The complexity of glucosinolates (GLS) in *Brassica carinata* and potential benefits for sustainable farming

Ted Stansly

Dr. David Wright & Dr. Ian Small

University of Florida

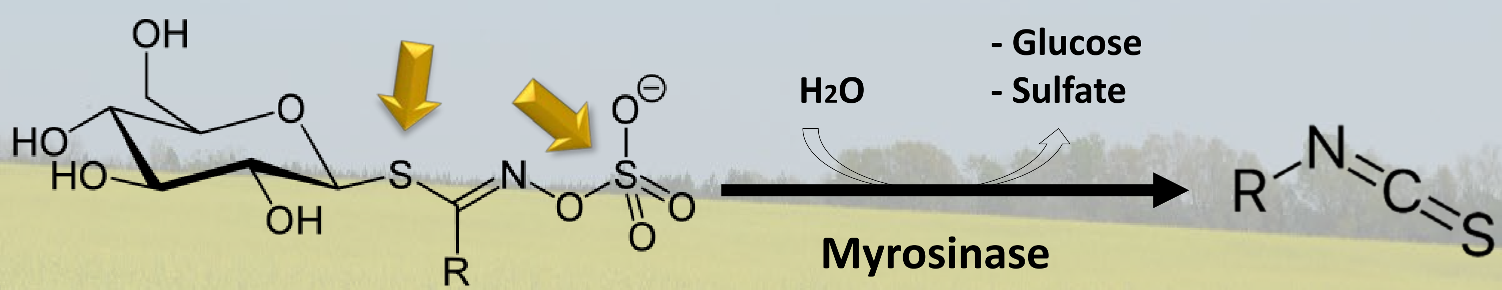
IFAS | NFREC – Quincy, FL

SPARC 2018

Gluco·sinol·ate (GLS)

- A **sulfur** and nitrogen-rich 2˚ metabolite for defense against herbivory and soil-borne pathogens

- Over 130 described molecules with same backbone, but vary in function, location, and concentration
(Fahey et al., 2001; Tripathi & Mishra, 2007)



Glucosinolate (GLS)

Isothiocyanate (ITC)

Classes of Glucosinolates

Class	AA Precursor	Stability	Location
Aliphatic	Alanine, Leucine, Isoleucine, Methionine	High	Seed>sprout>shoot>root
Indole	Tryptophan	Low-Med	Roots and shoots
Aromatic	Phenylalanine and Tyrosine	Low	Roots (mostly)

Bhandari et al., 2015

- Aliphatic-GSL can be anti-nutritional and reduce palatability to livestock (Cartea and Velasco, 2008; Fahey et al., 2001; Mawson et al., 1993)
- Indole and aromatic glucosinolates play important roles in plant defense (Clay et al., 2009; Hopkins et al., 2009)

Sulfur for Oilseed Brassica

- Brassica oilseed crops have a **higher demand** for sulfur partially due to the production of GLS
  (McGrath and Zhao, 1999)

- Since 1980's atm sulfur concentration decreased **87%**
  (www.epa.gov)

