Novel Methods for Controlling Trichothecene Contamination of Grain and Improving the Climate Resilience of Food Safety and Security Programs

USDA Agricultural Research Service

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Peoria IL

Impact of Trichothecene Producing Cereal Pathogens

Food Safety

- Inhibition of protein synthesis
- Growth Retardation
- Reproductive Disorders
- Immunosuppression
- Feed Refusal Vomiting

Impact of Trichothecene Producing Cereal Pathogens

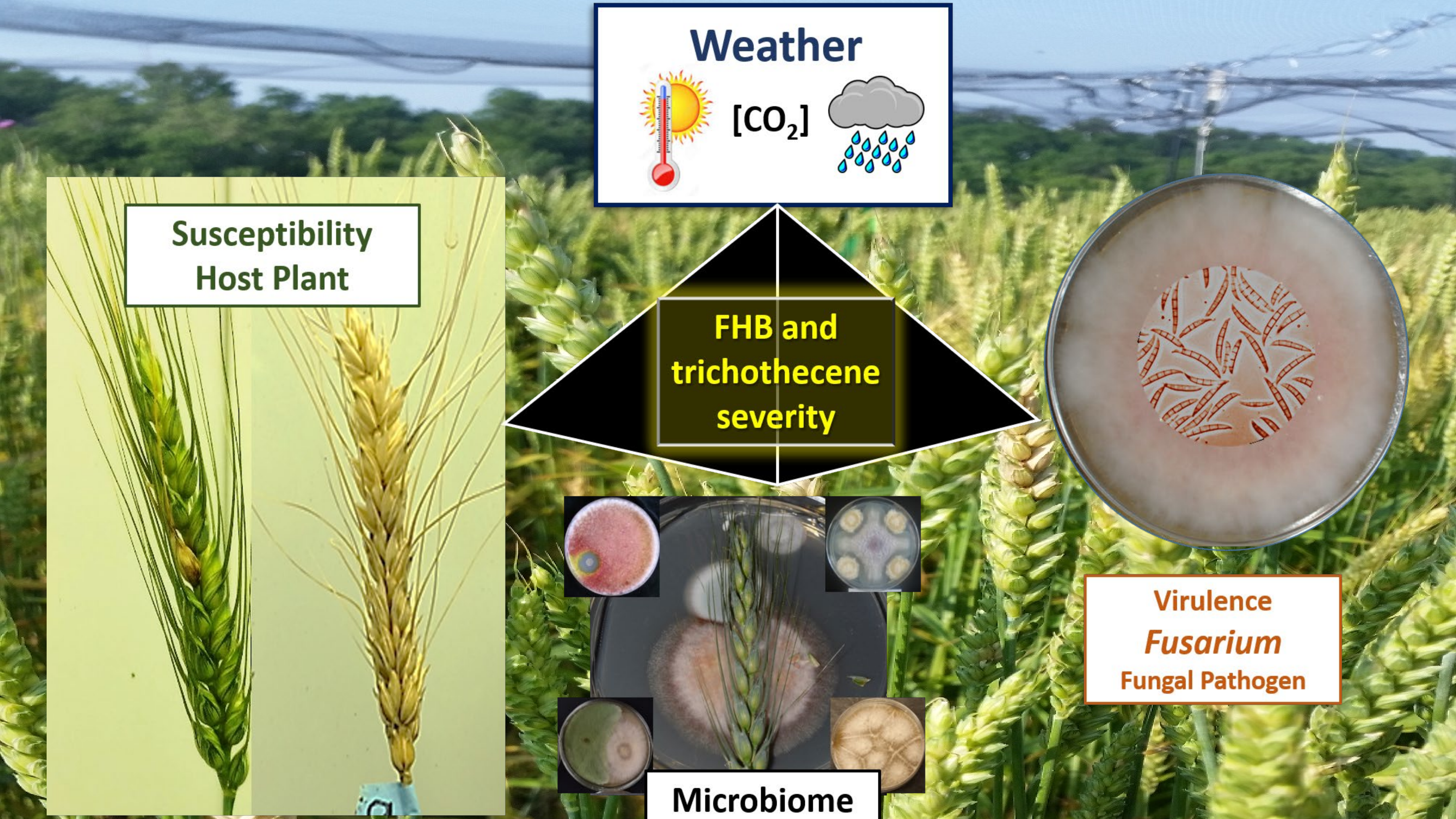
Food Security

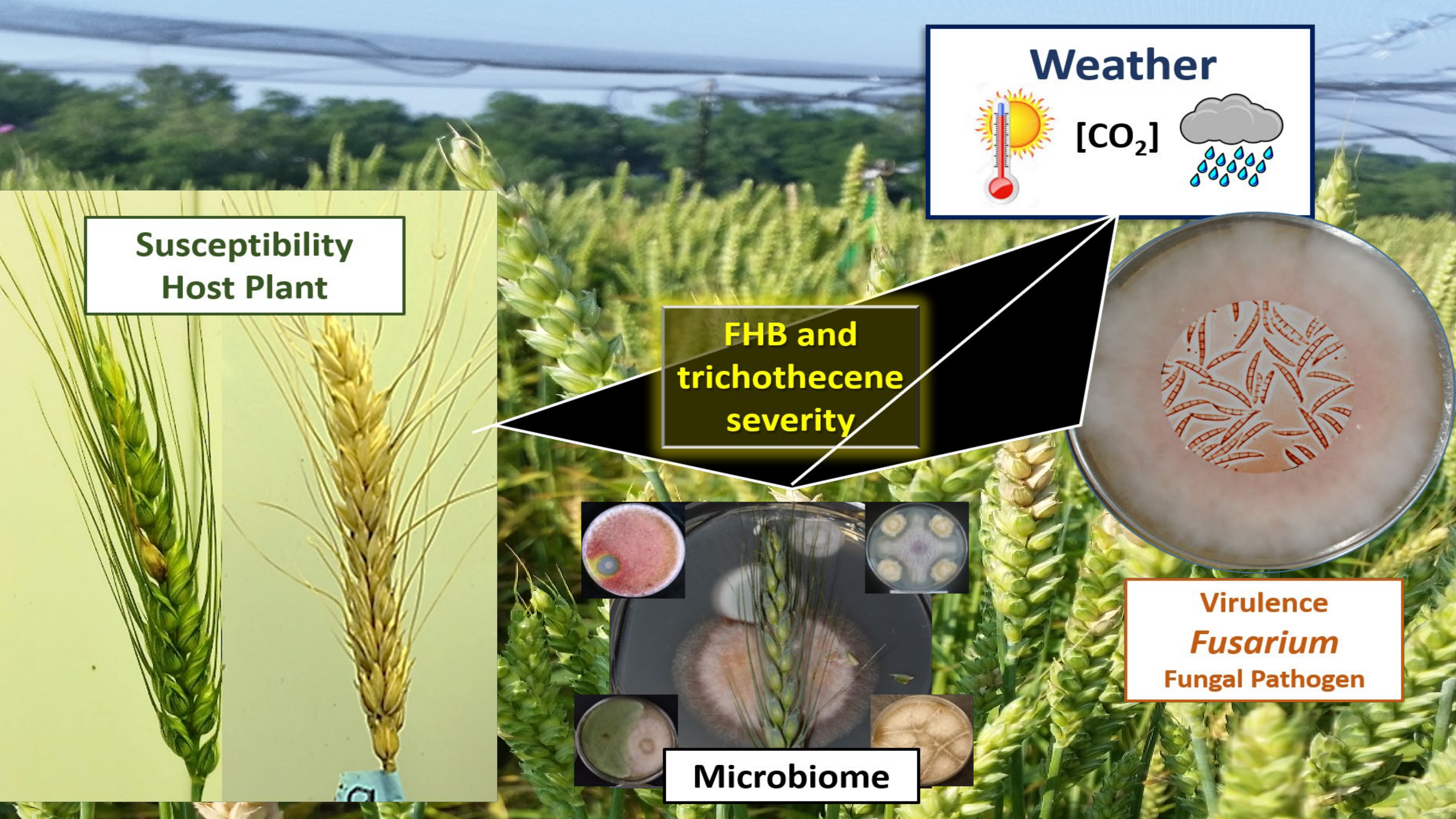
Value of yield forgone:

- **Wheat:** $1,176,000,000
- **Barley:** $293,000,000
- **Corn:** $500,000,000

~ $2 Billion Annually Loss

(Mueller et al., 2016; Wilson et al., 2018)


