Fusarium graminearum Population-Specific Differences During Wheat Infection

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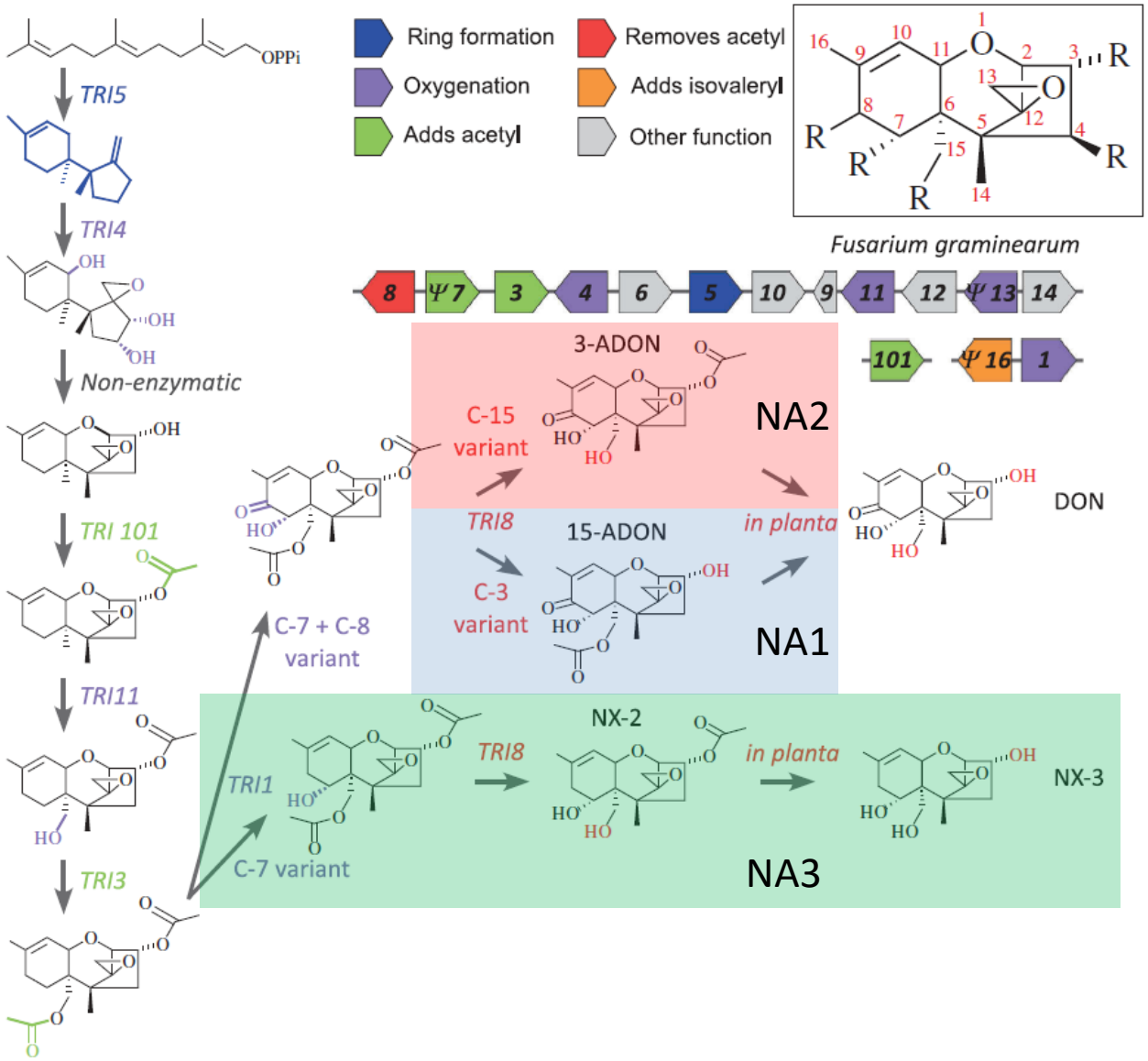
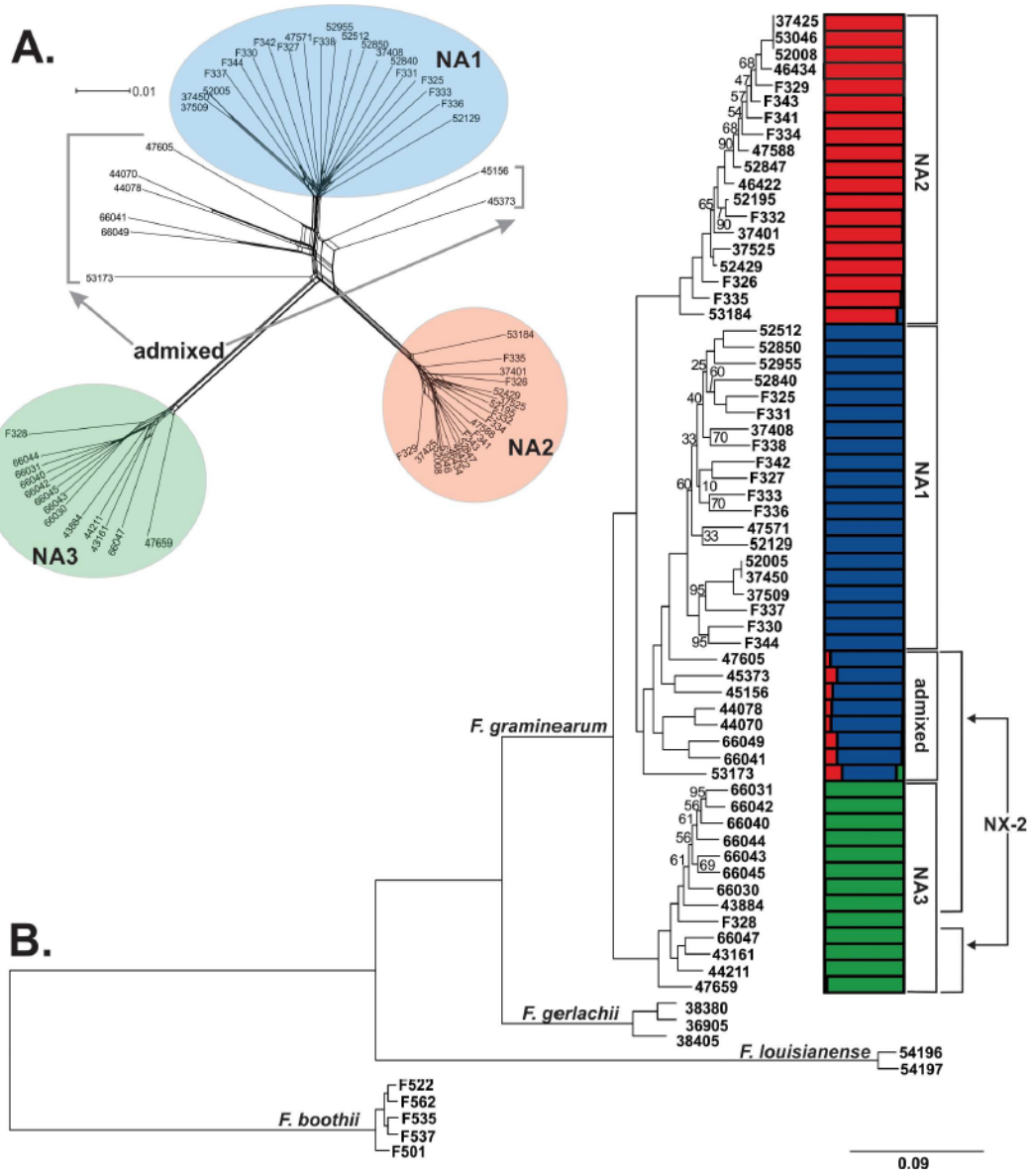
Susan P. McCormick and Matthew G. Bakker

Three North American Populations of *F. graminearum*



(Bakker et al., 2018)



(Kelly and Ward, 2018)

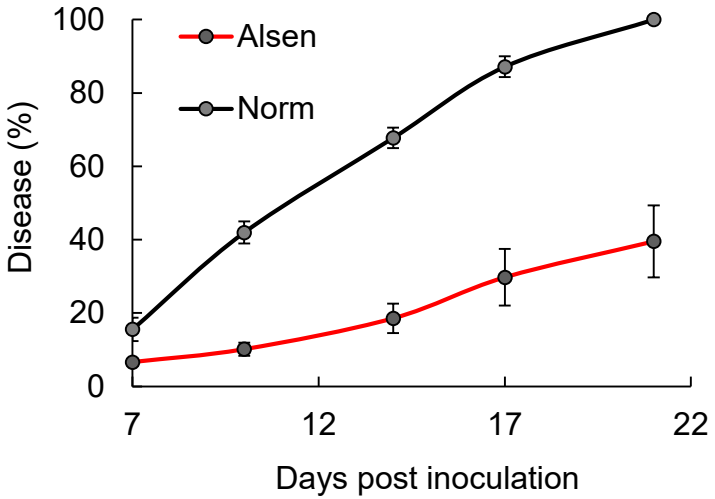
Three North American Populations of *F. graminearum*

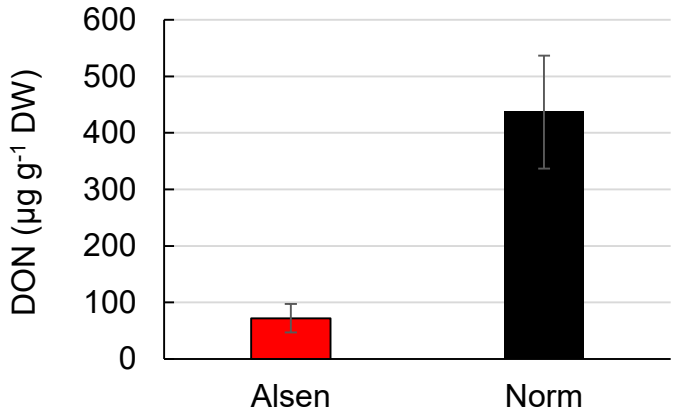
Genetic Background	Chemotype in culture	Chemotype in planta
NA1	15-ADON	DON
NA2	3-ADON	DON
NA3	NX-2	NX-3

Are there population specific differences during wheat infection?

