

Genetic mapping of resistance to *Aphanomyces* root rot in alfalfa

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Aphanomyces root rot



Courtesy E. Holub

Seedling "corpses"
Acute phase



Courtesy C. Grau

Adult plants lack lateral and fibrous roots, no nodules. Foliage stunted, yellow. Chronic phase.

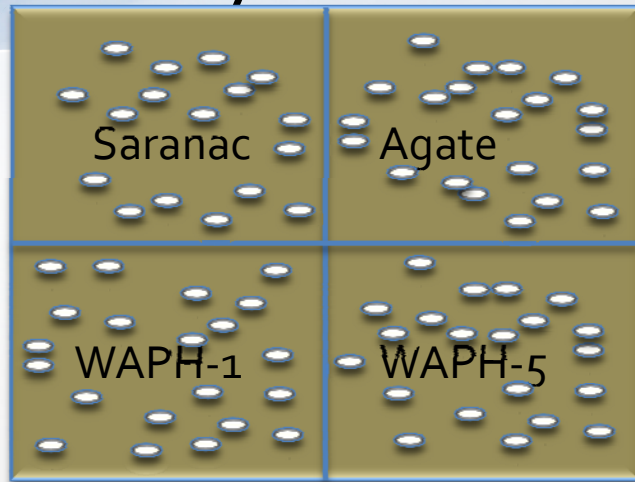
Aphanomyces root rot



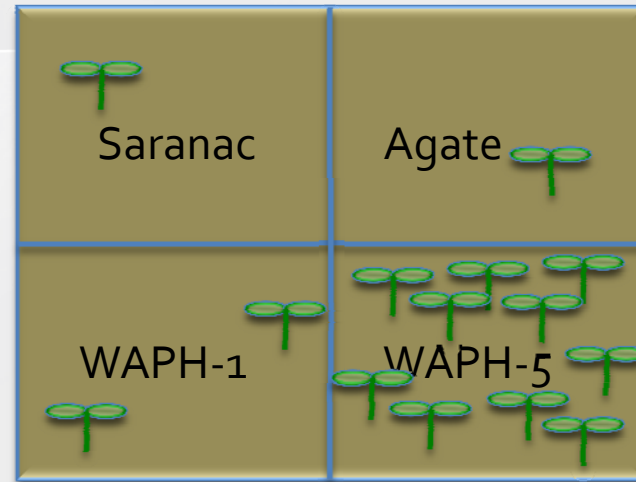
Research questions

- Survey of MN and NY soils for APH and PRR
 - What races are found in alfalfa fields?
- Two races identified by host genetics
 - Can markers be identified linked to resistance for marker assisted breeding?
- Possible failure of race 1+2 resistant cultivars
 - Are there additional races?

Bioassay for APH



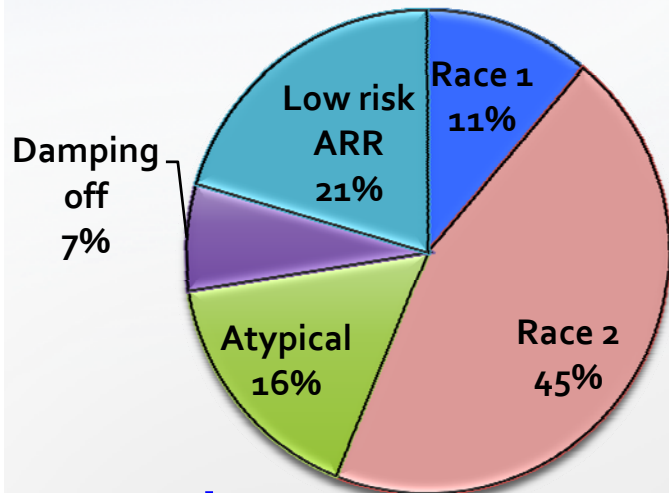
Plant checks in field soil sample.
After plants germinate, flood soil for 4 days.



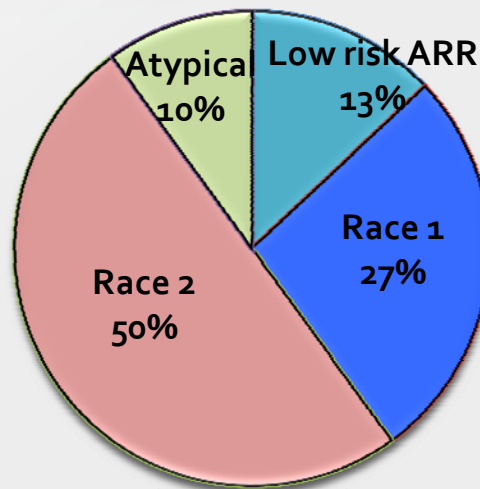
Score symptoms at 21 d after
planting.

