Ensuring food safety and security: Evaluating and utilizing plant/pest/pathogen phytochemical interactions.

William Hay, Ph.D.
William.Hay@usda.gov
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USDA
Agricultural Research Service

Presentation Overview

- Introduction
- Background Information
- Biopesticide synergy when combining plant flavonoids and entomopathogenic baculovirus
- Elevated [CO_2] alters wheat nutritional content and *Fusarium graminearum* growth and mycotoxin production on grain
- Closing remarks and Acknowledgements

Introduction

- Research Plant Physiologist – USDA ARS
- Part of the Mycotoxin Prevention and Applied Microbiology unit at the National Center for Agricultural Utilization Research (MPM/NCAUR) Peoria, IL.
- Research focus on the impact of climate change on cereal crop nutritional quality and plant susceptibility to mycotoxigenic fungal pathogens

USDA Agricultural Research Service

USDA – Mycotoxin Prevention and Applied Microbiology

- **Improve Food Safety**
  - Develop new technologies to detect mycotoxins and ensure safe food
- **Enhance Crop Production**
  - Research to prevent crop diseases and mycotoxin contamination
- **Ensure Global Food Security**
  - Research to promote climate resilient agriculture
- **Biotechnology and Innovation**
  - Microbial resources to enhance research and development worldwide

Research Synergy

- Variety of scientific disciplines
  - Plant Physiology
  - Plant Biochemistry
  - Entomology
  - Polymer Chemistry

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Biopesticide synergy when combining plant flavonoids and entomopathogenic baculovirus

William T. Hay[1], Robert W. Behle[2], Mark A. Berhow[3], Andie C. Miller[4] & Gordon W. Selling[1]

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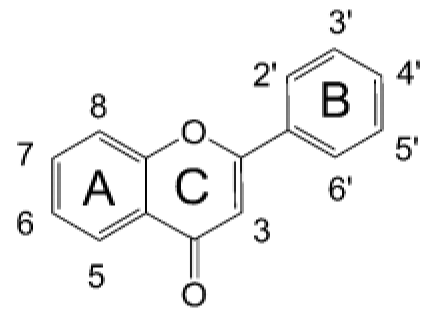
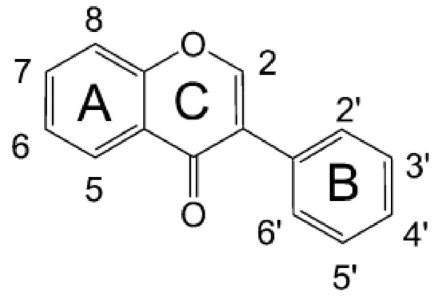
Plant secondary compounds: flavonoids

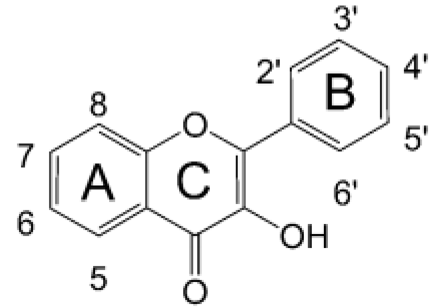
- Polyphenolic secondary metabolites
- Flavonoid compounds are involved in numerous biochemical processes including defense functions against insect pests
- Produced in response to biotic stress
  - Inhibit pathogen growth
  - Protect plant tissues from insect herbivory
    - Plant flavonoid-insect interactions can be highly species specific
- Also involved in soybean nodule formation
- Anti-oxidants



Flavone subclass backbone



Isoflavone subclass backbone



Flavonol subclass backbone

Phytochem Rev (2019) 18:241–272

Trichoplusia ni

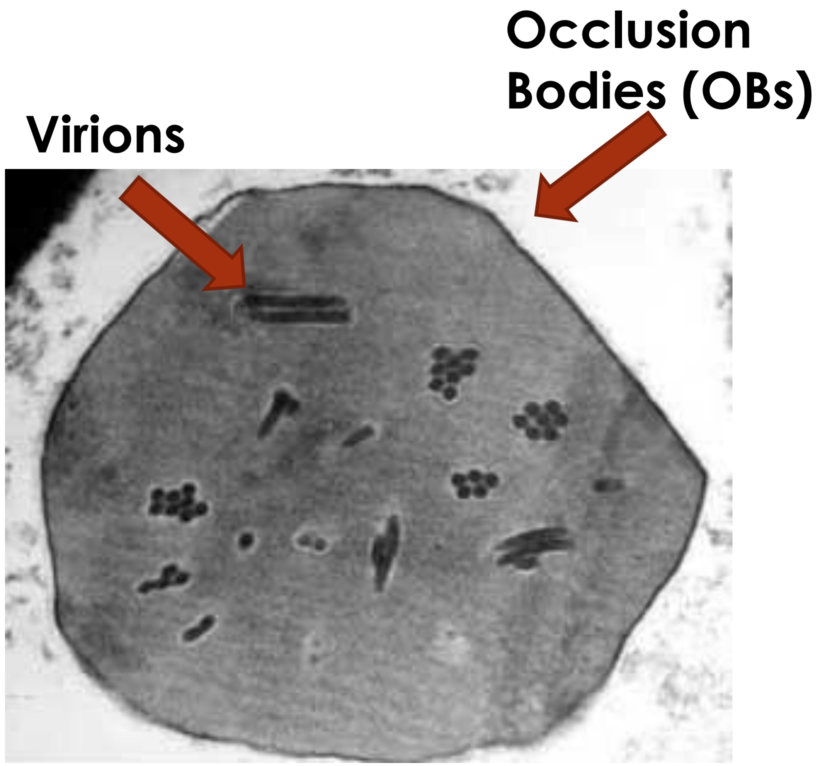
- Lepidopteran insect pest ‘Cabbage looper’
- Feeding damage to leaves
  - Cabbage
  - Beans
  - Lettuce
  - Spinach
  - Tomato
  - Cotton
  - Soybean

Larva

Adult

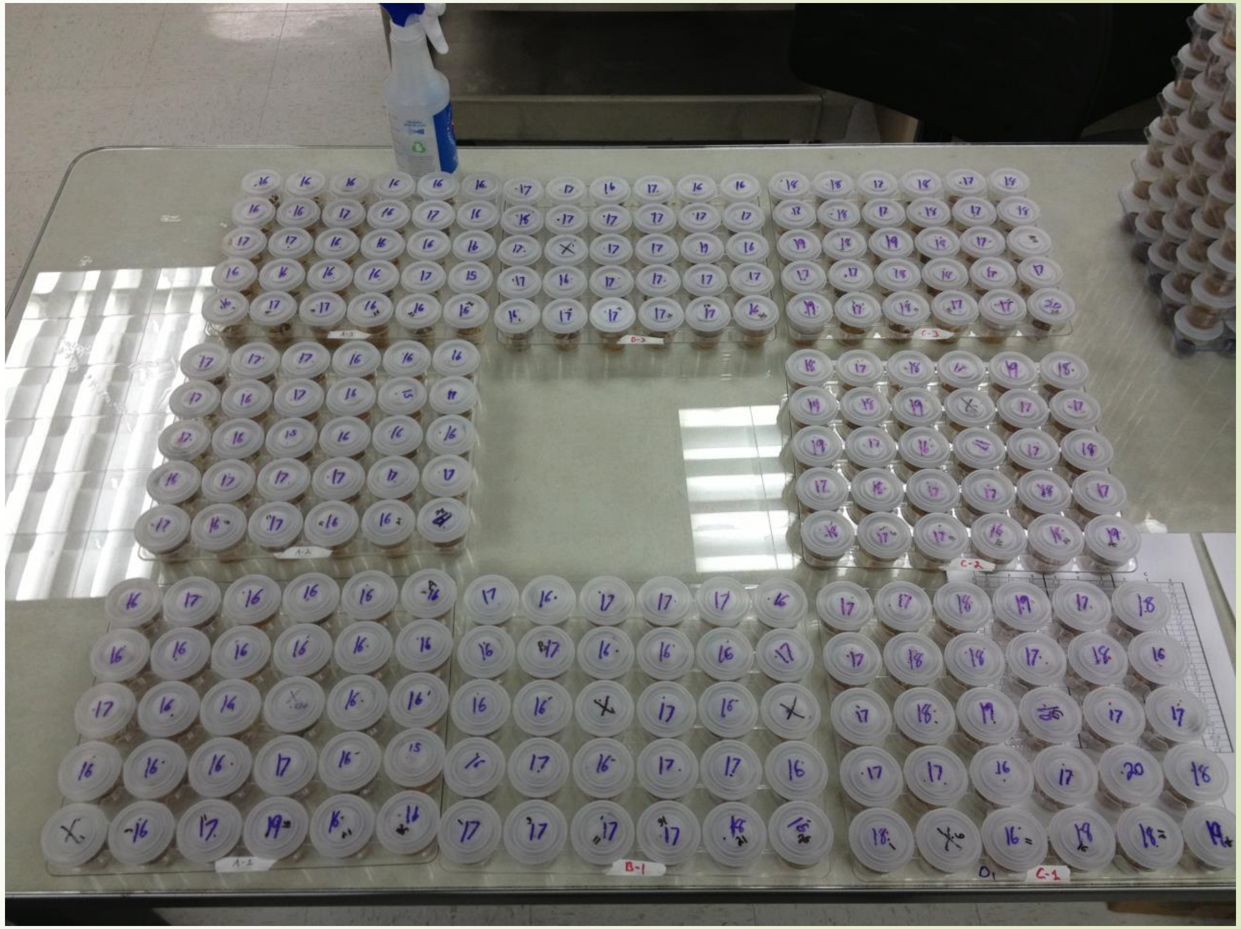
AfMNPV baculovirus

- Entomopathogenic virus
- Effective on a narrow range of insect pests
- After ingestion the virus reproduces in the insect
- Forms Occlusion bodies, large protein coat which contains and protects virions, or virus particles
- Widely available from commercial suppliers
- Virus is inactivated by UV or washed off during rain events