Weather
[CO_2]
Susceptibility
Host Plant
FHB and
trichothecene
severity
Virulence
Fusarium
Fungal Pathogen
Microbiome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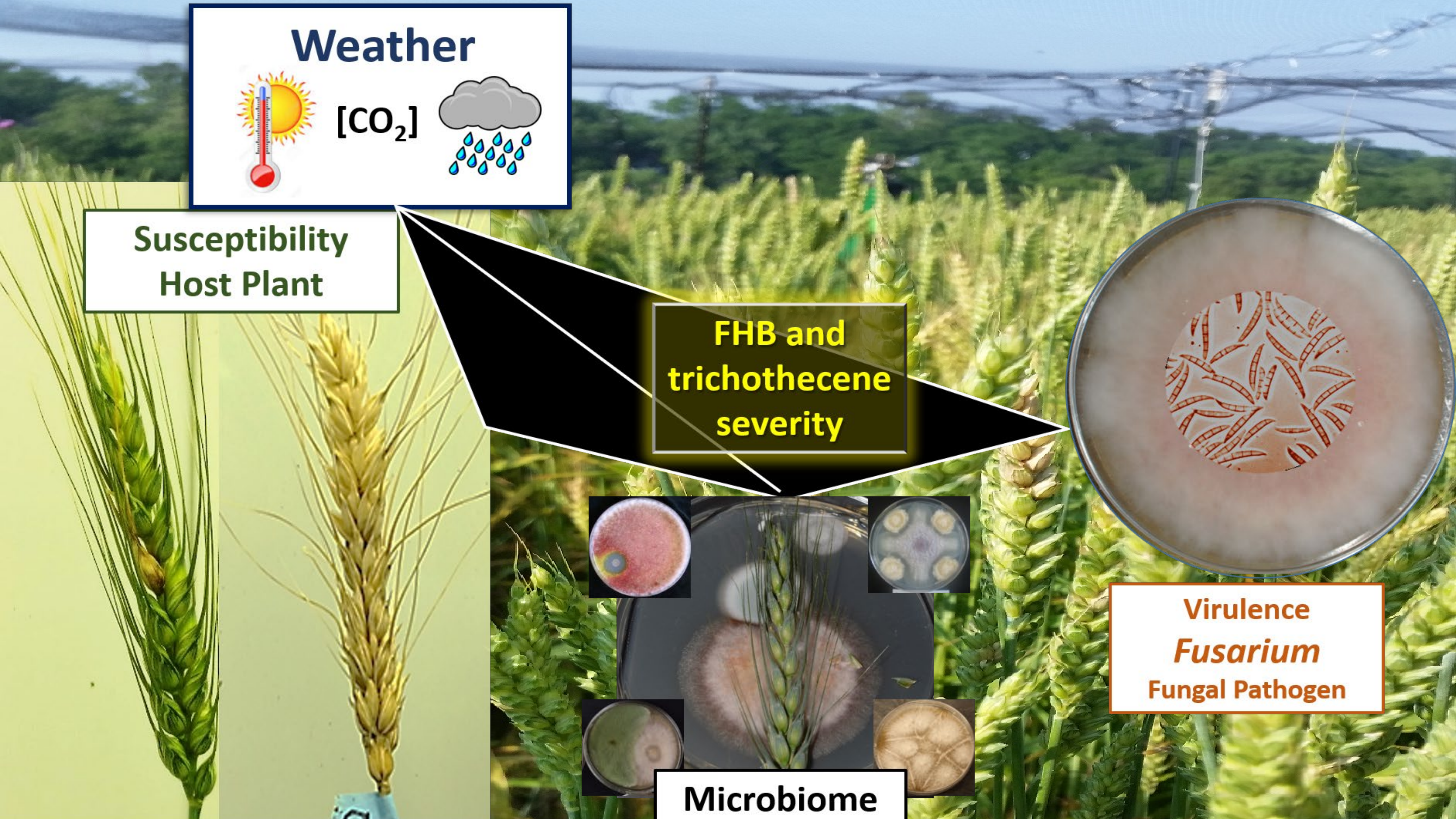
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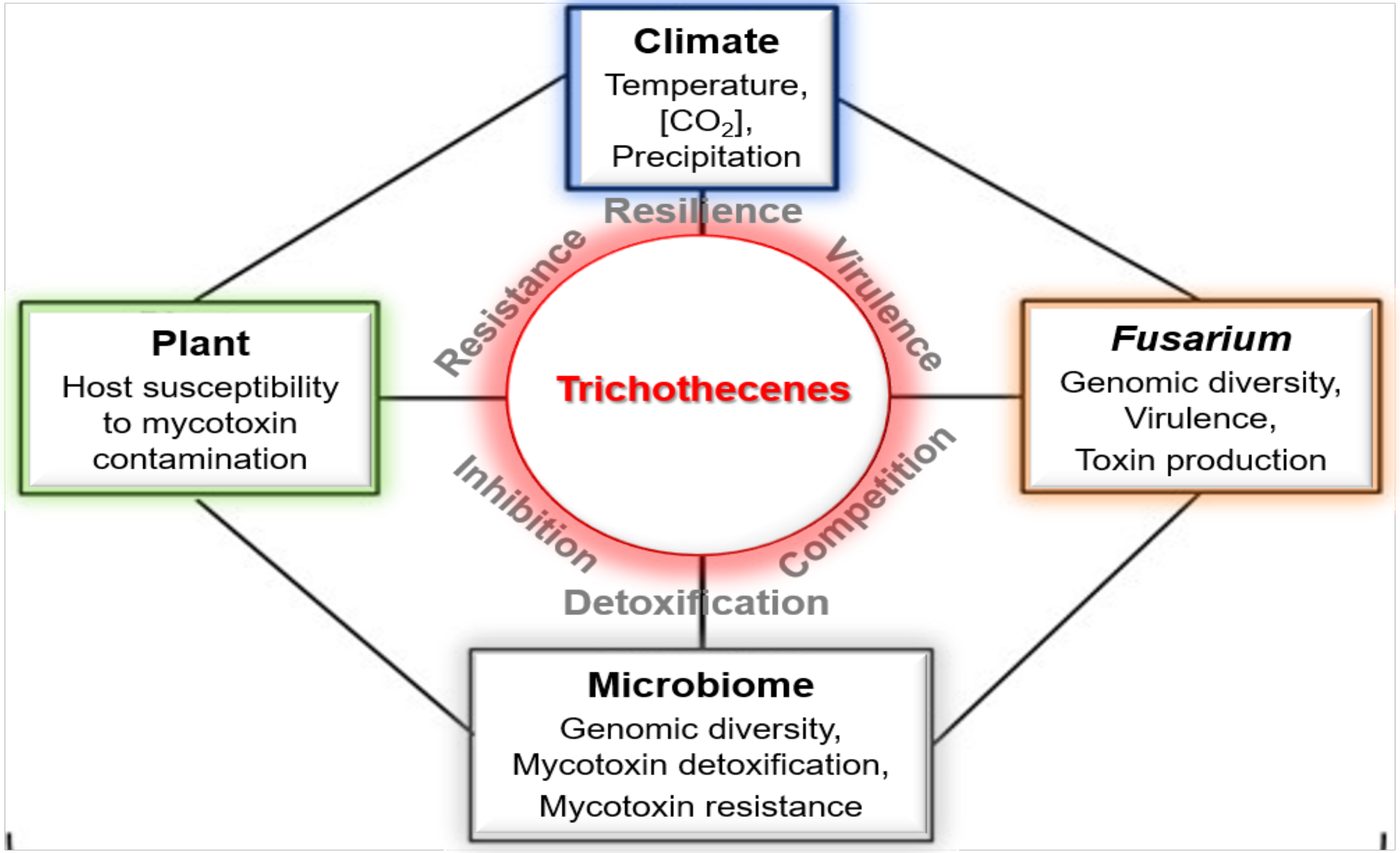
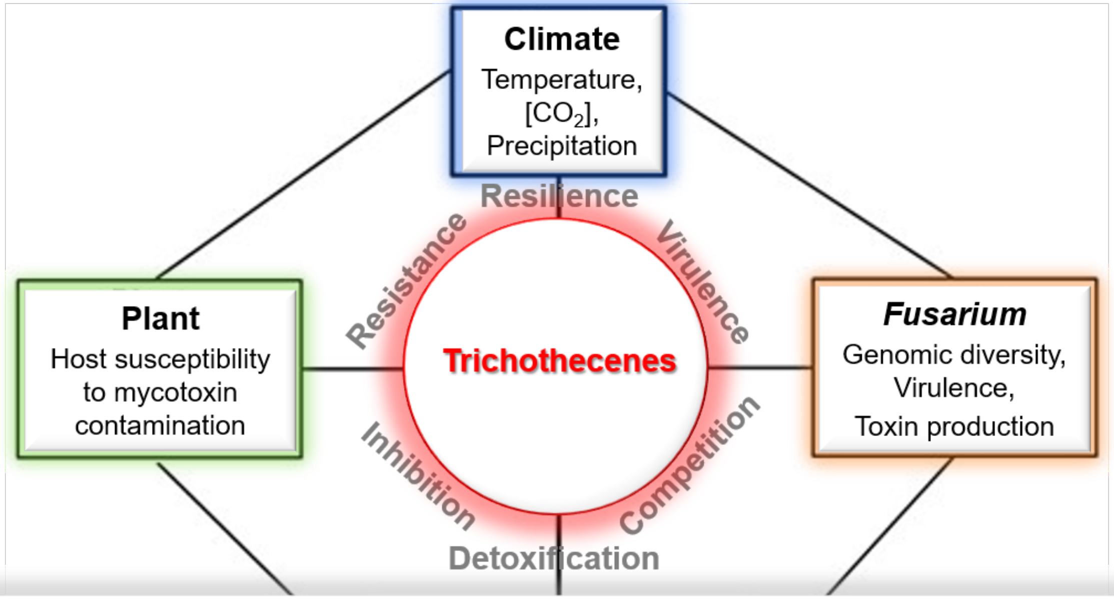
Holistic Ecological Approach to Understanding the Plant-Mycotoxin-Fungal Disease Triangle



Holistic Ecological Approach to Understanding the Plant-Mycotoxin-Fungal Disease Triangle (Microbiome)



Microbiome

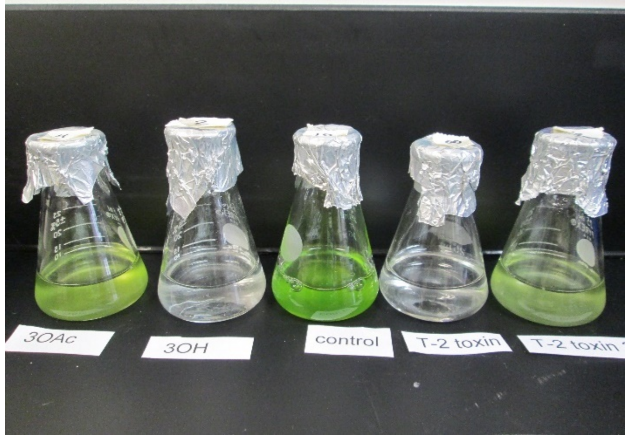
Objective: Identify and characterize microorganisms and microbial genes that can reduce trichothecene contamination of grain-based food and feed.

Trichothecene Resistance Mechanisms

Detoxification mechanisms of trichothecenes:

target key structural features tied to toxicity (do not degrade)

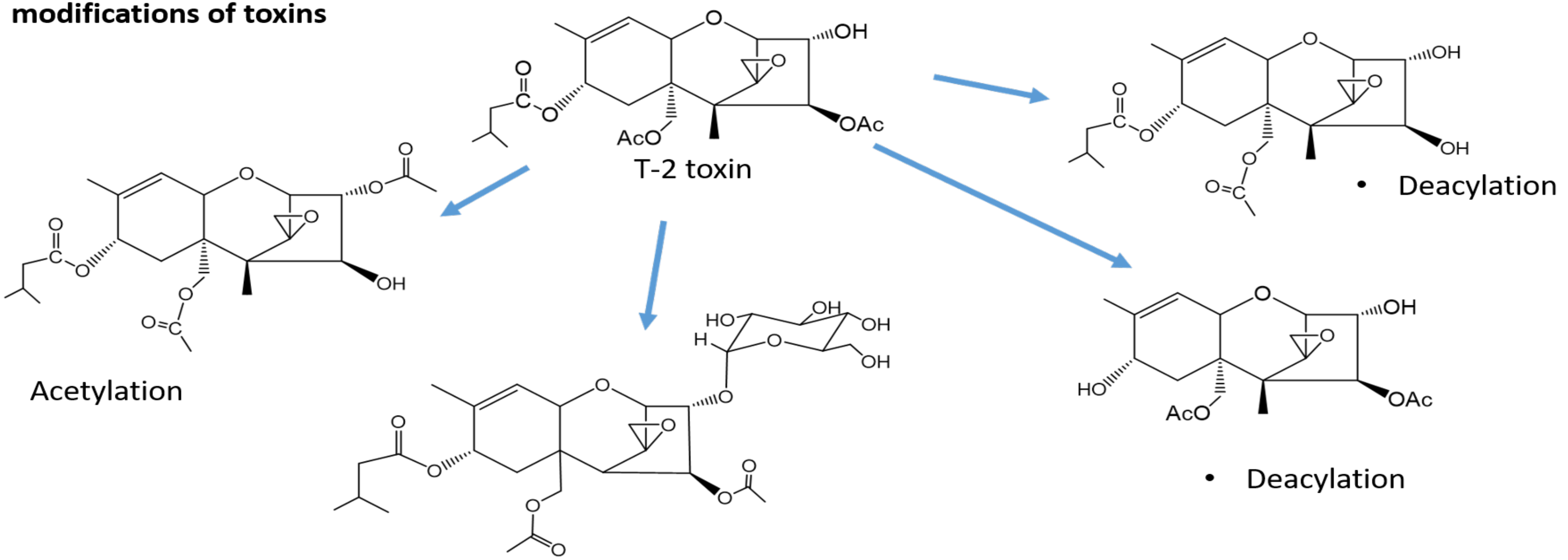
- Acetylation
- Glucosylation – in plants (allows for sequestration) and also in yeast and fungi
- Deacetylation followed by glucosylation
- Deacylation
- Oxygenation (addition of hydroxyl group)
- Oxidative transformations (3-keto)



Assess phytotoxicity with Chlamydomonas

Detoxification of T-2 toxin by *Trichomonascus* and *Blastobotrys* yeast

Testing for direct modifications of toxins

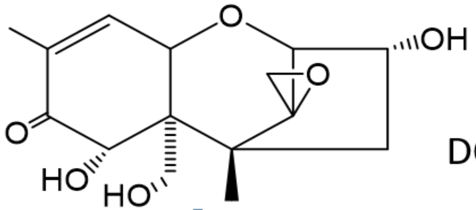


• Glycosylation

(Kurtzman and McCormick)

Detoxification transformations of DON

Testing for direct modifications of toxins

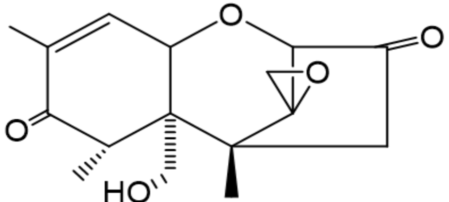


DON

(soil bacteria; laccase)

Laccase enzyme known for making radicals

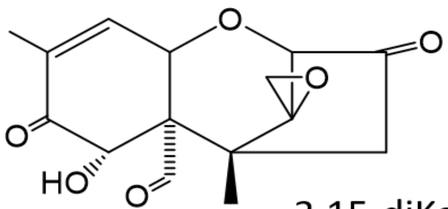
From fungus: *Trametes versicolor*



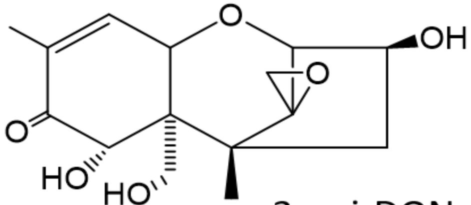
3-keto DON

(laccase)

(soil bacteria)



3,15-diKeto DON



3-epi-DON

- Oxidative transformations 3-keto

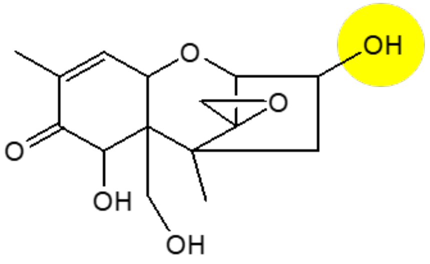
- Reduction

(Bakker and McCormick)

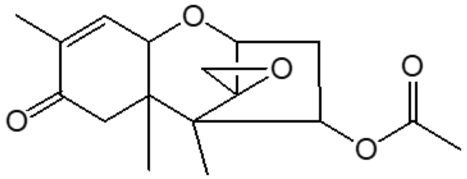
Search for novel trichothecene resistance mechanisms in fungi:

Absence of 3-hydroxyl in non-*Fusarium* trichothecenes indicate the producing fungi have a self-protection mechanism other than the *3-O*-acetylation

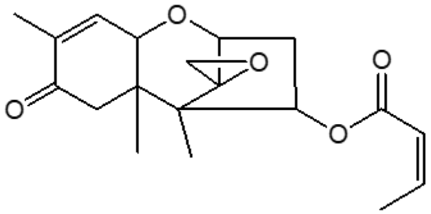
Fusarium graminearum
Cereal pathogen



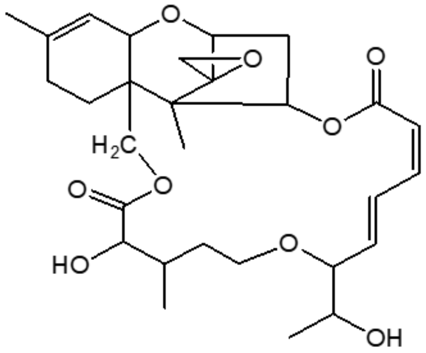
Microcyclospora tardicrescens
Apple pathogen



