Alfalfa to get back into planetary boundaries

2025 world alfalfa congress
FRANCE REIMS-CHÂLONS

Alfalfa and soil quality

Sokolović D. & Stepic M.

Parallel Session 2

FROM 03 › TO 06 nov. 2025

Alfalfa to get back into planetary boundaries

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FRANCE REIMS-CHÂLONS

Mapping and Validation of Resistance to *Aphanomyces euteiches* in Alfalfa

Steve Damon, Steve Wagner, Tara Tarnowski, Lindsey Rushford, Justin Valletta

FROM 03 › TO 06 nov. 2025

DLF – Seeds and Science

DLF

Dansk Landbrugs Frøselskab

English translation:
Danish Agricultural Seed Company

Danish based seed company founded in 1872

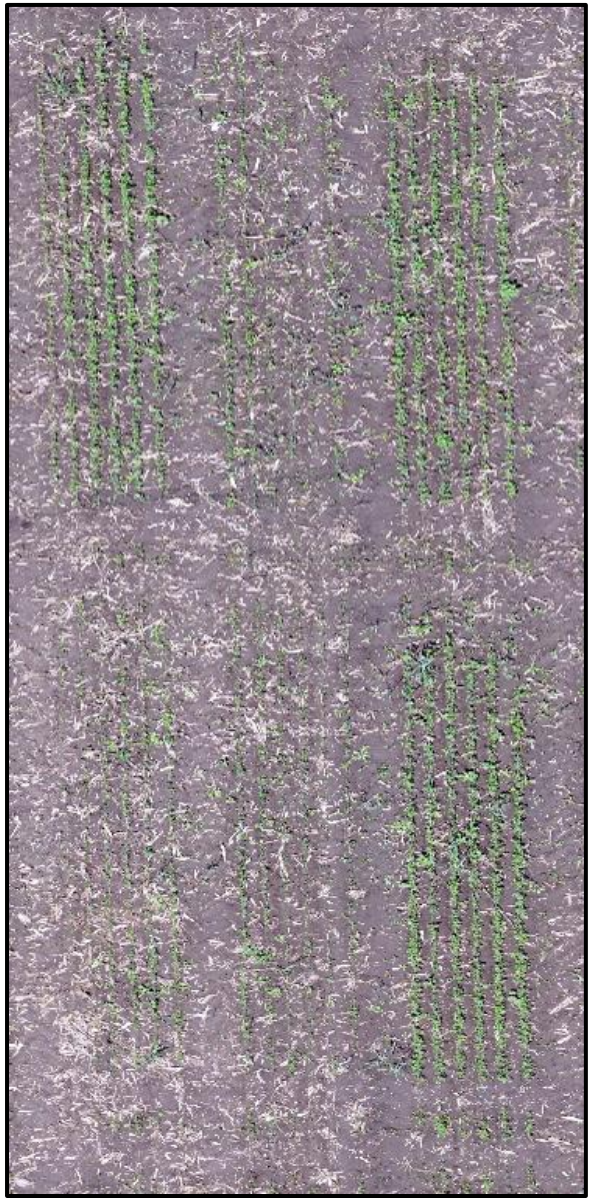
World market leader within temperate forage and turf seeds. Supplying to more than 100 countries

7th largest seed company in the world

Leading research and development program to deliver valued added products to benefit producers around the world

Why Work on Aphanomyces Root Rot?

- Most important disease in the Midwest dairy belt
- Climate extremes leading to excess soil moisture
- Progeny testing suggests dominance and simple inheritance
- Many isolates of race 2 being utilized in public and private sector research
- Questions around existence of race 3, in academia and the marketplace



Germplasm and Procedure

Fig 1a. Alfalfa seedlings inoculated with pipette tips containing agar plugs of *Aphanomyces euteiches* Race 2 and labeled with bird tags. Fig 1b. Resistant seedlings (blue circles) identified by green internode and susceptible seedlings (red circles) identified by necrotic lesion on internode.