	NA1	NA2	NA3
1	38746	37525	43161
2	38762	38763	43884
3	38811	38851	44211
4	38986	38964	47659
5	47571	38980	66030
6	52005	46422	66031
7	52512	47588	66037
8	52955	52008	66039
9	06-219	52195	66040
10	06-225	52429	66042
11	06-270	53046	66043
12	13MN1-6	00-588	66044
13	F333	12SD6-2	66045
14	F342	06-239	66047
15	F344	06-240	F322
	15-ADON	3-ADON	NX

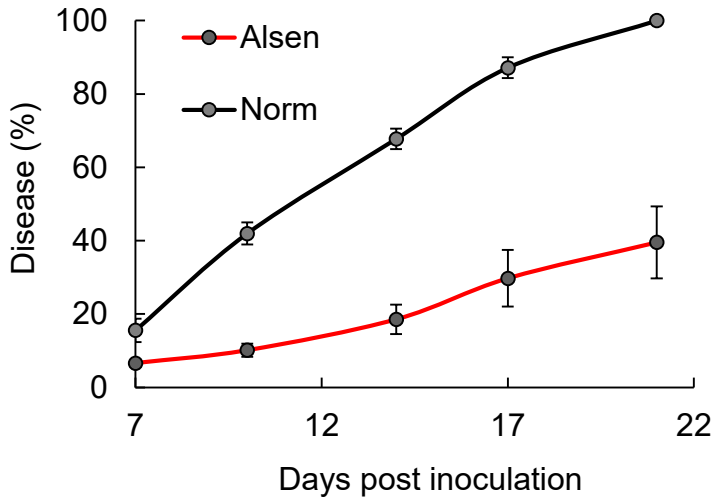
Population specific differences on moderately resistant wheat variety Alsen

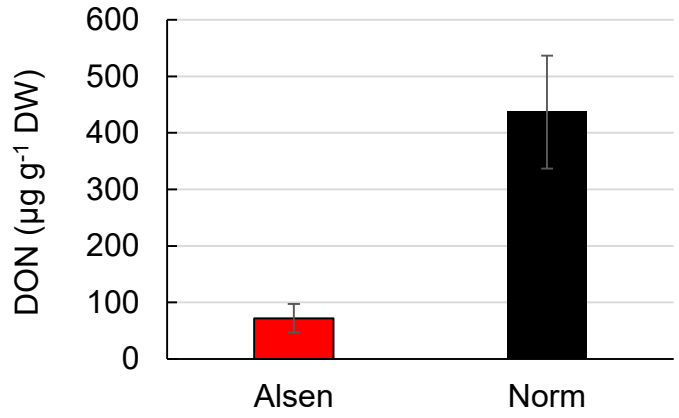




- **Moderately resistant variety Alsen** contains loci derived from the Chinese spring wheat cultivated variety Sumai 3 (Bai, 1996)
  - *Fhb1* confers type II resistance
  - *Fhb5* confers type I resistance

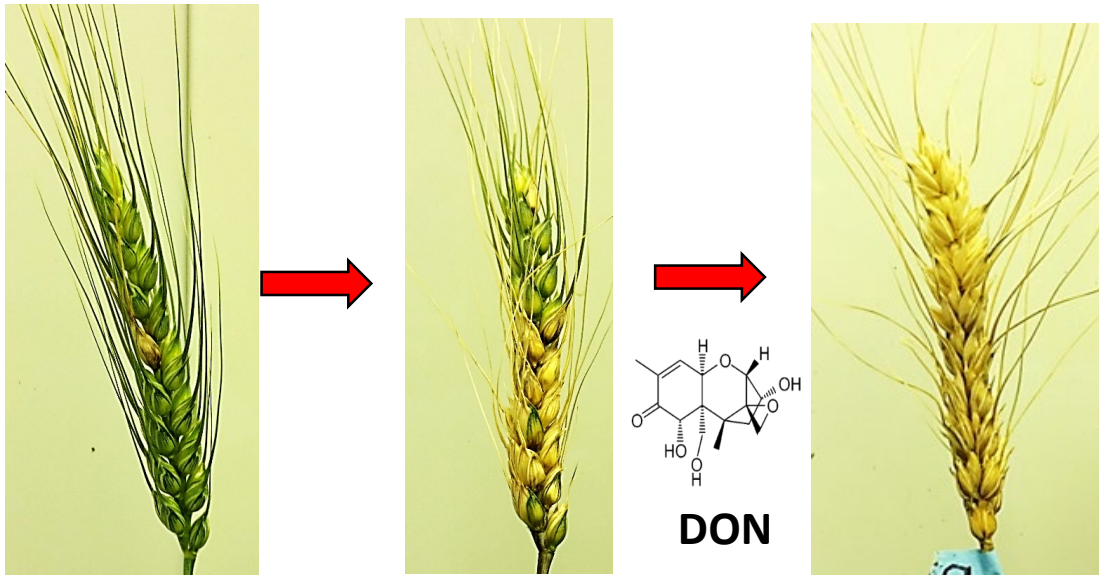
Population specific differences on moderately resistant wheat variety Alsen





- **Moderately resistant variety Alsen** contains loci derived from the Chinese spring wheat cultivated variety Sumai 3 (Bai, 1996)
  - *Fhb1* confers type II resistance
  - *Fhb5* confers type I resistance

- **Type I: Resistance to initial infection**
- **Type II: Resistance to spread**



Experimental Design

	NA1	NA2	NA3
1	38746	37525	43161
2	38762	38763	43884
3	38811	38851	44211
4	38986	38964	47659
5	47571	38980	66030
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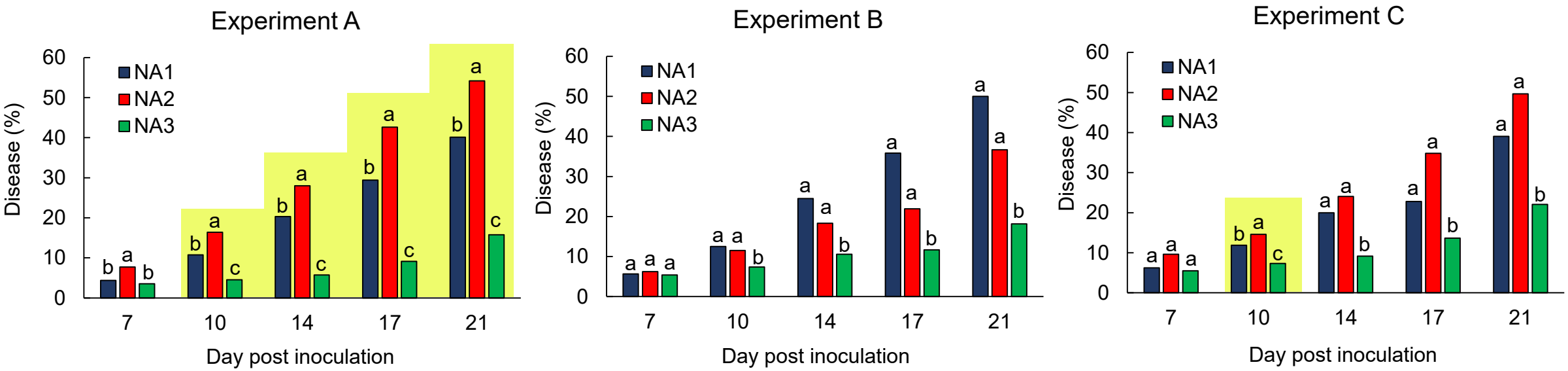
12	13MN1-6	00-588	66044
13	F333	12SD6-2	66045
14	F342	06-239	66047
15	F344	06-240	F322

- **6 flowering Alsen wheat heads per strain**
- **Total of 90 heads per population**

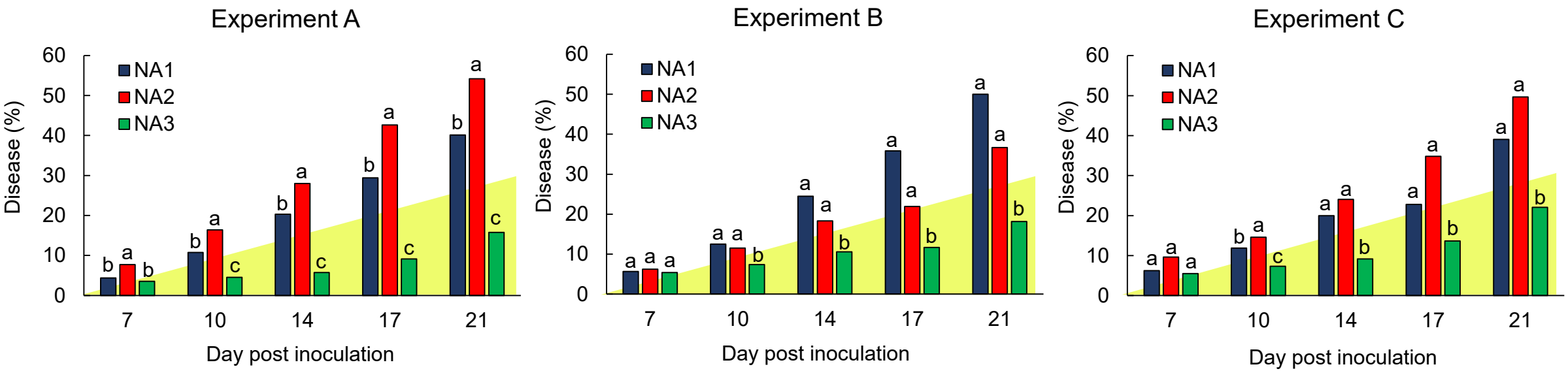
- **Destructive sampling of 1 head per strain at timepoints Day 7, 14 and 21 for toxin and molecular analyses.**
- **Combined three random heads so that n=5 at each timepoint**

Are there population specific differences during infection of moderately resistant wheat?

