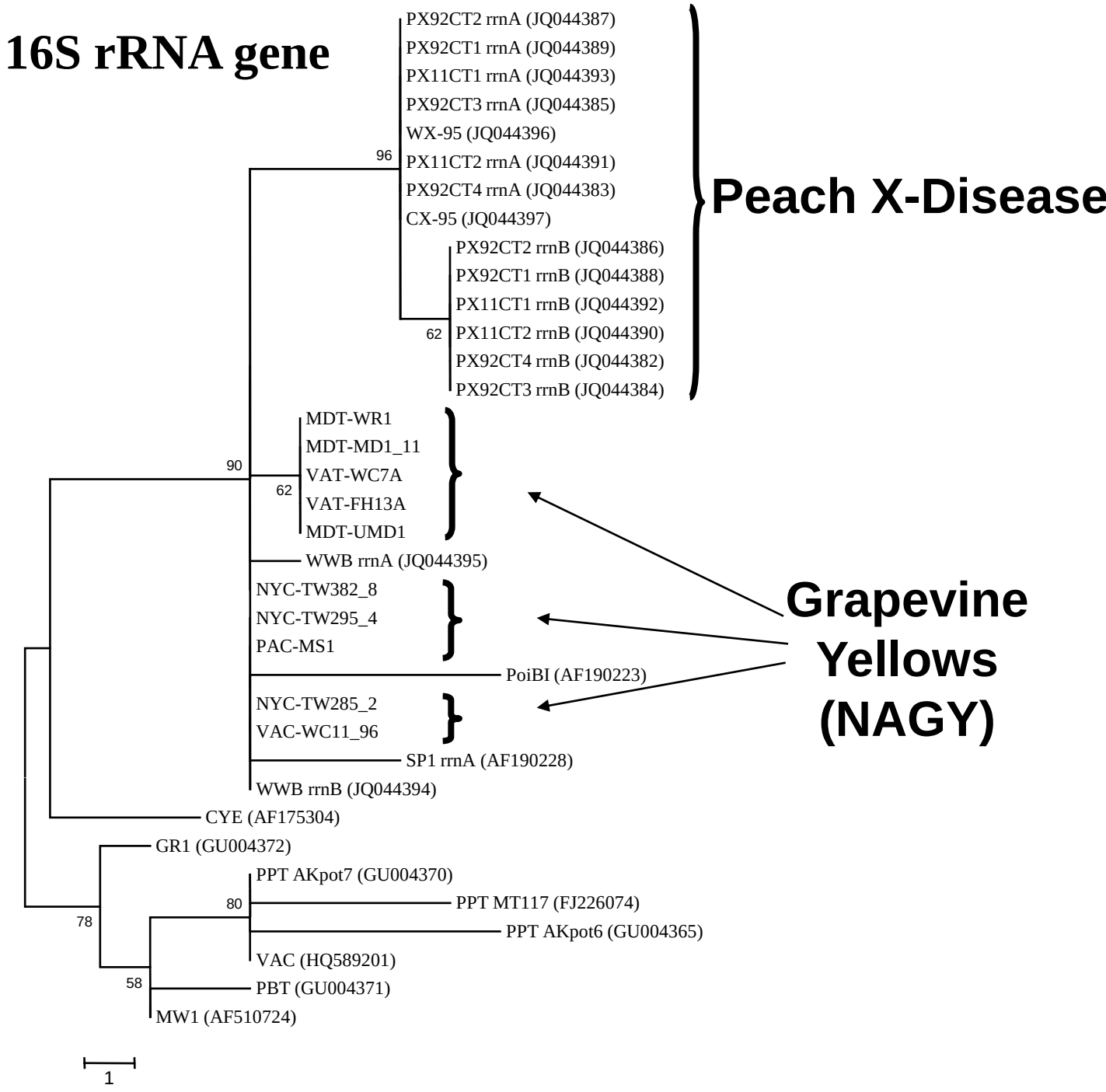
FUTURE

Insect transmission

coupled with

Molecular markers distinguishing the phytoplasma lineages

Phylogenetic analysis distinguishes NAGY strains from '*Ca.* Phytoplasma pruni', cause of X-disease.