Cultivar	PRR	APH Race 1	APH Race 2
Saranac	S	S	S
Agate	R	S	S
WAPH-1	R	R	S
WAPH-5	R	R	R

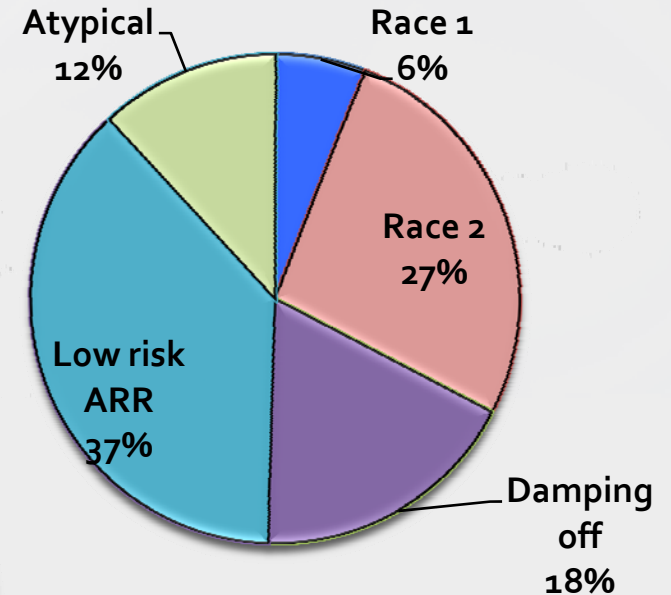
Race 2 is most common



Minnesota
N=44



New York
N=40



Wisconsin
N=227

Gibbs, 2009

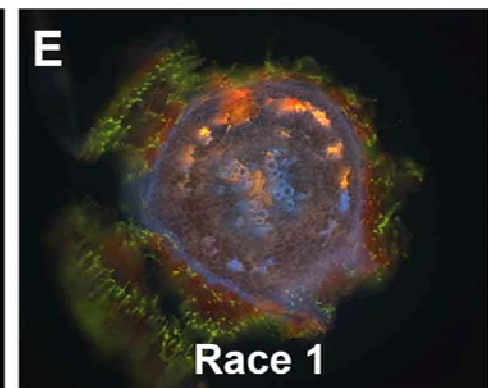
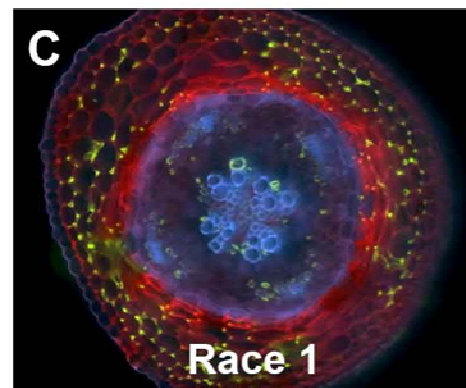
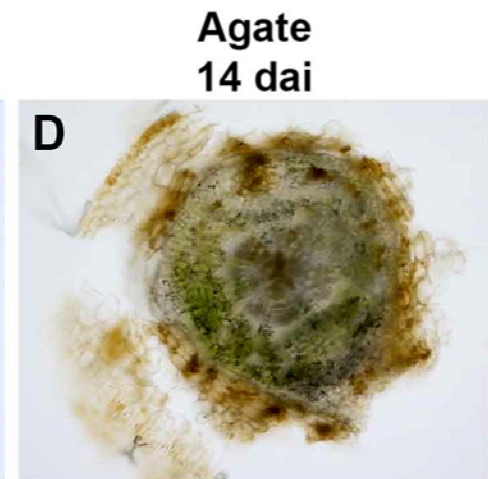
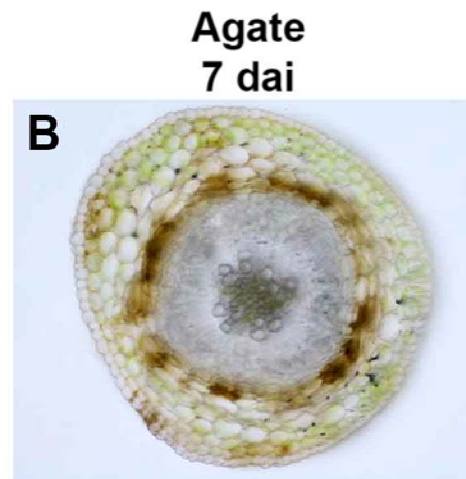
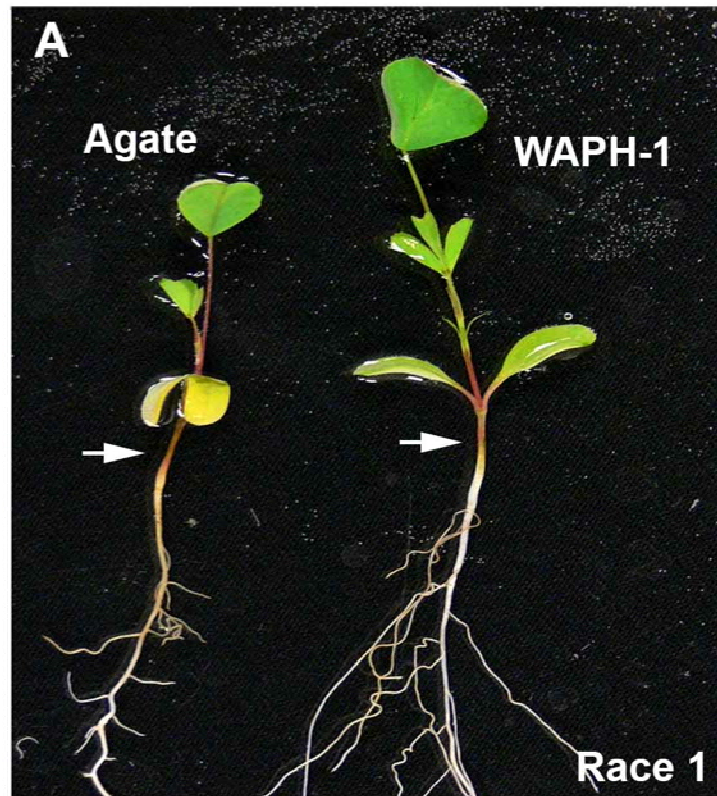
Results from atypical soils