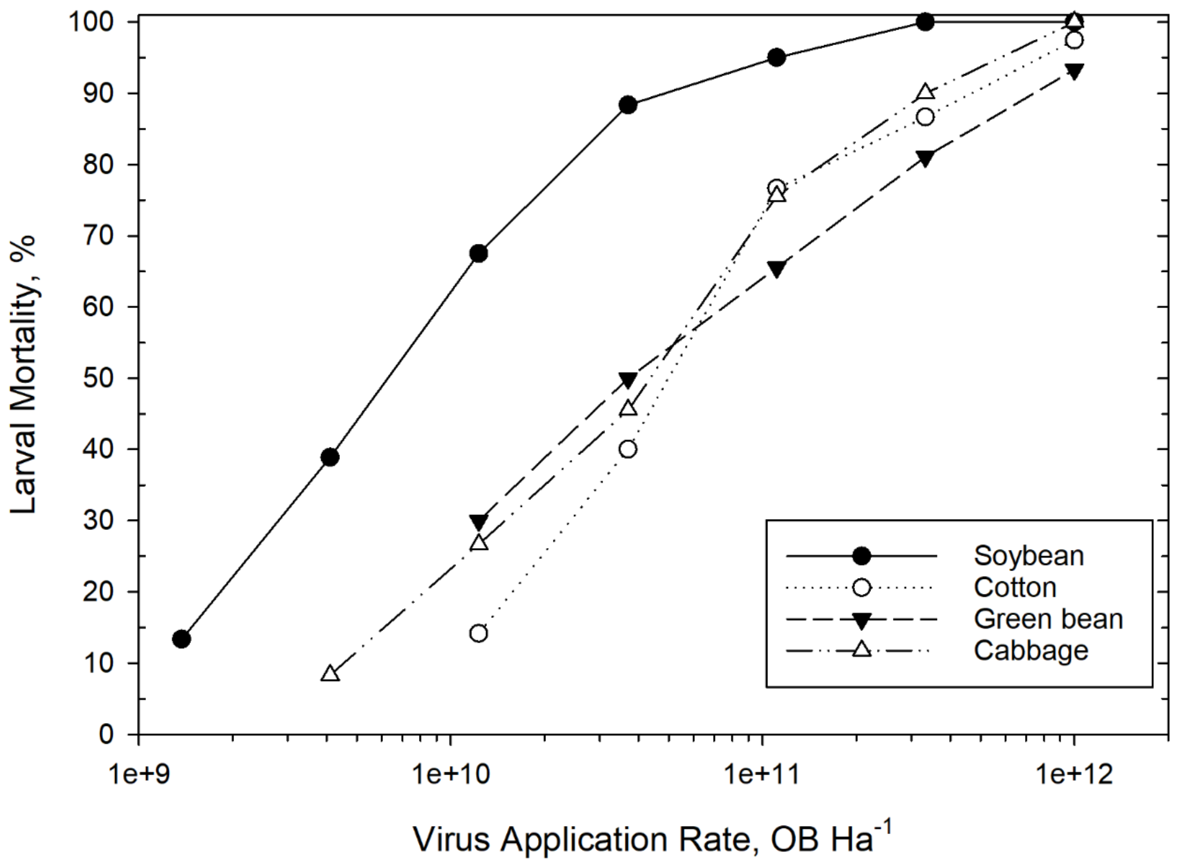


Method – Insecticidal activity of AfMNPV applied to crops

- Applied AfMNPV baculovirus to **cotton**, **cabbage**, **green bean**, and **soybean** in a dosage response assay: Greenhouse
  - Use of a research track sprayer to simulate field rates and conditions
- Leaf disks were cut and larvae were placed onto the disks for 24 hours
- Then transferred to sealed cups with General Purpose Lepidoptera Diet (GPLD)
- Larval mortality was recorded for an additional 6 days (one week total)
- Experiments were replicated a minimum of three times



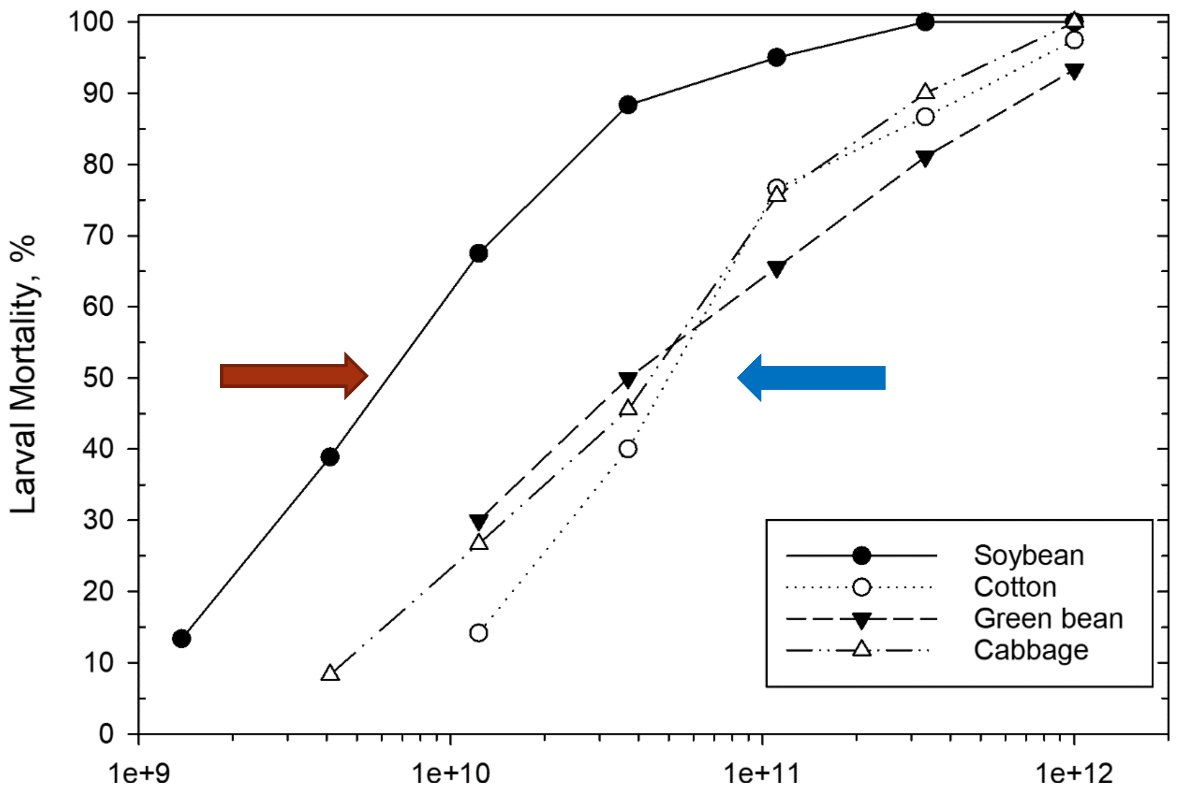
Insecticidal Activity



Insecticidal activity based on dosage-response of AfMNPV against *T. ni* neonates when applied to crop plants

Insecticidal Activity

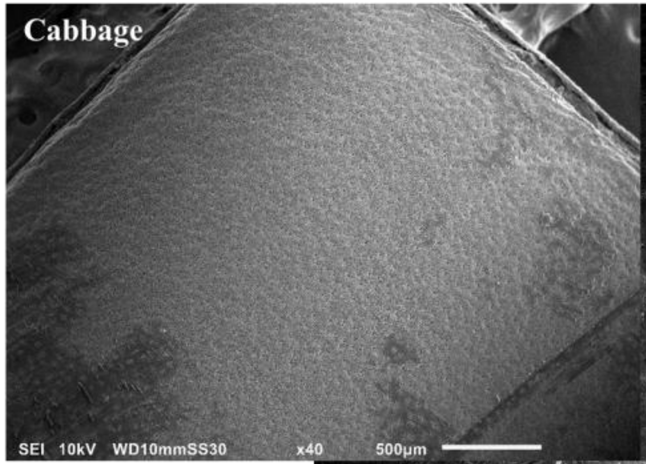
- Increased *T. ni* susceptibility to baculovirus on soybean (6 fold increase)
- Significantly different from green bean, despite being from the same family, Fabaceae
- What was the cause of the enhanced activity?

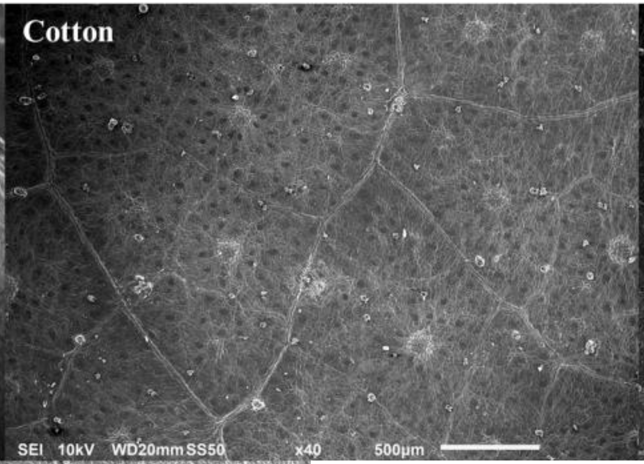


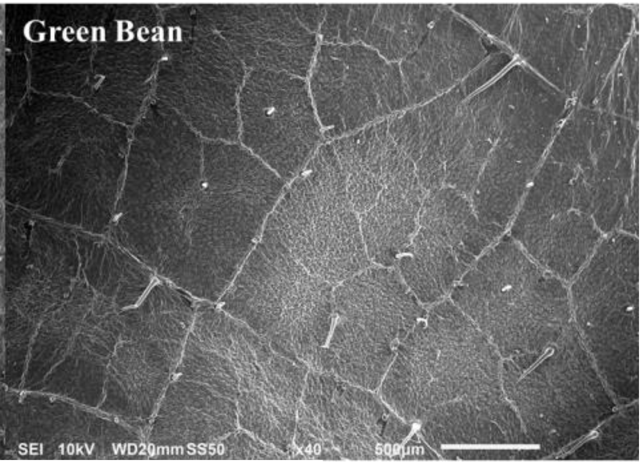
Plant	LC_{50}	Upper CL	Lower CL	Intercept	Slope	Heterogeneity	χ^2	*df*	n
Soybean	1.77 a	2.19	1.39	−0.331	1.570	0.60	3.02	5	720
Cabbage	9.95 b	12.33	7.92	−0.603	1.521	0.74	2.98	4	510
Green bean	10.73 b	32.31	5.61	−0.418	1.034	0.15	0.45	3	450
Cotton	13.17 b	18.92	9.69	−0.679	1.592	1.53	4.59	3	599

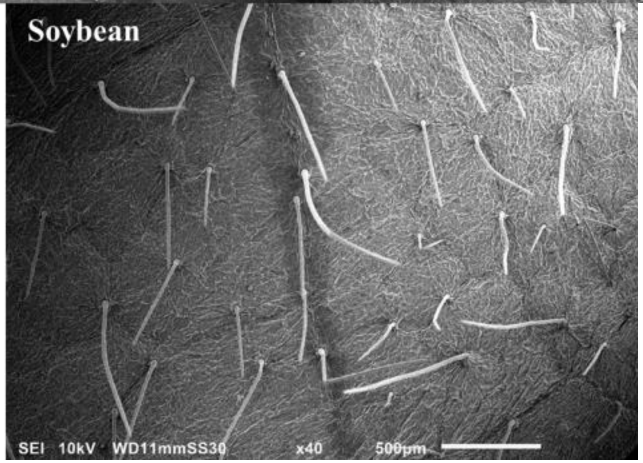
Insecticidal activity based on dosage-response of AfMNPV against *T. ni* neonates when applied to crop plants