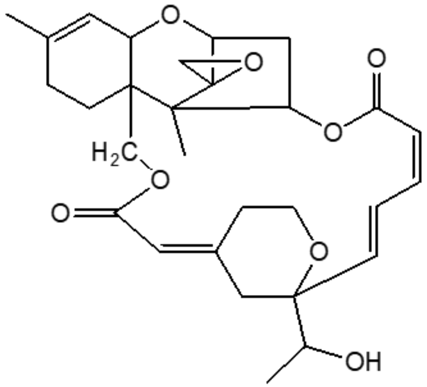
Trichothecium roseum
Saprotroph/plant pathogen



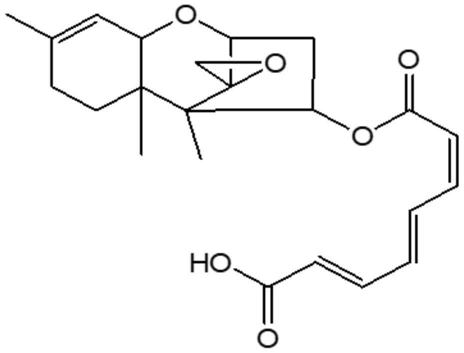
Myrothecium roridum
Melon pathogen



Stachybotrys chartarum
Saprotroph, in damp buildings



Trichoderma arundinaceum
Saprotroph, biocontrol activity



cDNA libraries

↓

DON sensitive yeast

↓

Screen for resistance

↓

Identify resistance gene

(Proctor)

Examination of content of trichothecene biosynthetic (*TRI)* genes in trichothecene-producing fungi: some *TRI* genes are unique to *Fusarium*

Fungus	TRI Gene 1	TRI Gene 3	TRI Gene 4	TRI Gene 5	TRI Gene 6	TRI Gene 7	TRI Gene 8	TRI Gene 9	TRI Gene 10	TRI Gene 11	TRI Gene 12	TRI Gene 13	TRI Gene 14	TRI Gene 16	TRI Gene 17	TRI Gene 18	TRI Gene 21	TRI Gene 22	TRI Gene 23	TRI Gene 101
Fusarium graminearum						Ψ						Ψ		Ψ						
Fusarium longipes																				
Fusarium sporotrichioides																				
F. incarnatum-equiseti complex																				
Aspergillus hancockii																				
Beauveria bassiana																				
Cordyceps confragosa																				
Microcyclospora tardicrescens																				
Myrothecium roridum																				
Spicellum ovalisporum																				
Spicellum roseum																				
Stachybotrys chartarum																				
Stachybotrys chlorohalonata																				
Trichoderma brevicompactum																				
Trichoderma arundinaceum																				
Trichothecium roseum																				

(Proctor et al., 2018)

TRI14 is present in all trichothecene-producing fungi, but is not required for biosynthesis: is *TRI14* a resistance gene?

Fungus	TRI Gene 1	TRI Gene 3	TRI Gene 4	TRI Gene 5	TRI Gene 6	TRI Gene 7	TRI Gene 8	TRI Gene 9	TRI Gene 10	TRI Gene 11	TRI Gene 12	TRI Gene 13	TRI Gene 14	TRI Gene 16	TRI Gene 17	TRI Gene 18	TRI Gene 21	TRI Gene 22	TRI Gene 23	TRI Gene 101
Fusarium graminearum						Ψ						Ψ		Ψ						
Fusarium longipes																				
Fusarium sporotrichioides																				
F. incarnatum-equiseti FIESC 12																				
Aspergillus hancockii																				
Beauveria bassiana																				
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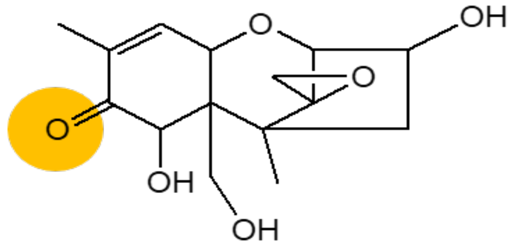
Trichoderma brevicompactum																				
Trichoderma arundinaceum																				
Trichothecium roseum																				

Plan: heterologously express *TRI14* in yeast to determine whether it confers trichothecene resistance

(Proctor et al., 2018)

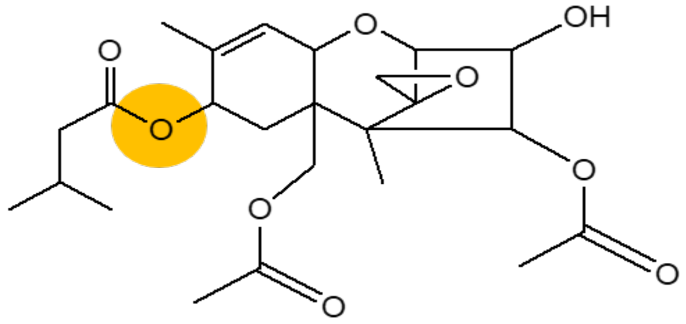
Trichothecene C8 oxygenation has evolved independently at least three times

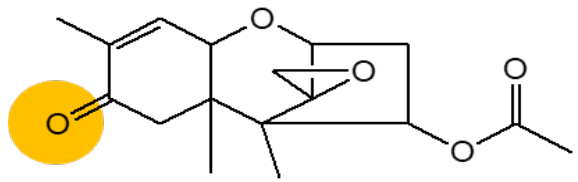
Fusarium
Deoxynivalenol



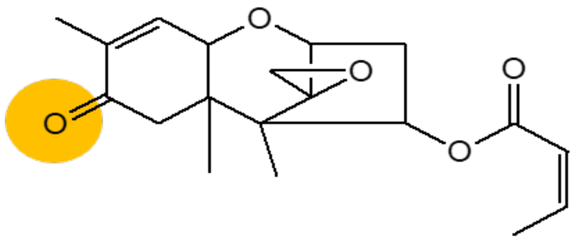
TRI1

Fusarium
T-2 toxin





Trichothecium
TRI?



Microcylospora
TRI24

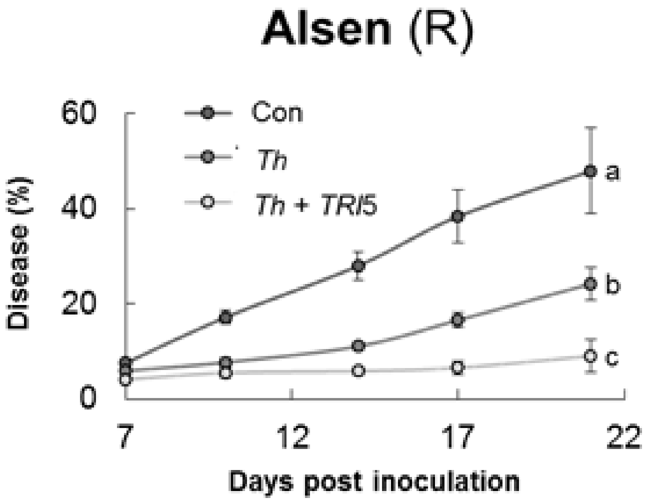
- Suggests a selective advantage to fungi in at least some environments.
- Understanding what the advantage is, will aid in the development control strategies

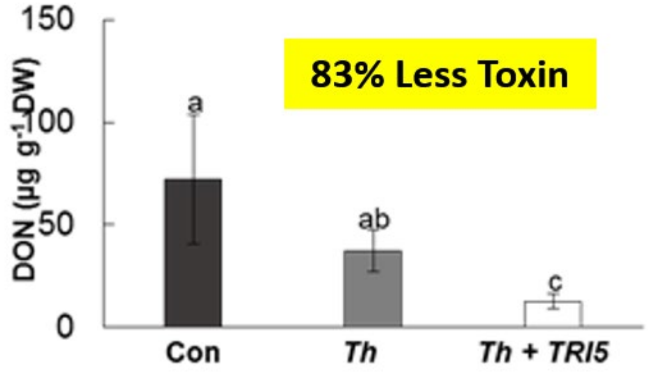
(Proctor et al., 2018)

Trichoderma harzianum + *TRI5* emits the volatile trichodiene which stimulates plant defenses and down regulates DON production d

Con *Th* *Th+TRI5*

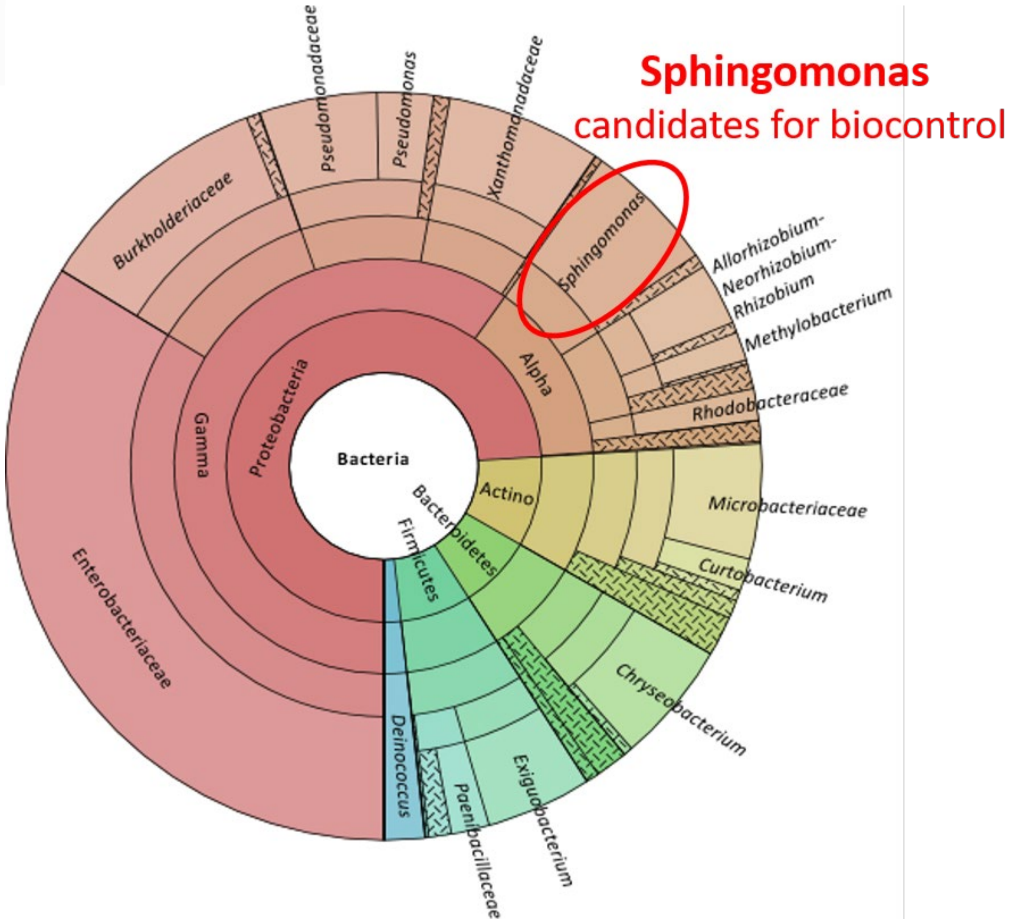
Con= *Fg* only

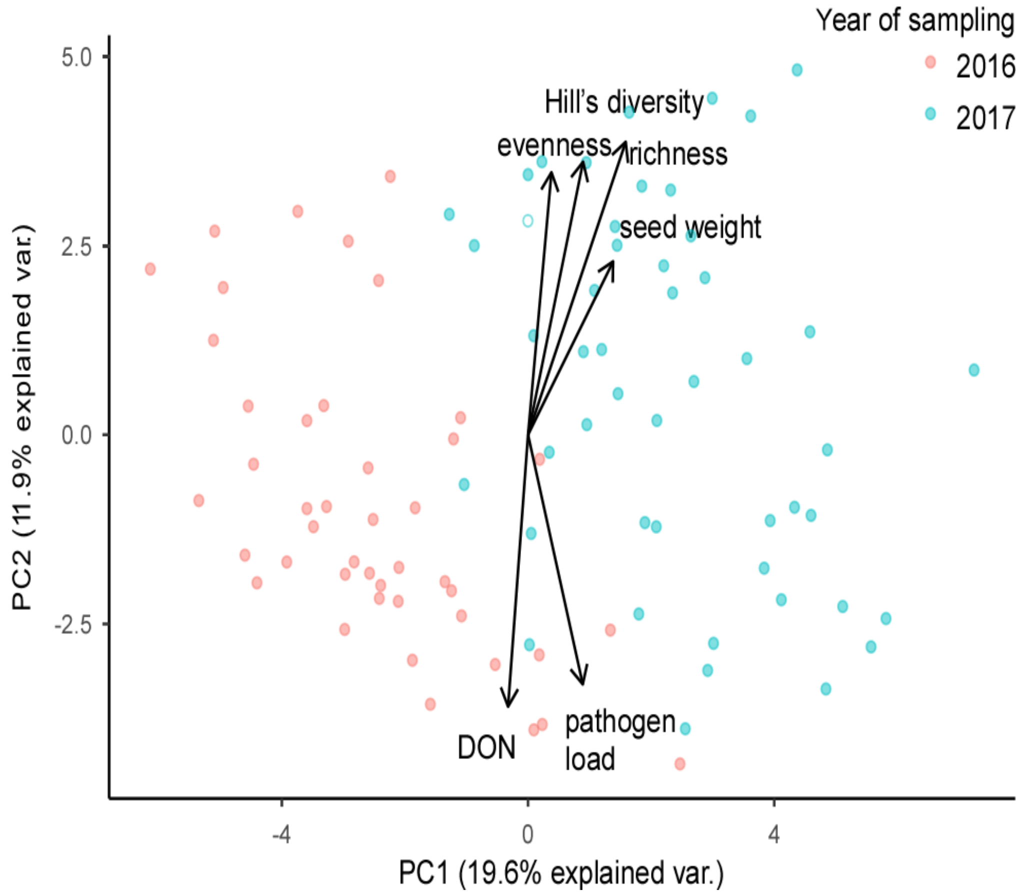




(Vaughan)