SNPs distinguish Grapevine Yellows Strains

from

X-disease Strains

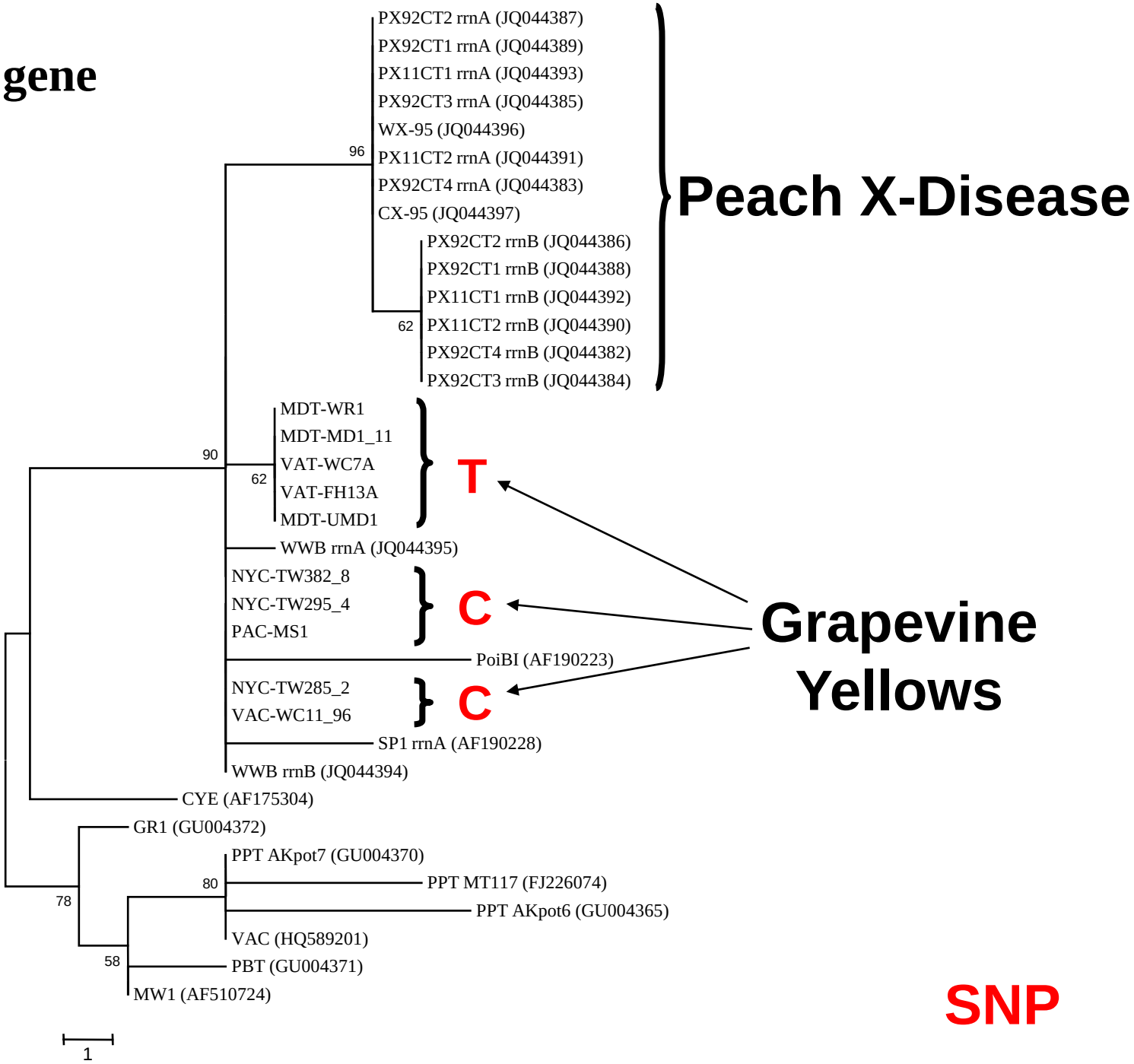