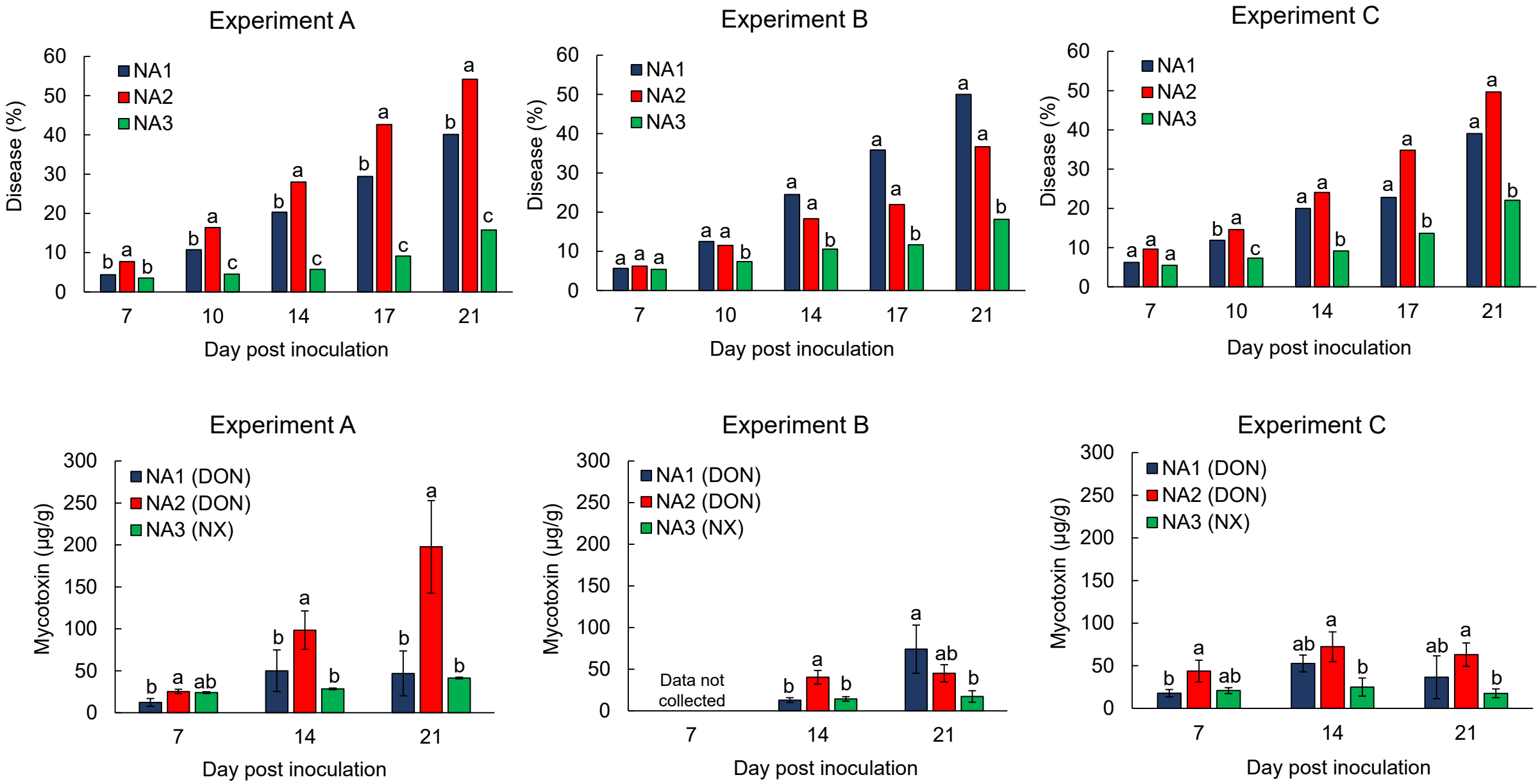


Data from 3 experiments were analyzed to compare 3 populations (NA1, NA2, and NA3) for % diseased florets as a function of time (7, 10, 14, 17, and 21 days) using weighted regression analysis.

Are there population specific differences during infection of moderately resistant wheat?



NA1 or NA2 > NA3


Experiment A
Disease (%)
NA1
NA2
NA3
Day post inoculation
Experiment B
Disease (%)
NA1
NA2
NA3
Day post inoculation
Experiment C
Disease (%)
NA1
NA2
NA3
Day post inoculation
Experiment A
Mycotoxin (µg/g)
NA1 (DON)
NA2 (DON)
NA3 (NX)
Day post inoculation
Experiment B
Mycotoxin (µg/g)
NA1 (DON)
NA2 (DON)
NA3 (NX)
Data not collected
Day post inoculation
Experiment C
Mycotoxin (µg/g)
NA1 (DON)
NA2 (DON)
NA3 (NX)
Day post inoculation

Why does NA3 cause less disease?

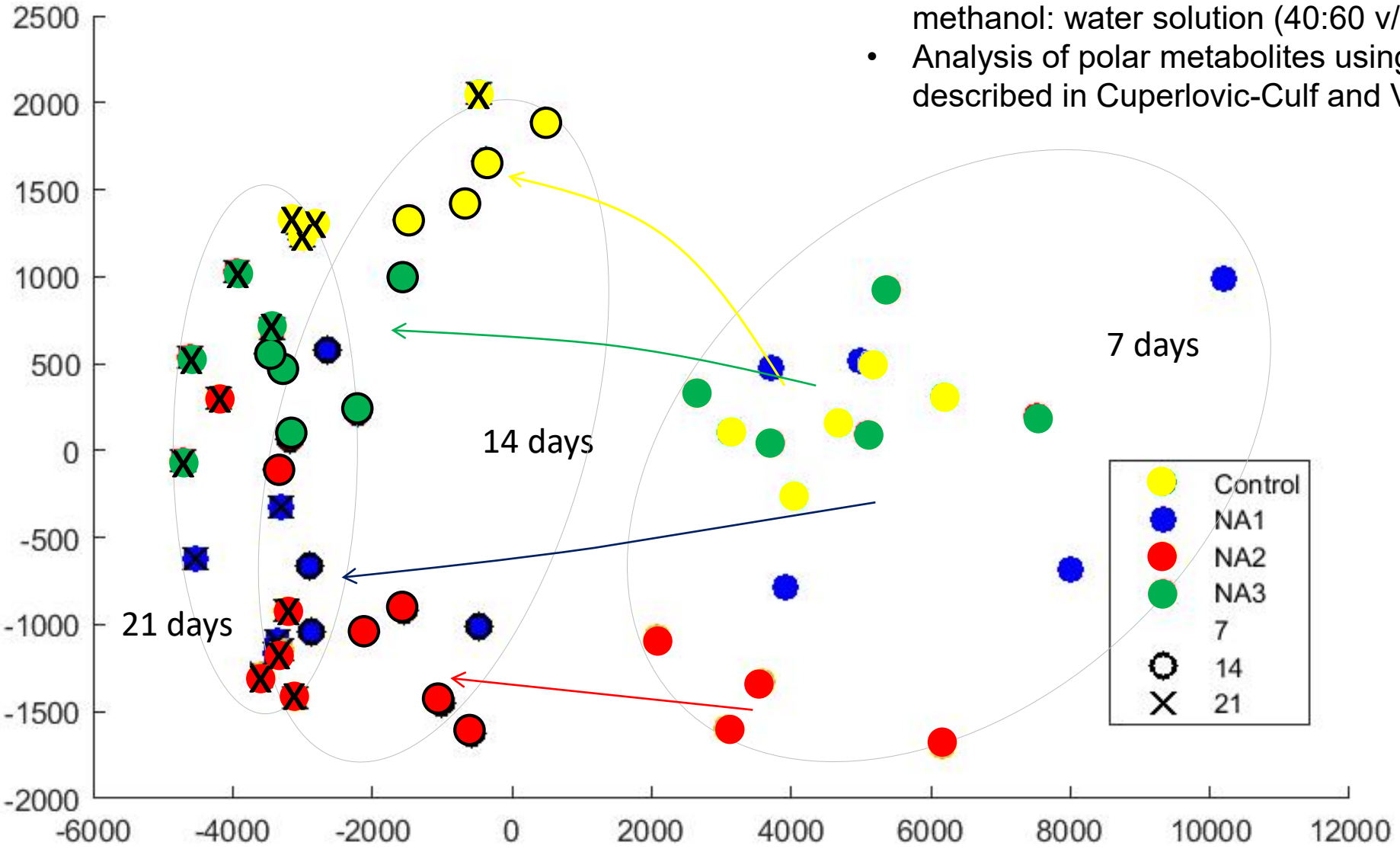
Hypotheses:

