



BREEDING
Insight



Identification of SNP markers associated with resistance to *Aphanomyces* root rot in alfalfa

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Aphanomyces root rot (*Aphanomyces euteiches*)

- Important seedling damping off and root rot pathogen of pea (*Pisum*), alfalfa, snap bean (*Phaseolus*), clover (*Trifolium*)
 - Persists in soil for many years
 - Favored by wet soil conditions, heavy soils
 - Stobilurin fungicides can protect alfalfa seedlings

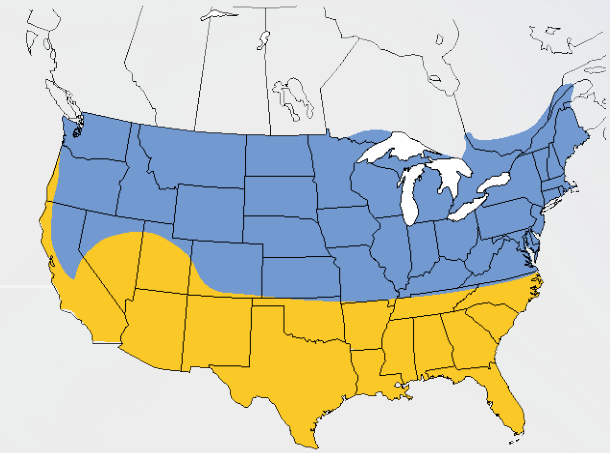


Aphanomyces root rot affects seedling and adult alfalfa plants



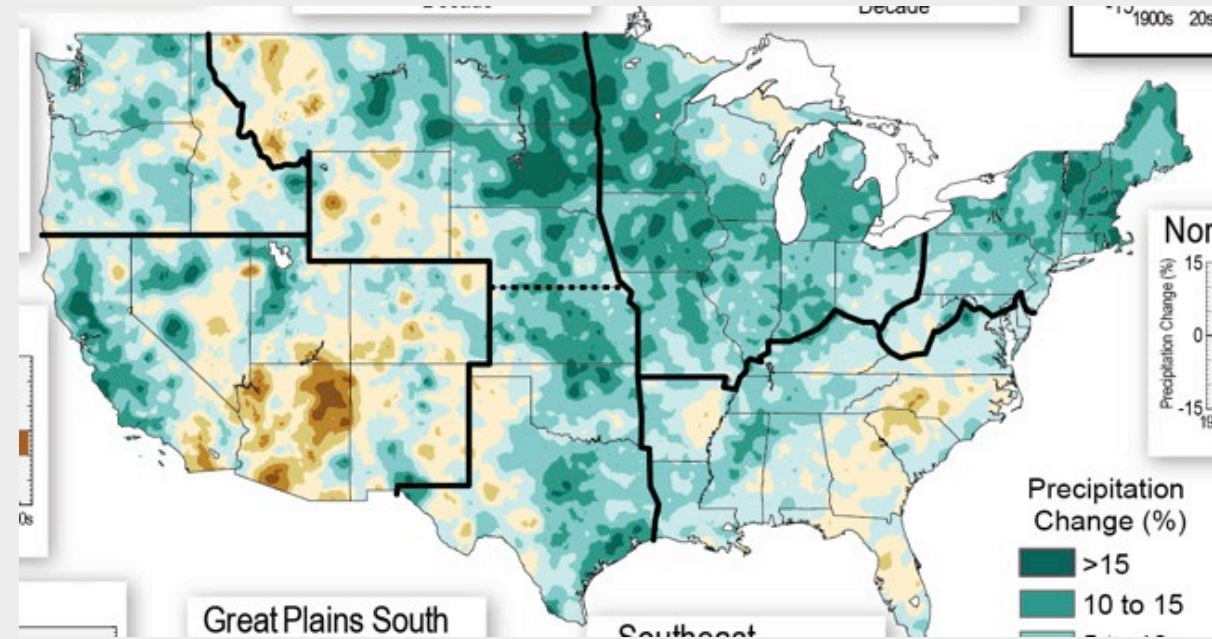
Seedling "corpses"
Acute phase

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



Wet soil syndrome

- Poor seedling emergence due to
 - Seed rot (*Pythium* species, *Fusarium* species)
 - Seedling damping-off (*Phytophthora medicaginis*, *Rhizoctonia solani*)
 - Seedling root rot (*Pythium* species, *P. medicaginis*, *Aphanomyces euteiches*, + others)
- Poor nodulation
- Hypoxia (low oxygen concentration)