Design of pathogen – specific detection

ALSO

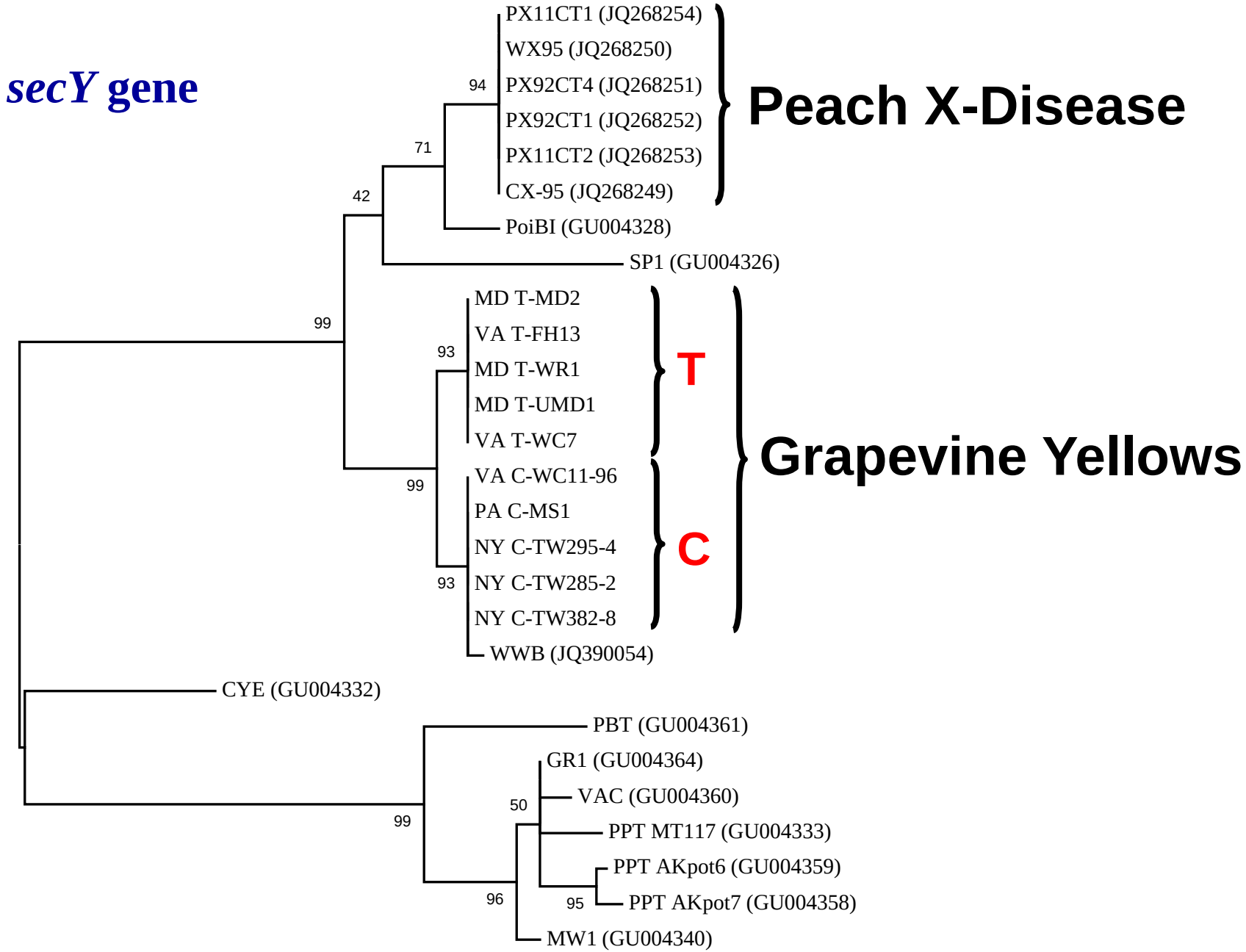
SNPs distinguished 2 lineages

among strains causing NAGY.