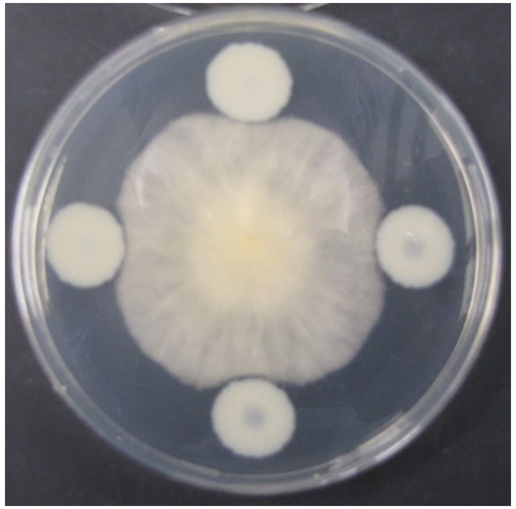
Microbiome characteristics relate significantly to toxin content and pathogen biomass within wheat kernels

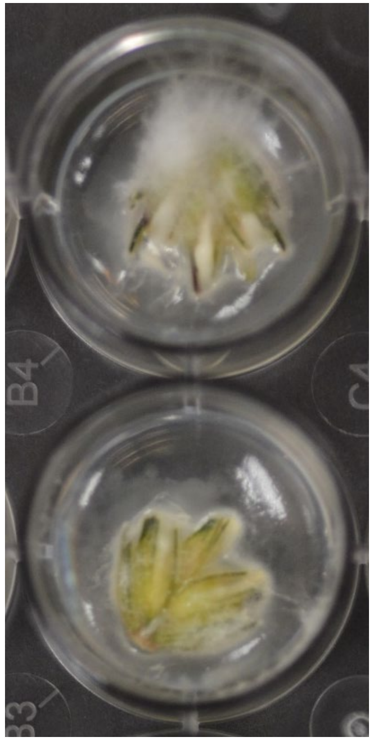


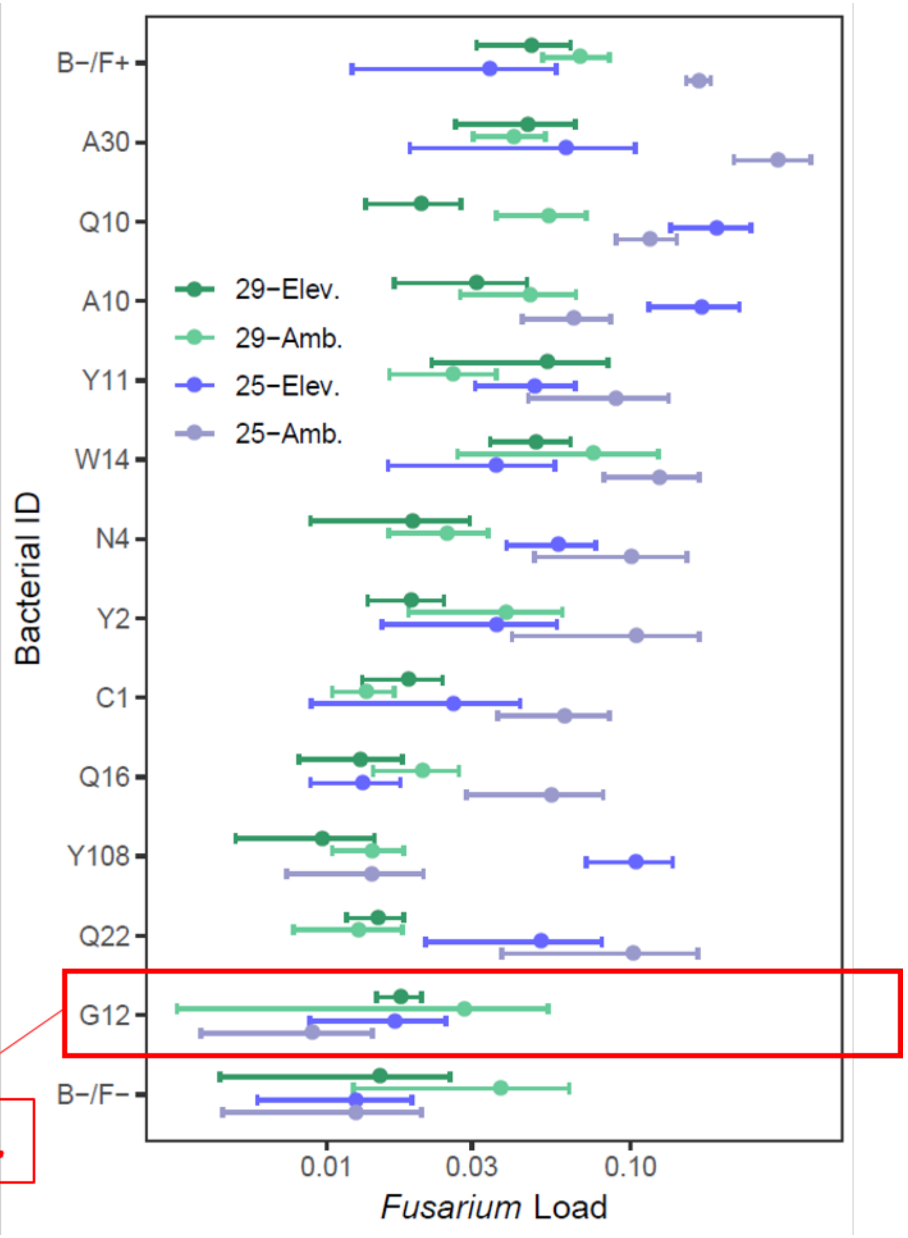


(Bakker and McCormick, 2019)

The efficacy of biocontrol microbes antagonistic to *Fusarium* varies with environmental conditions



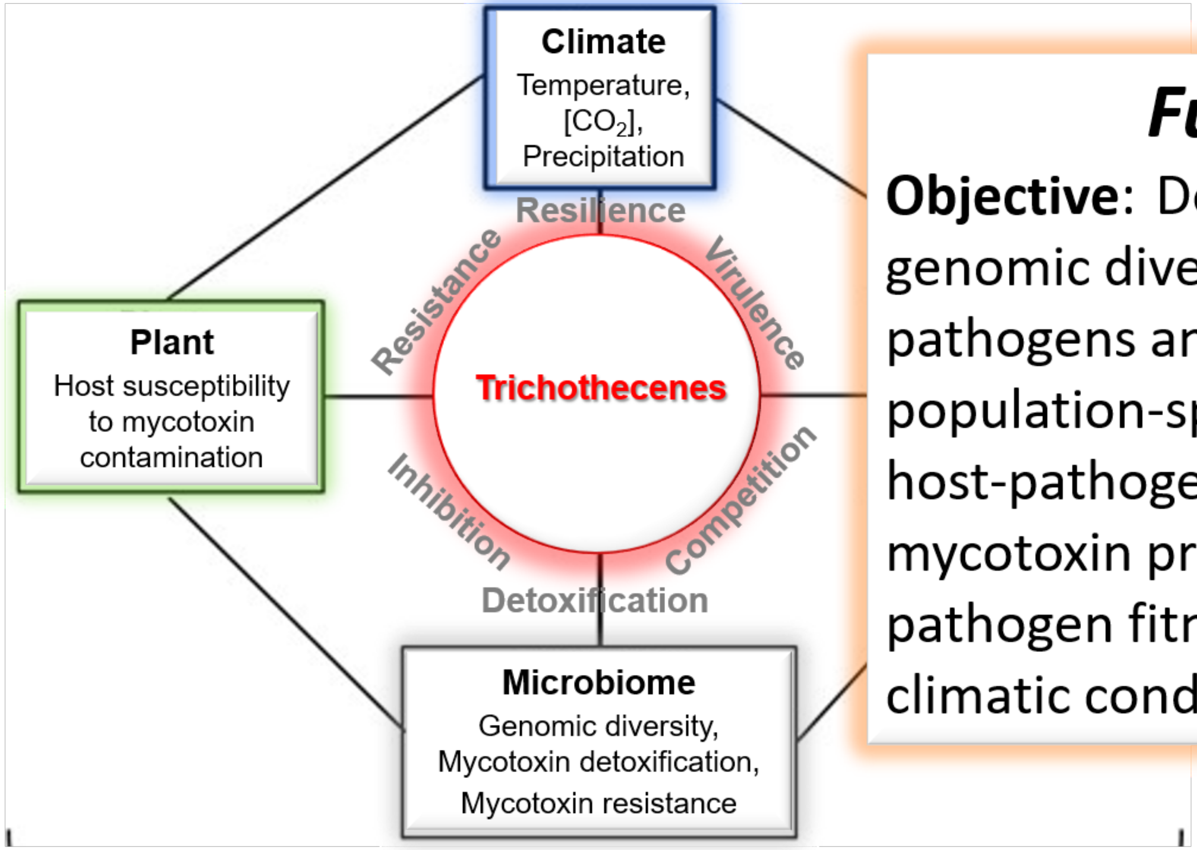




G12= *Terribacillus sp.*

(Whitaker and Bakker, 2019)

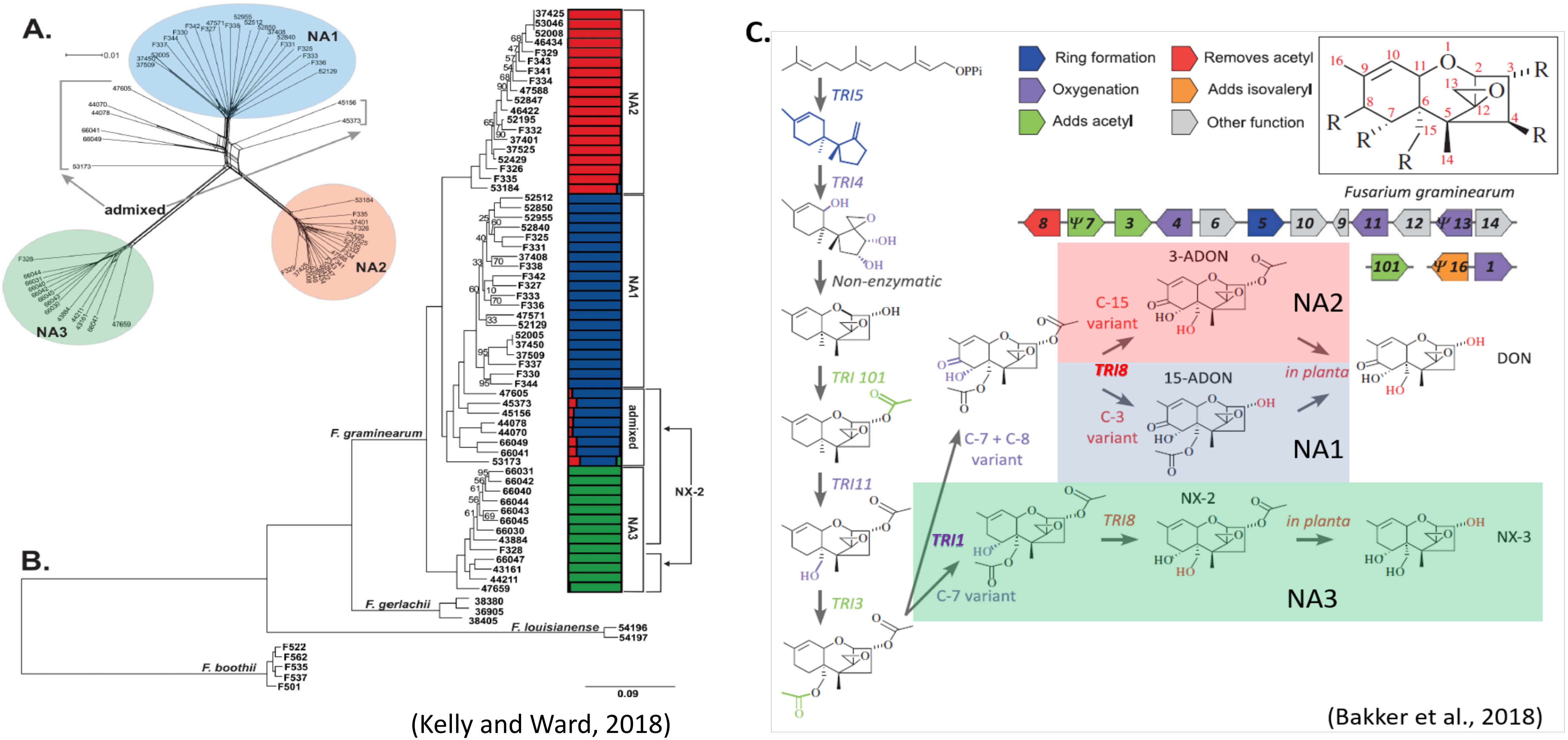
Holistic Ecological Approach to Understanding the Plant-Mycotoxin-Fungal Disease Triangle (Fusarium)



Fusarium

Objective: Determine the genomic diversity of FHB pathogens and identify population-specific differences in host-pathogen interactions, mycotoxin production, or pathogen fitness under different climatic conditions.