Aphanomyces root rot



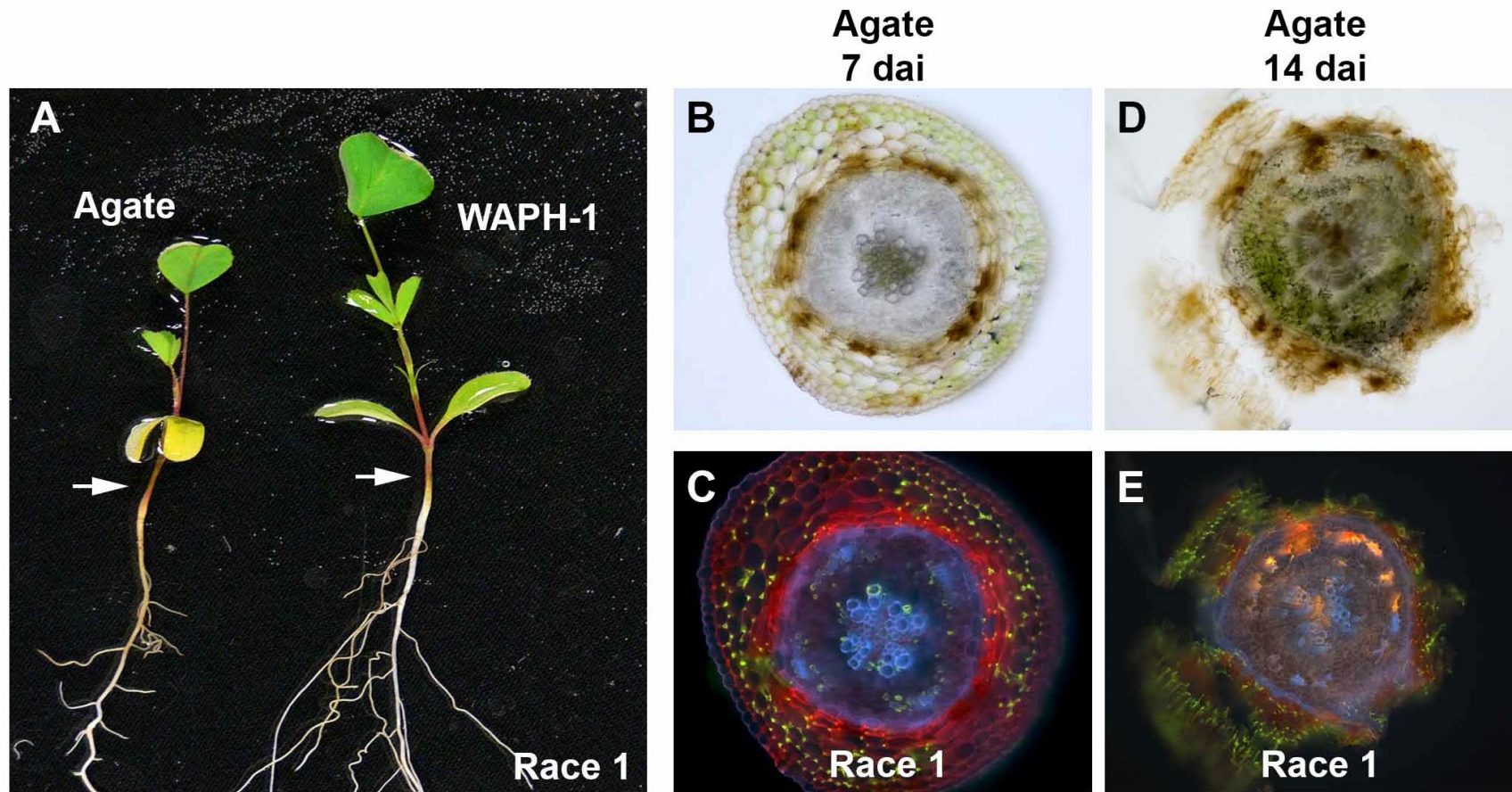
Development of APH resistant checks

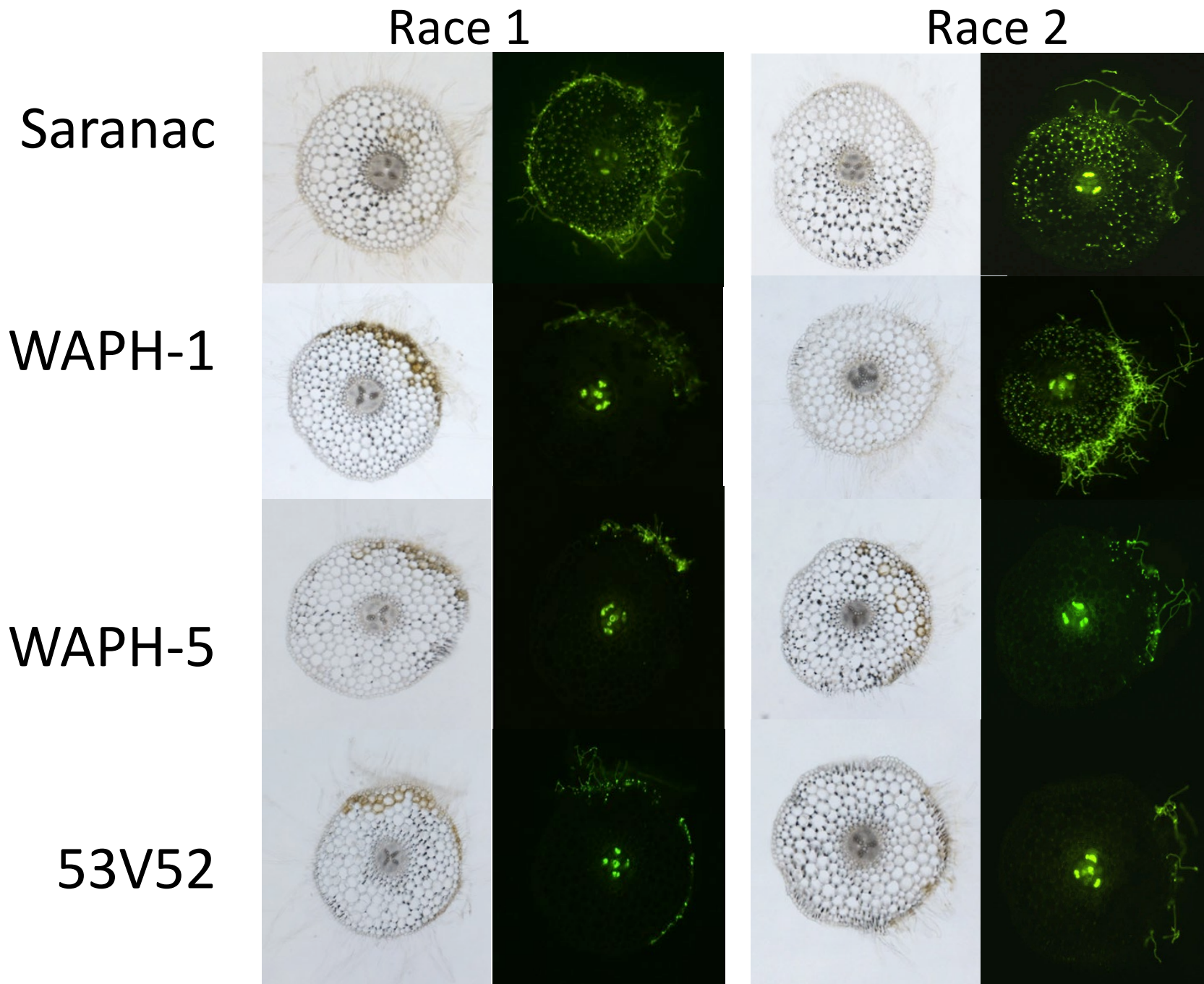
- Dr. Craig Grau (U Wisc) (1990's)
- WAPH-1: two cycles of selection from named cultivars
 - Cycle 1: 50 plants selected from 11 cultivars in Marshfield and 50 plants selected from 11 cultivars after inoculation
 - Cycle 2: 50 APH race 1 resistant plants from C₁ x 3 'Apollo' and 1 MSR193 (PRR resistant)
- Registration of WAPH-1: Crop Sci. 32:287-288.
- Commercial cultivars with race 1 resistance selected from elite breeding materials