Differences in leaf anatomy -Trichomes









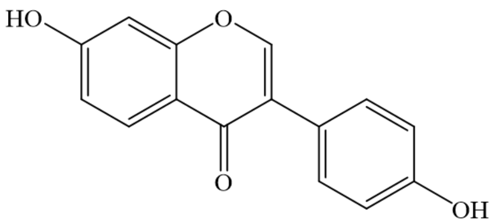
Mortality: 64.6%

Mortality: 58.3%

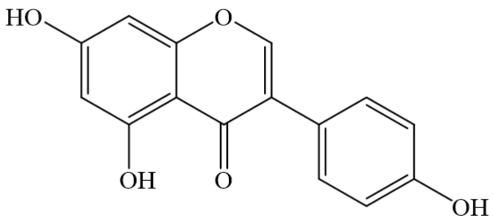
No Significant difference: P=0.52

Leaf Chemistry

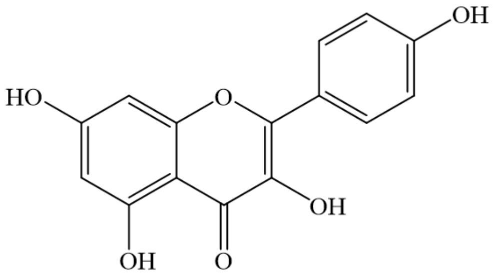
- Chemical differences between the plant species are likely to be extremely numerous
- Focus on leaf phenolic composition: known to be plant defense compounds. Identified by HPLC and verified by LC-ESI-MS
- We identified three flavonoids present exclusively in soybean (amongst the plants investigated)
- Artificial diets were then produced with each flavonoid compound at leaf level concentrations



Daidzein



Genistein



Kaempferol

	(μM/g)	(μg/g)
Daidzein	0.678 ± 0.004	172 ± 1
Genistein	0.684 ± 0.009	185 ± 3
Kaempferol	4.832 ± 0.004	1382 ± 2

Leaf level concentrations of flavonoids – diet incorporation

- The virus free flavonoid diets did not cause an increase in larval mortality
- Alone the flavonoids at leaf level concentrations did not enhance baculovirus activity
- A combination of all three flavonoids at leaf level concentrations resulted in a significant enhancement of insecticidal activity

Diet incorporation	LC_{50}	Upper CL	Lower CL	Intercept	Slope	Heterogeneity	χ^2	*df*	n
GPLD only	3061 a	4050	2380	−5.975	1.714	1.31	17.06	13	450
Genistein	2736 a	3857	2028	−4.277	1.244	1.20	15.61	13	450
Daidzein	2638 a	4103	1796	−4.706	1.376	2.23	28.94	13	450
DMSO, surfactant	2363 a	2967	1912	−5.119	1.517	0.89	11.53	13	451
Kaempferol	2307 a	2884	1862	−5.280	1.570	0.89	11.56	13	449
Combination	1666 b	2317	1217	−4.890	1.518	1.82	23.60	13	450

High concentration flavonoid diet incorporation

- Even at higher concentrations (3.5-6.5x leaf level) the virus free flavonoid diets still did not cause an increase in larval mortality
- Each flavonoid at higher concentrations synergistically improved virus potency
  - Daidzein: 1.5x Control (GPLD)
  - Genistein: 2.3x Control (GPLD)
  - Kaempferol: 4.3x Control (GPLD)

$$\text{Cotoxicity factor} = 100 \times \frac{(\text{observed\%mortality} - \text{expected\%mortality})}{(\text{expected\%mortality})}$$

Diet incorporation	LC_{50}	Upper CL	Lower CL	Intercept	Slope	Heterogeneity	χ^2	*df*	*n*
GPLD only	2669 a	5051	1148	−5.167	1.508	6.25	81.27	13	445
DMSO, surfactant	2431 a	4224	1197	−5.024	1.484	4.77	61.97	13	443
Daidzein 6.5x	1758 b	2140	1451	−5.627	1.734	0.93	2.779	3	450
Genistein 6.5x	1196 b	2466	299	−4.368	1.419	7.19	93.40	13	441
Kaempferol 3.5x	628 c	1213	140	−4.019	1.436	4.91	63.77	13	445

Synergy when combining plant flavonoids and entomopathogenic baculovirus

- Where a positive factor of ≥20 indicates synergistic potentiation, and a value of <20 to >0 indicates additive potentiation
- Daidzein, kaempferol, and genistein were found to synergistically improve virus activity
  - Daidzein: Cotoxicity factor - **20**
  - Genistein: Cotoxicity factor – **60**
  - Kaempferol: Cotoxicity factor - **150**

$$Cotoxicity\ factor = 100 \times \frac{(observed\%mortality - expected\%mortality)}{(expected\%mortality)}$$

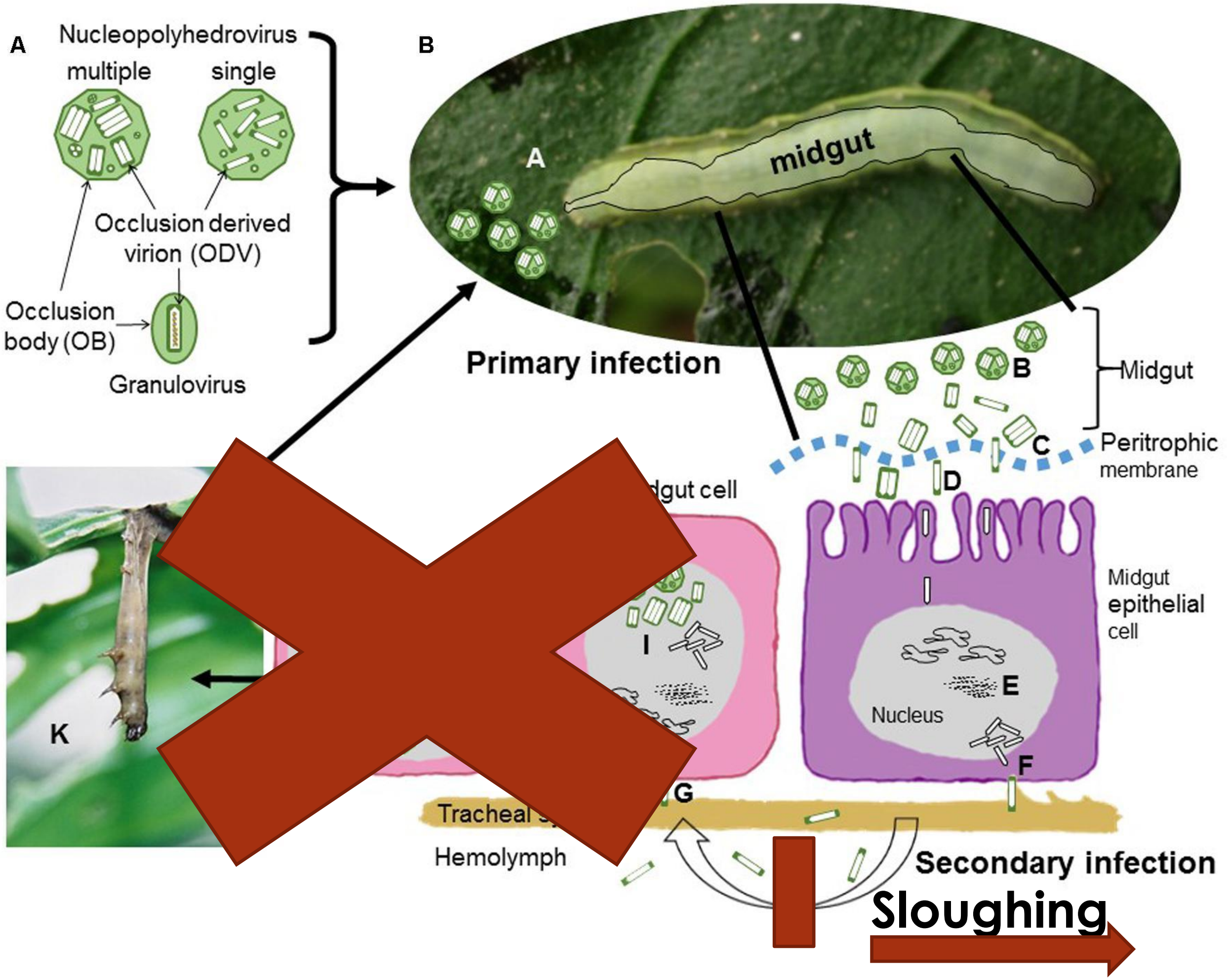
Sayed, A. M. & Behle, R. W. Evaluating a dual microbial agent biopesticide with Bacillus thuringiensis var. kurstaki and Beauveria bassiana blastospores. *Biocontrol science and technology* **27**, 461–474 (2017).

Shaalan, E. A.-S., Canyon, D. V., Younes, M. W. F., Abdel-Wahab, H. & Mansour, A.-H. Synergistic efficacy of botanical blends with and without synthetic insecticides against Aedes aegypti and Culex annulirostris mosquitoes. *Journal of vector ecology* **30**, 284–288 (2005).

Sun, Y.-P. & Johnson, E. Analysis of joint action of insecticides against house flies. *Journal of economic entomology* **53**, 887–892 (1960).

Inhibition of baculoviral infection

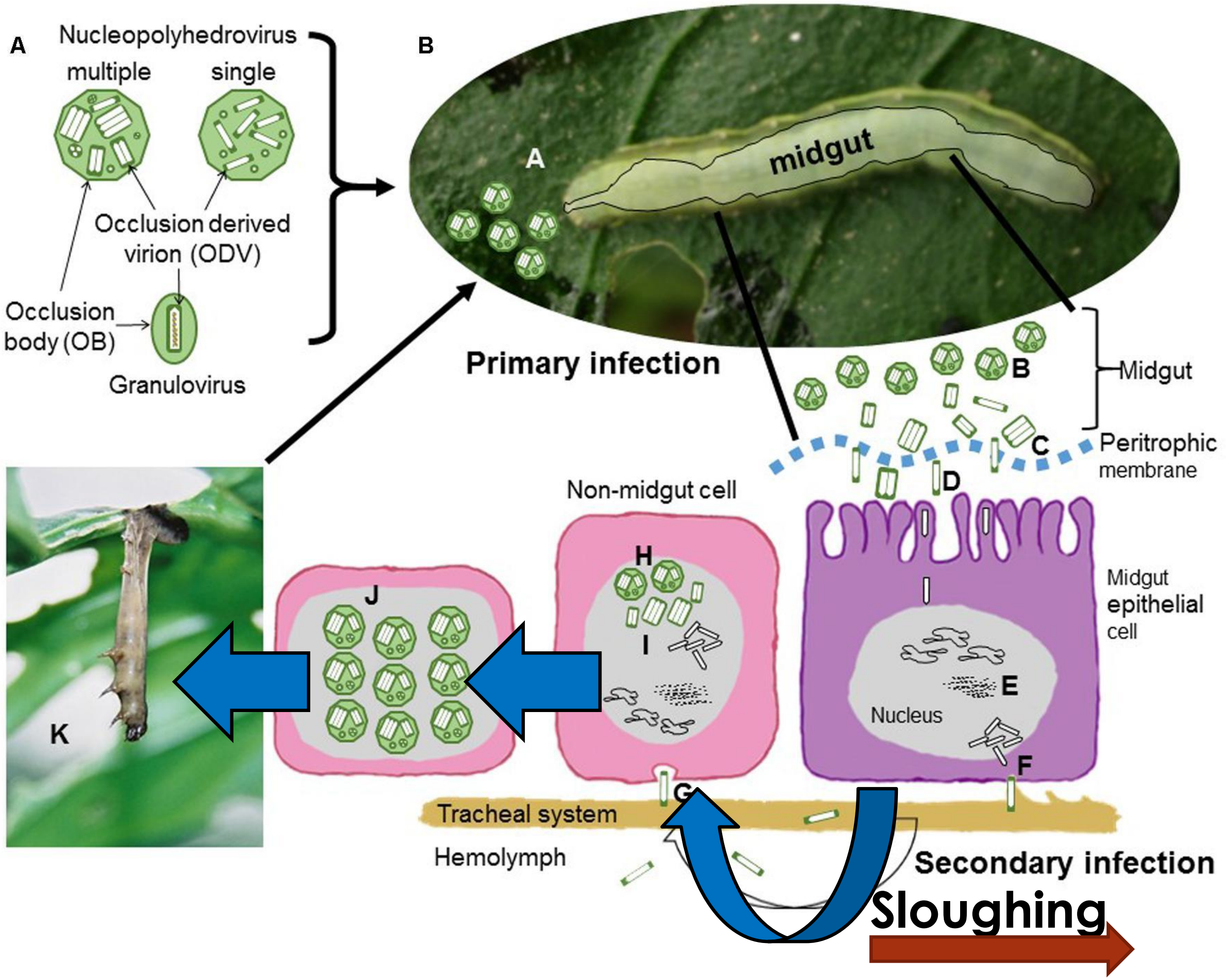
- The simplistic insect immune system relies on rapid apoptosis and premature cell lysis
- Early onset of apoptosis in infected insect cells can dramatically reduce virus concentrations (50 fold)
- Oxidative stress also reduces baculovirus activity and insect death by promoting midgut cell sloughing
- Rapid sloughing of infected midgut epithelial cells before the establishment of a systemic infection reduces viral susceptibility