Three North American Populations of *F. graminearum*



(Kelly and Ward, 2018)

(Bakker et al., 2018)

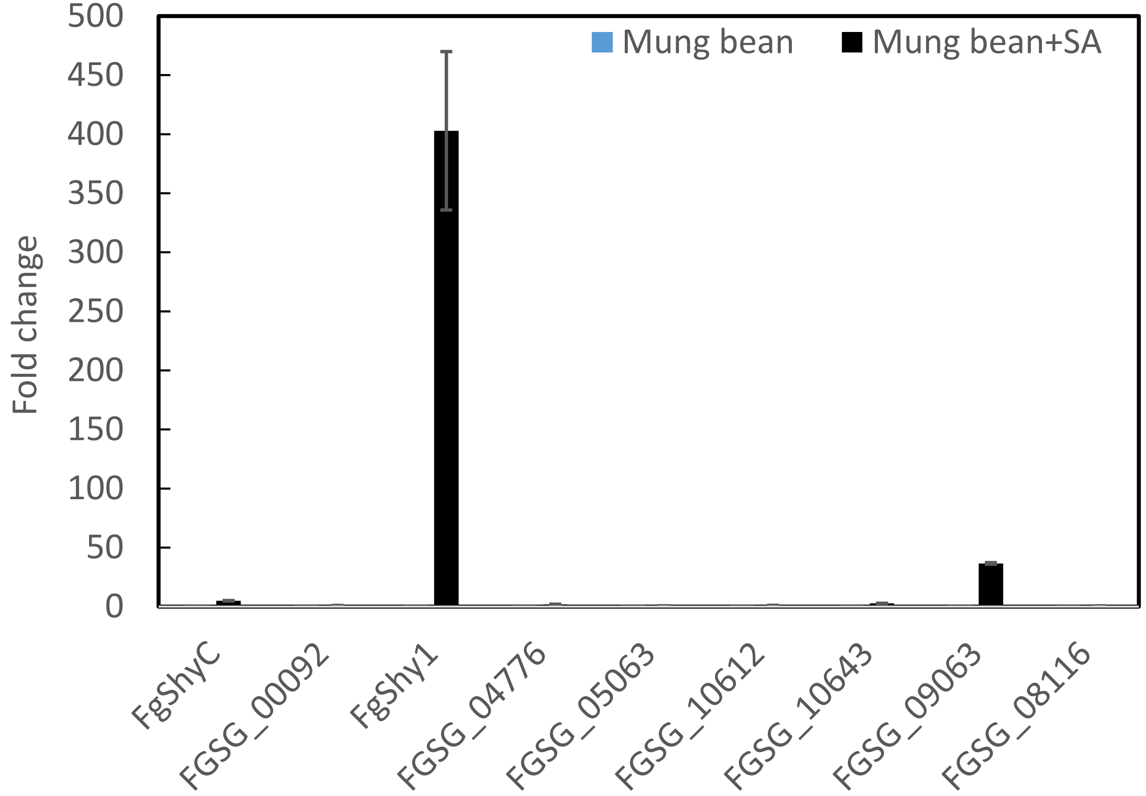
Genomic Signatures of Adaptive Divergence

Plant Path (7 loci)	Antagonism (3 loci)	Ecological Adaptation (2 loci)
Degradative enzymes	Heterokaryon incompatibility	Photolyase
Adhesin	Chitinases	Cryptochrome
Trichothecene toxin cluster	**Ecp2 effector with a chitinase domain**	Perithecial pigment (PKS1)
		DEAD DNA/RNA helicase

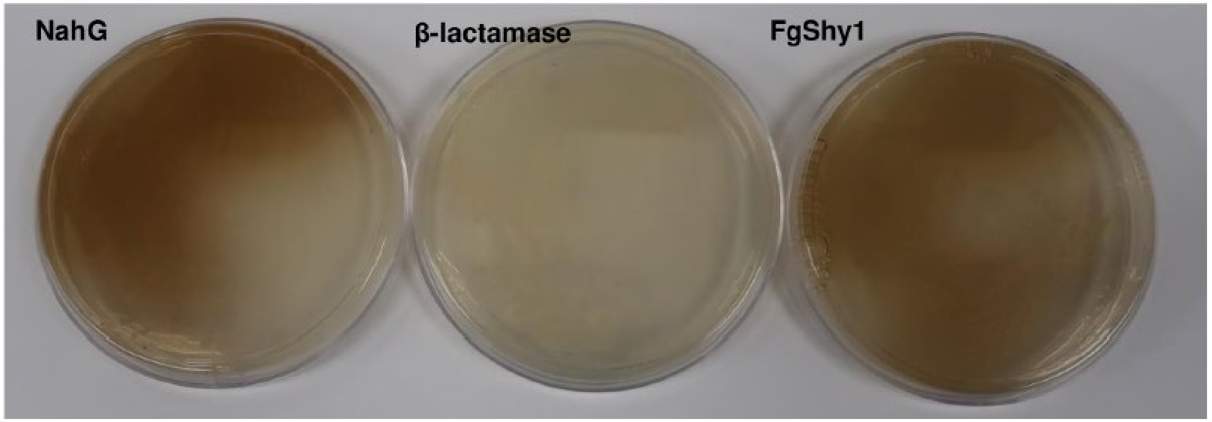
Essential for adaptation to local climate conditions

(Kelly and Ward, 2018)

Characterization of *FgShy1* salicylate hydroxylase



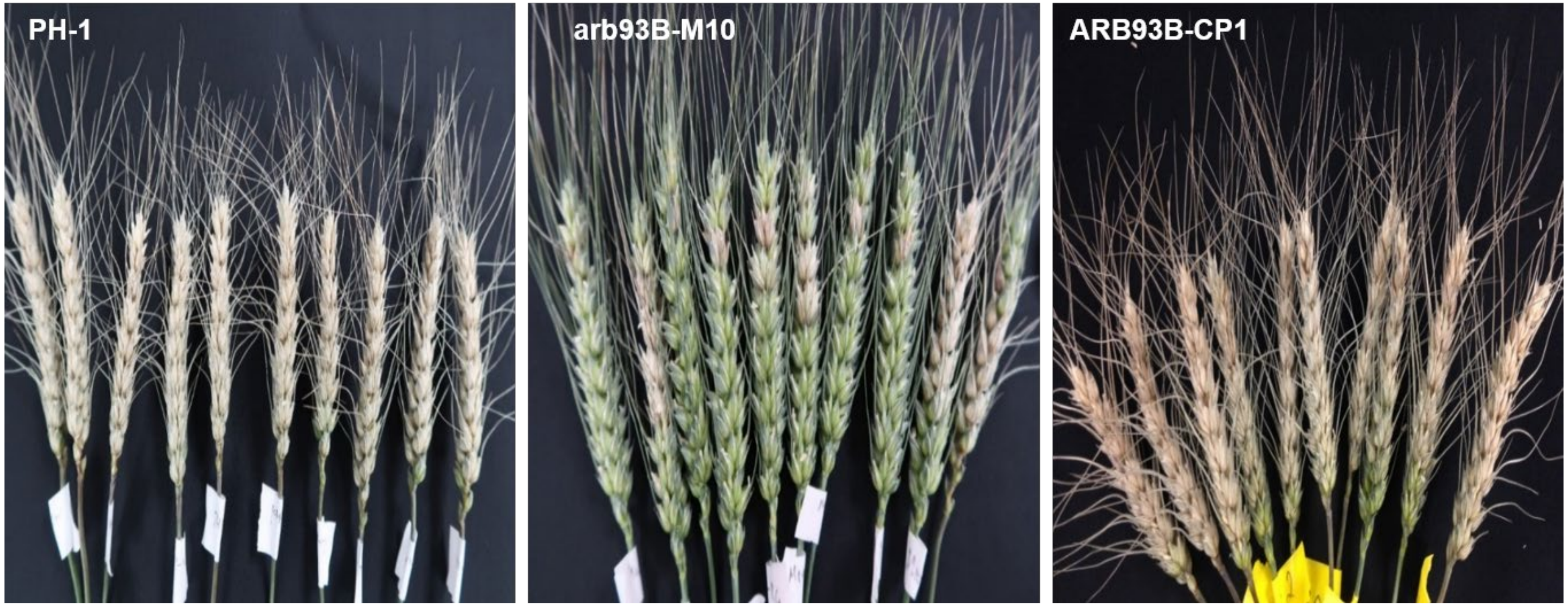
Analysis of salicylate hydroxylase candidates induction gene expression in mung bean liquid medium containing 1mM SA.
FgShy1 is highly induced by addition of exogenous SA.



Salicylate Hydroxylase Activity Assay demonstrating salicylate hydroxylase activity of heterologously-expressed FgShy1 in *E.coli.* Development of brown color, indicating conversion of SA to catechol,

(Hao et al., 2019)

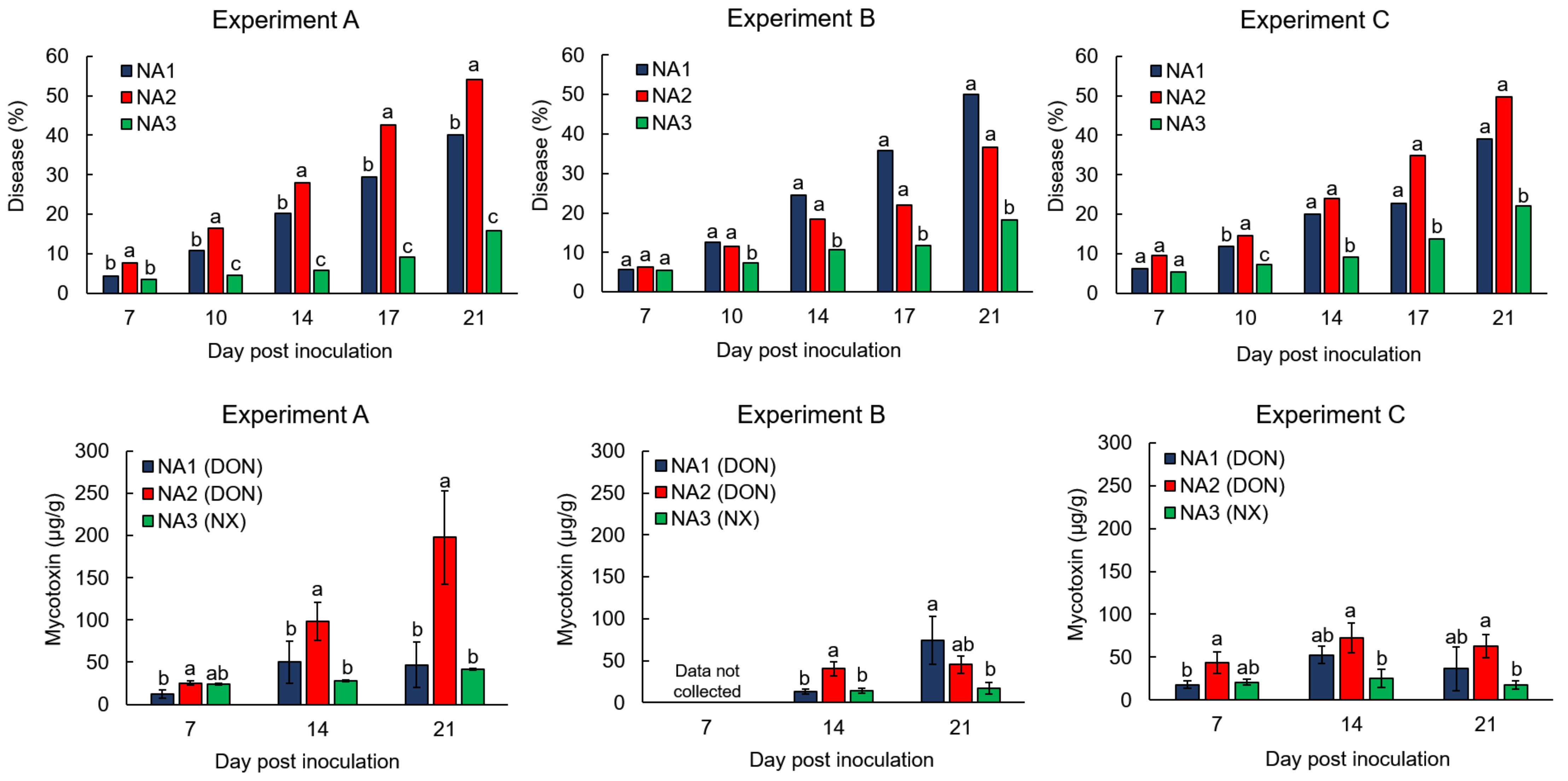
F. graminearum arabinanase (Arb93B) enhances wheat head blight susceptibility by suppressing plant immunity