Development of race 1+2 resistance: WAPH-5

- Race 1 resistant cultivars had significant *Aphanomyces* root rot
 - *Pythium* root rot was also observed
- ~300 Plant Introductions were screened for resistance to NC-1 (race 2)
 - PI 468018 ('Grimm,' Canada), PI 439006 ('Hamadany,' Syria), and PI 464781 (Turkey) were intermated with breeding populations derived from WAPH-1. Selections also were made for resistance to *P. medicaginis*.
 - Three cycles of phenotypic recurrent selection in the greenhouse (WI-98) and two cycles in race 2 infested fields at the UW Marshfield research station.
- NAAIC 1994, "Aphanomyces Root Rot of Alfalfa - Looking Back 10 Years"
- Race 2 resistance in commercial cultivars developed from elite materials by each company
- Is resistance in WAPH-5 distinct from resistance in commercial cultivars?

APH Susceptible Reaction



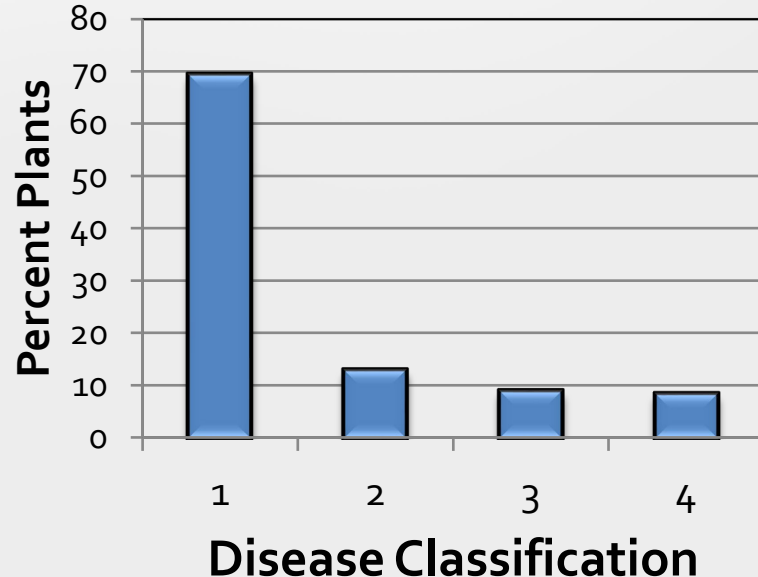


Hypersensitive response indicates defense is triggered by NBS-LRR type resistance gene; secondary defenses.

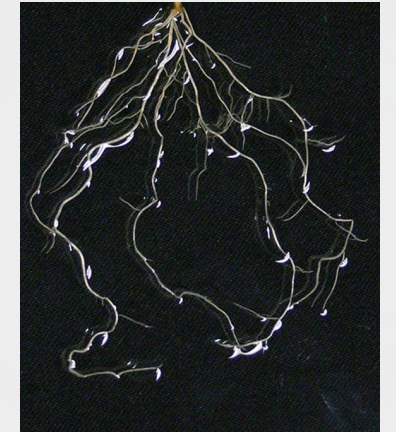
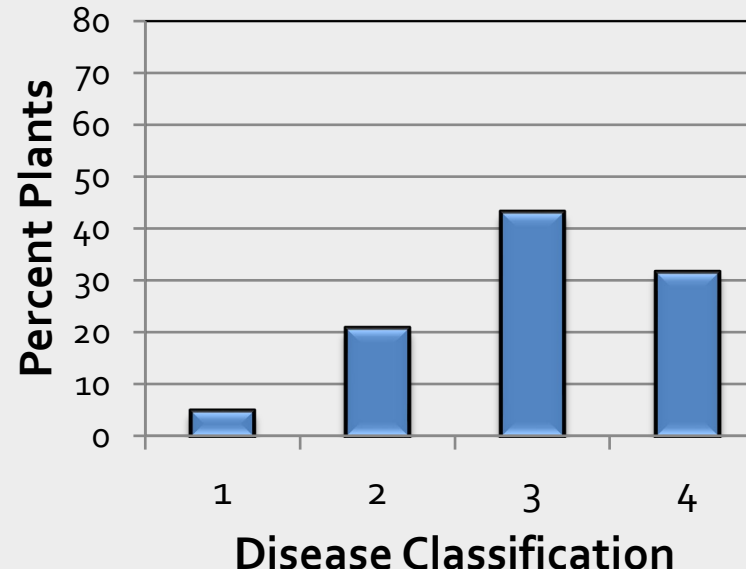
Developing mapping populations for APH

- Selected resistant and susceptible seedlings
 - WAPH-5 (race 1+2), 53V52 (race 1+2)
 - Retested with vegetative cuttings