16S rRNA gene

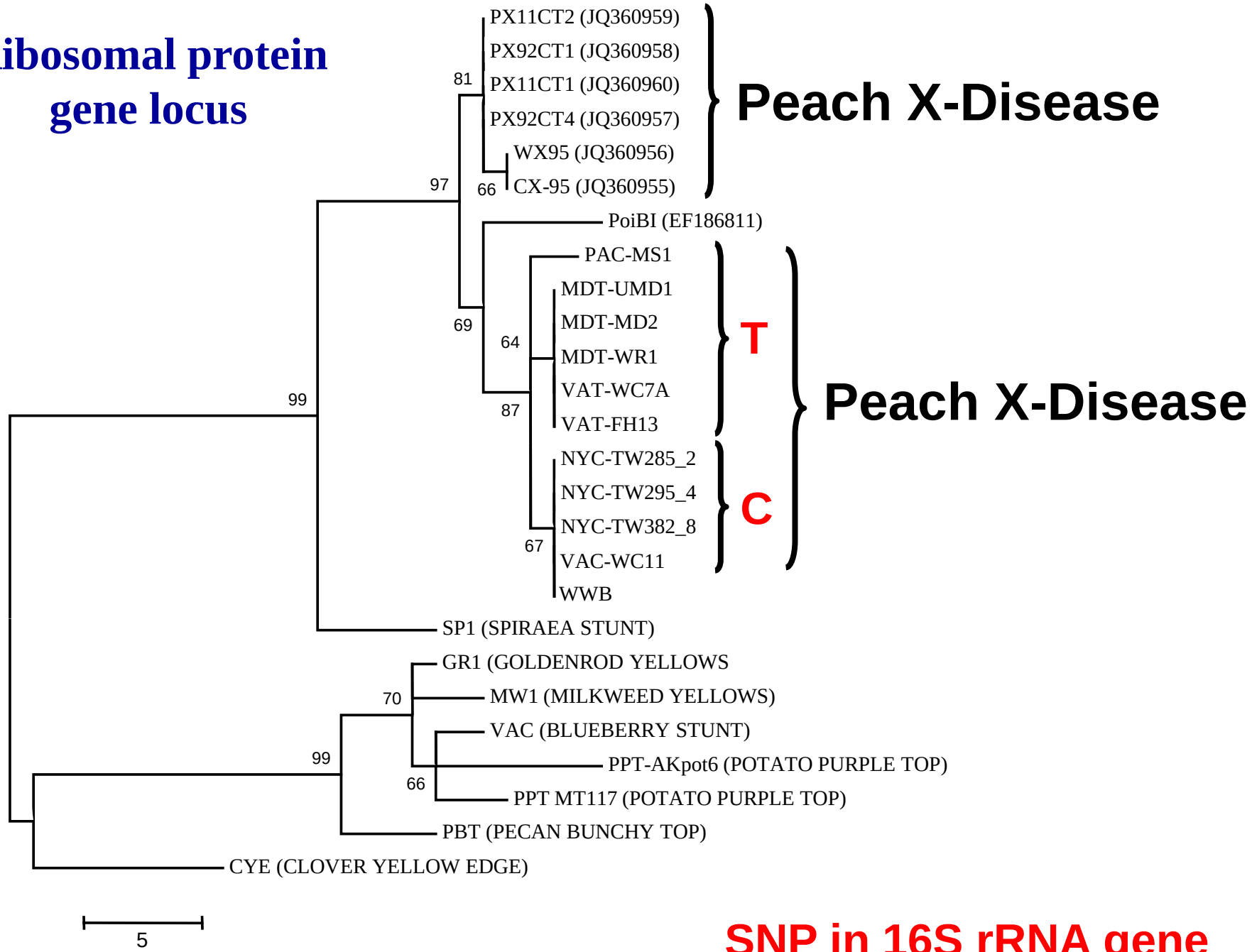


SNP in 16S rRNA gene is a predictor of lineage-specific divergence in other genes among NAGY phytoplasma strains