Williams, T., Virto, C., Murillo, R., & Caballero, P. (2017). Covert infection of insects by baculoviruses. *Frontiers in microbiology*, *8*, 1337.

Antioxidant Capacity

- All three of the identified flavonoids are known anti-oxidants
- Phytoestrogenic activity, with strong affinity to estrogen receptors
- Estrogen is anti-apoptotic, anti-inflammatory, and inhibits ROS production
- A reduction or delay in apoptosis and epithelial sloughing would increase the rate of viral production in the *T. ni* larvae and result in greater insect mortality



Williams, T., Virto, C., Murillo, R., & Caballero, P. (2017). Covert infection of insects by baculoviruses. *Frontiers in microbiology*, *8*, 1337.

Take-aways

- The individual flavonoid compounds did not cause *T ni.* mortality in no-virus assays when incorporated into artificial insect diet.
- The soybean flavonoid compounds were found to synergistically improve baculovirus activity against *T. ni.*
- Synergy suggests a potential plant breeding objective to improve plant insect resistance concurrent with an integrated pest management system.
- Additional plant biochemistries to identify, as daidzein, genistein, and kaempferol only partially account for the enhanced viral potency

Ongoing work

- Utilizing USDA Soybean Germplasm collection (USDA-ARS at the University of Illinois) and soybean collection at NCAUR (Peoria, IL)
- Identifying differences in soybean genotypes and plant biochemistries, evaluate LC_{50} values
- Developing adjuvants to enhance biocontrol potency
  - Field testing
- Goal: improve food security

Rising CO_2 and Food Security

- Elevated CO_2 alters plant primary metabolism and grain nutritional content
- Grain composition impacts pathogen growth and toxin production
- Effects are cultivar and fungal species specific

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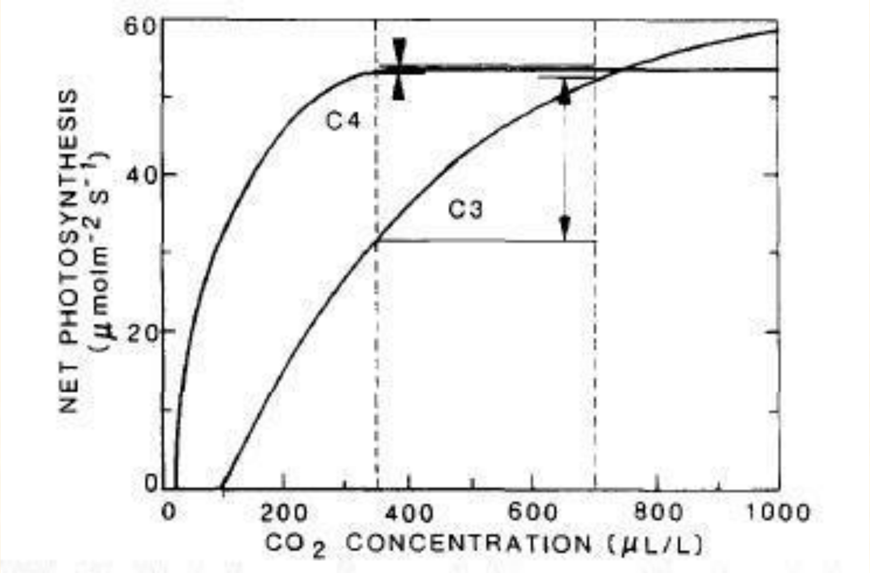
Changes in Wheat Nutritional Content at Elevated [CO_2] Alter *Fusarium graminearum* Growth and Mycotoxin Production on Grain

William T. Hay,* Susan P. McCormick, Milagros P. Hojilla-Evangelista, Michael J. Bowman, Robert O. Dunn, Jennifer M. Teresi, Mark A. Berhow, and Martha M. Vaughan

Cite This: *J. Agric. Food Chem.* 2020, 68, 6297–6307 Read Online



As atmospheric CO_2 concentration increases, C3 plants become more efficient

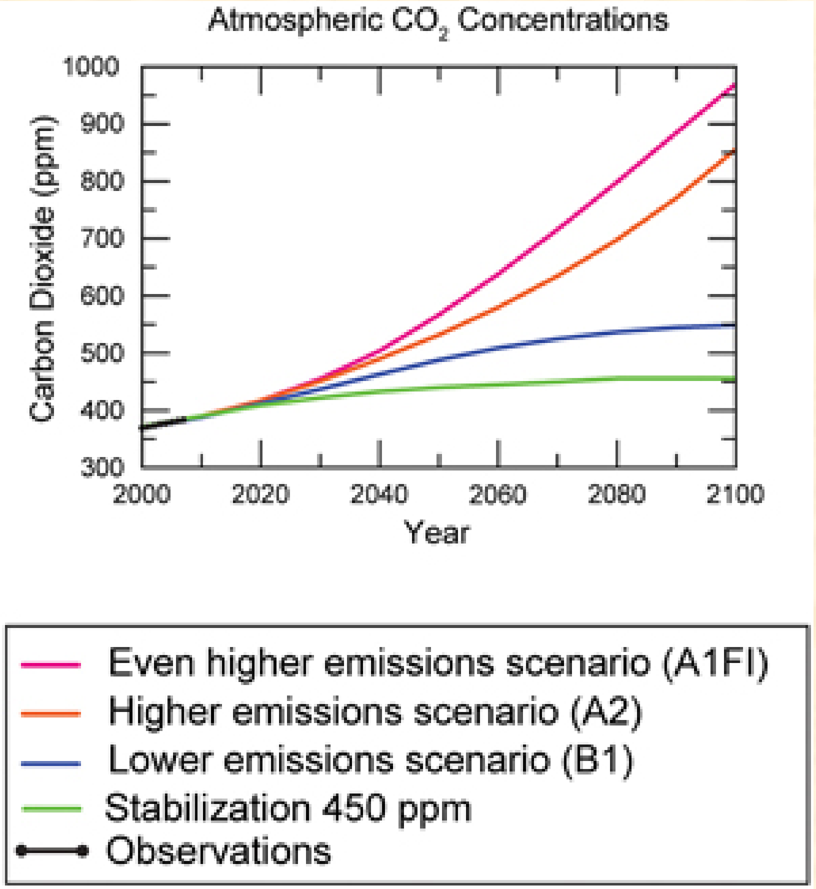
- Greater net photosynthetic rate
- Less photorespiration

C4 plants (such as corn) possess CO_2 concentrating mechanisms

- Increasing $[CO_2]$ in the bundle sheath

At elevated CO_2, C3 plants also have higher water use efficiency

- Improved water status

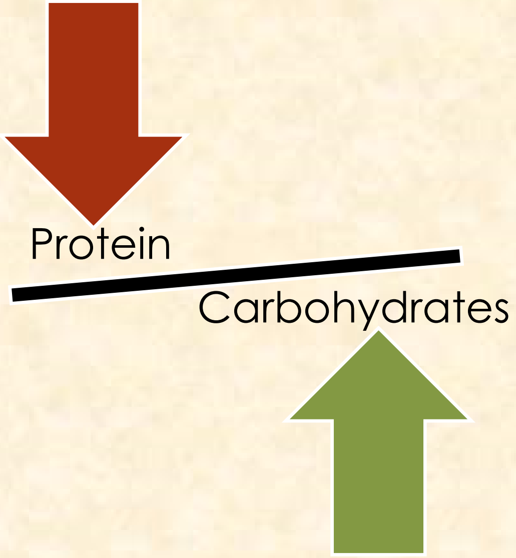


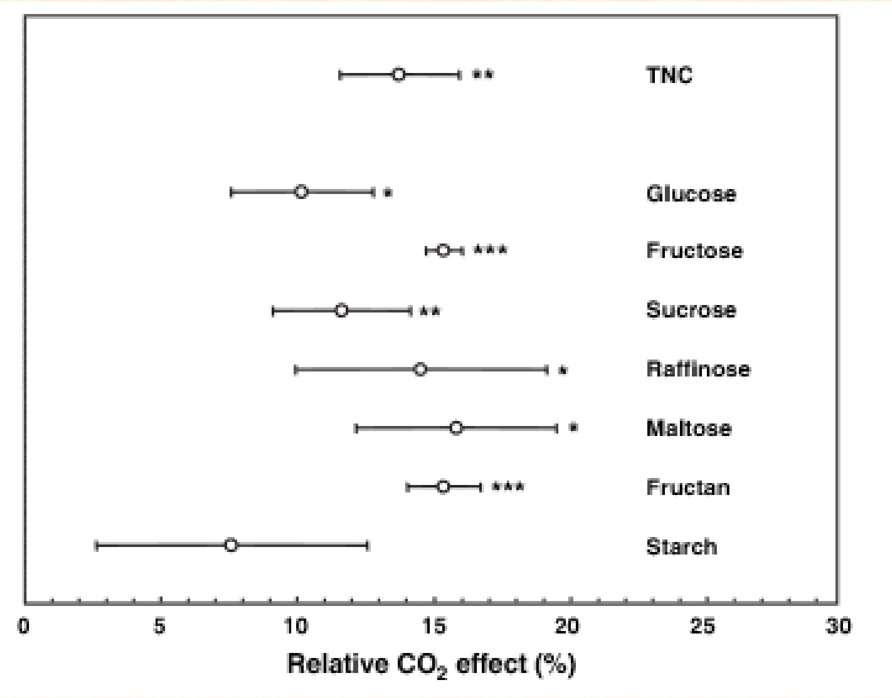
Nutrient dilution in C3 plants at elevated CO_2

Increased carbon assimilation causes an increase in the abundance of CHO compounds

- Plants still limited by mineral nutrient availability

As plant carbohydrate concentration increases, the concentration of Protein decreases





Taub, et al., *Global Change Biology* 14.3 (2008): 565-575.