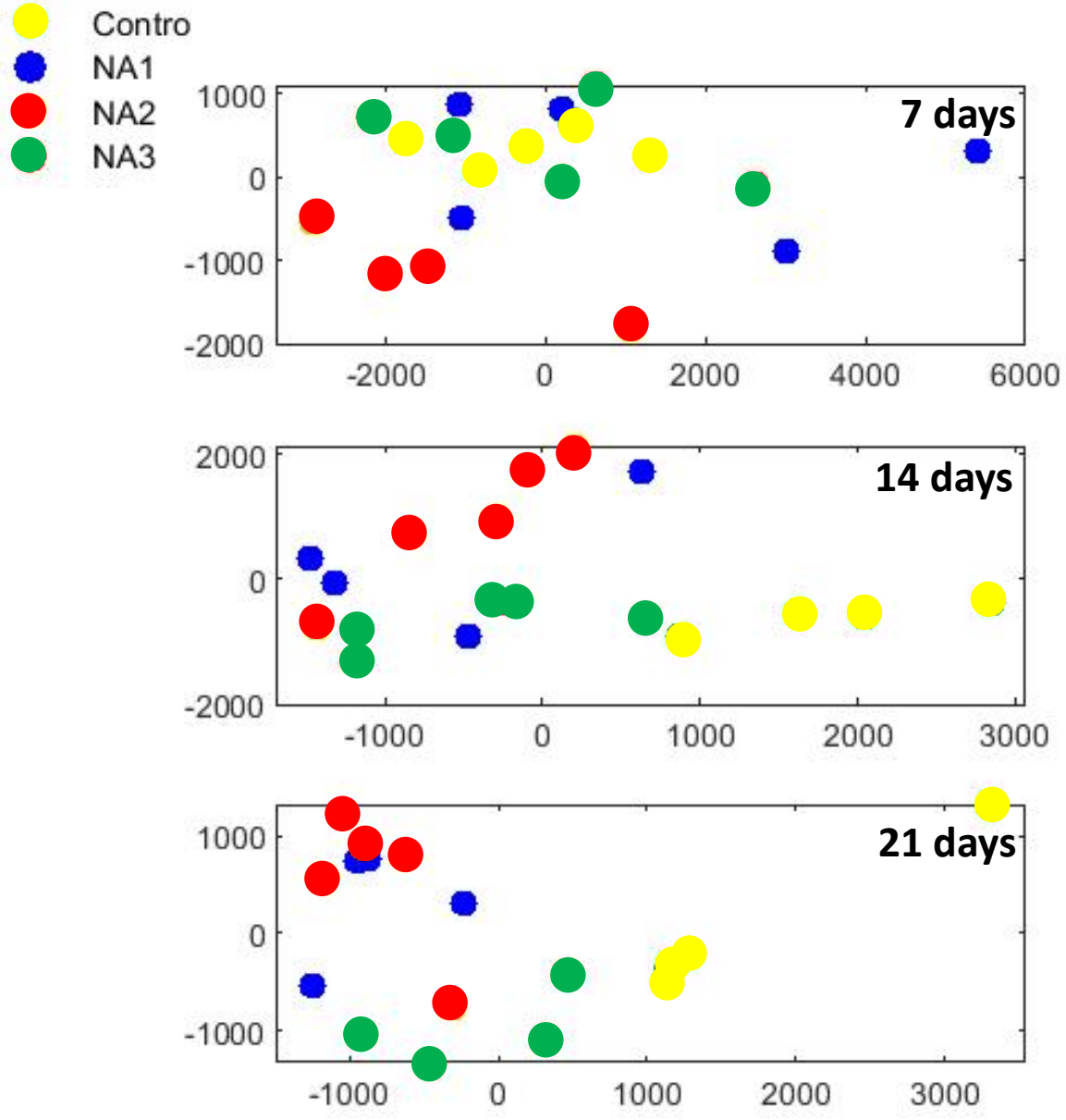
1. Host plant response against NA3 strains is stronger and more effective at controlling disease spread.

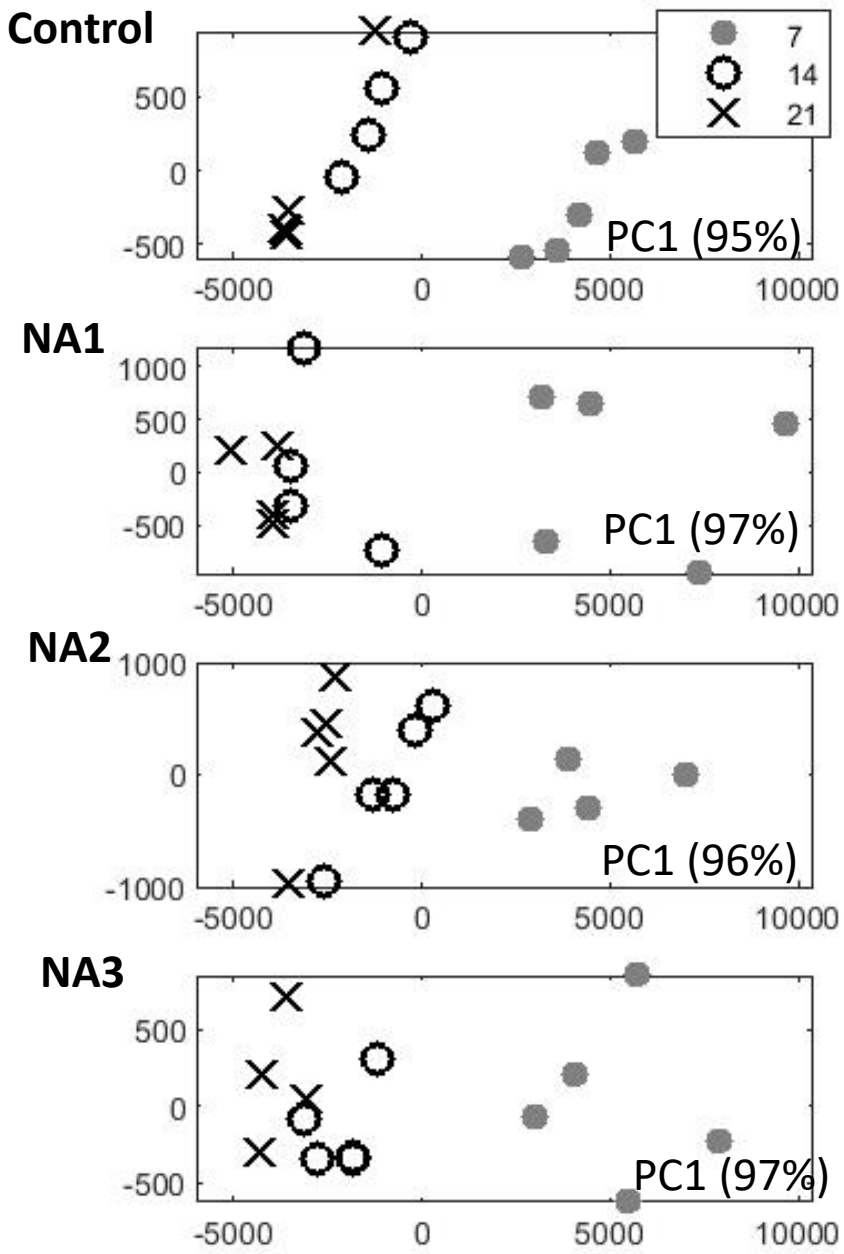
2. NA3 strains are less virulent.

Metabolomic Analysis (NMR)

- Metabolite Extraction from plant tissues using methanol: water solution (40:60 v/v)
- Analysis of polar metabolites using NMR methods described in Cuperlovic-Culf and Vaughan et al., 2018