State	Soil	WAPH-1 resistant plants (%)	WAPH-5 resistant plants (%)	Predominant race
MN	GRE	6	15	Undetermined
MN	JIM	0	5	Undetermined
MN	WAS	14	28	Undetermined
NY	CAR	13	32	Undetermined
NY	ROY	0	21	Undetermined

60 pure Aphanomyces strains tested

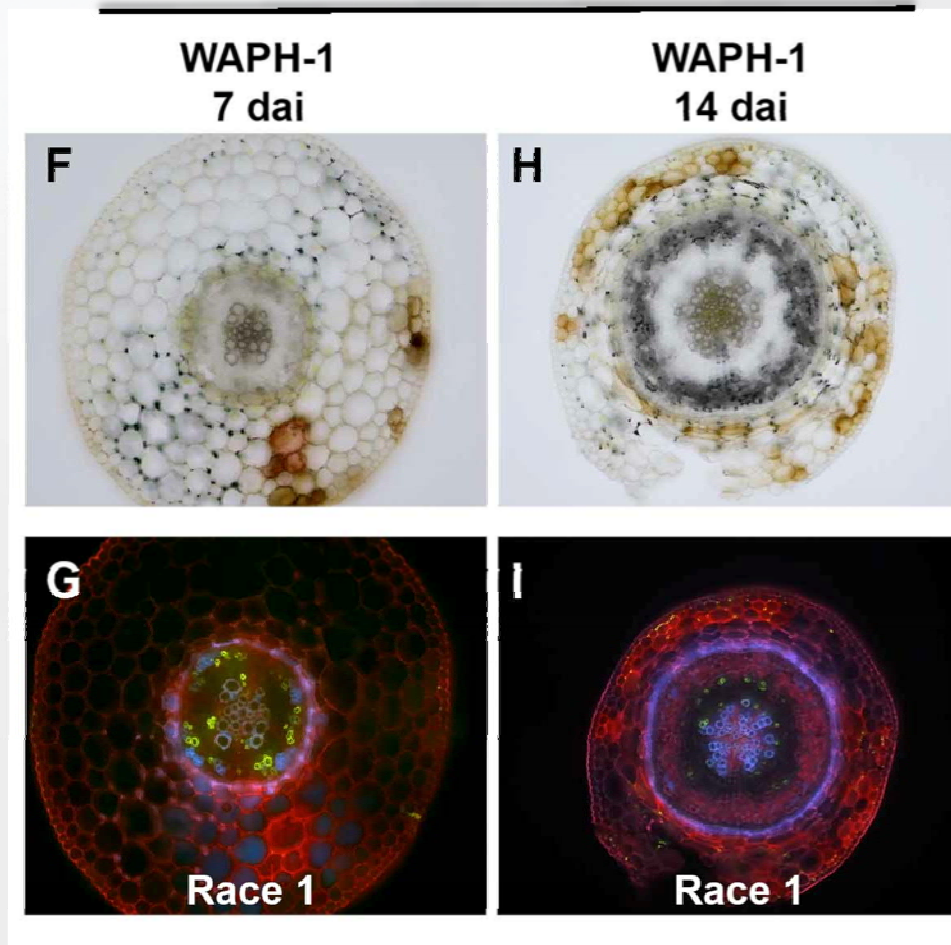
- 4 check cultivars, 4-6 reps, 30 seeds/rep
- All strains read as either race 1 or race 2
- Resistance in WAPH-5 was not overcome
- What is the nature of resistance?