- Capture data on individual seedling plants
- Improve survivability of susceptible seedlings
- High correlation to standard protocol

Four biparental populations

A142156/C16-432

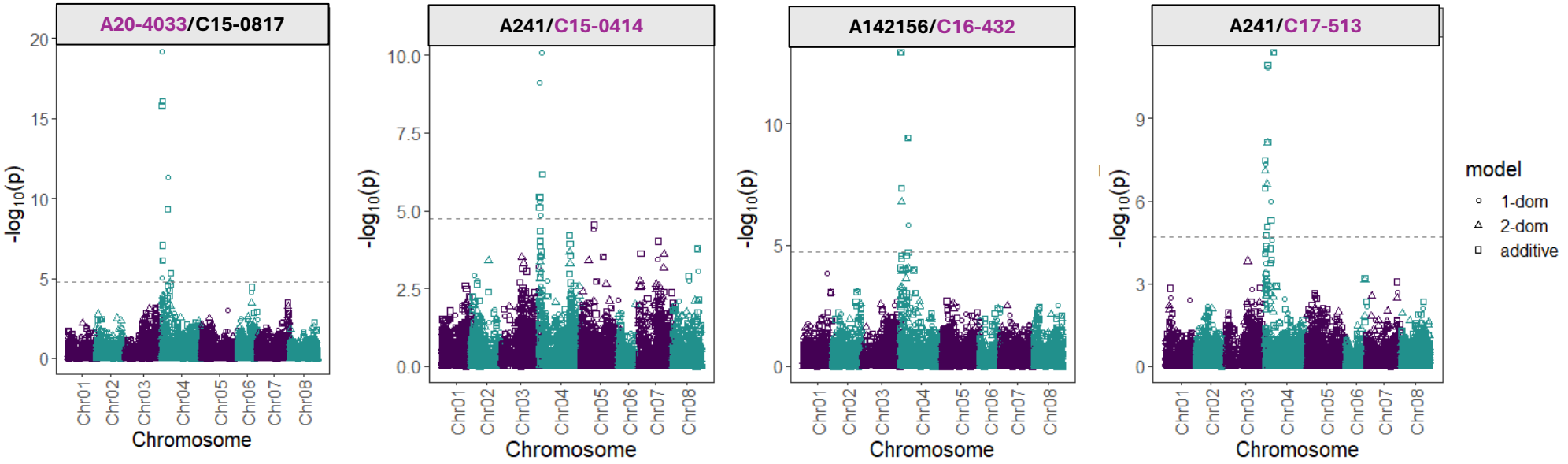
A241/C17-513

A241/C15-0414

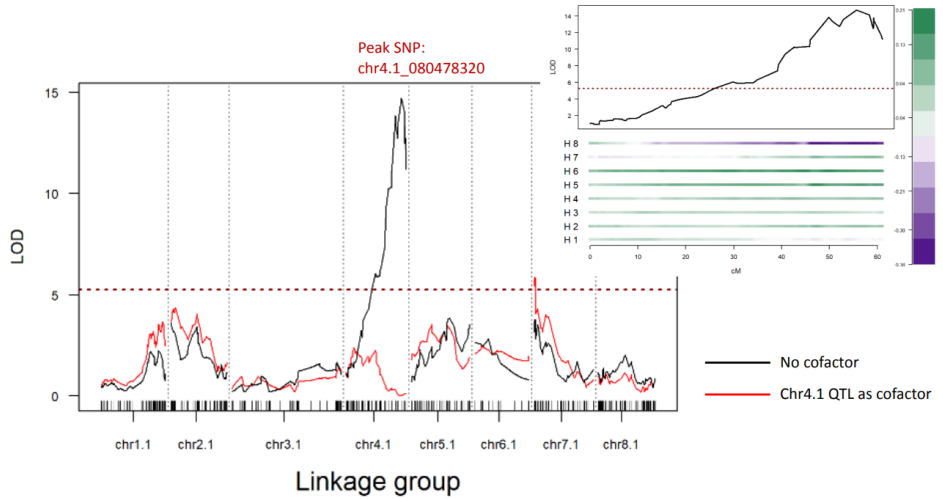
A20-4033/C15-0817

Tissue sampling for DNA isolation at Corteva

Results



- All resistance sources mapped to only one location on chromosome 4
- This is the WAPH-5 resistance according to Deb Samac (NAAIC, 2024)