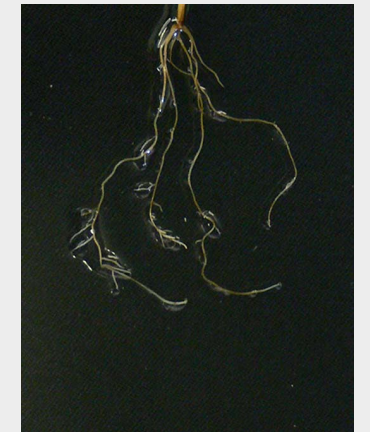
53V52 Line 85 race 1: 82% R,
18% S



53V52 Line 85 race 2: 26% R,
74% S



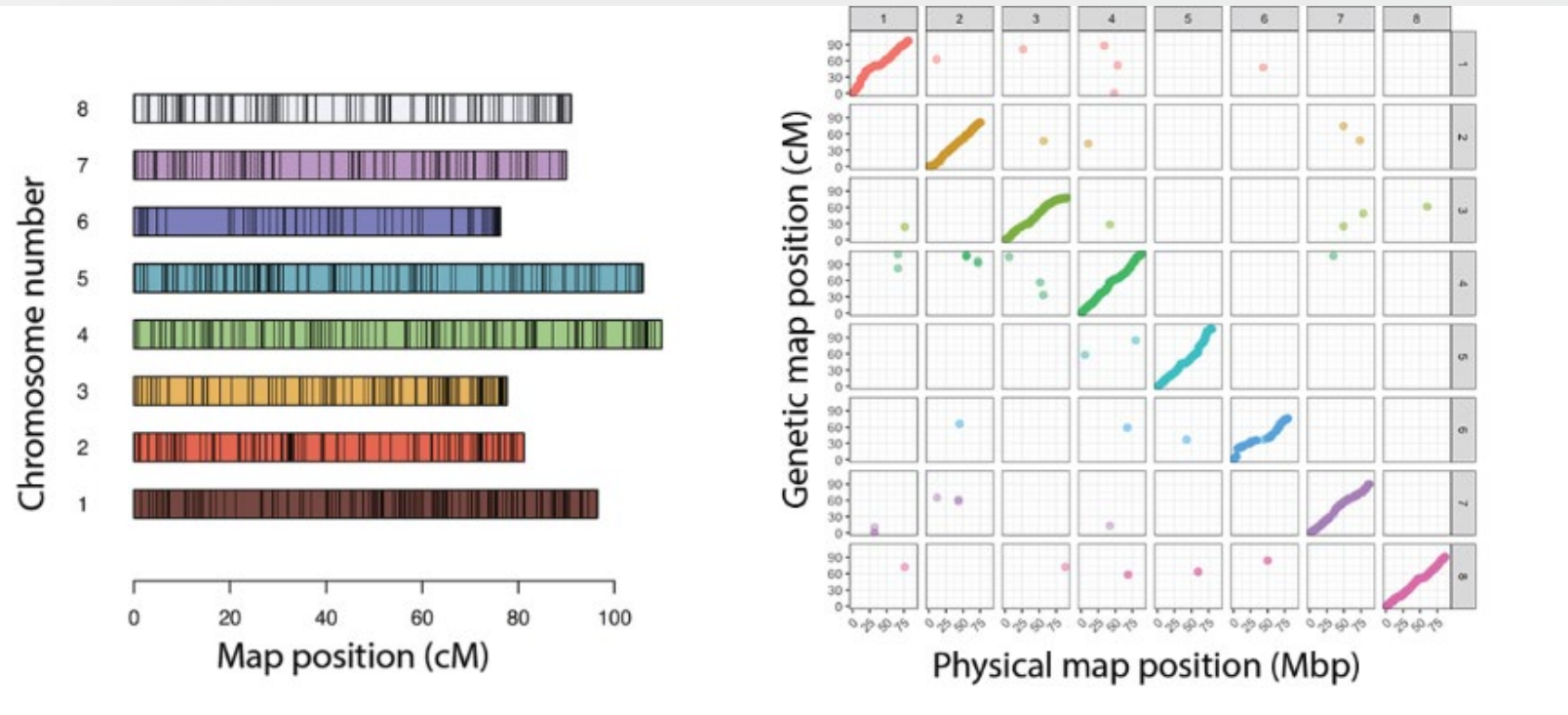
Resistant



