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

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Presentation Overview

- Introduction
- Background Information
- Biopesticide synergy when combining plant flavonoids and entomopathogenic baculovirus
- Elevated $[\text{CO}_2]$ 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 – 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
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

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Flavone subclass backbone

Isoflavone subclass backbone

Flavonol subclass backbone

**Trichoplusia ni**

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

![Larva](image1)

![Adult](image2)
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?

<table>
<thead>
<tr>
<th>Plant</th>
<th>LC$_{50}$</th>
<th>Upper CL</th>
<th>Lower CL</th>
<th>Intercept</th>
<th>Slope</th>
<th>Heterogeneity</th>
<th>$\chi^2$</th>
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<td>1.39</td>
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<td>0.60</td>
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<td>Green bean</td>
<td>10.73 b</td>
<td>32.31</td>
<td>5.61</td>
<td>$-0.418$</td>
<td>1.034</td>
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<td>9.69</td>
<td>$-0.679$</td>
<td>1.592</td>
<td>1.53</td>
<td>4.59</td>
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Insecticidal activity based on dosage-response of AfMNPV against *T. ni* neonates when applied to crop plants.
Differences in leaf anatomy - Trichomes

Mortality:
- Cabbage: 64.6%
- Cotton: 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.

<table>
<thead>
<tr>
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<th>(μM/g)</th>
<th>(μg/g)</th>
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<tr>
<td>Daidzein</td>
<td>0.678 ± 0.004</td>
<td>172 ± 1</td>
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<tr>
<td>Genistein</td>
<td>0.684 ± 0.009</td>
<td>185 ± 3</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>4.832 ± 0.004</td>
<td>1382 ± 2</td>
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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

<table>
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<tr>
<th>Diet incorporation</th>
<th>LC$_{50}$</th>
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<th>Lower CL</th>
<th>Intercept</th>
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<td>3061 a</td>
<td>4050</td>
<td>2380</td>
<td>−5.975</td>
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<td>Genistein</td>
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<td>3857</td>
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<td>−4.277</td>
<td>1.244</td>
<td>1.20</td>
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<td>Daidzein</td>
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<td>4103</td>
<td>1796</td>
<td>−4.706</td>
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<td>2.23</td>
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<td>DMSO, surfactant</td>
<td>2363 a</td>
<td>2967</td>
<td>1912</td>
<td>−5.119</td>
<td>1.517</td>
<td>0.89</td>
<td>11.53</td>
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<tr>
<td>Kaempferol</td>
<td>2307 a</td>
<td>2884</td>
<td>1862</td>
<td>−5.280</td>
<td>1.570</td>
<td>0.89</td>
<td>11.56</td>
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<tr>
<td>Combination</td>
<td>1666 b</td>
<td>2317</td>
<td>1217</td>
<td>−4.890</td>
<td>1.518</td>
<td>1.82</td>
<td>23.60</td>
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</table>
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)

\[
\text{Cototoxicity factor} = 100 \times \frac{(\text{observed}\%\text{mortality} - \text{expected}\%\text{mortality})}{(\text{expected}\%\text{mortality})}
\]

<table>
<thead>
<tr>
<th>Diet incorporation</th>
<th>LC\text{50}</th>
<th>Upper CL</th>
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<th>Intercept</th>
<th>Slope</th>
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<th>$\chi^2$</th>
<th>df</th>
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<td>Daidzein 6.5x</td>
<td>1758 b</td>
<td>2140</td>
<td>1451</td>
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<tr>
<td>Genistein 6.5x</td>
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<td>2466</td>
<td>299</td>
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<td>1.419</td>
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<td>441</td>
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<td>Kaempferol 3.5x</td>
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<td>1.436</td>
<td>4.91</td>
<td>63.77</td>
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</table>
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

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


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.

Antioxidant Capacity

- All three of the identified flavonoids are known antioxidants.
- 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.