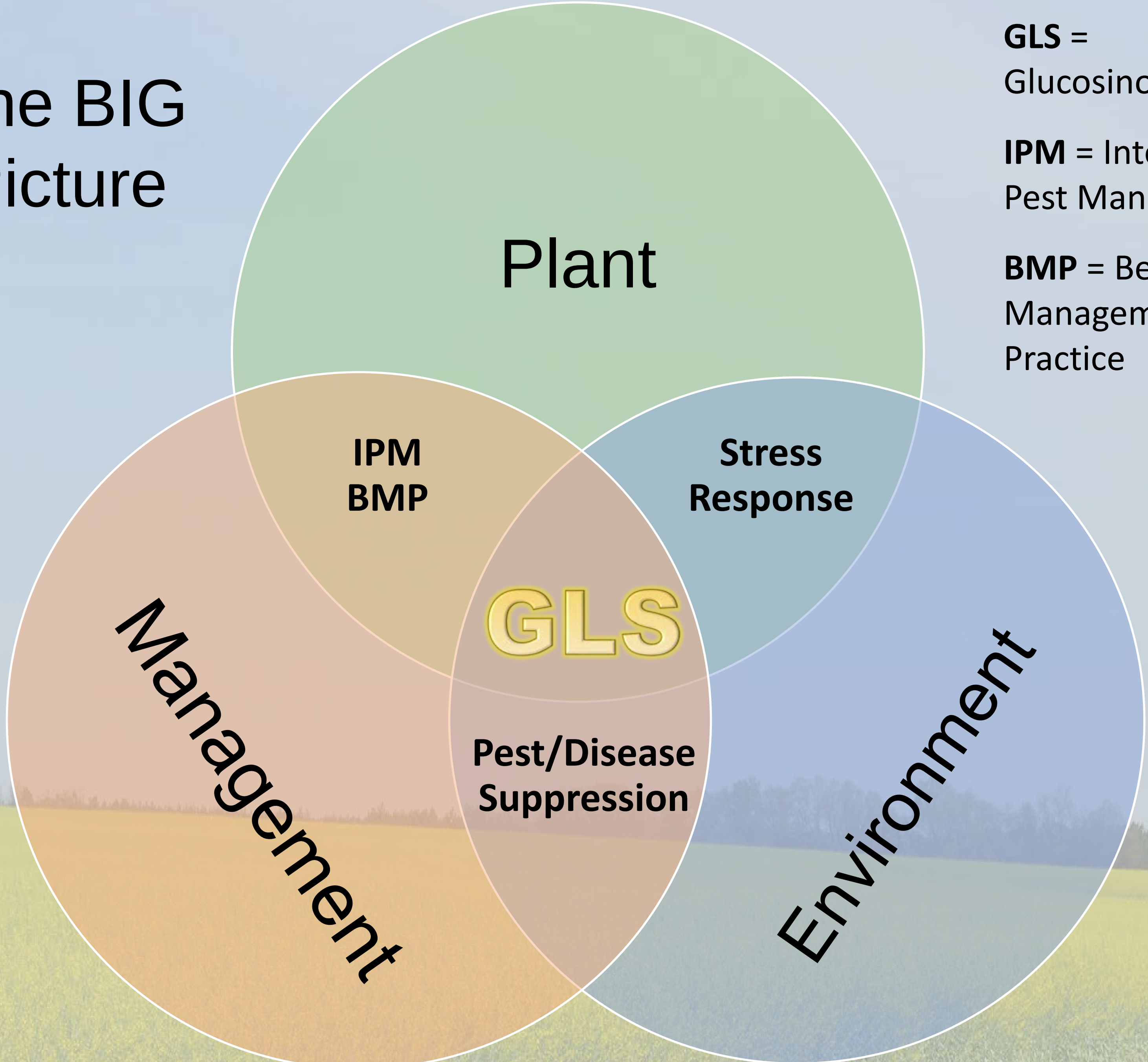
- Sulfur soil tests (i.e. SO_4 -S) remains largely unreliable due to the complexities of sulfur mobility in soil profiles
  (Dinkins and Jones, 2013; Horneck, 2001)

- Total GLS are decreased with limitation to sulfur availability in brassica. In particular, it has down-regulated **aliphatic-GLS** producing genes
  (K. L. Falk, J. G. Tokuhisa, J. Gershenzon, 2007)

Providing Ecosystem Services

- Isothiocyanates (ITC) suppress many types of plant pathogens, including nematodes via **biofumigation** (Matthiessen & Kirkegaard, 2006)
- Suppression remained for months after ITCs have dissipated from the soil profile (Papavizas 1966; Lewis and Papavizas, 1971; Warton et al, 2003; Weerakoon et al., 2011)
- ITCs are strongly sorbed by the organic matter, but breakdown influenced by chemical, physical and biological properties (Gimsing & Kirkegaard)