SNP in 16S rRNA gene

Ribosomal protein gene locus



SNP in 16S rRNA gene

This information will be used to develop rapid, specific, accurate diagnostic methods which will enable:

- Field diagnostics, clean plants, APHIS activities

..and will also enable:

- Epidemiology of two NAGY diseases
  - Identify insect vectors
  - Identify additional plant hosts
- Develop management strategies based on the epidemiological information

ACKNOWLEDGEMENTS

We are grateful to all the vineyard managers and owners who permitted us to enter their vineyards and collect leaf samples for this work.

Robert E. Davis, Ellen L. Dally, Yan Zhao, Ing-Ming Lee, Tony K. Wolf, LeAnn Beanland, Joseph A. Fiola, Mark Chien, and Hans C. Walter-Peterson

ARS research on grapes continues with . . .

New virus-like disease in Oregon on Pinot Noir

Bob Martin, USDA, ARS, Corvallis

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Symptoms on Cabernet Franc in Southern Oregon

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Race-specificity: selection on local scale

Selection for powdery mildew virulence on *V. labrusc*

PI	ID		Geneva, NY	Fredonia, NY
588128	Vergennes	*V. labrusca*	0	7
588145	GBC 12	*V. labrusca*	2	5
588165	Alba	*V. labrusca*	0	4
588173	GBC 2	*V. labrusca*	2	5
588277	Jersey Muscat	*V. labrusca*	1	6
588307	Champagne	*V. labrusca*	1	6
~~588583~~	~~Grem-4~~	~~*V. labrusca*~~	~~6~~	~~7~~
594349	Alexander	*V. labrusca*	0	5
	Average		1.3	5.5
			R	S

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Race-specificity due to effector variation

Science AAAS

Genome Expansion and Gene Loss in Powdery Mildew Fungi Reveal Tradeoffs in Extreme Parasitism

Pietro D. Spanu, *et al.*

Science **330**, 1543 (2010);
DOI: 10.1126/science.1194573

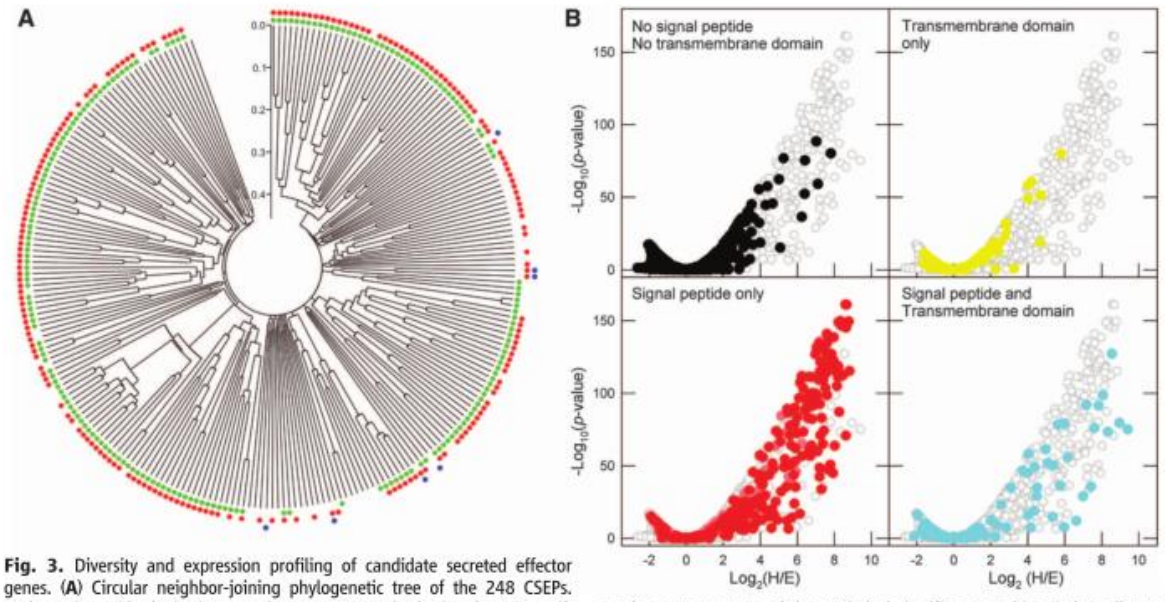
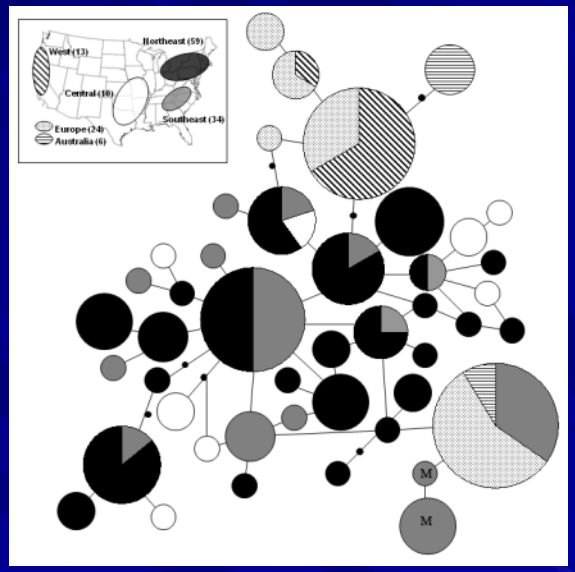