F. graminearum arabinanase (Arb93B) mutant reduces FHB

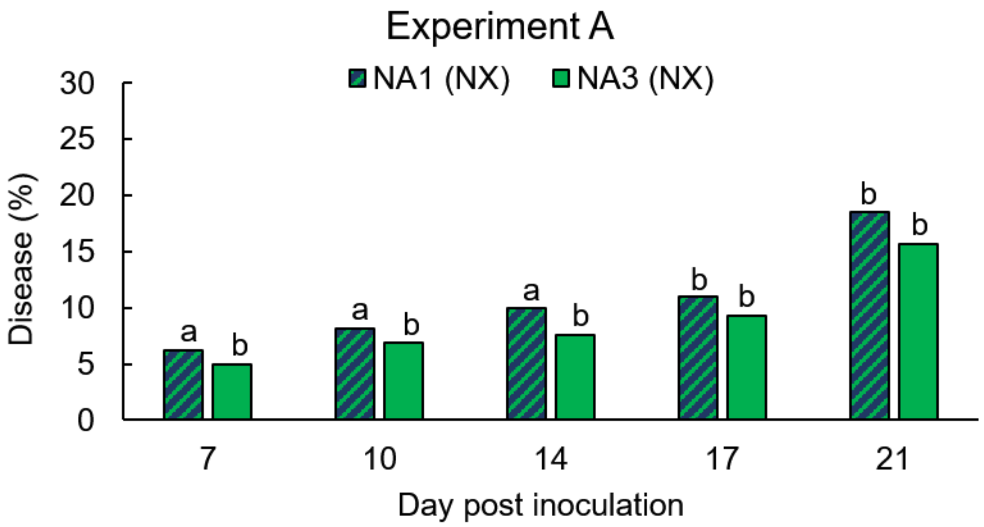
(Hao et al., 2019)

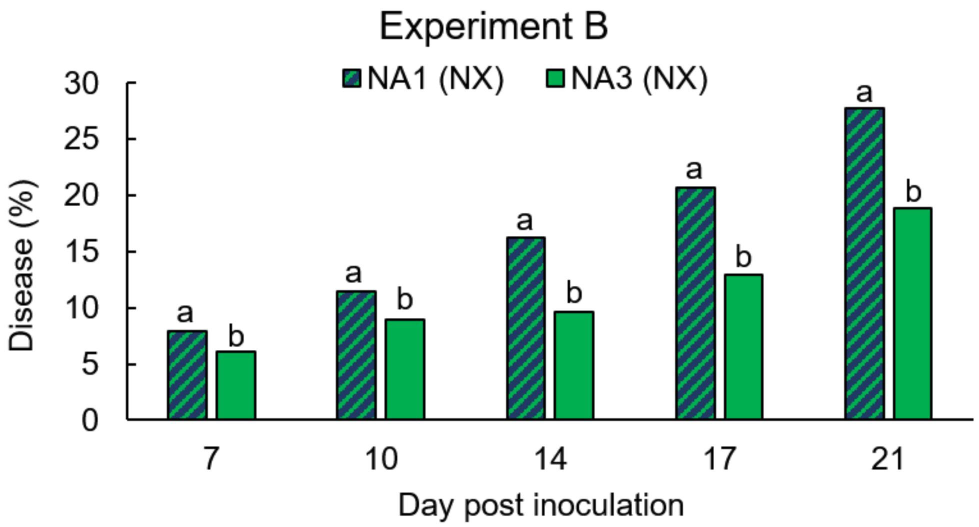
Population Specific Differences in Disease



(Vaughan and Ward et al.)

Population genetic background influences disease development





- **NX toxin a contributing factor**
- **NA1 genetic background is more virulent than NA3**

Hypothesis:

1) NA3 has an effector that stimulates plants defenses more strongly

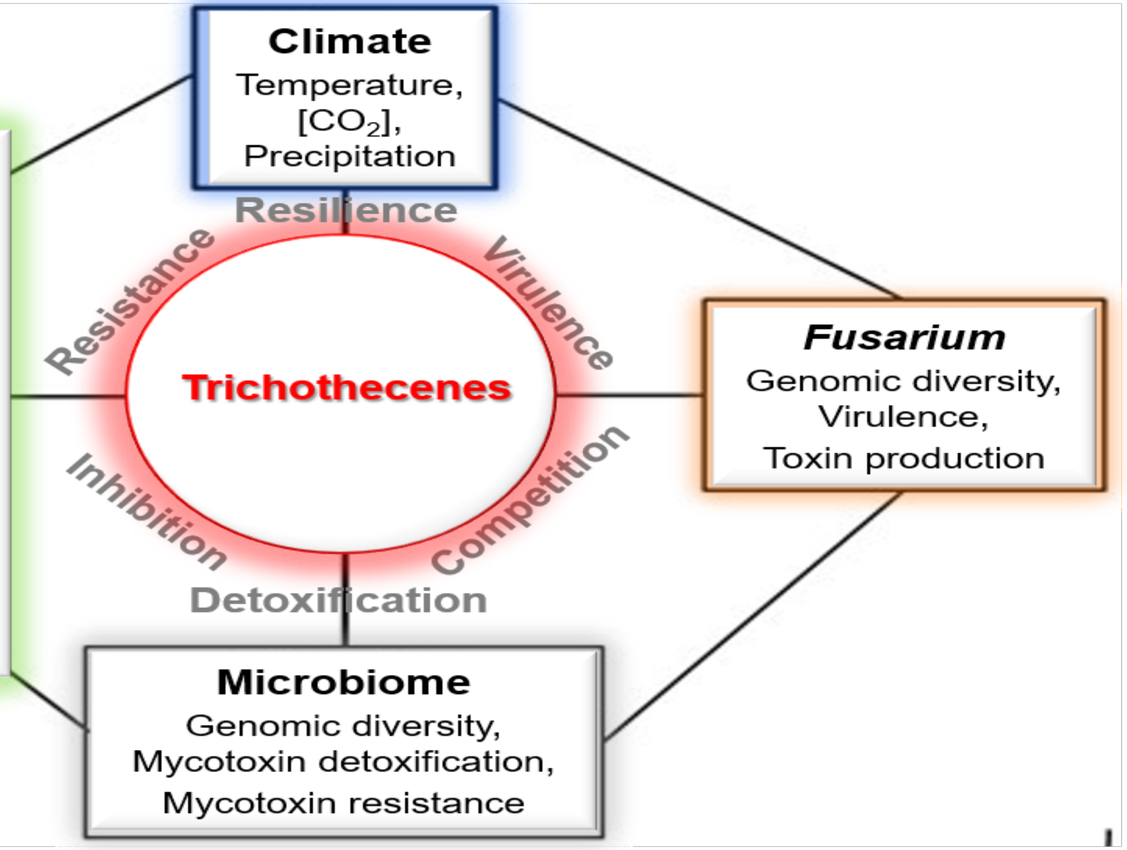
2) NA1 has an effector that down regulates host defenses that NA3 is missing

(Vaughan and Ward et al.)

Holistic Ecological Approach to Understanding the Plant-Mycotoxin-Fungal Disease Triangle (Plant)

Plant

Objective: Determine the effects of climate change on susceptibility of wheat to contamination by trichothecenes and other *Fusarium* mycotoxins.



Effects of elevated CO_2 level on the metabolic response of resistant and susceptible wheat to *F. graminearum* infection

$1x[CO_2]$ = 400 ppm

$2x[CO_2]$ = 800 ppm

FHB susceptible and resistant Spring Wheat varieties:

- **Norm and Alsen**

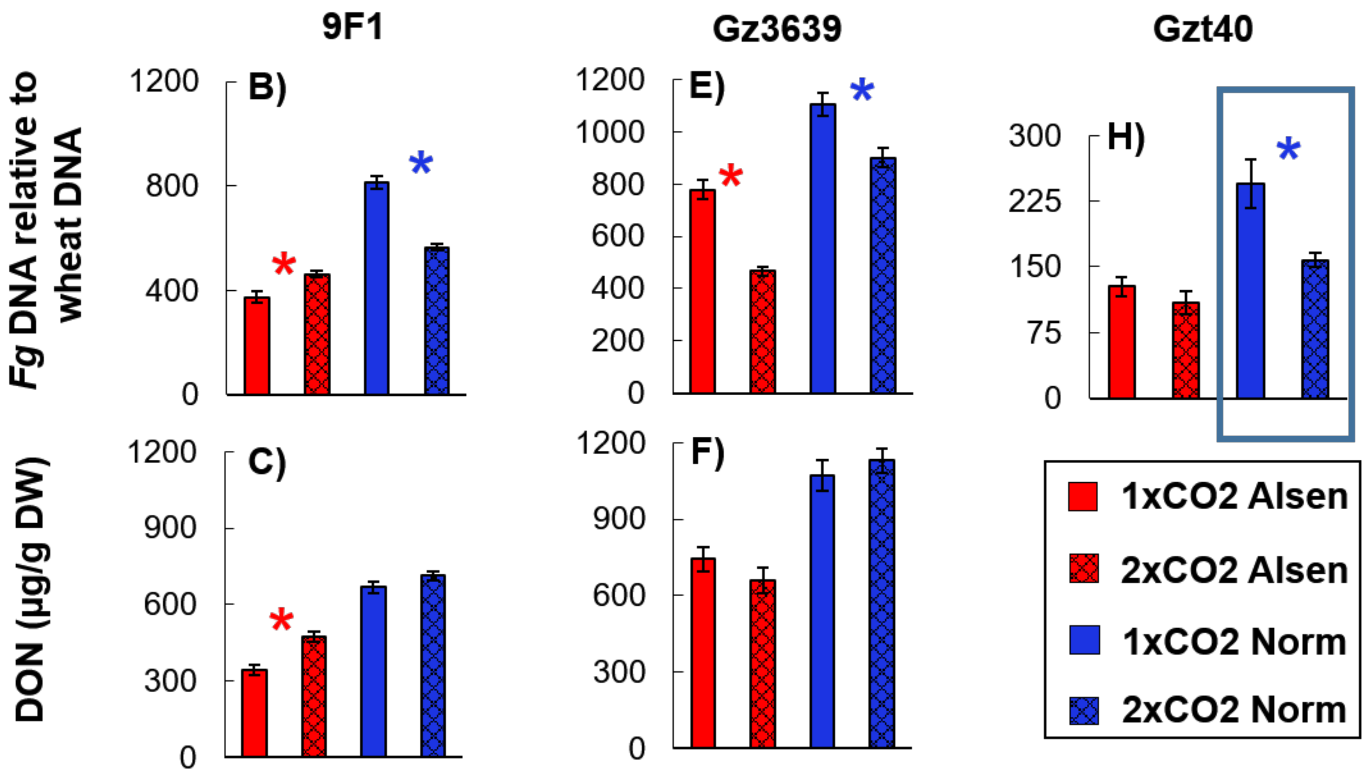
***F. graminearum* strains:**

- **9F1 DON+**
- **Gz3639 DON+**
- **Gzt40 DON-**

Dip inoculation method

- **Evaluated 7 pdi**

Effects of elevated *CO2* level on the metabolic response of resistant and susceptible wheat to *F. graminearum* infection



The effects of elevated CO_2 are dependent on both the *F. graminearum* strain and the wheat variety

Resistant variety Alsen:

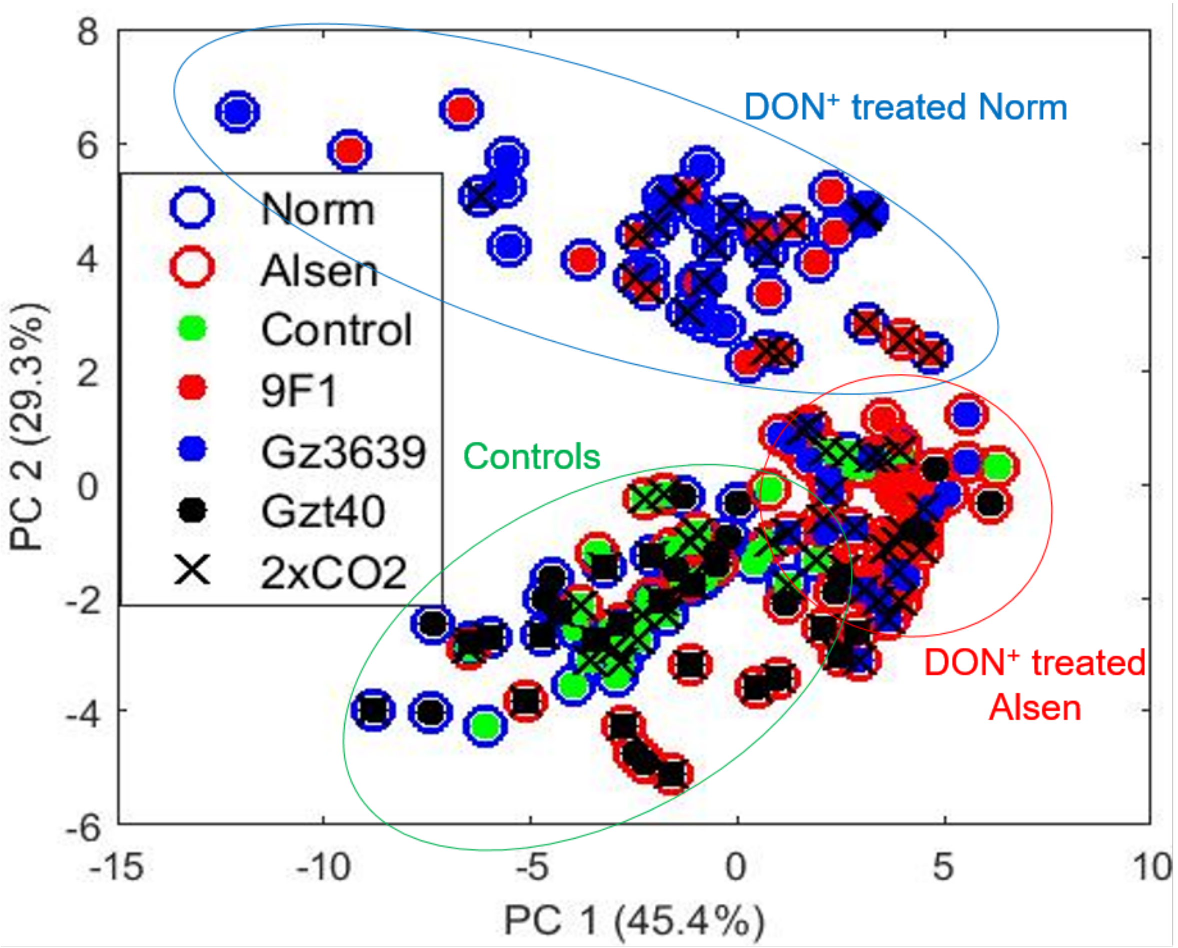
- Effect dependent on *Fg* strain
- Compromised resistance to 9F1 biomass and DON
- Enhanced resistance Gz3639 biomass but no change in DON

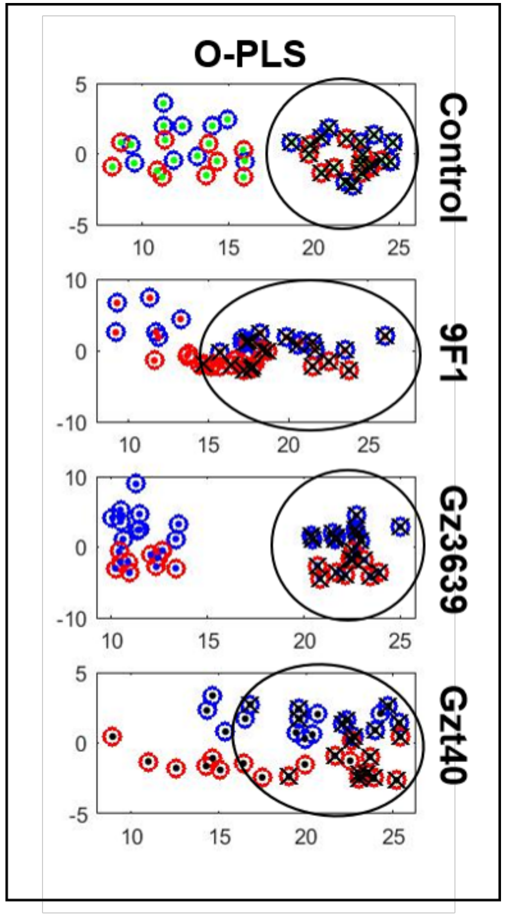
Susceptible variety Norm:

- enhanced resistance to pathogen biomass accumulation
- no change in DON
- DON production per unit *Fg* biomass was increased
- **Enhanced resistance not dependent on DON**

(Cuperlovic-Culf and Vaughan et al., 2018)

Non-Targeted Metabolomic Analysis

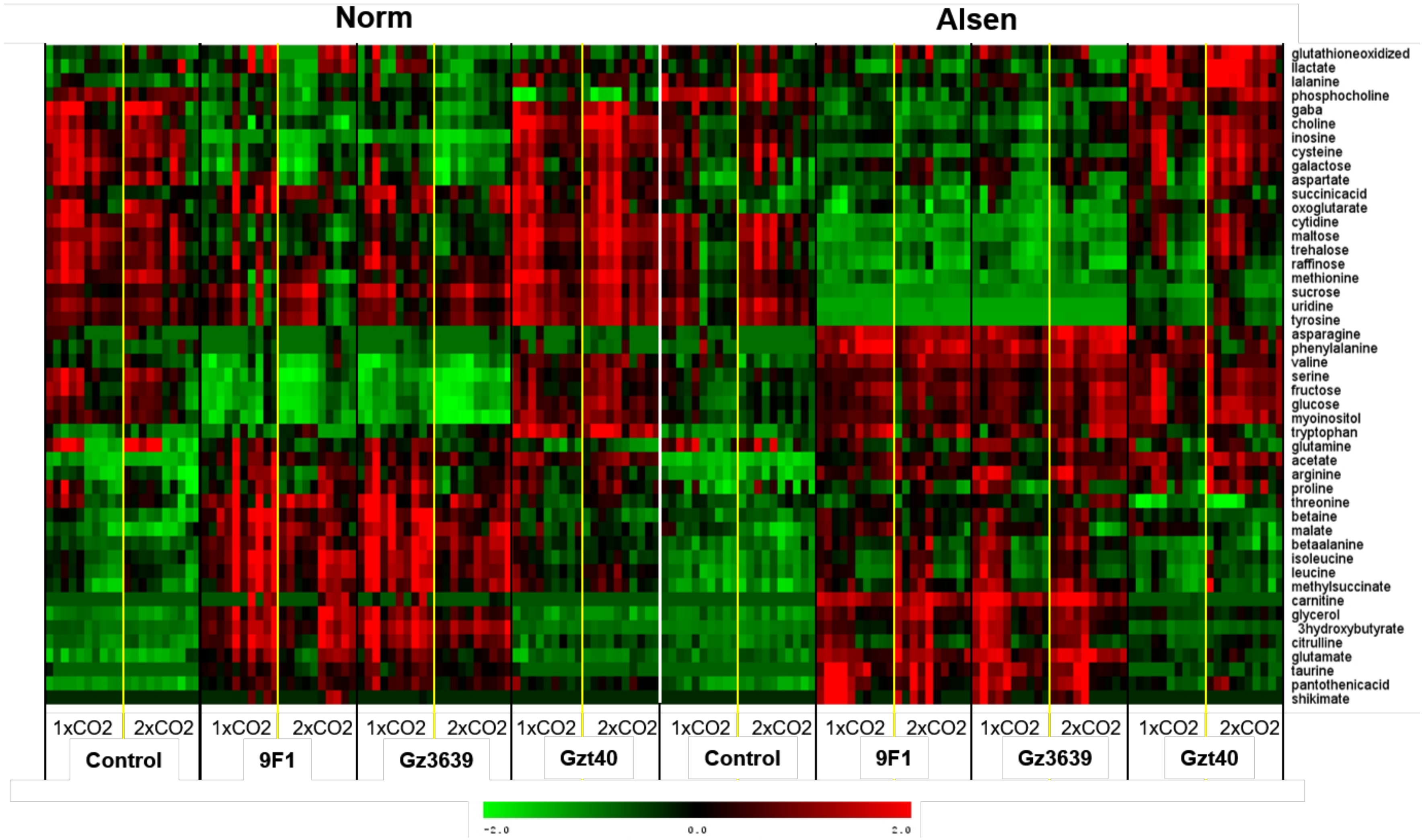




Principal component analysis (PCA) of 1D NMR spectra illustrated variances in the metabolic profiles across all samples

(Cuperlovic-Culf and Vaughan et al., 2018)

Relative Concentration of Targeted Metabolites



45 metabolites

Metabolomic Analysis

- **Metabolic pathways that contribute to the primary source of FHB resistance, are upregulated in *Fg* inoculated Alsen relative to Norm at both CO2 concentrations.**

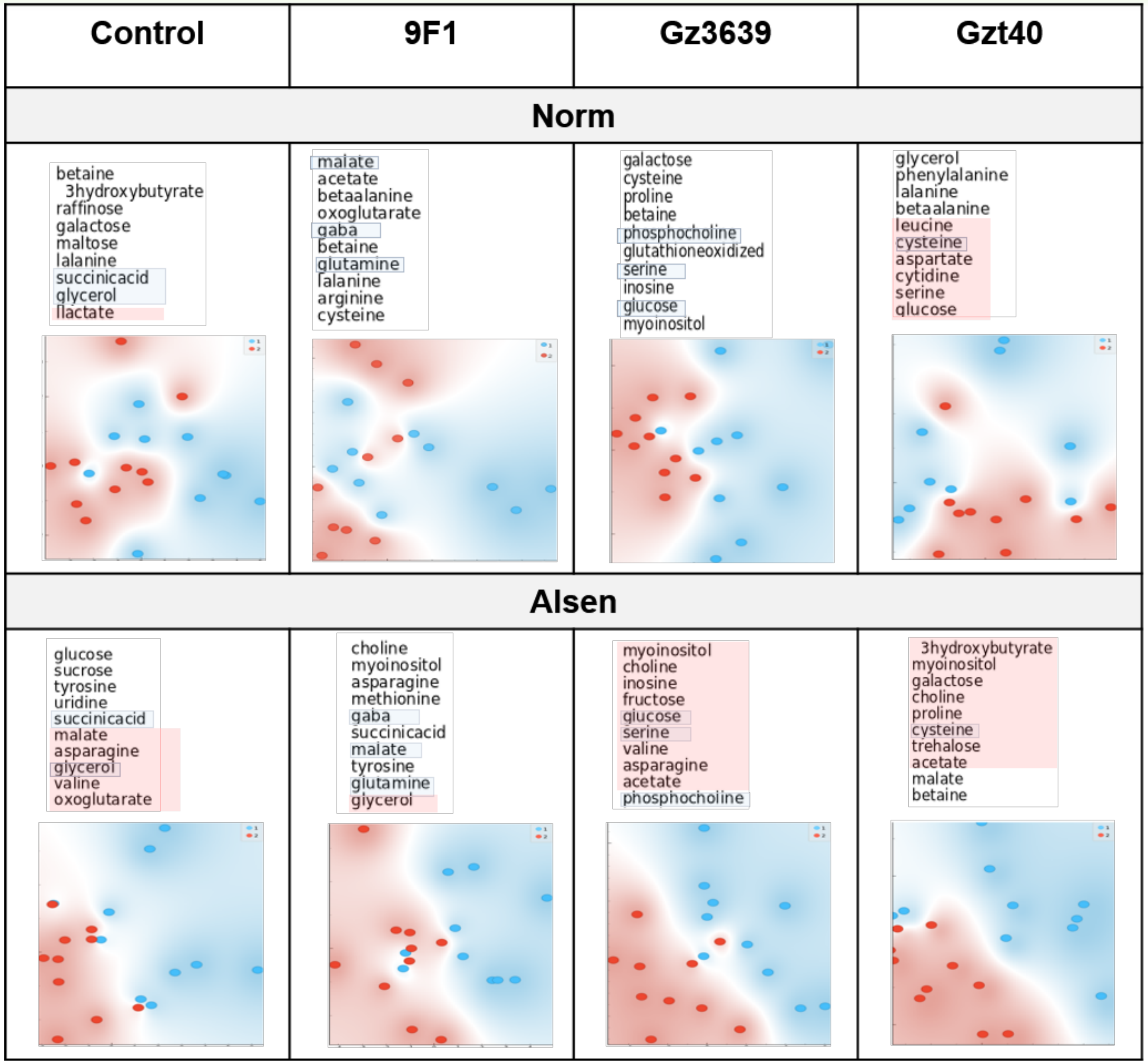
- **Fhb loci containing varieties will likely remain more resistant to FHB than non Fhb-containing varieties even at elevated CO_2.**