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

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

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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 CO₂

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

Wheat (C3) produces far more carbohydrates under elevated CO₂
Wheat grain subsequently loses protein concentration at elevated CO₂

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
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 \( Fhb7 \)

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
Elevated CO$_2$ has been reported to increase the severity of FHB

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

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

<table>
<thead>
<tr>
<th></th>
<th>Alsen</th>
<th></th>
<th>Norm</th>
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<td></td>
<td>1x</td>
<td>2x</td>
<td>1x</td>
<td>2x</td>
</tr>
<tr>
<td>[CO$_2$]</td>
<td></td>
<td></td>
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<tr>
<td>% Fat</td>
<td>0.58 ±0.15</td>
<td>0.53 ±0.10</td>
<td>0.87 ±0.18</td>
<td>0.76 ±0.12</td>
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<tr>
<td>% Protein</td>
<td>17.48 ±0.30</td>
<td><strong>11.40 ±0.23</strong></td>
<td>18.96 ±0.31</td>
<td><strong>15.41 ±0.24</strong></td>
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<tr>
<td>% Carbohydrate</td>
<td>78.42 ±1.24</td>
<td><strong>84.72 ±0.84</strong></td>
<td>76.18 ±1.78</td>
<td><strong>80.13 ±1.47</strong></td>
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<tr>
<td>% Crude Fiber</td>
<td>1.16 ±0.13</td>
<td>1.31 ±0.06</td>
<td>1.46 ±0.22</td>
<td>1.49 ±0.32</td>
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<tr>
<td>% Ash</td>
<td>2.36 ±0.04</td>
<td><strong>2.04 ±0.03</strong></td>
<td>2.53 ±0.18</td>
<td><strong>2.21 ±0.05</strong></td>
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Moderately resistant cultivar was impacted more than the susceptible cultivar
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$_2$
Amino Acids %

Were changes in protein due to dilution?

Majority of amino acid %’s were unaffected by elevated CO₂

However, both varieties had alterations in the same five amino acids in response to elevated CO₂
Mineral Content

Greater loses in moderately resistant Alsen
- P, Ca, Zn, Fe, Cu

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

Phosphorus 11%

Calcium 22%

Zinc 30%

Iron 21%

Copper 25%

Moderately Resistant Alsen

Susceptible Norm
Water Soluble Carbohydrates

- Alsen cultivar had significant increases in glucose and maltose at elevated CO₂
- 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\textsubscript{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>
<thead>
<tr>
<th></th>
<th>Gz3639</th>
<th></th>
<th>9F1</th>
<th></th>
<th>Gz3639</th>
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<tr>
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<td>2x</td>
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<td>TRI1</td>
<td>13 ± 3</td>
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<td>23 ± 4</td>
<td>32 ± 3</td>
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<tr>
<td>TRI4</td>
<td>15 ± 4</td>
<td>19 ± 4</td>
<td></td>
<td>26 ± 7</td>
<td>41 ± 6</td>
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<tr>
<td>TRI5</td>
<td>9 ± 2</td>
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<td>13 ± 2</td>
<td>21 ± 3</td>
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<td>TRI6</td>
<td>9 ± 2</td>
<td>18 ± 4</td>
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<td>17 ± 3</td>
<td>27 ± 3</td>
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</table>

Table 1. Expression of Trichothecene Biosynthetic (TRI) Genes Was Influenced by Compositional Differences in Wheat Grain Grown at 1x or 2x [CO₂]
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. graminearum* 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
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

<table>
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<th>Cultivar</th>
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<td>S2</td>
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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
Acknowledgements

- Martha Vaughan
- Jen Teresi
- Keegan McConnel
- Jacob Brown
- Nathan Kemp
- Christine Poppe
- Kim Ascherl
- Susan McCormick
- Stephanie Folmar
- Gordon Selling
- Kelly Utt
- Mark Berhow
- Mila Hojilla-Evangelista
- Robert Dunn
- Gary Grose
- Mike Bowman
- Jeffery Byars
- Steven Lyle
- AJ Thomas
- Jim Anderson
- Bob Behle
- Andie Miller
- Erica Goett
Questions?