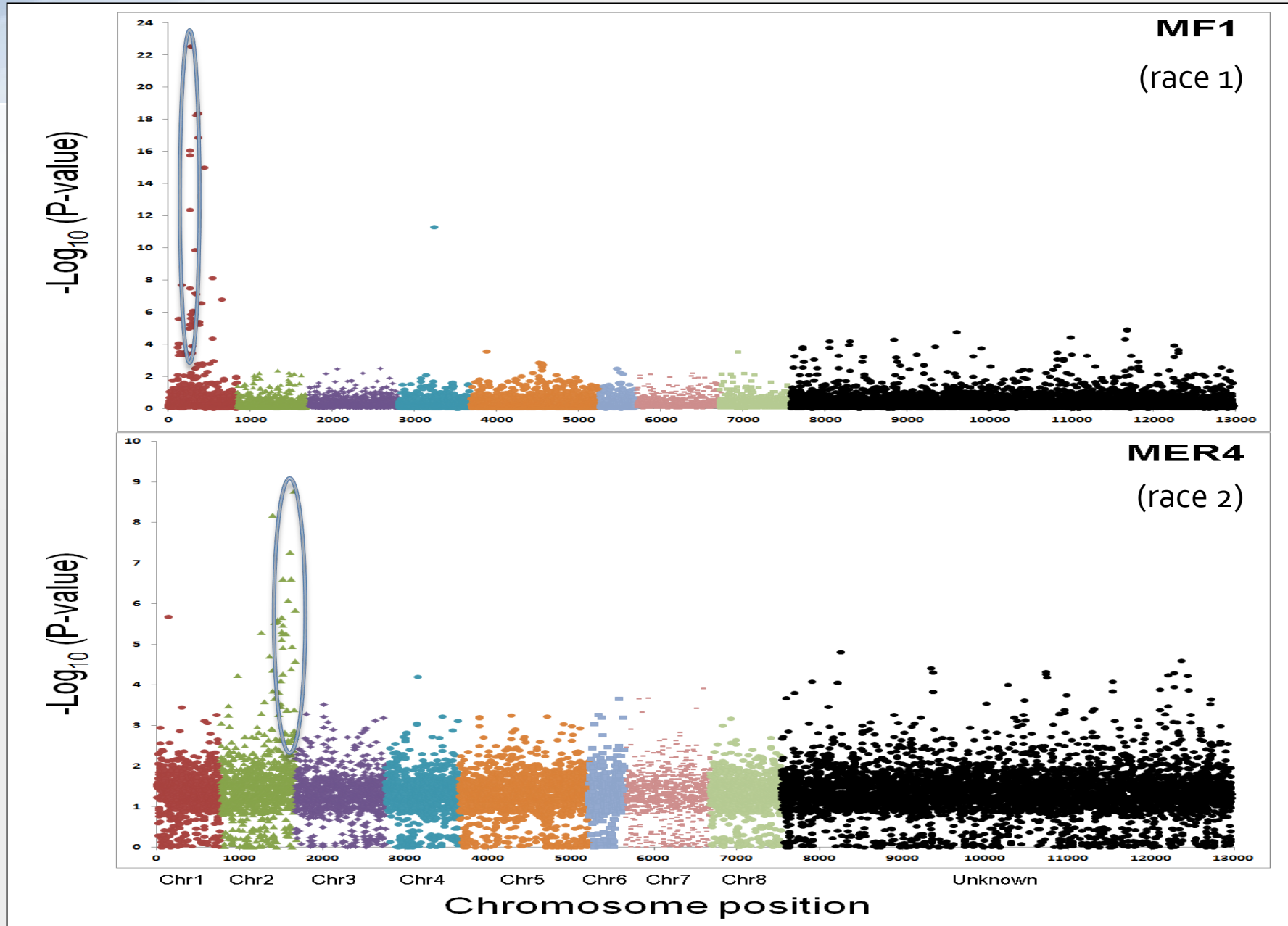
Susceptible

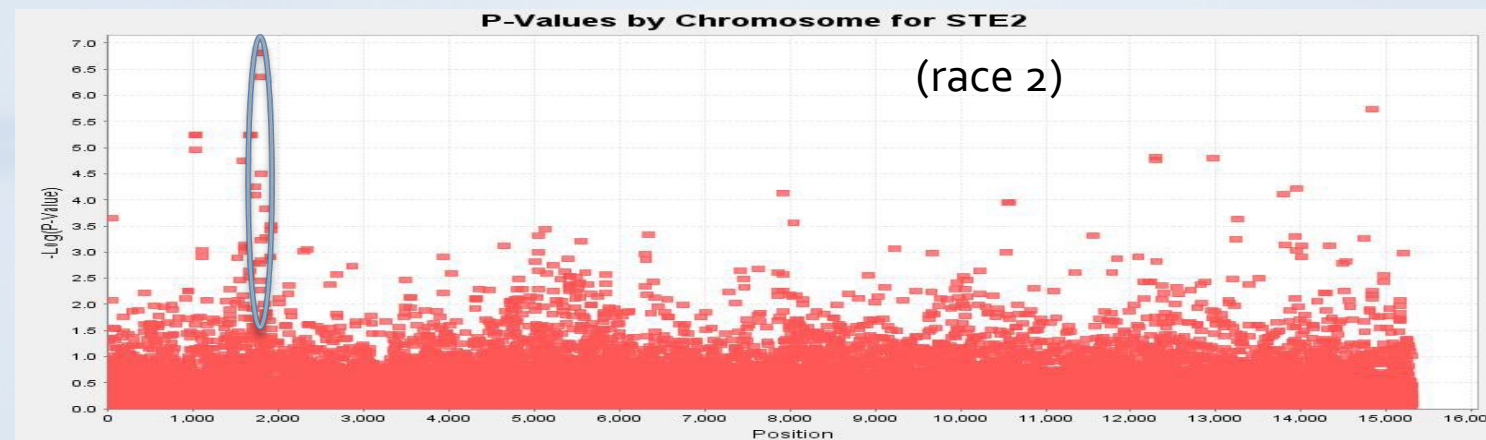
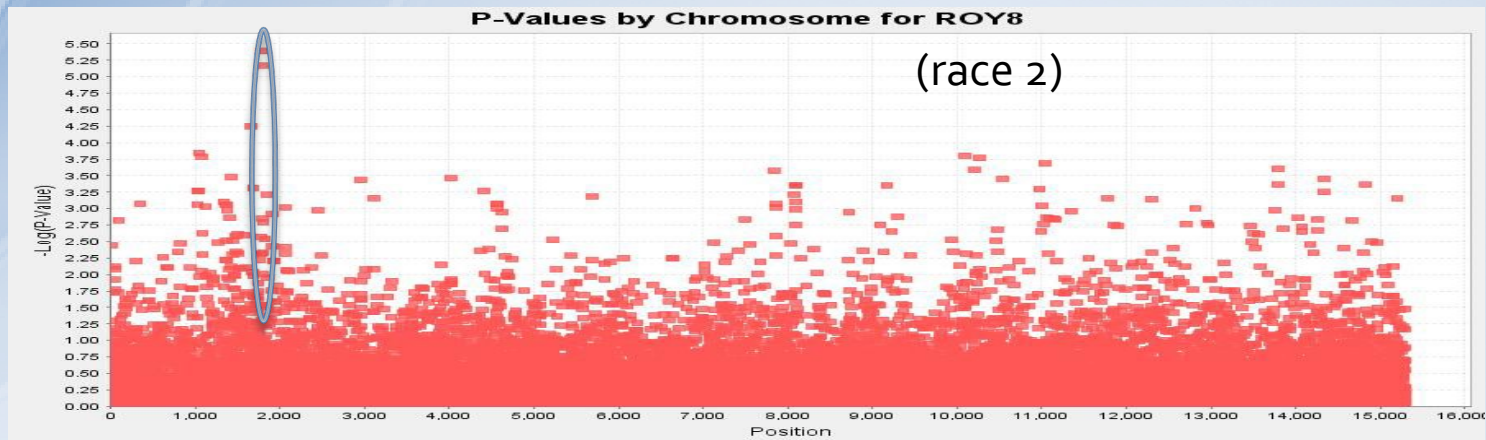
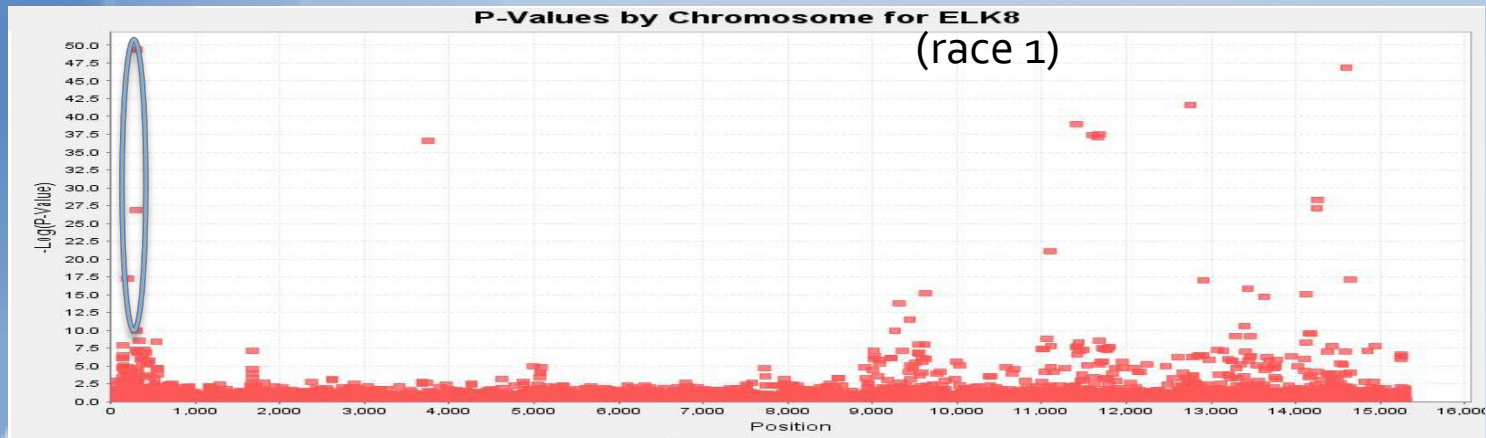
Mapping resistance loci