APH Susceptible Reaction



APH Resistant Reaction

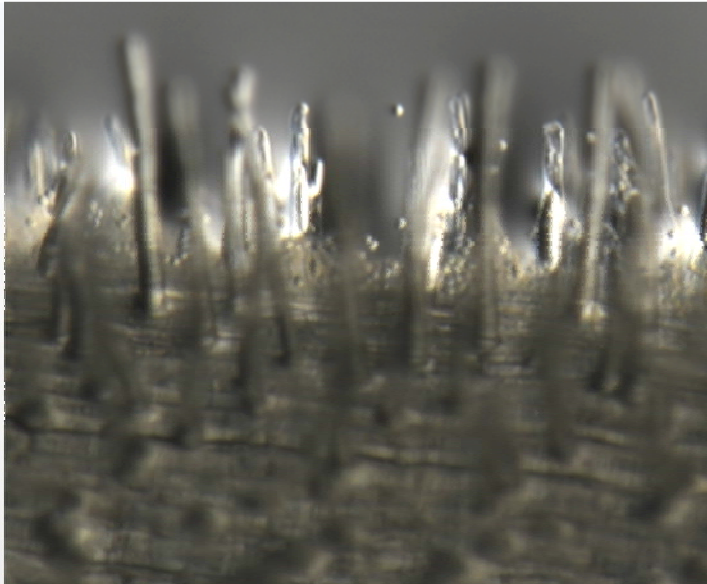
Race-specific resistance



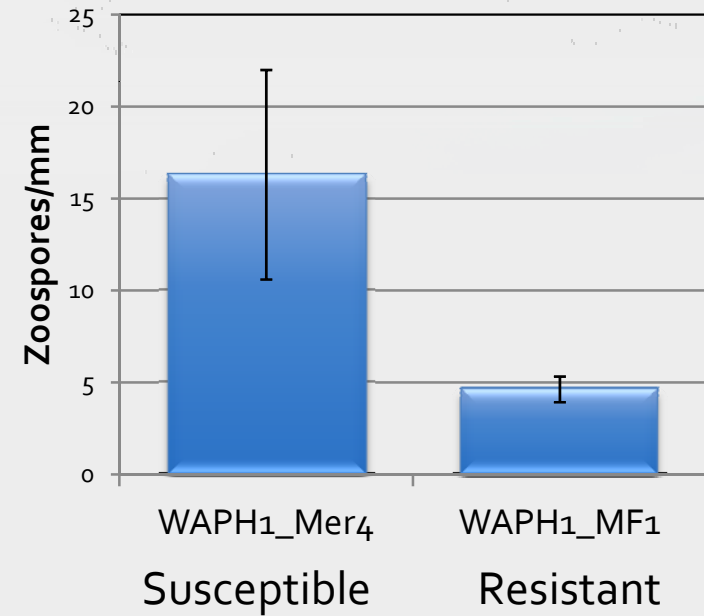
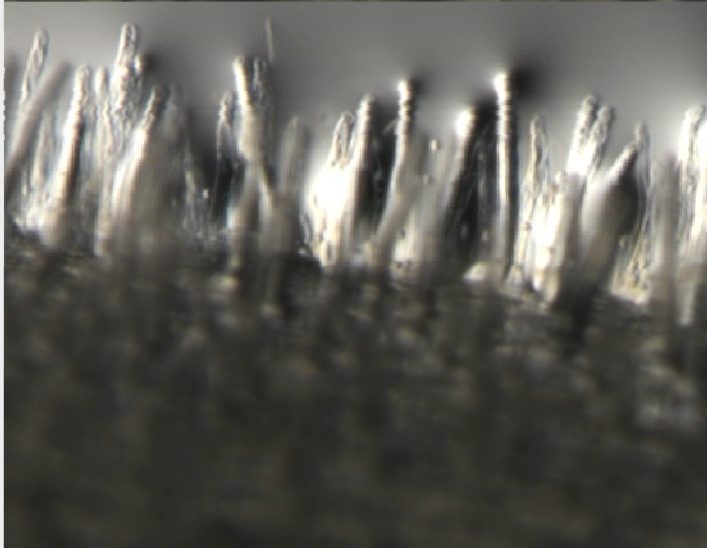
Hypersensitive response indicates defense is triggered by NBS-LRR type resistance gene.

Pre-infection resistance?