Wheat (C3) produces far more carbohydrates under elevated CO_2

Wheat grain subsequently loses protein concentration at elevated CO_2

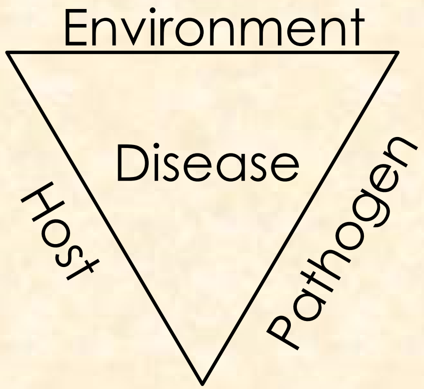
Climate Resilience

What is climate resilience?

- The capacity to withstand and adapt to climate variability

What is climate resilience in agriculture?

- Ability of an agricultural cropping system to withstand and adapt to climate induced:
  - Abiotic stresses
  - Biotic stresses



Food security and mycotoxigenic fungal pathogens

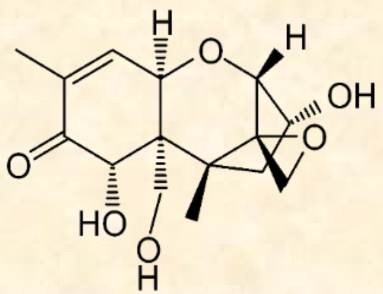
Fusarium head blight (**FHB**): fungal disease of cereals

Infects wheat after flowering (anthesis), contaminating wheat grain with mycotoxins.

- Primarily *Fusarium graminearum* in NA
- Infection is heavily dependent on weather conditions and plant developmental timing

Deoxynivalenol (DON)

- Fungal secondary metabolite



Virulence factor: Causes plant cell death and enables the fungus to spread throughout the wheat head.

DON accumulation reduces grain yield, quality, and suitability as food/feed due to potential health hazards.

- Feed refusal
- Immunosuppression
- Organ damage

No true resistance to FHB

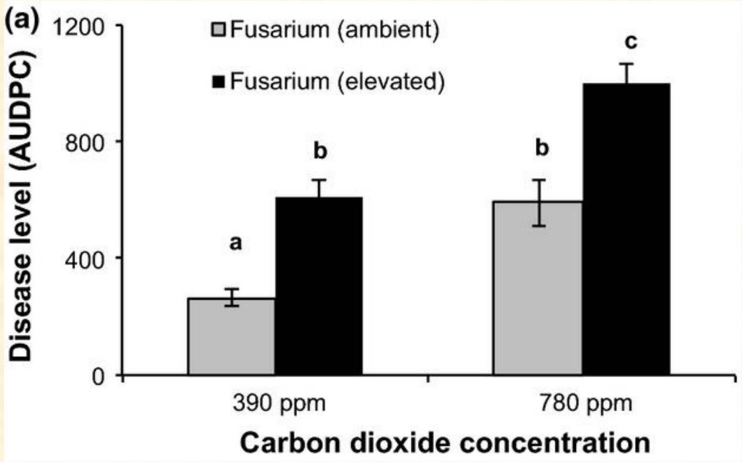
There is currently no FHB resistant variety of wheat.

- Moderately resistant wheat can limit the spread of the fungal pathogen

Resistance traits are primarily derived from the Sumai 3 cultivar.

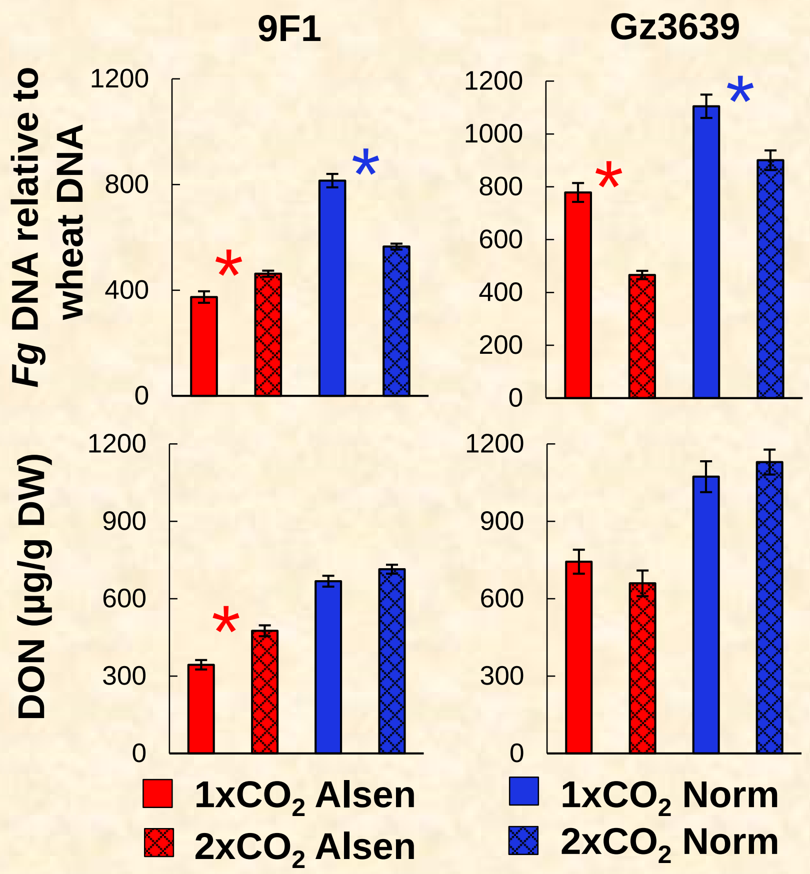
- Released in 1970
- FHB resistance predominantly due to the gene *Fhb1*, but also *Fhb2*, *Fhb4*, *Fhb5*, and *Fhb7*

Elevated CO_2 has been reported to increase the severity of FHB



(Vary et al., 2015)

However, this effect was observed to be dependent on both the variety of the host plant and the *F. graminearum* strain



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

It is difficult to untangle the direct impact of elevated CO_2 on the host, pathogen, and host x pathogen interaction.

Impact of Elevated CO_2

- **Objective**: Determine whether changes in wheat grain composition due to elevated CO_2 directly impact *F. graminearum* growth and mycotoxin production
  - Use of growth chambers configured for CO_2 enrichment
    - Ambient (**1x**) set to 400 ppm, elevated (**2x**) 800 ppm
      - Alsen – Moderately resistant
      - Norm – Susceptible
    - Seed collected for testing

Growth Chamber Experiments

- Grain Characteristics
  - Protein
  - Carbohydrate
  - Mineral
  - Fatty Acids
- Pathogen Characteristics
  - Growth
  - Toxin Production
  - Expression of toxin biosynthetic pathway

Growth at elevated CO_2 altered grain nutritional content of wheat

	Alsen		Norm	
$[CO_2]$	1x	2x	1x	2x
% Fat	0.58 ±0.15	0.53 ±0.10	0.87 ±0.18	0.76 ±0.12
% Protein	17.48 ±0.30	↘ **11.40* ±0.23**	18.96 ±0.31	↘ **15.41* ±0.24**
% Carbohydrate	78.42 ±1.24	↗ **84.72* ±0.84**	76.18 ±1.78	↗ **80.13* ±1.47**
% Crude Fiber	1.16 ±0.13	1.31 ±0.06	1.46 ±0.22	1.49 ±0.32
% Ash	2.36 ±0.04	↘ **2.04* ±0.03**	2.53 ±0.18	↘ **2.21* ±0.05**

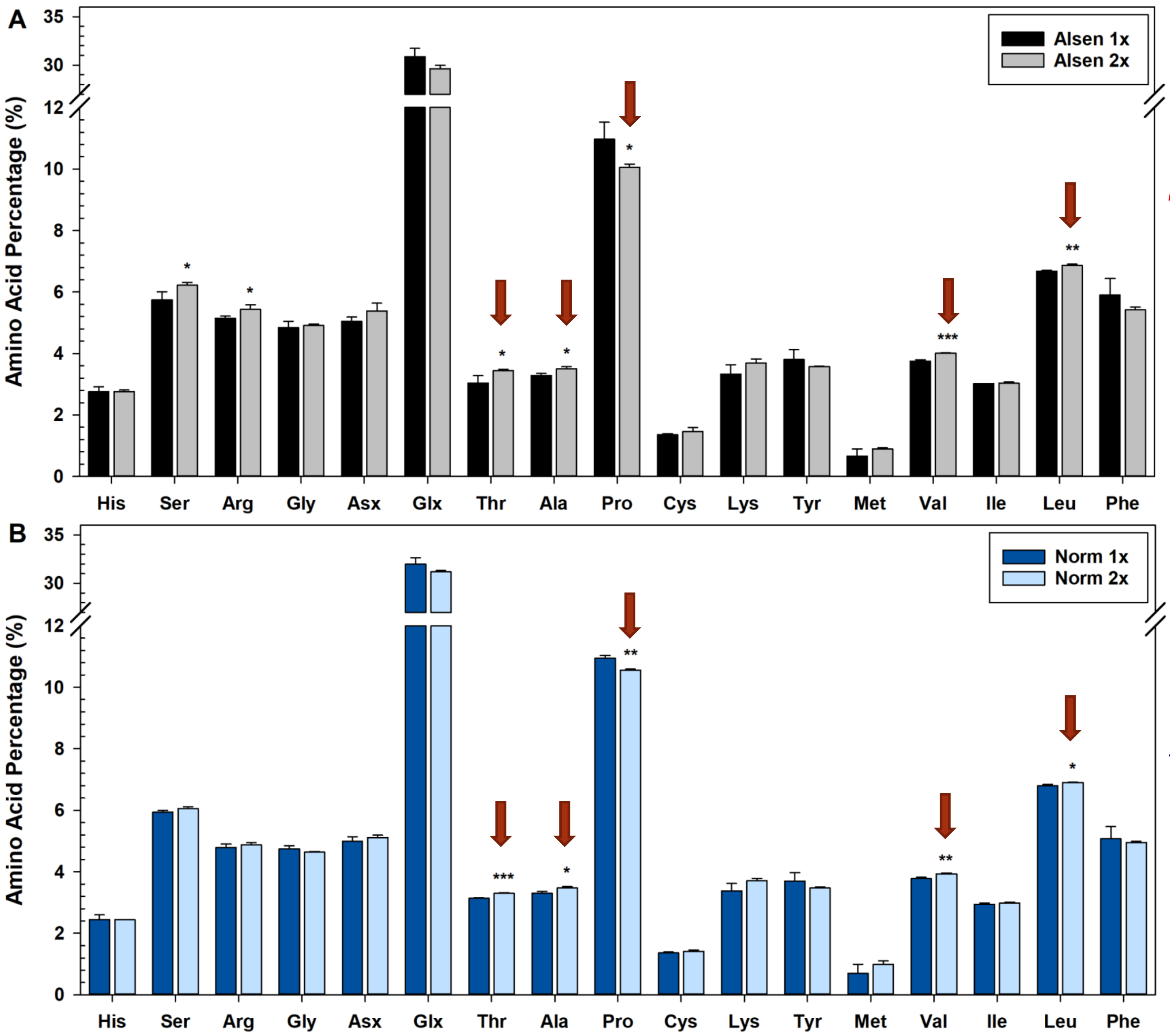
Moderately resistant cultivar was impacted more than the susceptible cultivar

Amino Acids %

Were changes in protein due to dilution?