Unsupervised Principle Components Analysis

Further analysis of Host Defense Response Experimental Design

	NA1	NA2	NA3
1	38746	37525	43161
2	38762	38763	43884
3	38811	38851	44211
4	38986	38964	47659
5	47571	38980	66030
6	52005	46422	66031
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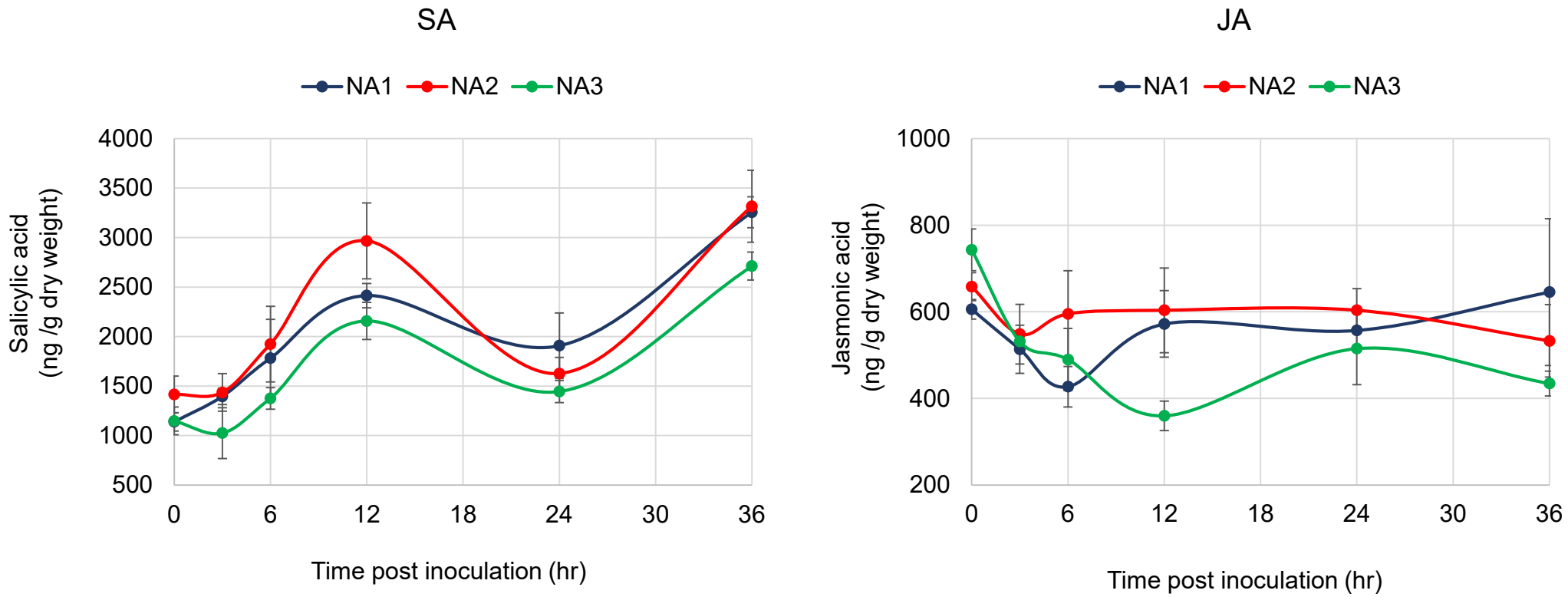
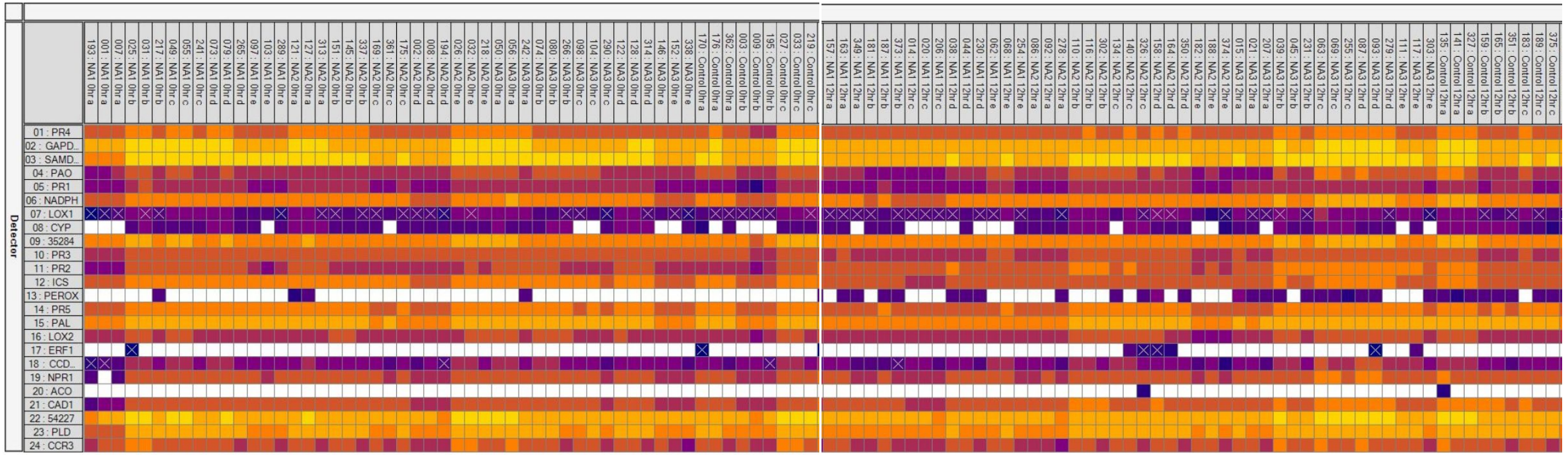
12	13MN1-6	00-588	66044
13	F333	12SD6-2	66045
14	F342	06-239	66047
15	F344	06-240	F322

- Conducted whole head inoculations (via dip method)
- Collected samples at Early Time points:
  - 0, 3, 6, 12, 24, 36 hr
- 15 representative strains from each population
  - (NA1, NA2, NA3)
- 6 flowering Alsen wheat heads per strain
- Total of 90 heads per population
- Destructive sampling of 1 head per strain at each timepoint.
- Combined three random heads so that n=5 at each timepoint
- Phytohormone and Transcriptional Analyses

Phytohormone response to *F. graminearum* populations



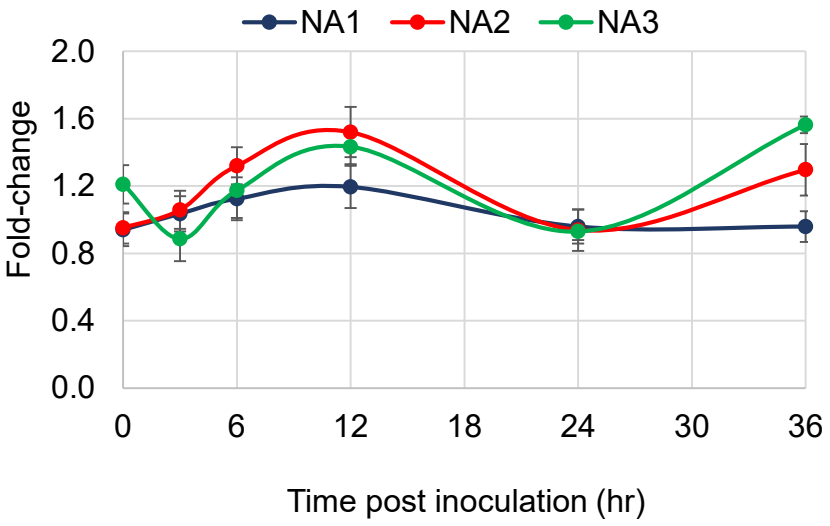
Transcriptional Analysis of Host Defense Response Genes



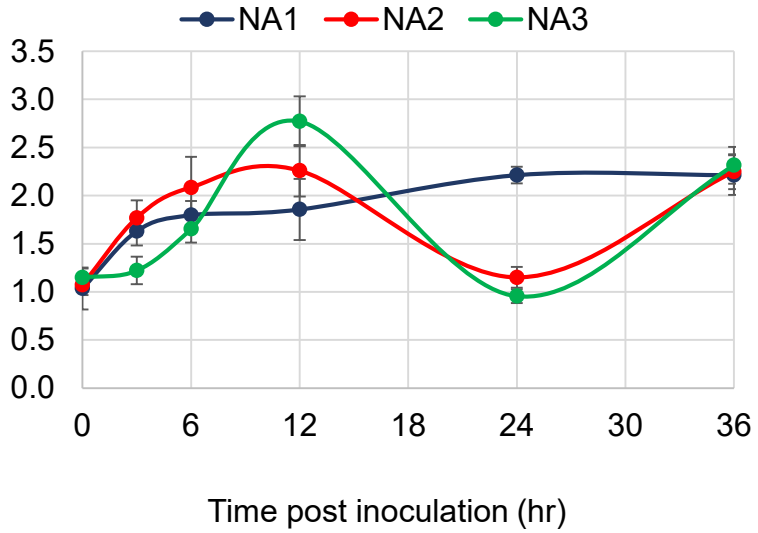
- 20 selected defense related genes
- Time points: 0, 3, 6, 12, 24, 36 hr post whole head inoculation method
- 15 representative strains from each population (NA1, NA2, NA3)

Transcription of Host Defense Response Genes

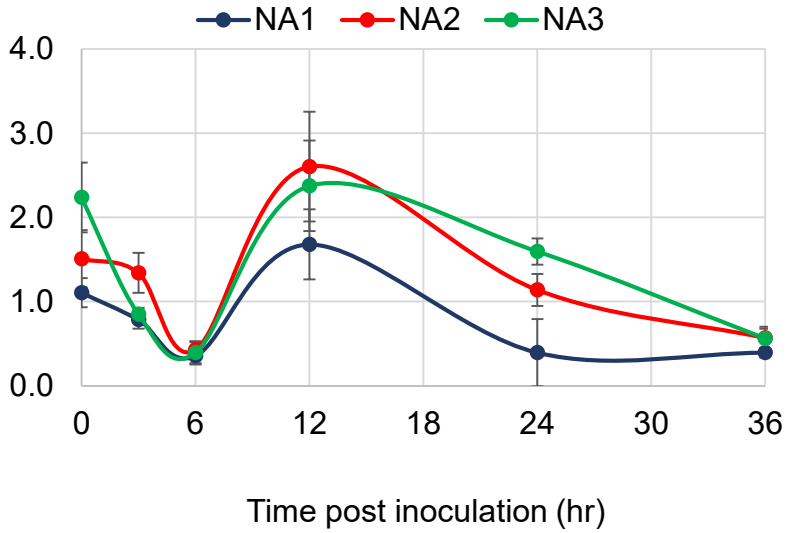
Phenylalanine ammonia lyase (PAL)



Nonexpressor of PR genes 1 (NPR1)