Susceptible



Resistant

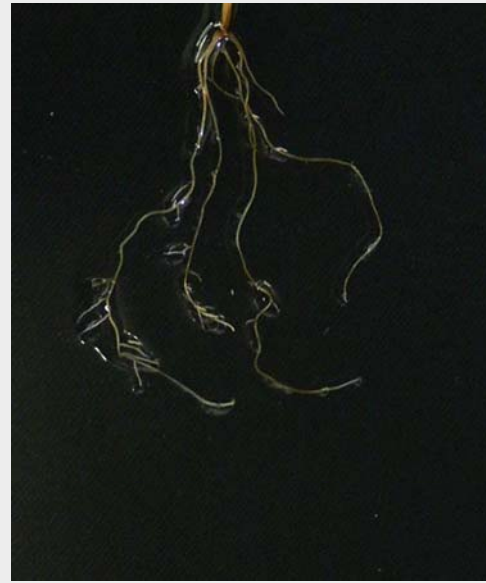


Developing MAS for APH

- Selected resistant and susceptible seedlings
 - WAPH-1 (race 1), WAPH-5 (race 1+2), 53V54 (race 1+2)
 - Retested with vegetative cuttings
- Symptoms correlated with oospores counts and Aphanomyces DNA content

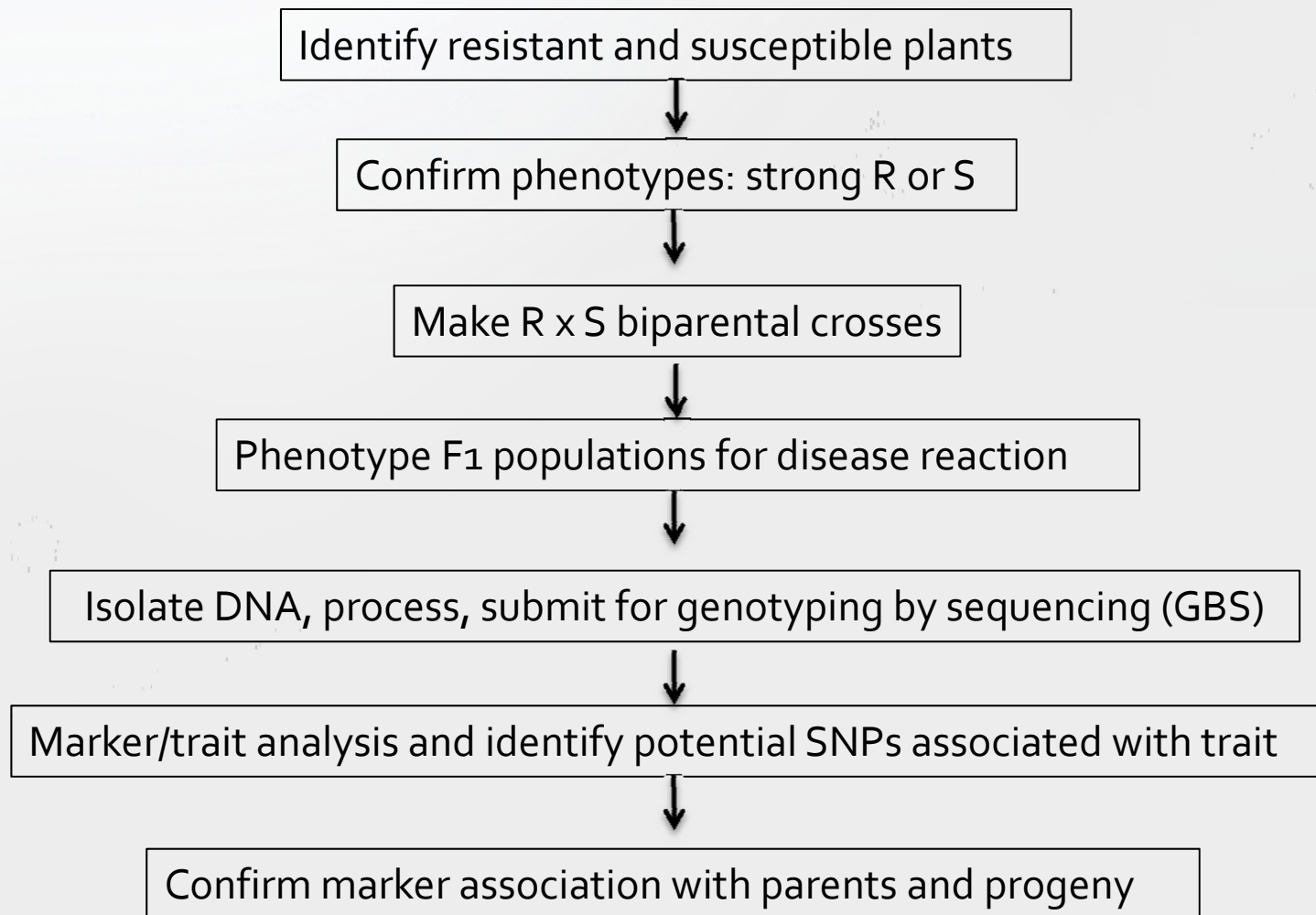


Resistant