Majority of amino acid %'s were unaffected by elevated CO_2

However, both varieties had alterations in the same five amino acids in response to elevated CO_2



Moderately Resistant Alsen

Susceptible Norm

Amino Acids %

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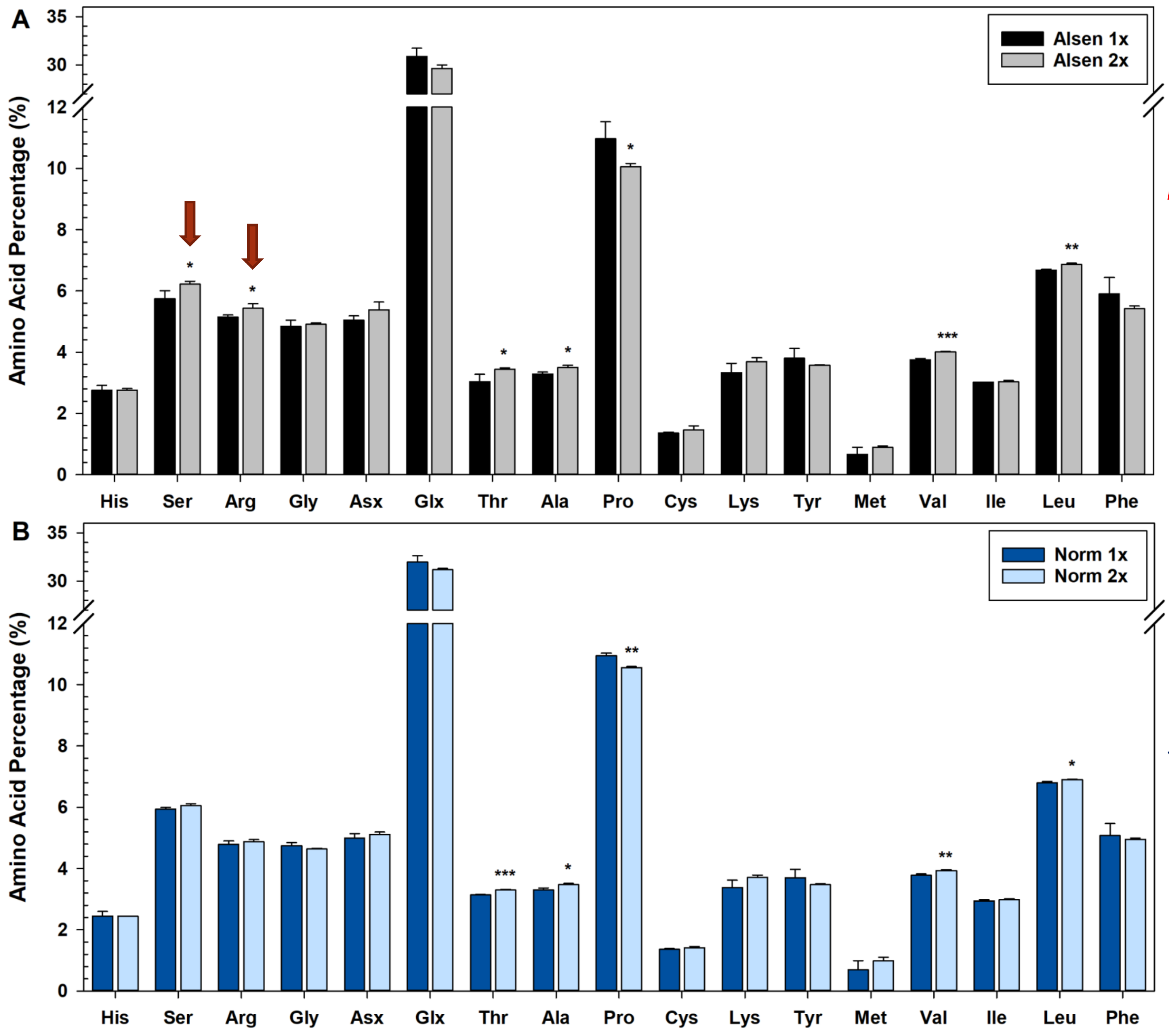