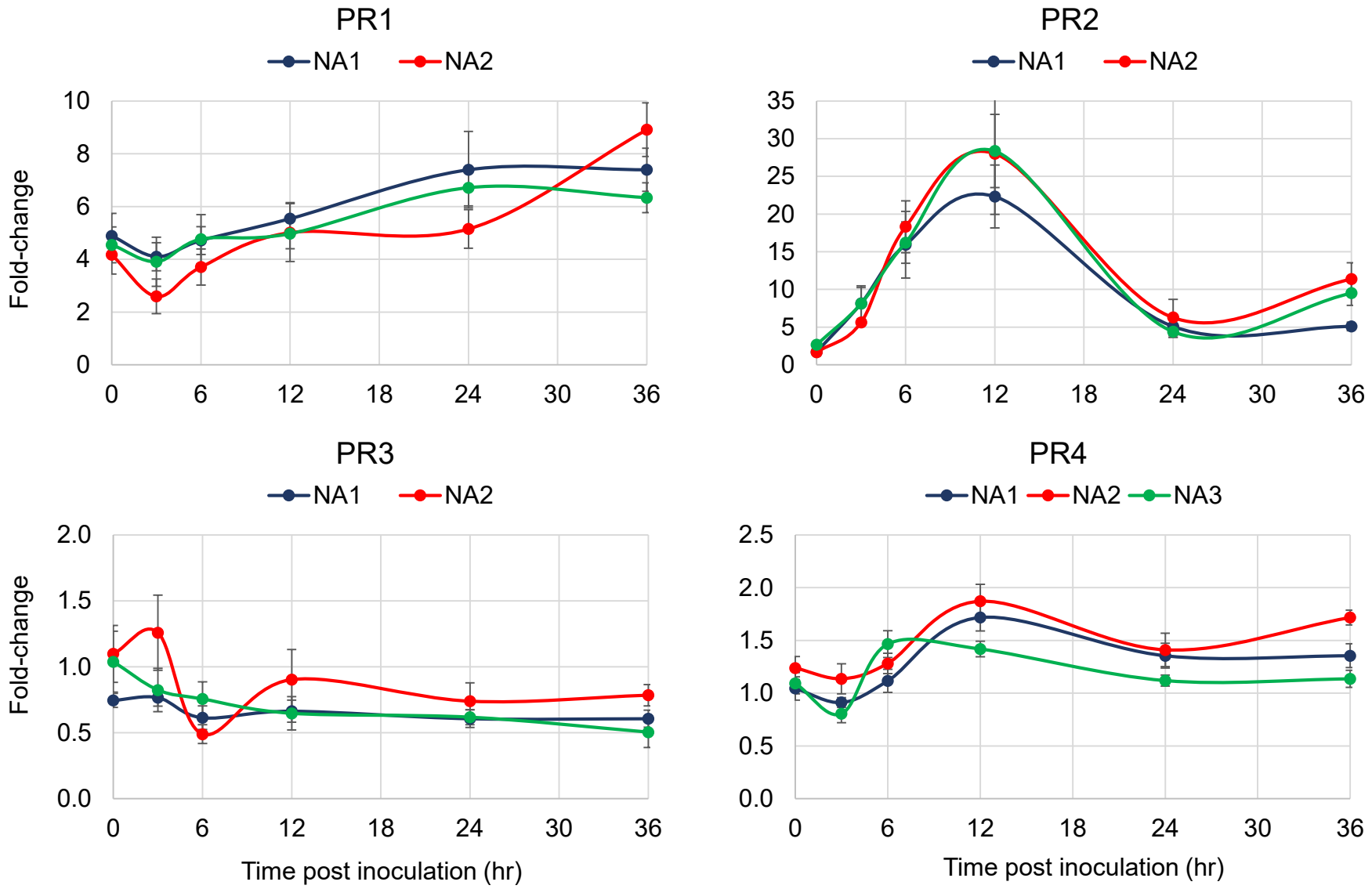
Lipoxygenase 2 (LOX2)



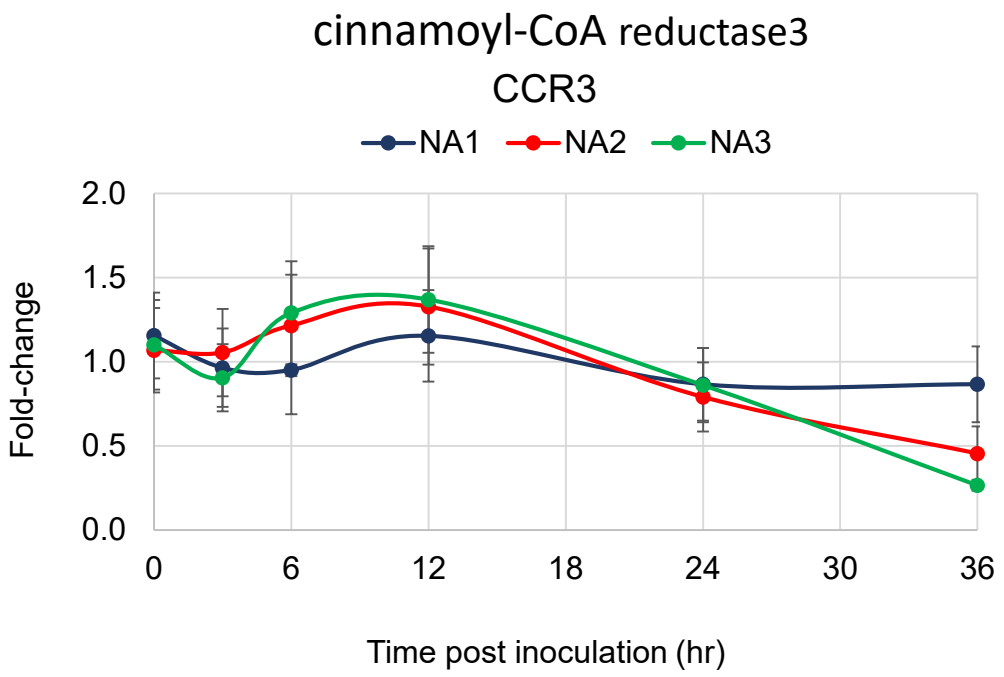
Involved in SA pathway

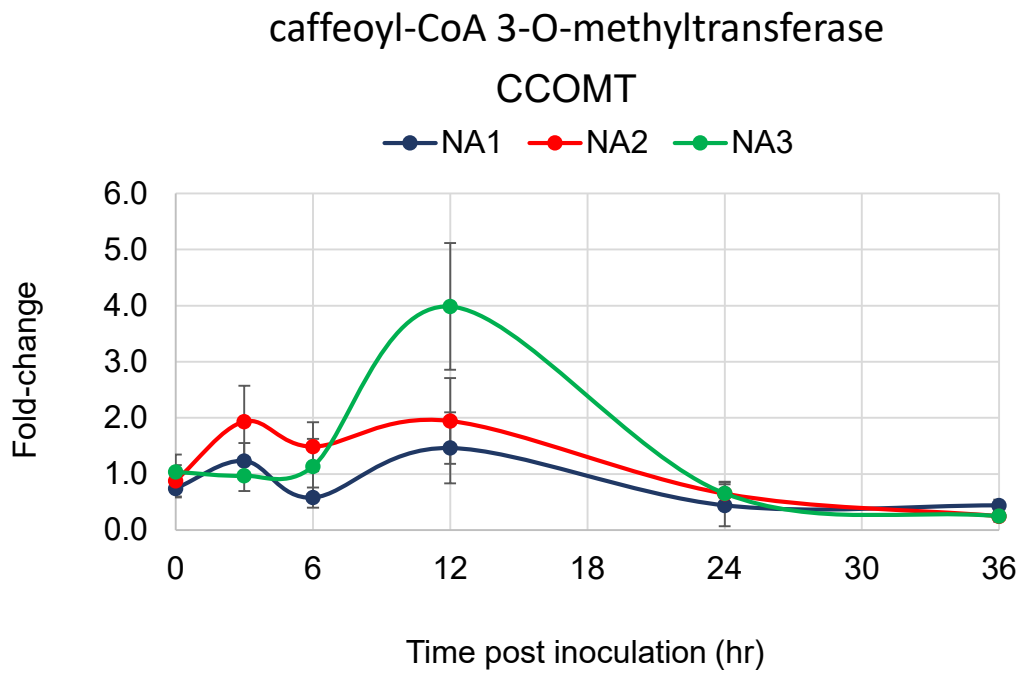
Involved in JA pathway

Transcription of Pathogenesis Related (PR) genes



Transcription of genes involved in lignin deposition





Why does NA3 cause less disease?

Hypotheses:

1. Host plant response against NA3 strains is stronger and more effective at controlling disease spread.

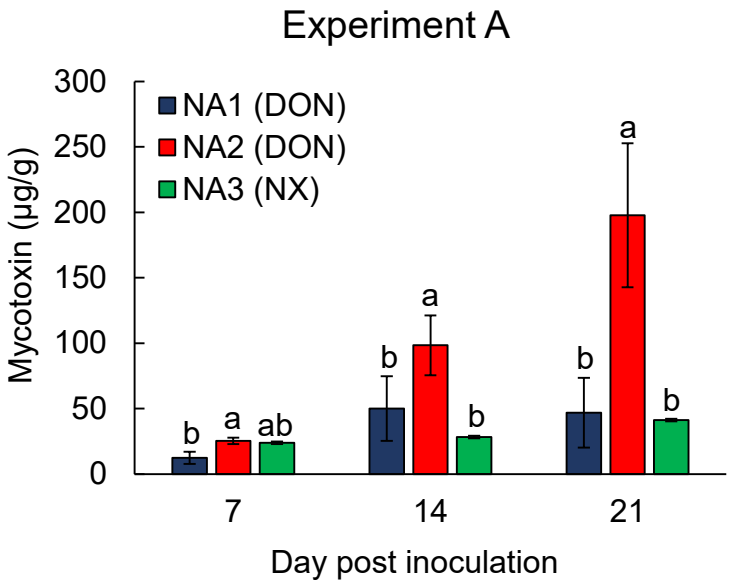
2. NA3 strains are less virulent.

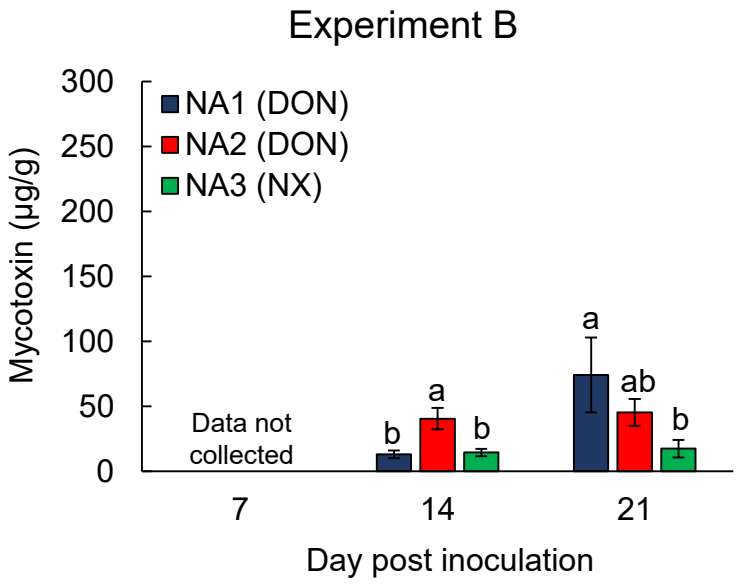
Are NA3 strains less virulent?