Results

- Markers map to a region between 70 million and 90 million base pairs on Chromosome 4 (Genome assembly from Chen et al, 2020)
- There is a cluster of plant disease resistance genes at this location (nucleotide-binding site Leucine-rich repeats)

SNP or gene	M. sativa Chr4.1
S_15342922	70,644,552
S_9141167	71,132,781
S_8557330	71,574,430
S_7552767	74,136,693
S_8884216	75,759,978
DaRT_1374366	80,478,320
NBS-LRR gene	81,267,912
NBS-LRR gene	81,562,968
NBS-LRR gene	81,751,894
NBS-LRR gene	81,885,910
NBS-LRR gene	81,926,798
NBS-LRR gene	82,044,176
NBS-LRR gene	82,110,915
NBS-LRR gene	82,124,222
NBS-LRR gene	82,136,485
NBS-LRR gene	82,179,313
NBS-LRR gene	82,190,181
NBS-LRR gene	82,220,956
NBS-LRR gene	82,295,457
S_10108080	84,163,501
S_24248838	84,173,012
S_24560229	85,652,501
NBS-LRR gene	85,863,756
S_18557967	87,535,923
S_24908062	88,443,672
S_587216	88,698,134
S_19246557	89,062,535
S_23642916	89,112,808
S_23641495	89,113,306
S_22092534	89,176,482
S_23545123	89,287,385
S_20025557	89,494,749

qPCR Assay Developed

WAPH-5 QTL Peak (Samac, 2024)

NBS-LRR Cluster (Ameline-Torregrosa et al, 2008)

qPCR Assay Developed

qPCR Assay Developed

Validation

A241/C15-0414

Marker	C15-0414
S_23641495	AACC
S_23642916	AATT
S_24248838	TTCC

Cross	Nulliplex (aaaa)				
Nulliplex (aaaa)	All N	Simplex (Aaaa)			
Simplex (Aaaa)	1S:1N	1D:2S:1N	Duplex (AAaa)		
Duplex (AAaa)	1D:4S:1N	1T:5D:5S:1N	1Q:8T:18D:8S:1N	Triplex (AAAa)	
Triplex (AAAa)	1D:1S	1T:2D:1S	1Q:5T:5D:1N	1Q:2T:1D	Qaud'plex (AAAA)
Quad'plex (AAAA)	All D	1T:1D	1Q:4T:1D	1Q:1T	All Q

- **A241/C15-0414 segregated 80/20 for resistance**
- **C15-0414 is Duplex for the resistance gene**
- **What happens if we polycross its duplex progeny?**