Moderately Resistant Alsen

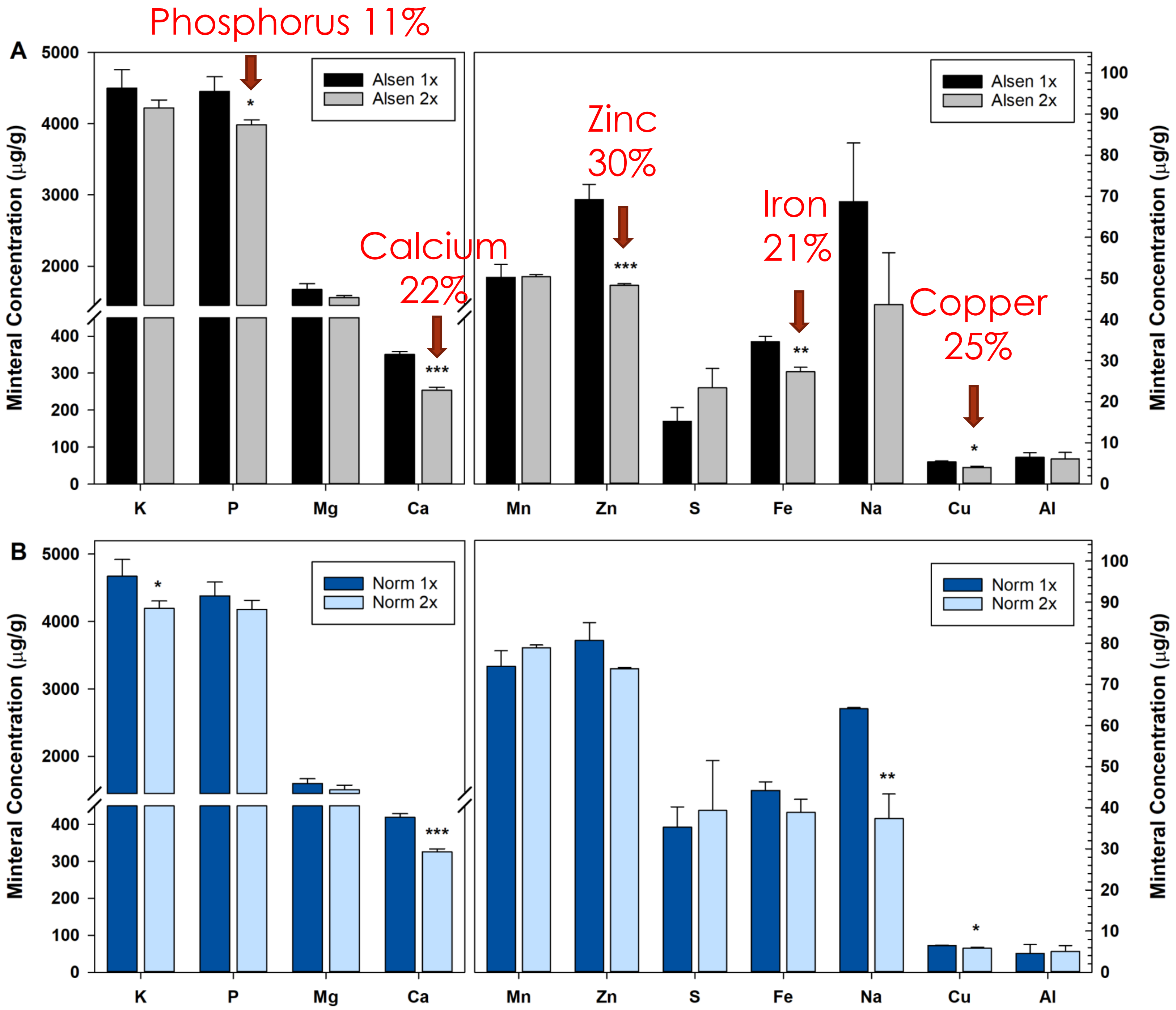
Susceptible Norm

Mineral Content

Greater loses in moderately resistant Alsen

- **P, Ca, Zn, Fe, Cu**

All are classified as essential mineral nutrients by the US-FDA

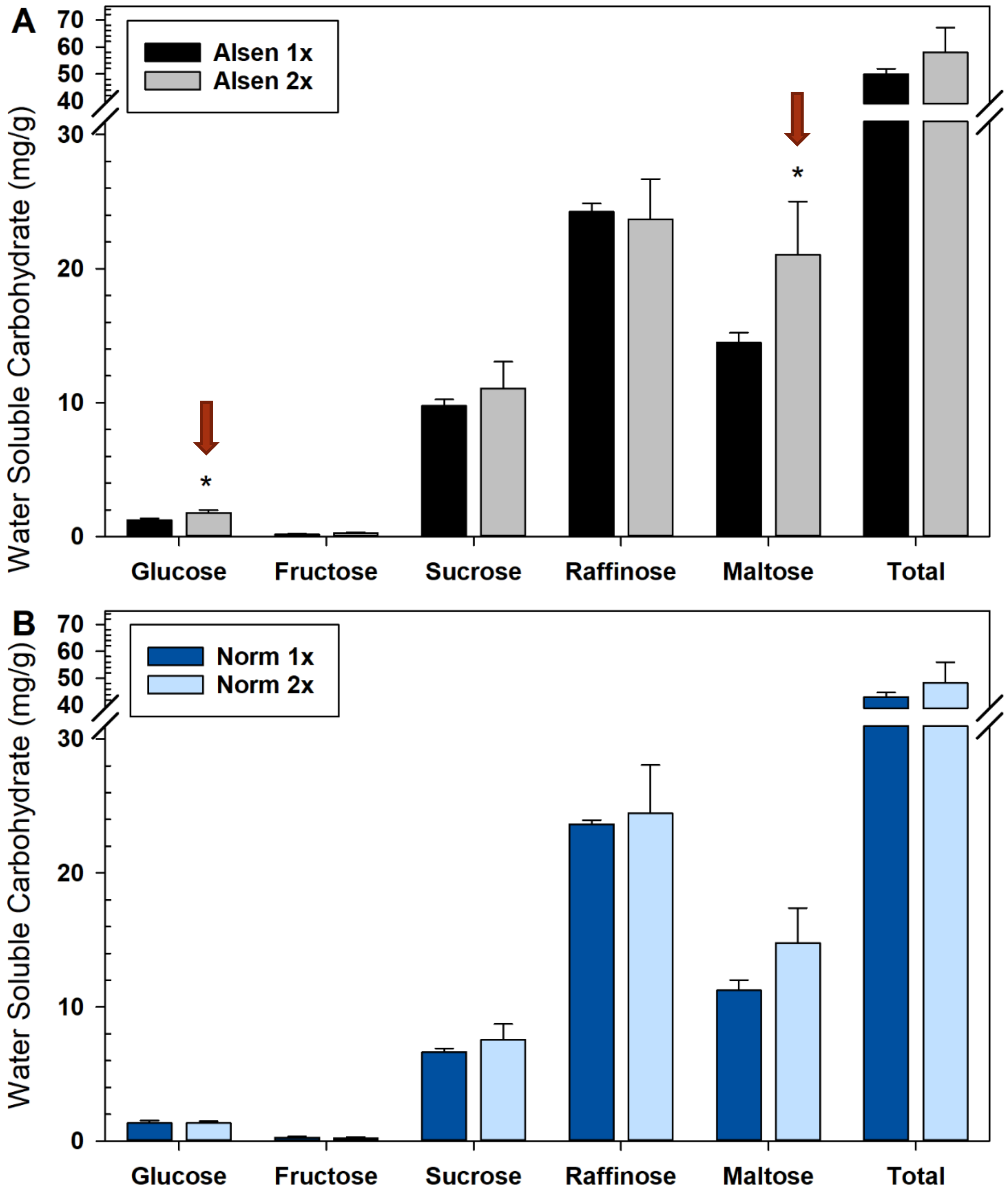


Moderately Resistant Alsen

Susceptible Norm

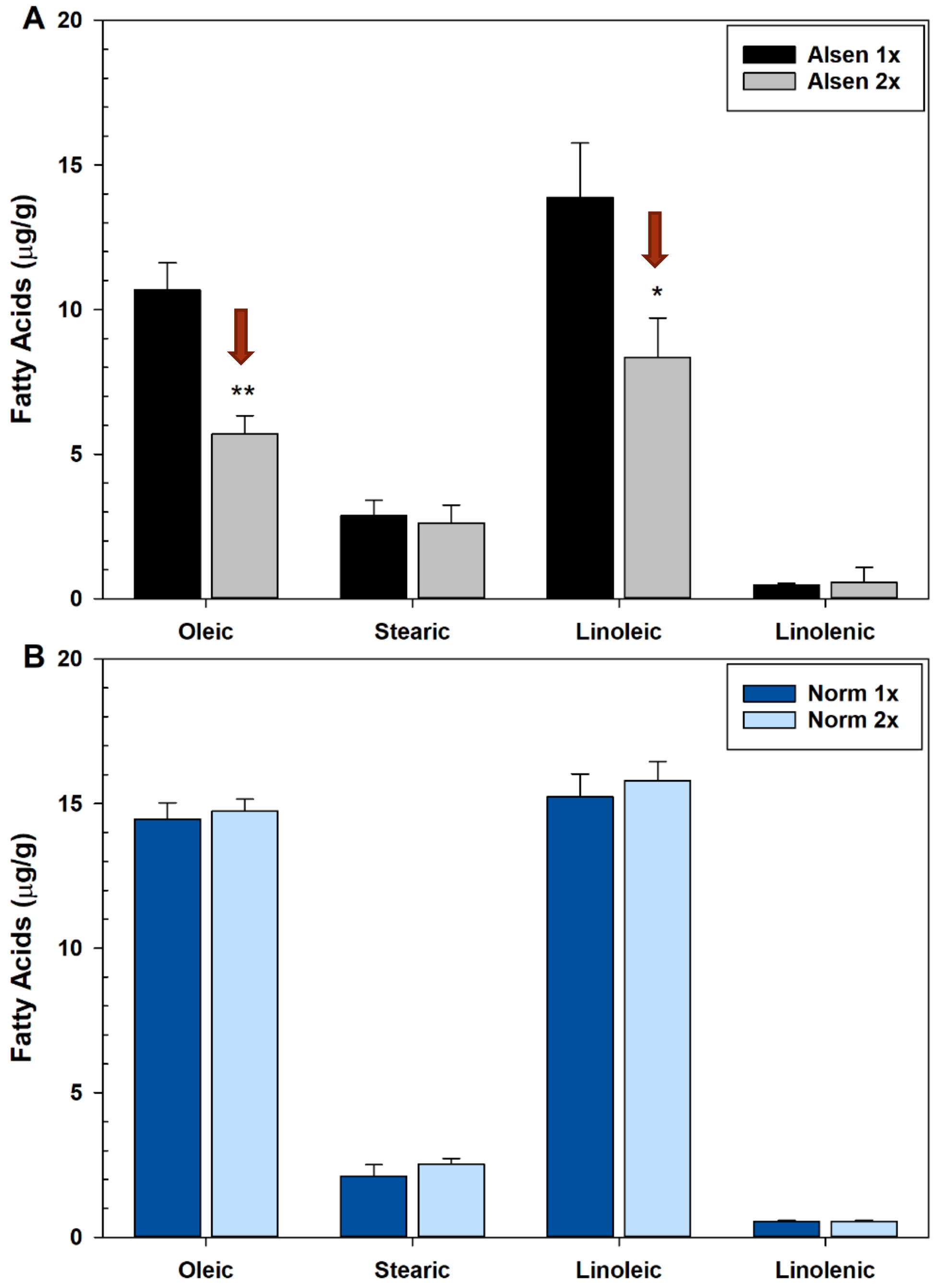
Water Soluble Carbohydrates

- Alsen cultivar had significant increases in glucose and maltose at elevated CO_2
- No change in water soluble carbohydrate concentration in Norm cultivar
- No change in the concentration of sucrose or raffinose: inducers of trichothecene biosynthesis



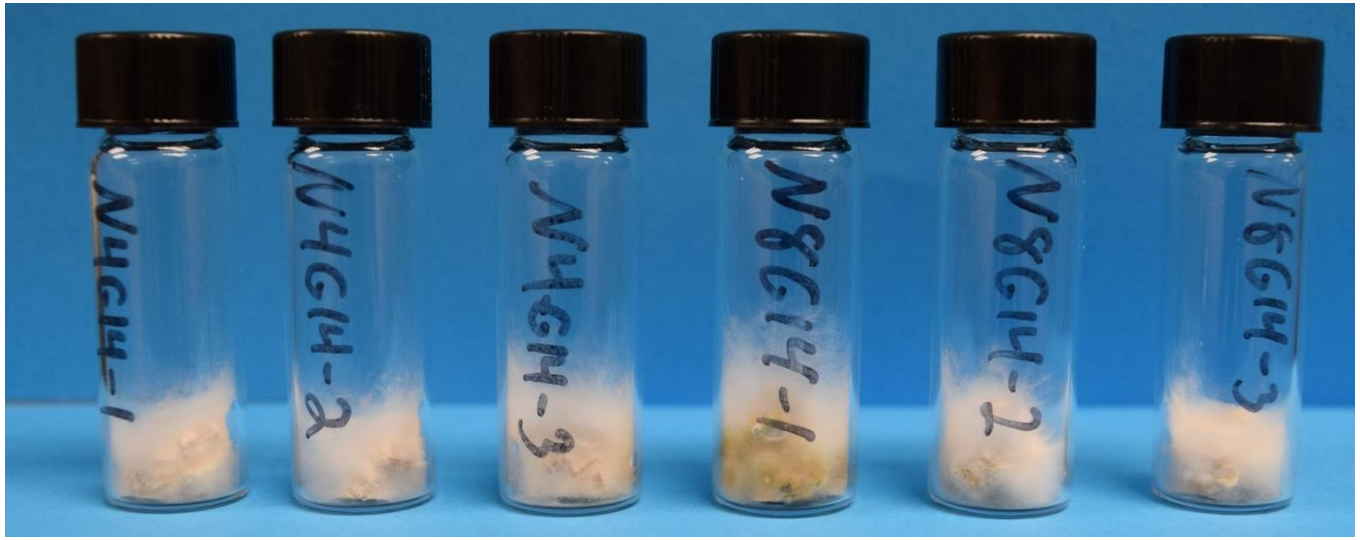
Fatty Acids

- Alsen cultivar had significant decreases in oleic and linoleic acids
- No change in fatty acid concentration in the Norm cultivar
- Oleic and linoleic acids are involved in plant resistance to fungal pathogens

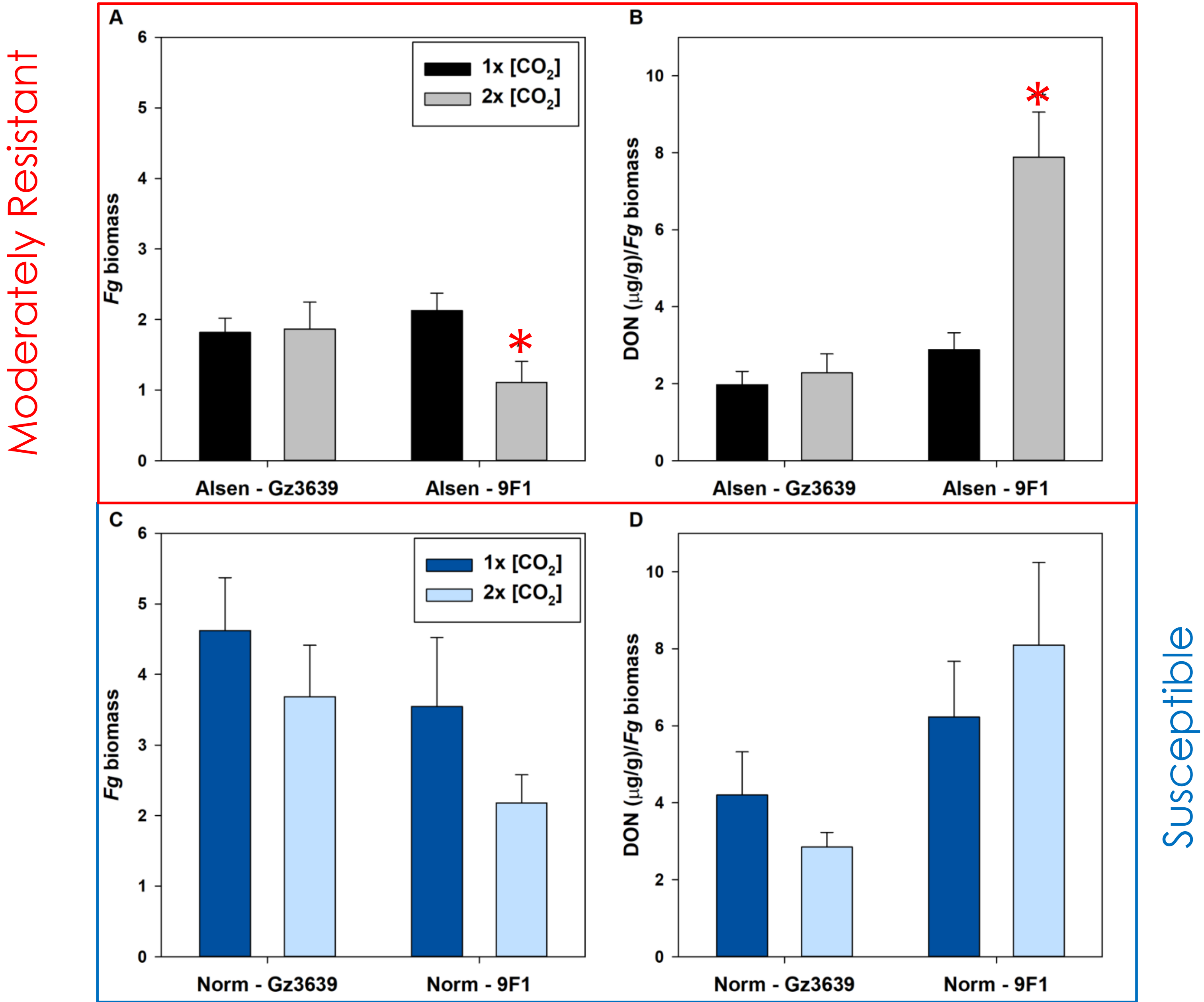


Approach: To evaluate the isolated effect of grain composition on *F. graminearum*