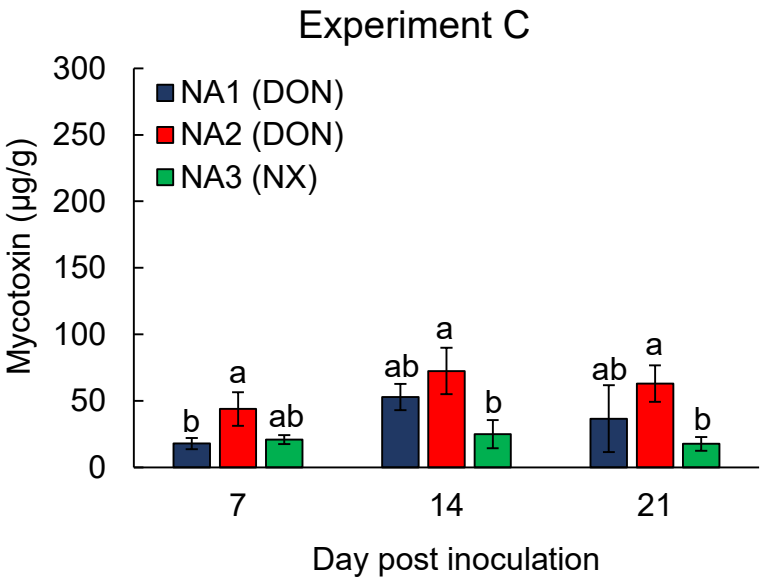
Hypothesis:

NA3 produces less toxin?

Not always significantly less







NA3 may be less virulent because of NX chemotype?

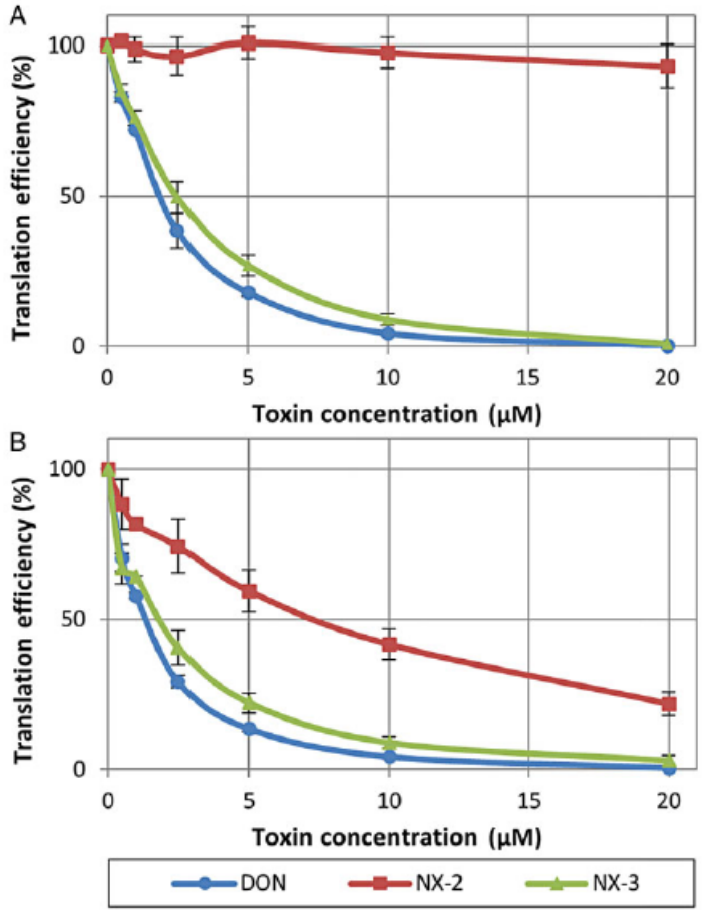


Fig. 3. *In vitro* toxicity of deoxynivalenol and the novel metabolites NX-2 and NX-3 in (A) rabbit reticulocyte lysate and (B) wheat germ extract based translation assays. Error bars show ± standard deviations.

Supporting Information Fig. 3. *Chlamydomonas reinhardtii* grown in the presence of 100 μM trichothecenes. Average culture doublings after 4 days were 4.0 (3-acetyl-deoxynivalenol—3-ADON), 0.2 (deoxynivalenol—DON), and 4.5 (NX-2), 1.5 (NX-3) for the novel compounds, compared to 4.8 for an acetone control.¶

(Varga et al., 2015)

NA3 may be less virulent because of NX chemotype?

Less-toxic rearrangement products of NX-toxins are formed during storage and food processing

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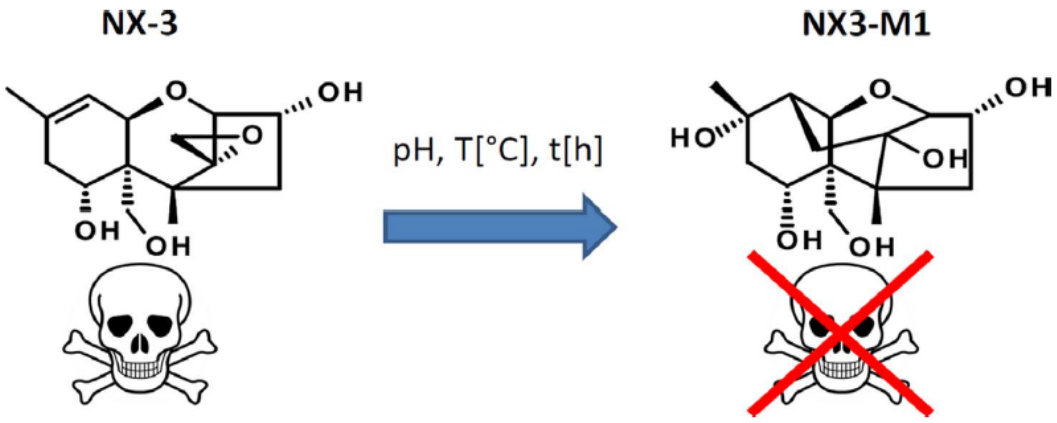
GRAPHICAL ABSTRACT



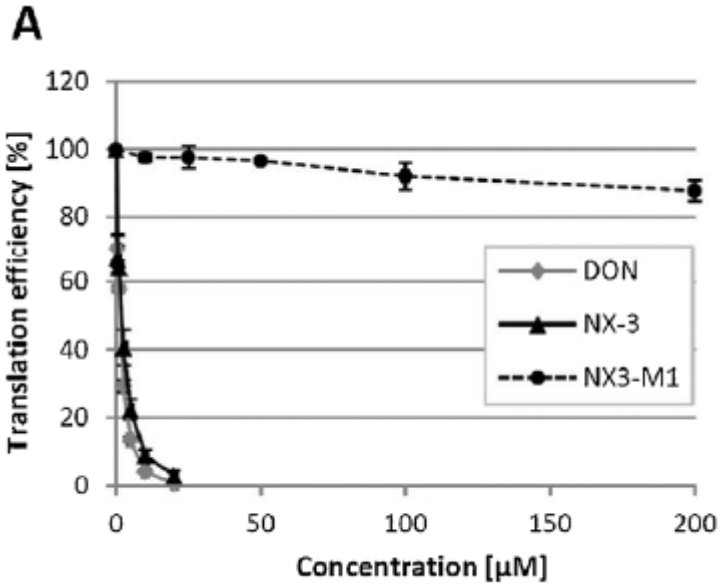


Fig. 2. *In vitro* toxicity of deoxynivalenol (DON) and NX-3 and NX3-M1 in A) wheat germ extract and B) rabbit reticulocyte lysate based translation assays. Error bars show standard deviations of replicates.

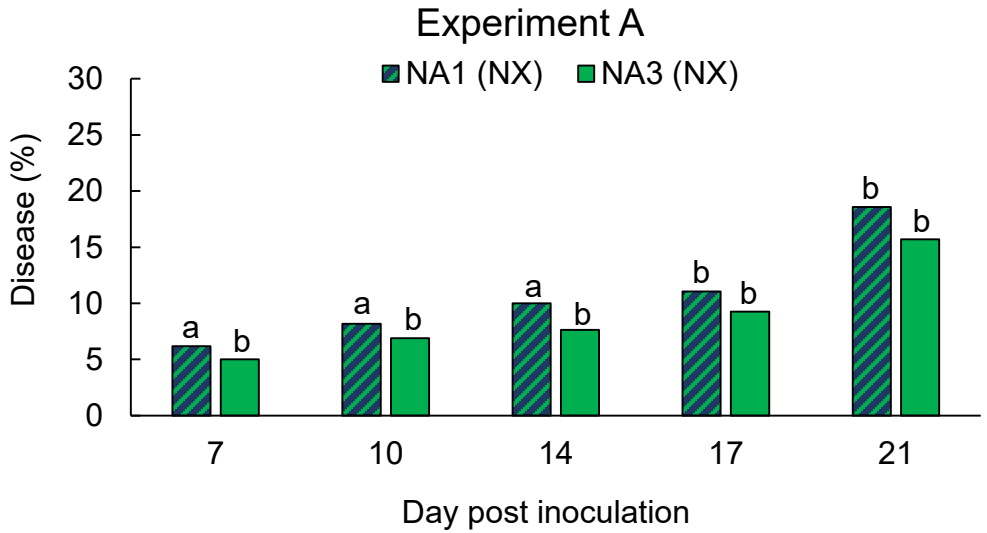
(Varga et al., 2018)