Validation

8 Duplex Progeny Plants

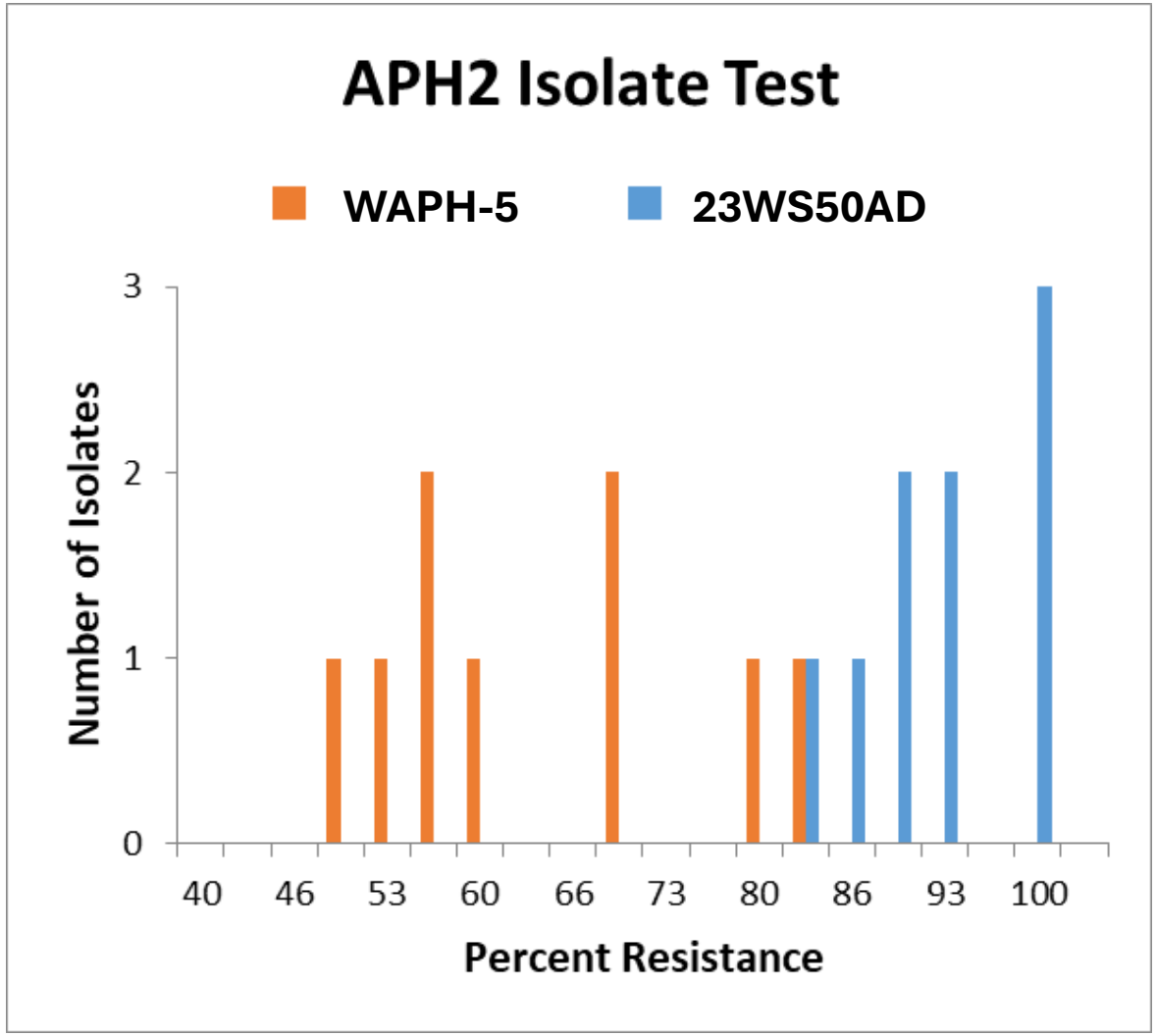
EA55483_002_43
EA55483_002_84
EA55483_003_2
EA55483_003_7
EA55483_003_13
EA55483_003_20
EA55483_003_31
EA55483_003_41

23WS50AD

Cross	Nulliplex (aaaa)				
Nulliplex (aaaa)	All N	Simplex (Aaaa)			
Simplex (Aaaa)	1S:1N	1D:2S:1N	Duplex (AAaa)		
Duplex (AAaa)	1D:4S:1N	1T:5D:5S:1N	1Q:8T:18D:8S:1N	Triplex (AAAa)	
Triplex (AAAa)	1D:1S	1T:2D:1S	1Q:5T:5D:1N	1Q:2T:1D	Qaud'plex (AAAA)
Quad'plex (AAAA)	All D	1T:1D	1Q:4T:1D	1Q:1T	All Q

- Phenotypic selection can't discern simplex from duplex
- Simplex cage/phenotypic selection: 75% Resistant
- Duplex cage/MAS: 98% Resistant

Validation



- Nine isolates of Aphanomyces race 2 were tested using standard protocol
- Simplex cage/phenotypic selection represented by WAPH-5 (industry standard check) Avg: 65% Resistant
- Duplex cage/MAS represented by 23WS50AD Avg: 90% Resistant
- MAS will lead to varieties able to tolerate high disease pressure in water-logged soils

Collaborators

- **Steve Damon**, DLF USA
- **Steve Wagner**, DLF USA
- **Lindsey Rushford**, UW Madison - Extension
- **Justin Valletta**, DLF USA
- **Tara Tarnowski**, Corteva Agriscience
- **Jamie Hadley**, Corteva Agriscience

SPECIAL THANKS

To the Corteva Agriscience High Throughput Genotyping Laboratory Johnston, IA