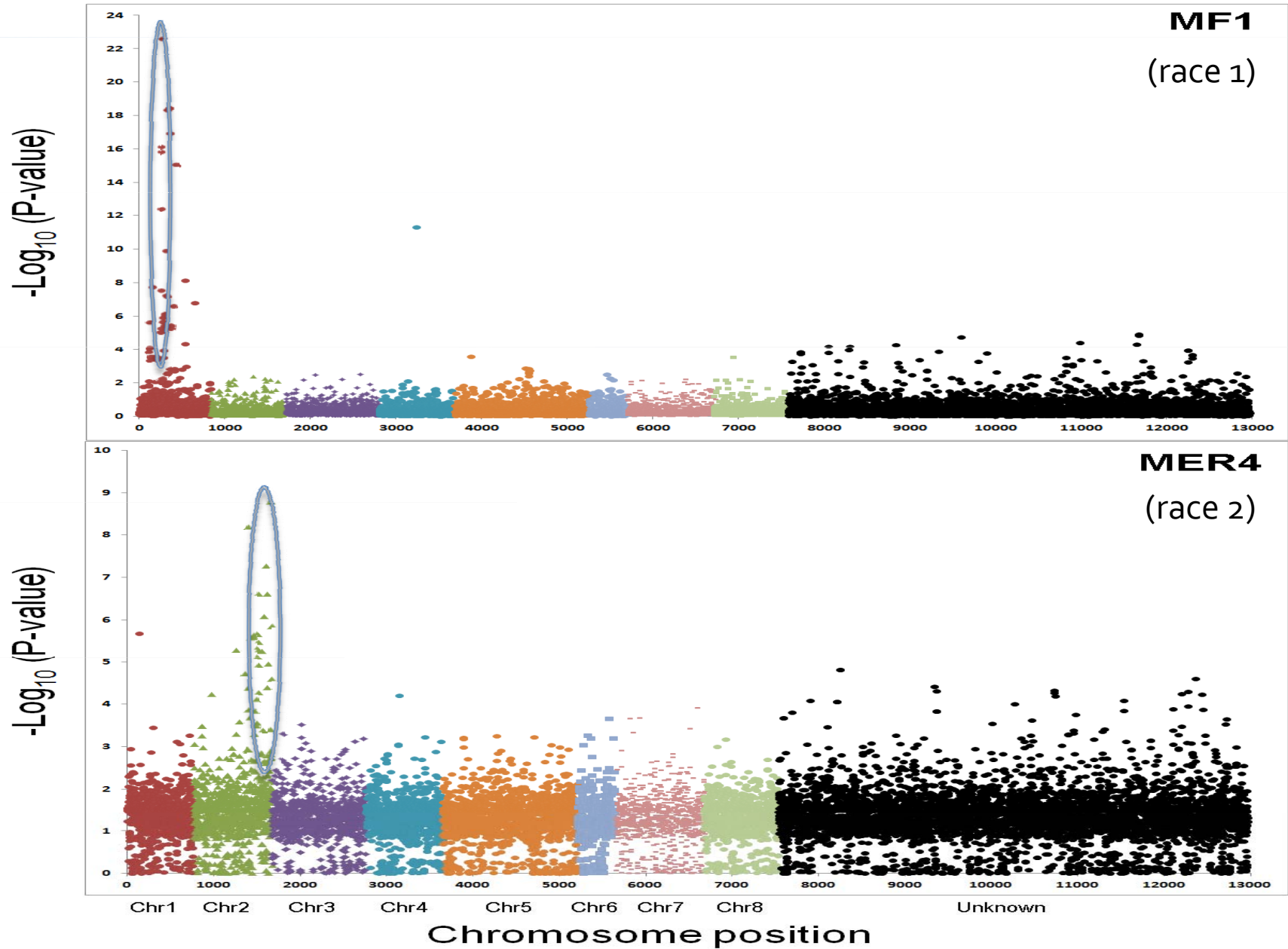


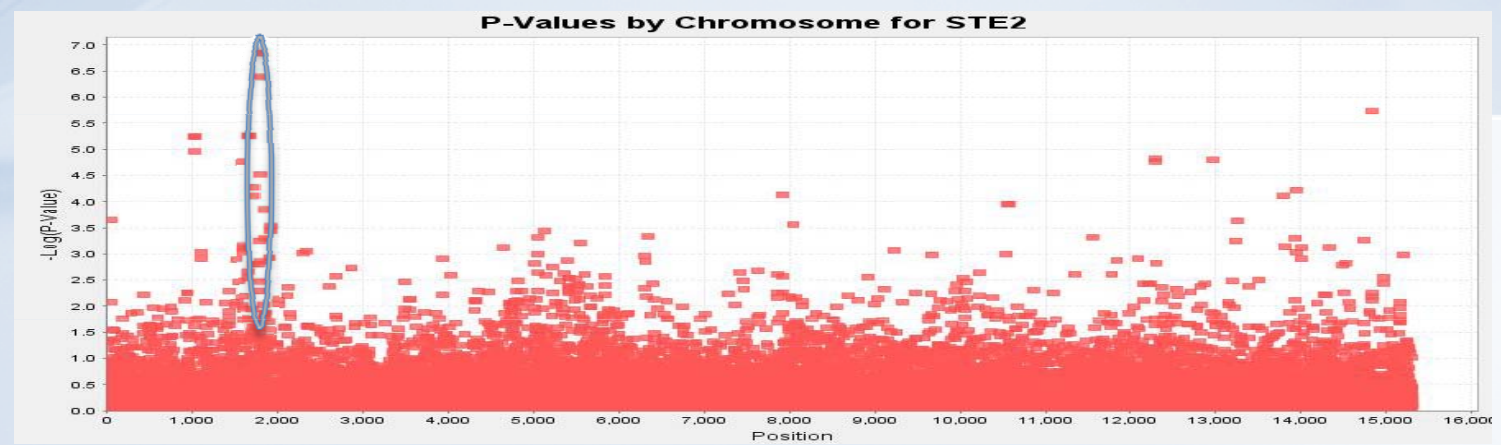
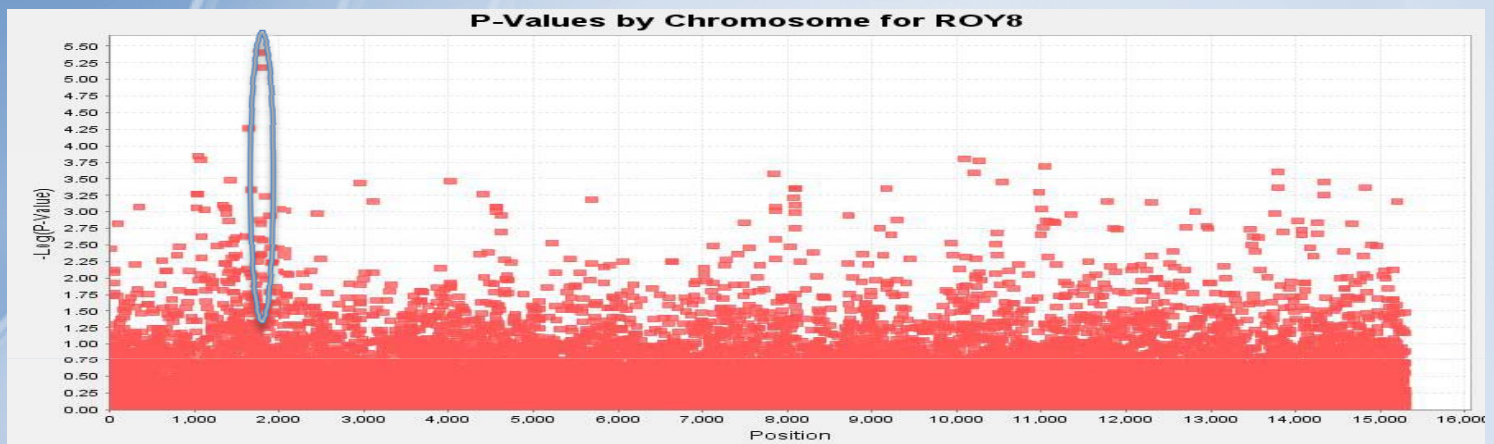
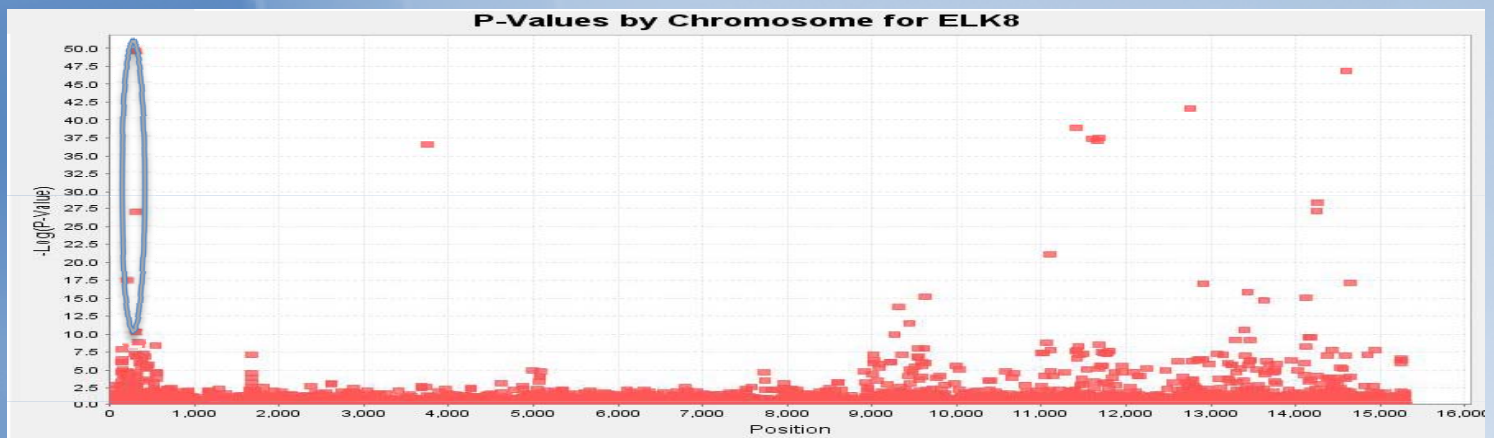
Susceptible