- Genotyping-by-sequencing (line 85; 53V52 F1 population)
- DaRTag markers (~3,000 SNP markers) developed by Breeding Insight
 - Line 85
 - Line 55/56 (WAPH-5 F1 population)

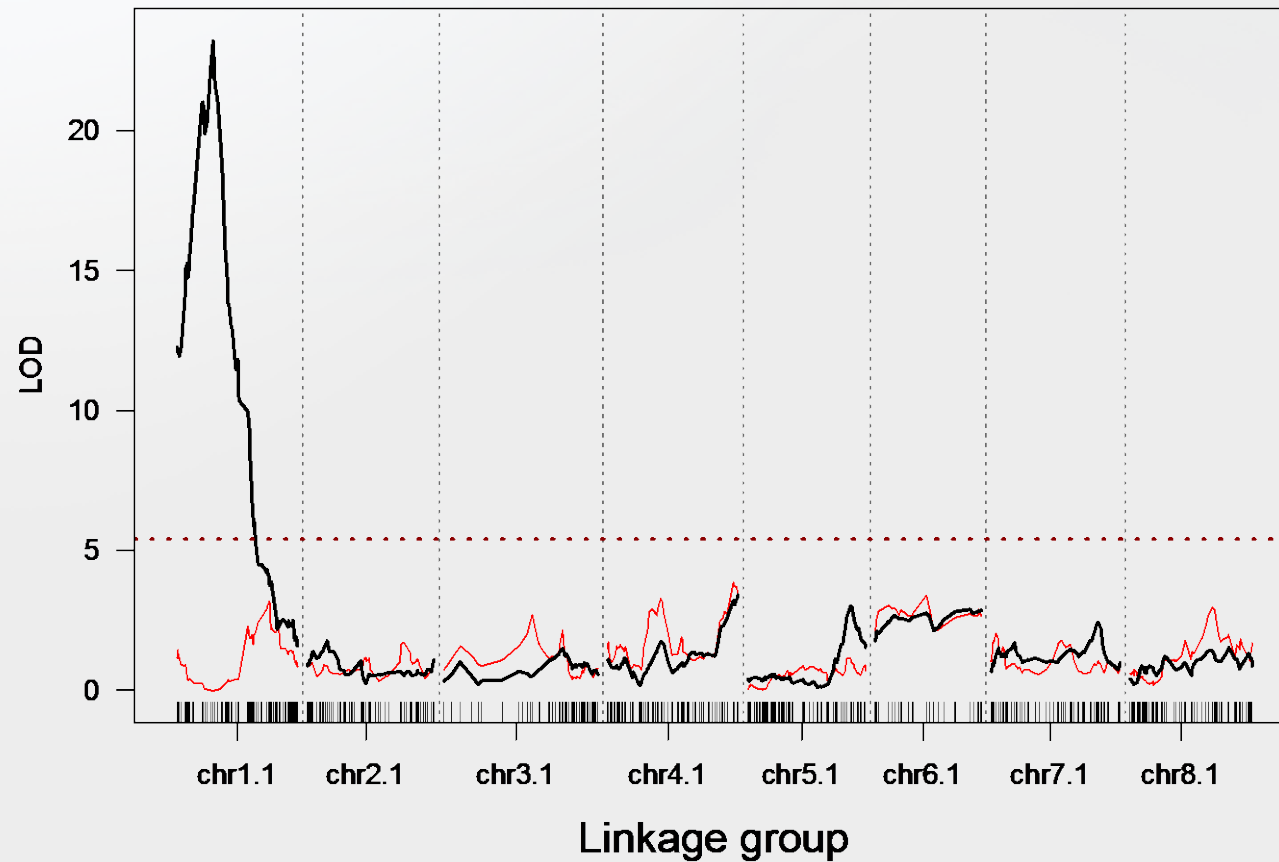


Genotyping-by-sequencing 53V52 population