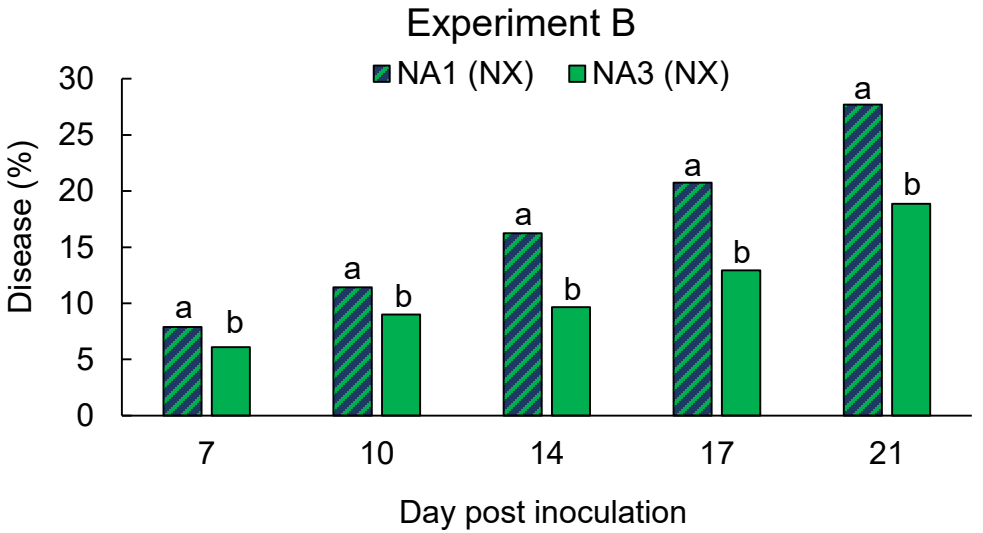
Are NA3 less virulent because of NX chemotype?

- 6 flowering Alsen wheat heads per strain
- Total of 48 heads per population
- Point inoculation method
- Tracked % disease progression

	Strain Name	Pop/Background	Chemotype
1	43161	NA3	NX
2	44211	NA3	NX
3	47659	NA3	NX
4	66030 (F277)	NA3	NX
5	66044 (F272)	NA3	NX
6	66047 (F268)	NA3	NX
7	66039 (F270)	NA3	NX
8	F322	NA3	NX
1	47605	NA1	NX
2	45373	NA1	NX
3	45156	NA1	NX
4	44078	NA1	NX
5	44070	NA1	NX
6	66049	NA1	NX
7	66041	NA1	NX
8	53173	NA1	NX

Population genetic background influences disease development

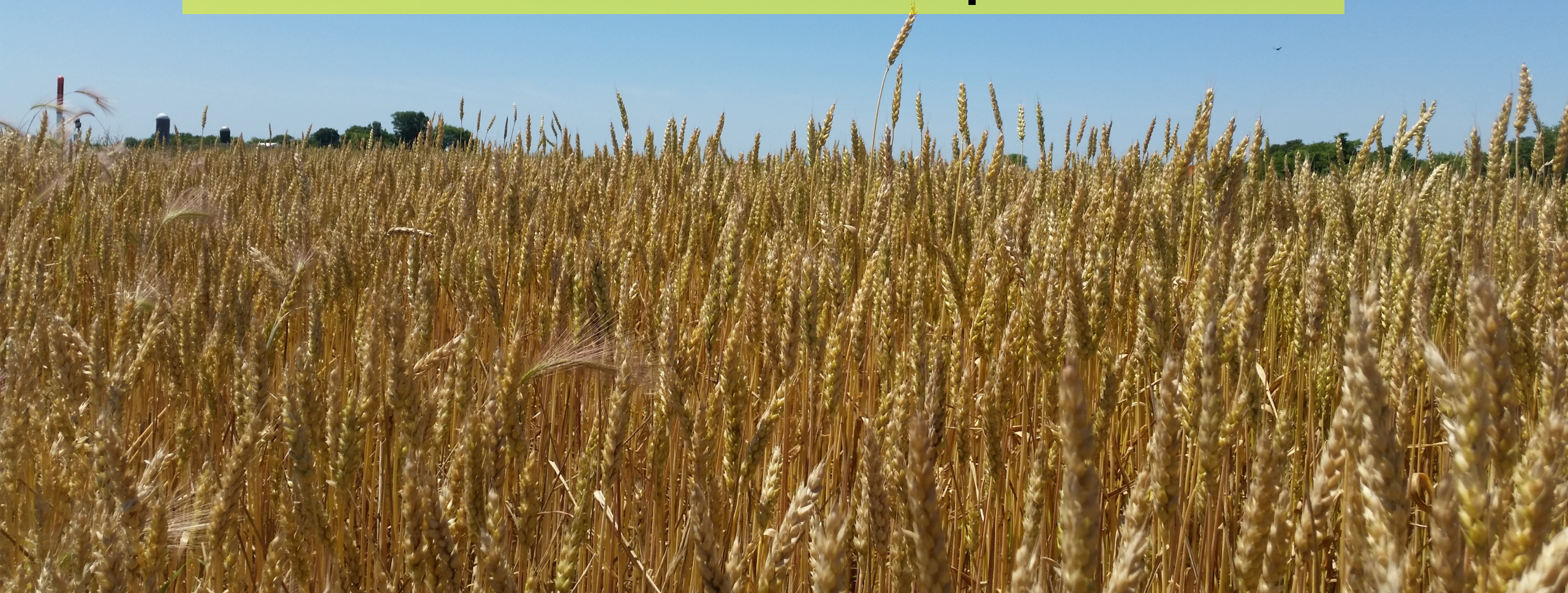




Data analyzed to compare 2 populations (NA1 and NA3) for % diseased florets as a function of time (7, 10, 14, 17, and 21 days) using weighted regression analysis.

Summary

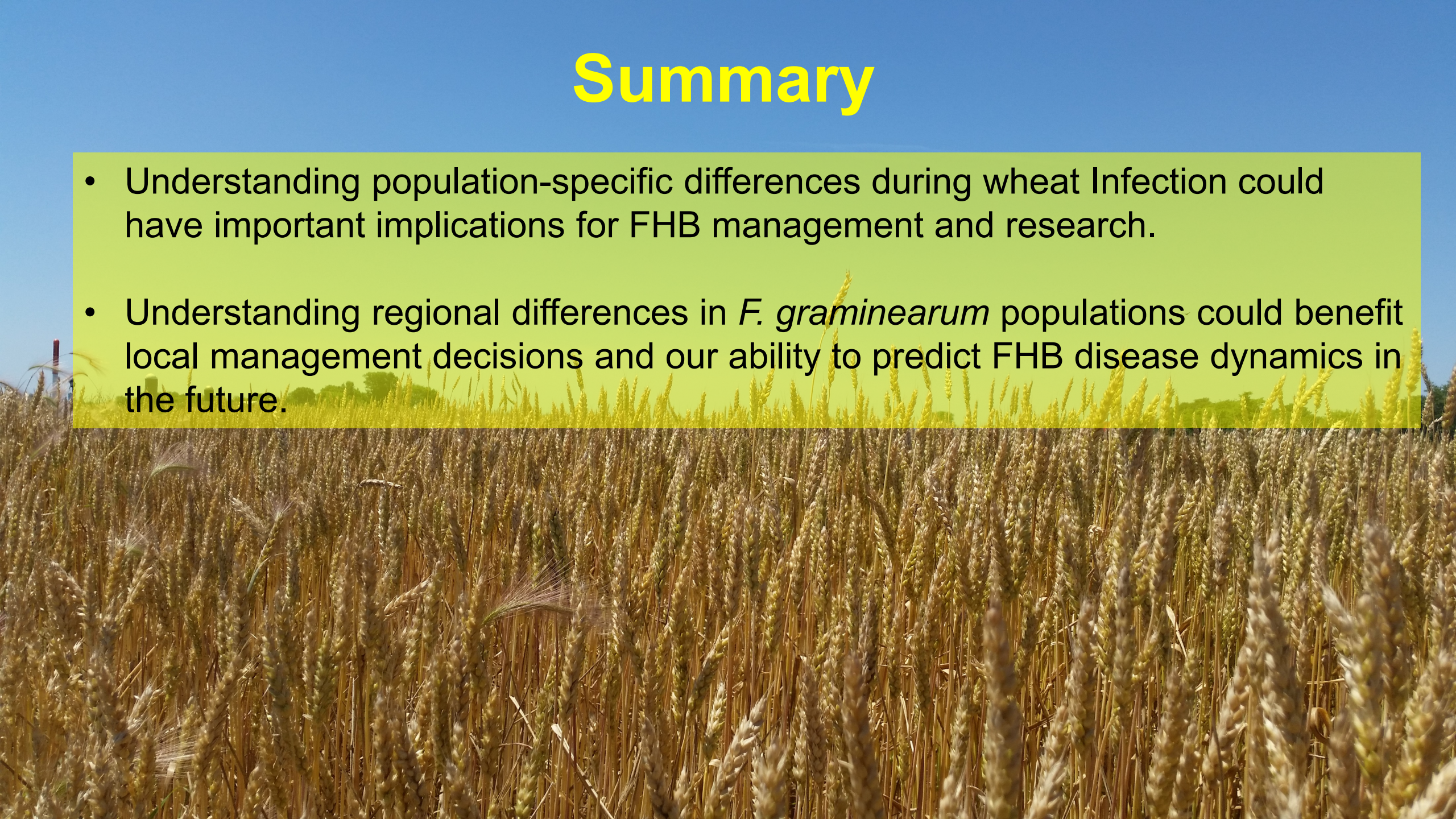
- *F. graminearum* population and chemotype influence FHB development.



Summary

- Understanding population-specific differences during wheat Infection could have important implications for FHB management and research.

- Understanding regional differences in *F. graminearum* populations could benefit local management decisions and our ability to predict FHB disease dynamics in the future.



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Stephanie Folmar
Christine Hodges

Statistics
Deb Palquest

Good things happen when we put our heads together