- Selected two *Fusarium graminearum* strains: Gz3639 and 9F1
- Wheat embryo was killed by freeze/thaw (5 cycles: -80 °C to 40 °C)
  - Elimination the confounding factors:
    - Plant defense responses
    - Direct CO_2 effect on the fungus



Changes in wheat nutritional content at elevated CO_2 were associated with strain and cultivar specific differences



Trichothecene biosynthesis pathways

- Trichothecene biosynthesis pathway
  - *TRI1*, *TRI4*, *TRI5* genes all encode enzymes essential for the biosynthesis of DON
  - *TRI6* encodes a transcription factor which controls other trichothecene biosynthesis genes, TRI6 is a self-regulating transcription factor, decreasing the expression under nutrient-rich conditions.
- The *F. graminearum* strain 9F1 had increased mycotoxin biosynthesis in response to the loss of wheat nutritional content in Alsen.
- Gz3639 strain also showed an increase in TRI6 expression, indicating that both strains were impacted by changes in nutritional content, but to different degrees.

Table 1. Expression of Trichothecene Biosynthetic (*TRI*) Genes Was Influenced by Compositional Differences in Wheat Grain Grown at 1× or 2× [CO_2][a]

	Alsen				Norm			
	Gz3639		9F1		Gz3639		9F1	
[CO_2]	1×	2×	1×	2×	1×	2×	1×	2×
TRI1	13 ± 3	21 ± 5	23 ± 4	32 ± 3†	55 ± 15	36 ± 11	40 ± 7	61 ± 15
TRI4	15 ± 4	19 ± 4	26 ± 7	41 ± 6†	58 ± 16	39 ± 11	47 ± 8	85 ± 25
TRI5	9 ± 2	12 ± 2	13 ± 2	21 ± 3*	35 ± 9	21 ± 8	25 ± 4	31 ± 16
TRI6	9 ± 2	18 ± 4*	17 ± 3	27 ± 3*	21 ± 5	13 ± 3	20 ± 3	26 ± 6

Single floret point inoculations of wheat

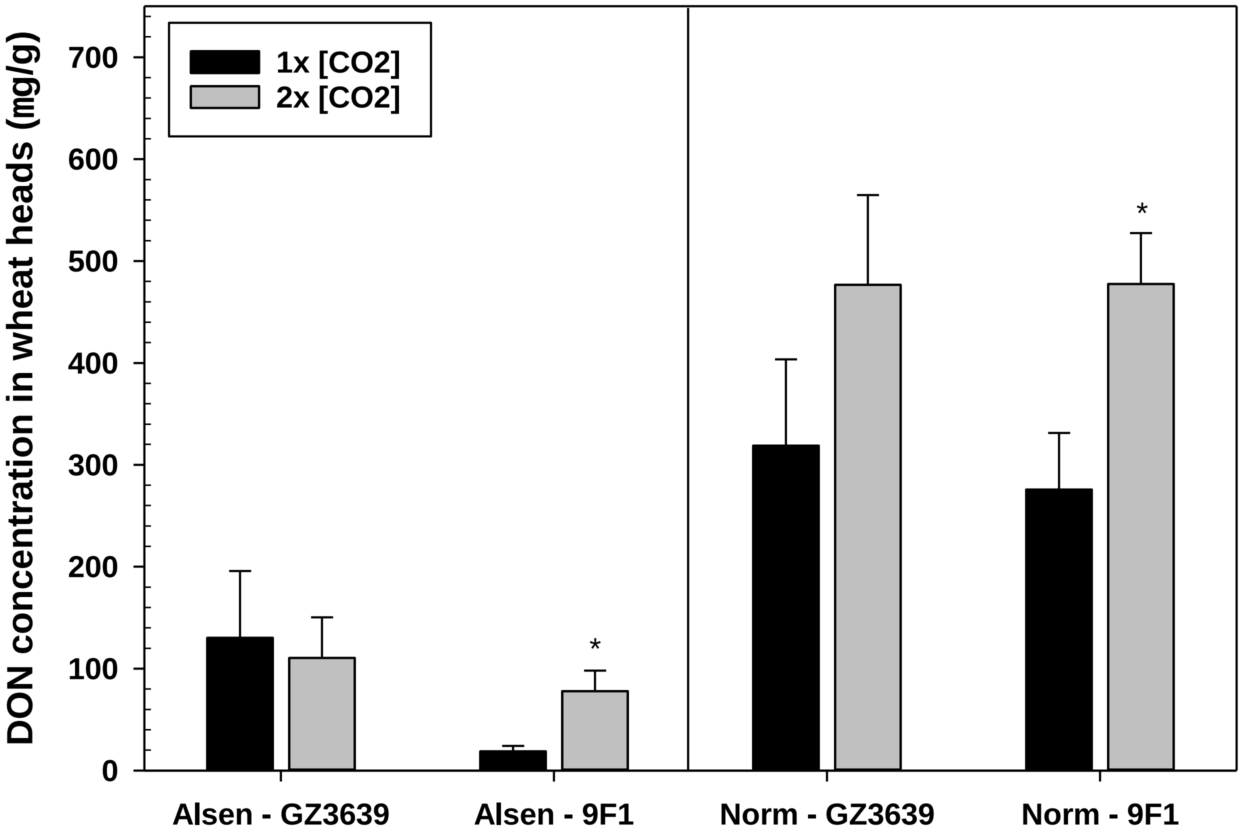
- Alsen and Norm were grown at both 1x and 2x current atmospheric CO_2 concentrations
- Then inoculated with *F. graminearum* strain Gz3639 and 9F1

Inoculation at anthesis (flowering)

Wheat head bleaching as disease progresses

Effect of Elevated CO_2: More Toxin-less nutrients

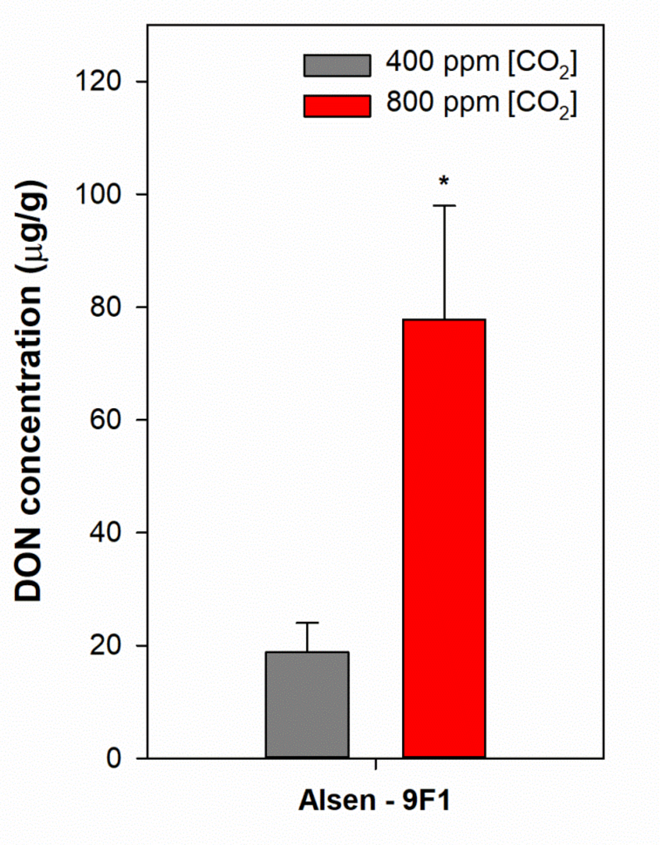
- Increased mycotoxin concentrations at elevated CO_2: 9F1
- No difference in the Gz3639 strain
- Consistent with results from seed trials



Effect of Elevated CO_2: More Toxin-less nutrients

- **Concern:** Moderately resistant wheat had a three-fold increase in toxin accumulation from *F. graminearium* 9F1

- **Is this increase in toxin and reduction in nutritional content consistent among moderately resistant wheat cultivars?**



Impact of Nutritional changes

- The FHB moderately resistant cultivar Alsen grain that had been grown at 2× [CO_2] had more severe losses in protein, fatty acid, and mineral contents
  - Resulted in a poorer fungal growth medium
- Nutrient stress increases the expression of virulence related genes and induces the accumulation of trichothecenes
  - Nitrogen limitation induces trichothecene biosynthesis
- 9F1 appears to be more sensitive to the nutrient limitations, as significant differences were detected in both biomass and DON production.

Food security concerns: questions for the future

Impact:

- Potential reduced efficacy of resistance factors in wheat currently considered moderately resistant to FHB
- Strain specific pathogenic advantage as nutrient content declines
- Wheat growers may be less likely to choose moderately resistant cultivars with reduced end use quality; increasing risk of FHB infection.
- Uncertainty of future food security and safety

Testing additional wheat cultivars

- In collaboration with Dr. James Anderson from the University of Minnesota
- Testing 15 wheat cultivars of varying FHB susceptibility
  - Scale is 1-9: 1 is 100% resistant (none currently), 9 is most susceptible

Cultivar	FHB Susceptability
S1	9
S2	9
S3	6
S4	6
S5	6
S6	5
MR1	4
MR2	4
MR3	4
MR4	4
MR5	3
MR6	3
MR7	3
MR8	3
MR9	2-3

Susceptible

Moderately Resistant

Dr. James Anderson
University of Minnesota

Future plans and goals

Objective: Identify markers that can be used by breeders to simultaneously target climate resilient mycotoxin resistance and high grain quality traits.

- Determine the climate resilience of FHB resistance for various wheat cultivars
- Determine the impact of elevated CO_2 on photosynthesis, water use efficiency, growth, development, yield, and nutrition
- Test grain quality of near isogenic lines and parent lines used for FHB resistance at elevated CO_2
- Identify additional *F. Gram* strains which may become more aggressive in future climatic conditions

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Questions?

William.Hay@usda.gov

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