Identify DNA markers associated with race-specific resistance to APH



Genotyping by sequencing to map R genes





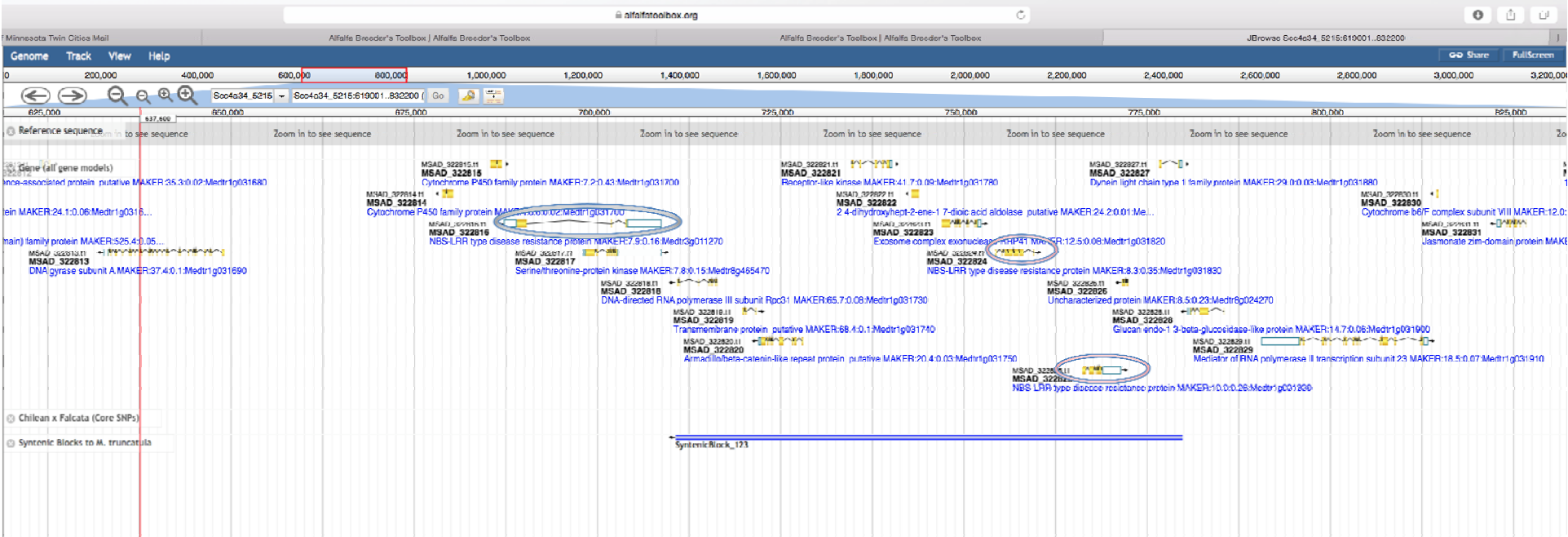
Reaction of strains on F₁ population (L85)

MER ₄	GRE ₅	ROY ₈	STE ₂	JIM ₁	Number of plants (of 269)
R	R	R	R	R	2
S	S	S	S	S	16
R	S	S	S	S	6
S	R	S	S	S	2
S	S	R	S	S	5
S	S	S	R	S	2
S	S	S	S	R	3

Suggests that there are multiple R genes in WAPH-5.

Intercross plants with resistance to a single strain to develop new checks.

Genes near MF-1 (race 1) marker



Pennsylvania field soil
Flooded for 4 days (6-9 days after planting)
Photo at 12 days after planting

Commercial
race 1
resistant
variety

WAPH-5
race 1+2 resistant
check

Susceptible
checks

Commercial
race 1+2
resistant
variety

WAPH-1 race 1
resistant check



Conclusions

- Resistance appears to be conditioned by dominant R-genes with additional pre- and post-infection defense mechanisms
- R-genes may be clustered on chromosome 1 and 2
- WAPH-5 may have multiple R-genes for APH
- Strains of *A. euteiches* with diverse virulences are found in field soil
- Developers of resistant varieties should use diverse isolates to have sufficient field resistance to *Aphanomyces* root rot

A wide-angle photograph of a lush green field, possibly a crop field, stretching to a distant horizon. The sky is filled with large, white, puffy clouds, with some darker, greyer clouds on the right side. The sun is visible through the clouds on the left, creating a bright glow. The text "Thank you!" is overlaid in the center of the image in a light green, outlined font.

Thank you!