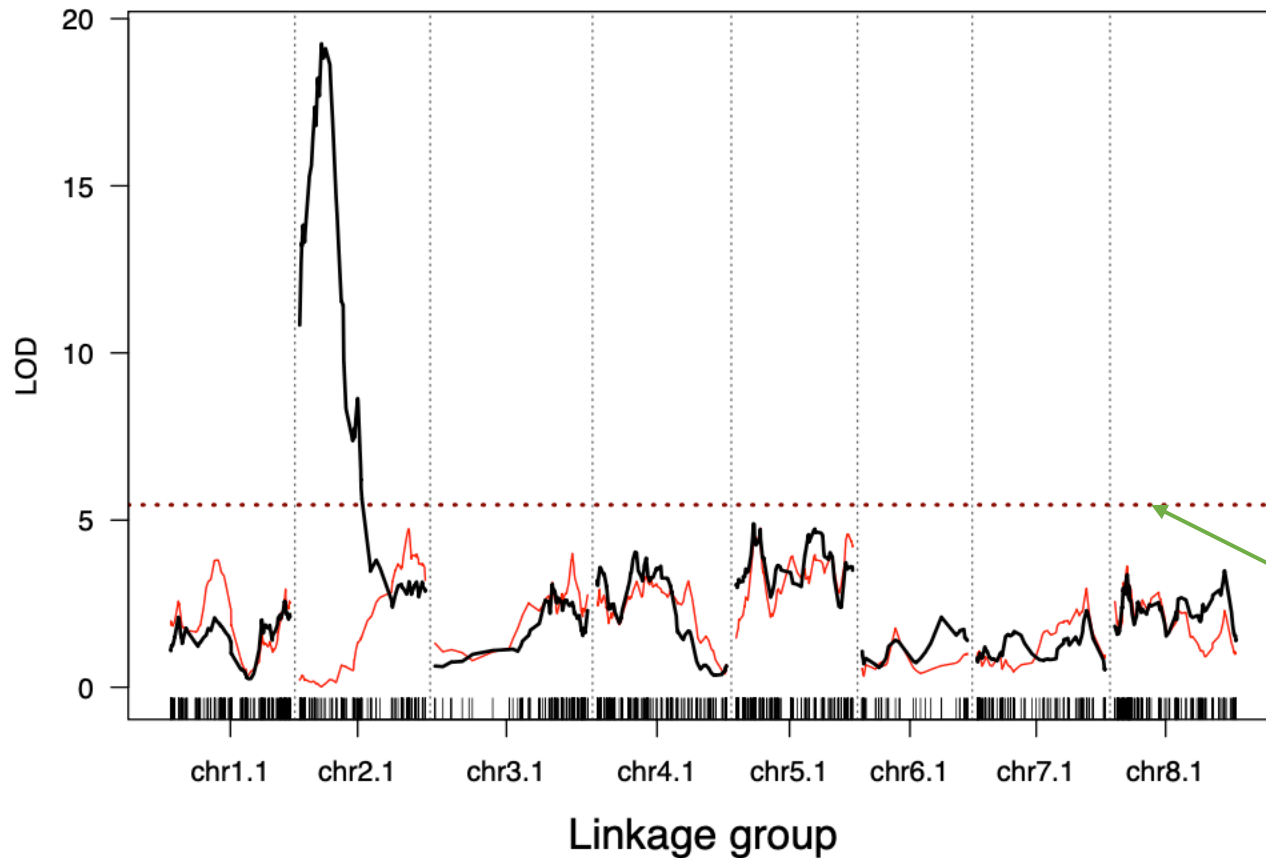




A major QTL was identified on chr1.1 for *Aphanomyces* race 1 in the 53V52 F1 population using DaRTag markers



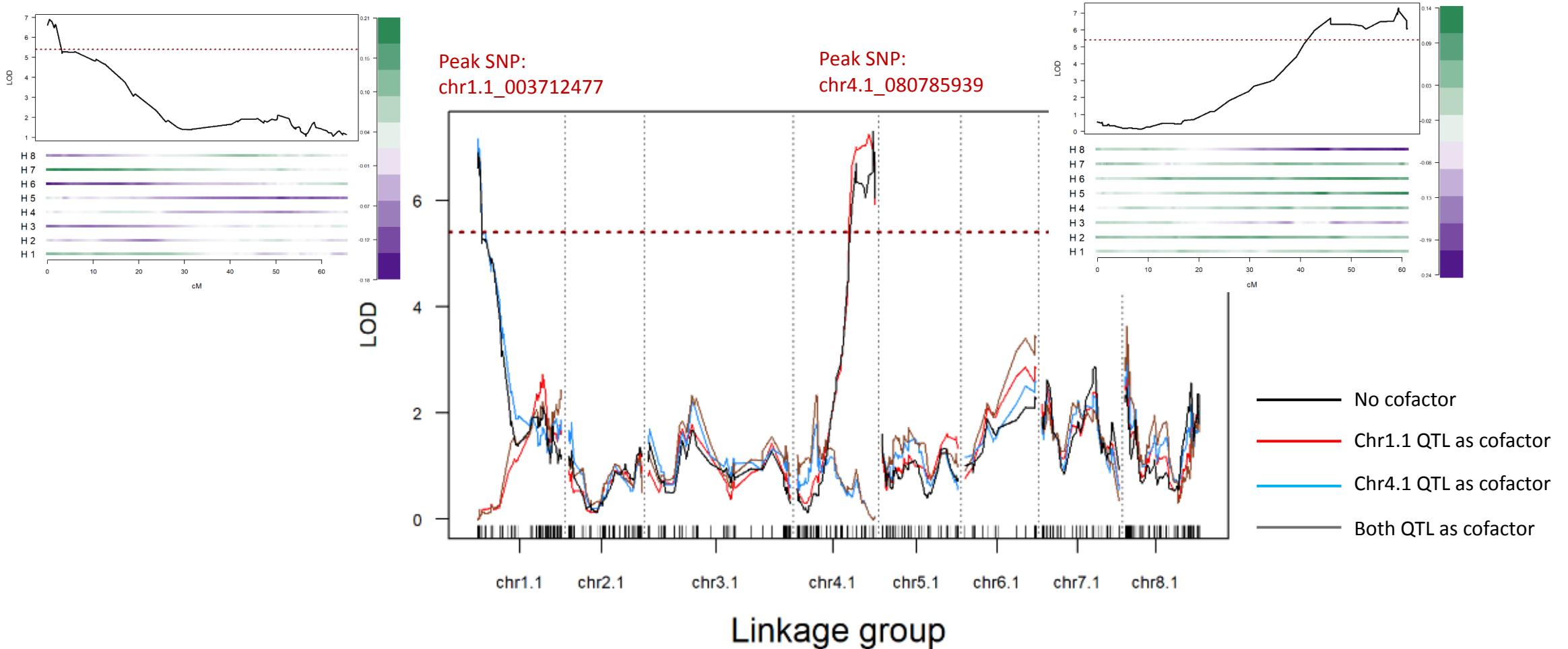
A major QTL was identified on chr2.1 using combined information from five stains (BLUEs)