- **Identification of FHB resistance markers that are not effected by CO_2 concentration differences**

  - **L-alanine**
  - **isoleucine**
  - **hydroxybutarate**
  - **myoinositol**

(Cuperlovic-Culf and Vaughan et al., 2018)

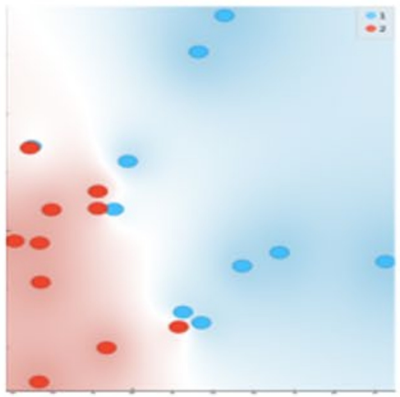
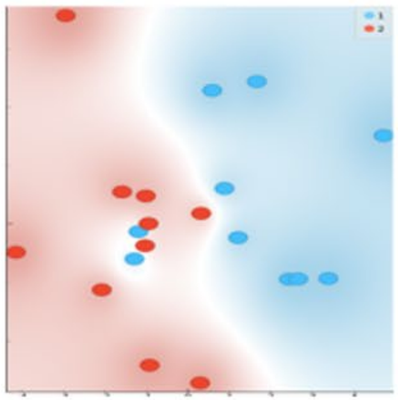
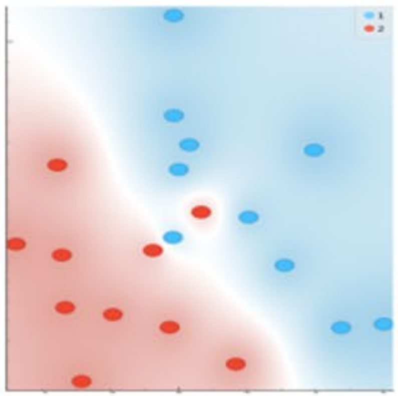
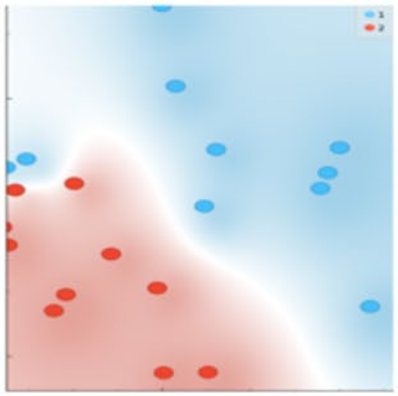
Metabolites with concentration differences at 1xCO2 vs 2xCO2



Metabolites with higher concentration in plants at $2xCO_2$ relative to $1xCO_2$

Metabolites that are significantly affected by CO_2 in both Norm and Alsen

(Cuperlovic-Culf and Vaughan et al., 2018)

Control	9F1 +biomass, +DON	Gz3639 -biomass, =DON	Gzt40
Alsen			
glucose sucrose tyrosine uridine succinicacid malate asparagine glycerol valine oxoglutarate	choline myoinositol asparagine methionine gaba succinicacid malate tyrosine glutamine glycerol	myoinositol choline inosine fructose glucose serine valine asparagine acetate phosphocholine	3hydroxybutyrate myoinositol galactose choline proline cysteine trehalose acetate malate betaine
			

Difference in asparagine, myoinositol and choline are dependent on the infecting *Fg* strain and may serve as markers for enhanced FHB susceptibility at elevated CO_2

(Cuperlovic-Culf and Vaughan et al., 2018)

Hypothesis:

The aggressiveness of *Fg* strains on Alsen is determined by-

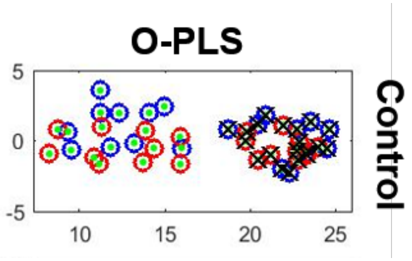
1) **its ability to inhibit the host from sequestering nitrogen in <u>asparagine</u>**

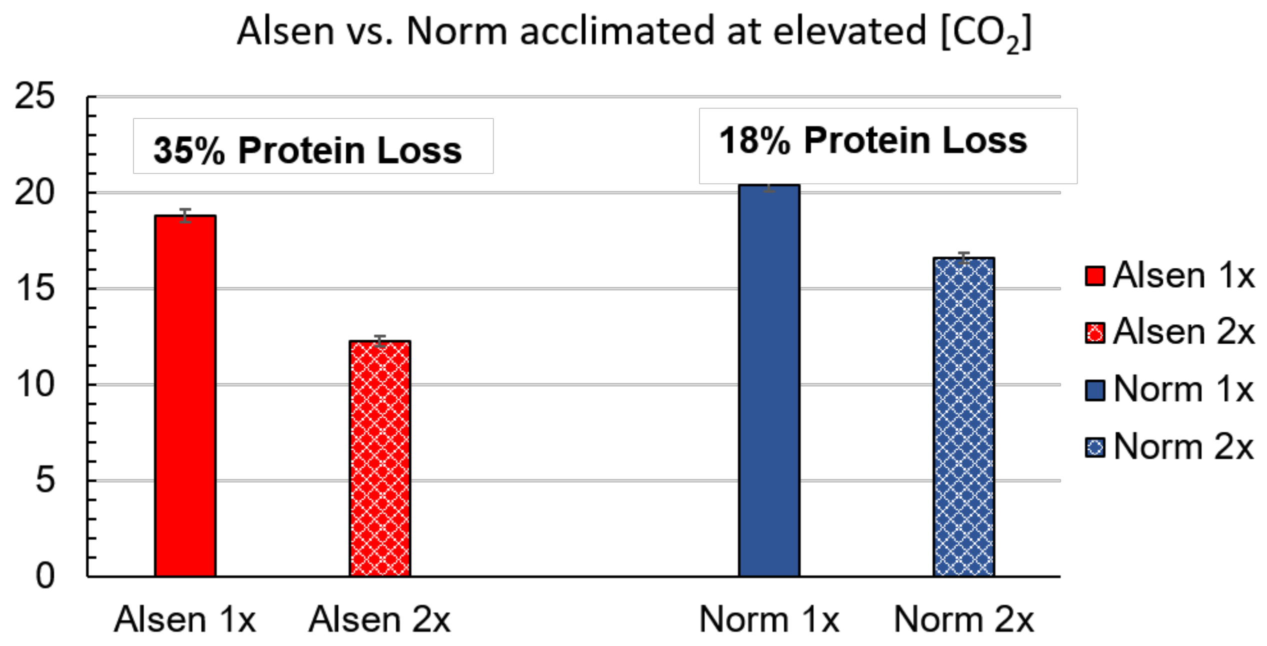
2) **its ability to the induction of <u>myoinositol</u>**

-and elevated CO_2 interferes with this process in a strain specific manner.

Do environmental conditions influence the expression of *Fg* effectors?

Growth at elevated CO_2 reduces grain protein content of FHB resistant variety significantly more than susceptible variety



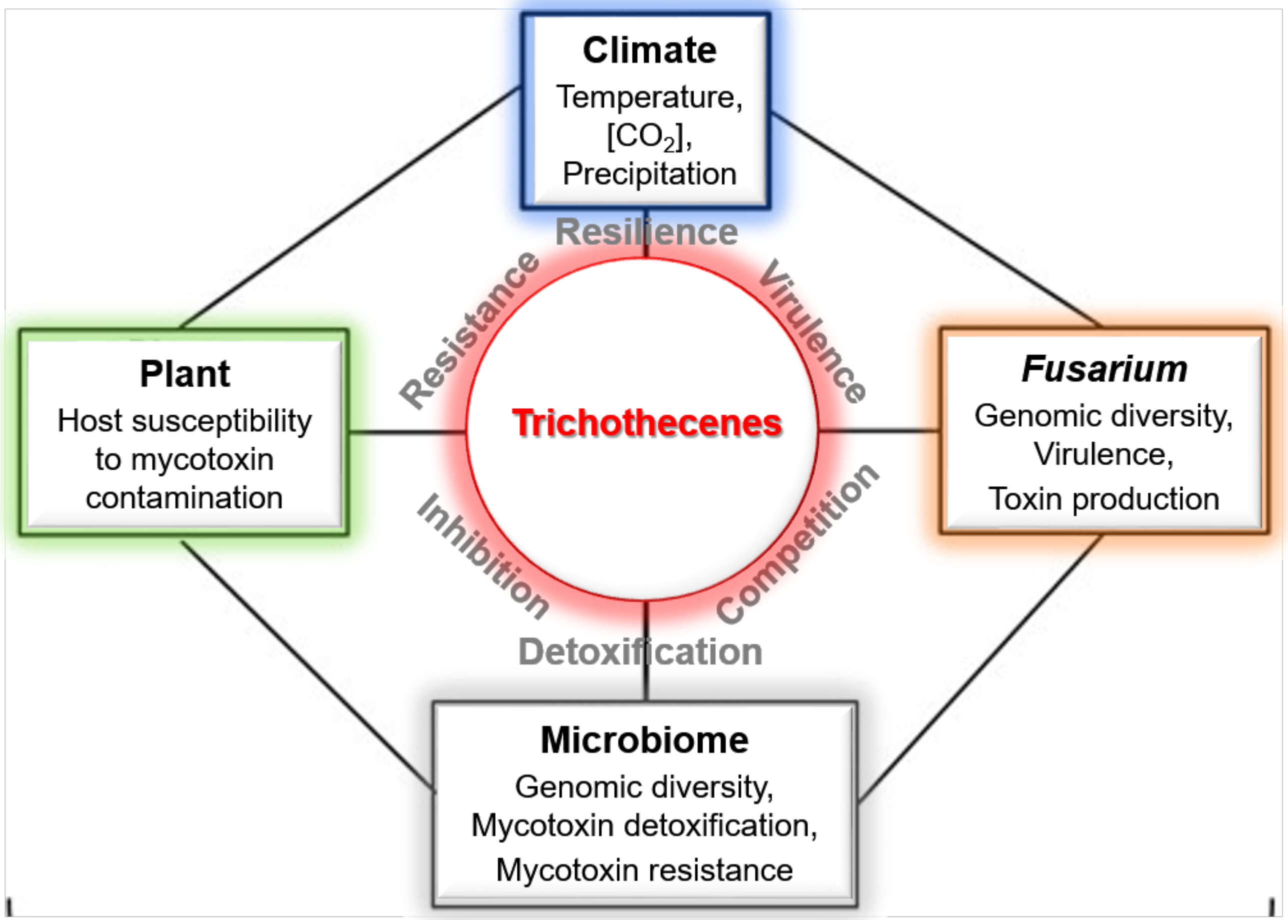


	% Moisture	% Fat	% Protein	% Carbohydrate	% Ash
Alsen T2 400 ppm CO2	11.03^{C} ±0.08	0.58^{AB} ±0.15	18.80^{B} ±0.32	77.10^{BC} ±1.28	2.36^{A} ±0.04
Alsen T2 800 ppm CO2	11.56^{B} ±0.14	0.53^{B} ±0.10	12.26^{D} ±0.25	83.86^{A} ±0.88	2.04^{C} ±0.03
Norm T2 400 ppm CO2	12.97^{A} ±0.13	0.87^{A} ±0.18	20.40^{A} ±0.33	74.74^{C} ±1.82	2.53^{A} ±0.18
Norm T2 800 ppm CO2	13.10^{A} ±0.06	0.76^{AB} ±0.12	16.58^{C} ±0.25	78.96^{B} ±1.49	2.21^{B} ±0.05

(Hay and Vaughan)

Future Objectives

Continuation of Holistic Ecological Approach



Future Objectives (2)

Continuation of Holistic Ecological Approach

1. **Develop climate resilient control strategies of FHB and DON.**
   - Determine the influence of environmental conditions and cultivars on *Fg* secondary metabolites and virulence factors
   - Identify climate resilient biocontrol microorganisms

2. **Screen microbial communities and identify novel detoxification mechanisms of trichothecenes**

3. **Determine the impact of elevated CO_2 and other abiotic factors on the nutritional quality of FHB moderately resistant parent lines being used in breeding programs.**

4. **Identify the strain specific and variety specific differences that result changes in FHB and DON severity under conditions of abiotic stress**

5. **Evaluate differences in host metabolic responses to *Fg* populations**

Future Objectives (3)

New Ideas:

Soliciting input directly from our Stakeholders

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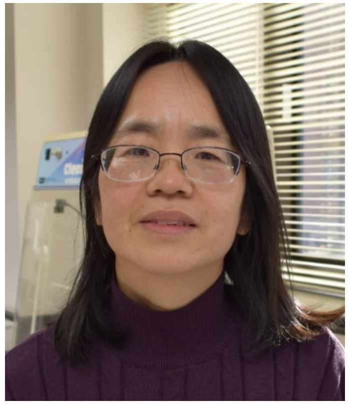
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Good things happen when we put our heads together