Fig. 3. Diversity and expression profiling of candidate secreted effector genes. **(A)** Circular neighbor-joining phylogenetic tree of the 248 CSEPs. Scale, amino acid substitutions per site; green, CSEPs harboring the YxC motif; blue, CSEPs conserved in *E. pisi* and/or *G. orontii*; red, CSEPs predominantly expressed in haustoria. **(B)** Global gene expression in haustoria (H) versus epiphytic structures (E). Relative abundance of each gene plotted versus the *p*-value as a measure of the statistical significance. White circles, all gene models; black, no SP and no TM domain; yellow, TM only; red, CSEPs; pink, genes with BLASTP hits in the National Center for Biotechnology Information nr database and SP only; light blue, both SP and TM domains.



PM genetic diversity Brewer & Milgroom

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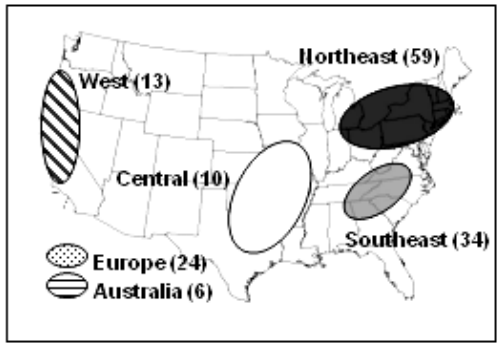
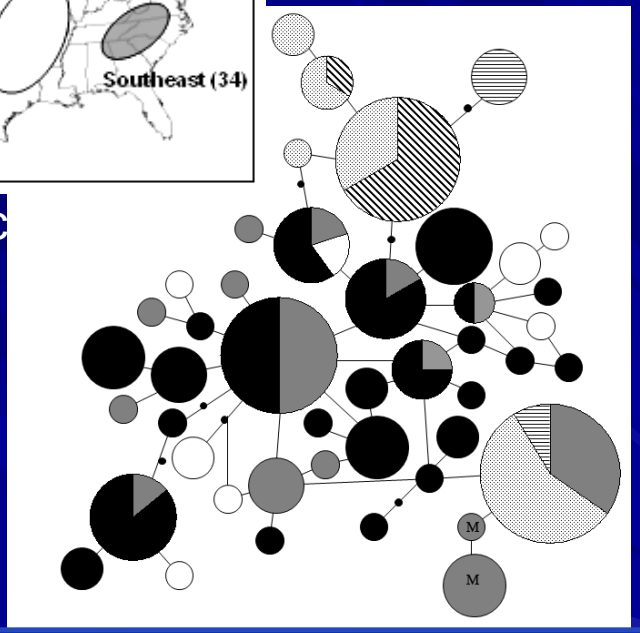
Natural variation in Geneva, NY: Heaven on Earth for powdery mildew genetics