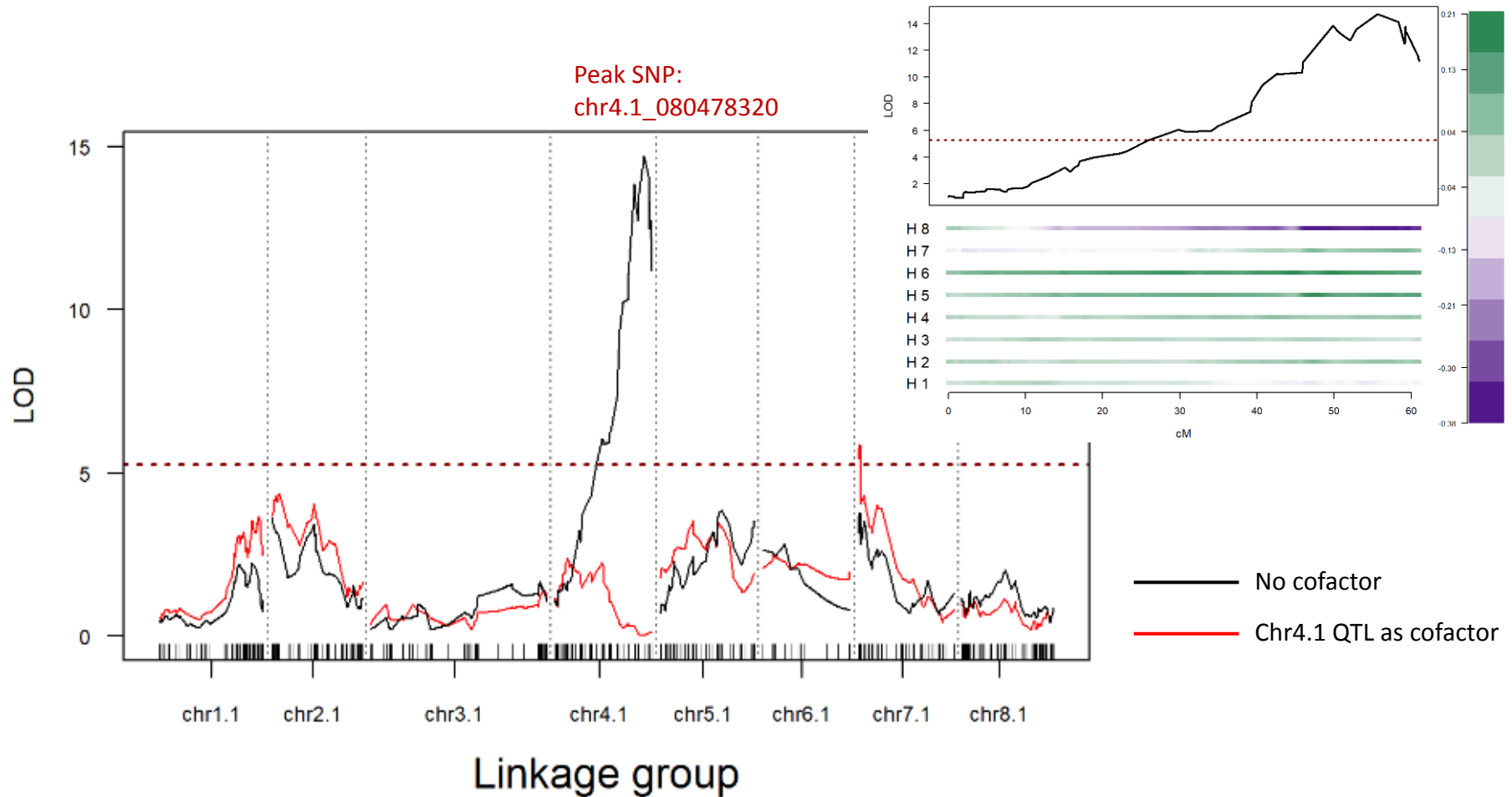
GBS and DaRTag markers identified the same loci for race 1 and race 2 resistance in the 53V52 population.

Significance thresholds

WAPH-5 population: QTL mapping identified resistance loci to *Aphanomyces* Race 1 on chr 1.1 & 4.1



WAPH-5: QTL mapping identified resistance loci to *Aphanomyces* Race 2 on chr 4.1 & 7.1



Summary of QTL mapping for Aphanomyces Race 1 and Race 2

Population	Disease (race/strain)	Chromosome	Peak SNP
52V52 F1	Aphanomyces Race 1	Chr1.1	chr1.1_019596141
52V52 F1	Aphanomyces Race 2 (BLUE, MER4, STE2)	Chr2.1	chr2.1_017012486
52V52 F1	Aphanomyces Race 2 (STE2)	Chr5.1	chr5.1_018083565
WAPH-5 F1	Aphanomyces Race 1	Chr1.1	chr1.1_003712477
WAPH-5 F1	Aphanomyces Race 1	Chr4.1	chr4.1_080785939
WAPH-5 F1	Aphanomyces Race 2	Chr4.1	chr4.1_080478320
WAPH-5 F1	Aphanomyces Race 2	Chr7.1	chr7.1_006496412

Race 2 resistance loci in WAPH-5 are distinct from resistance locus in 53V52.

Future goals

- Reduce the size of the QTLs through additional mapping studies
- Identify potential candidate R genes through transcript analysis
 - Verify genes through gene editing and/or transgenic expression
- Develop PCR based markers (or a mini-DaRTag array)
 - Identify plants with different race 1 and race 2 resistances
 - Determine the source of race 2 resistance in WAPH-5
- Evaluate disease resistance with the combination of chr2 and chr4 race 2 resistance



A wide-angle photograph of a lush green field, likely a crop field, stretching to a distant horizon. The sky is filled with large, white, fluffy clouds, with some darker, greyish clouds on the right side. The overall scene is bright and open. The text "Thank you!" is centered in the middle of the image in a white, sans-serif font with a thin green outline.

Thank you!