ARS-PGRU germplasm collection



PM genetic diversity Brewer & Milgroom



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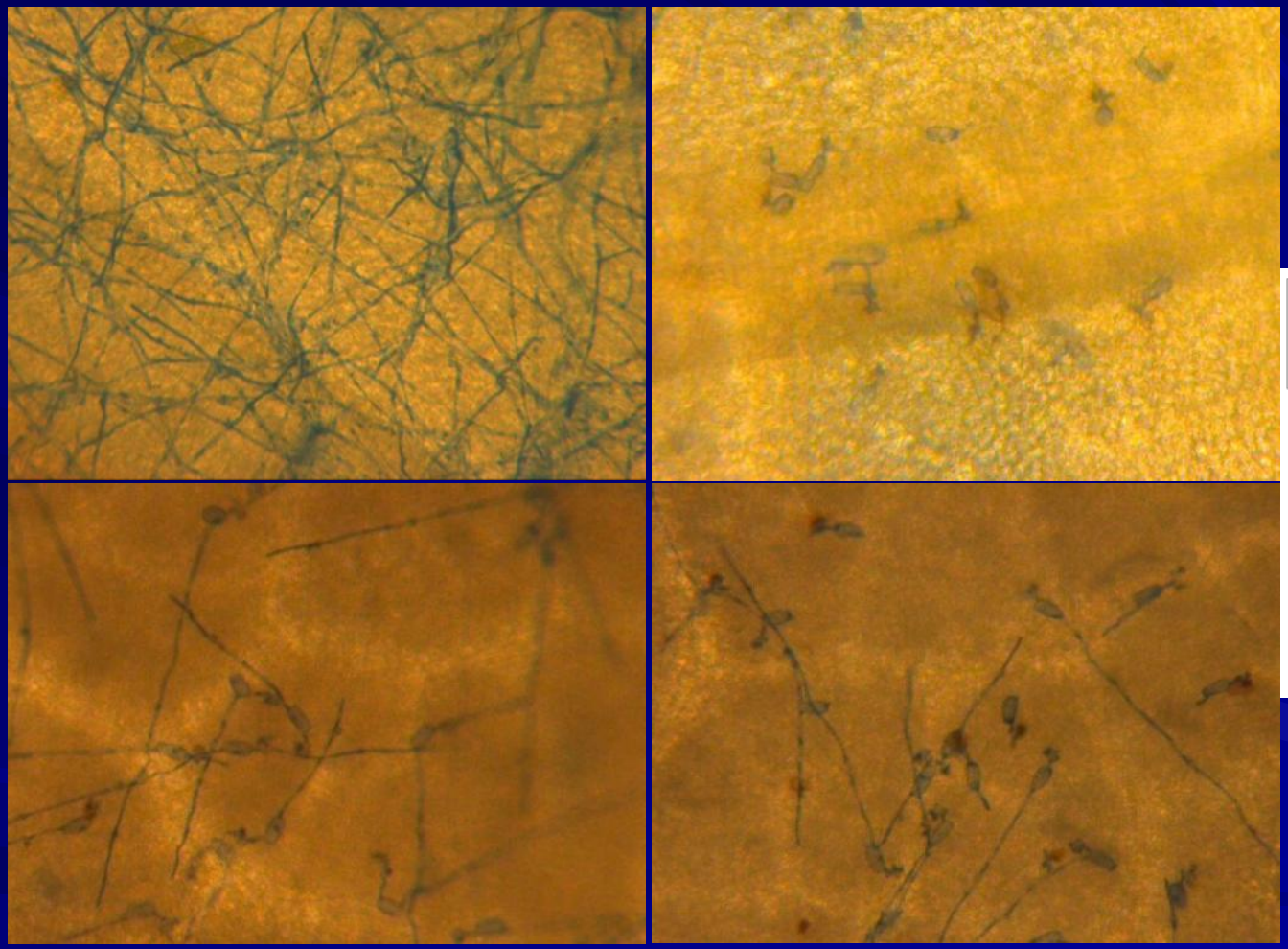
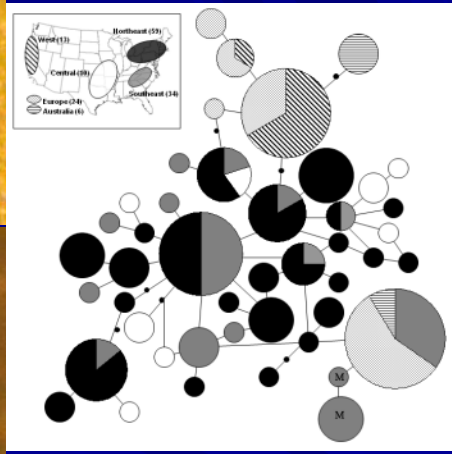
How far can you grow?





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How far can you grow?
Different answer for different genotypes

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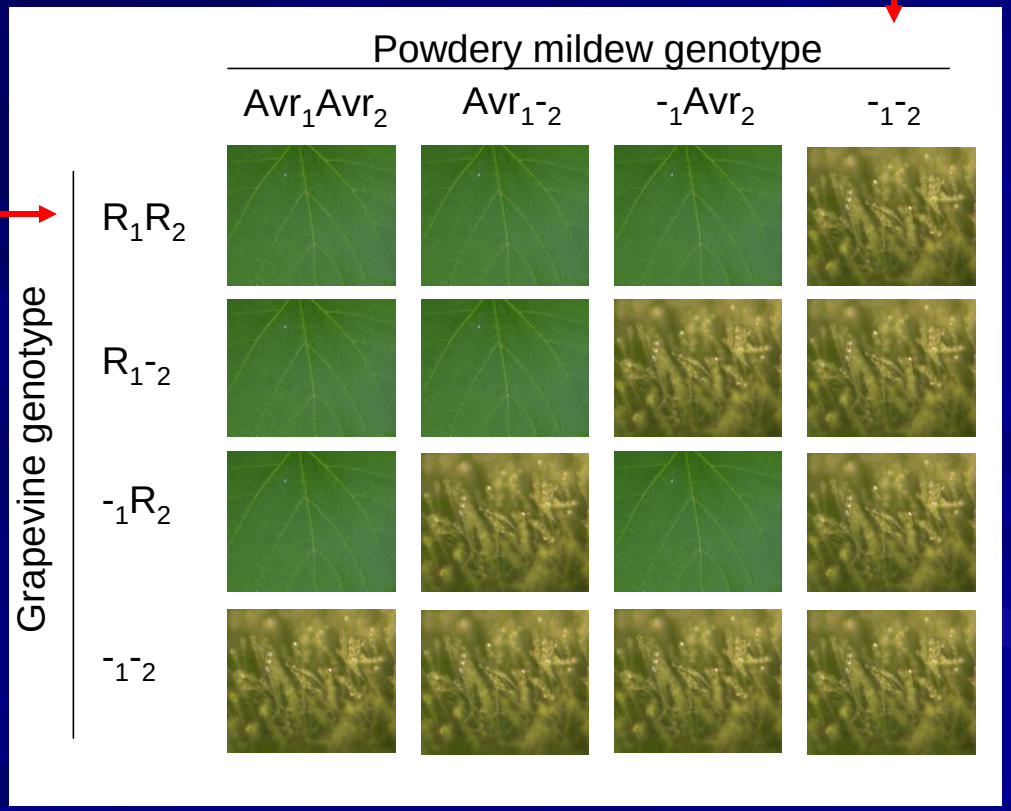
Durable?



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