The BIG Picture



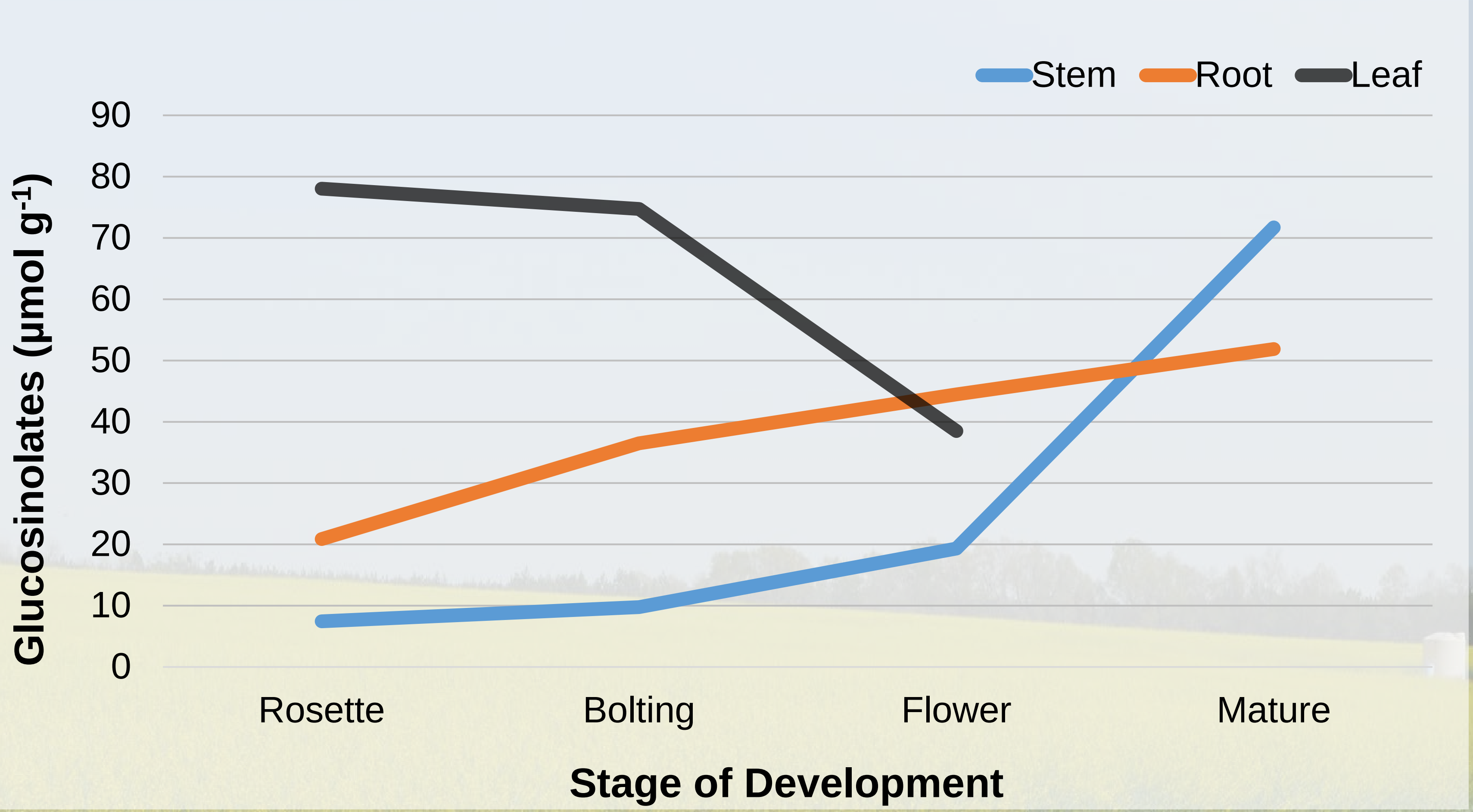
GLS = Glucosinolates

IPM = Integrated Pest Management

BMP = Best Management Practice

Total GLS in Carinata (2016)

Genotype: AAC-A110



Objectives

1. Determine the effects of sulfur nutrition on *B. carinata* and *B. napus* seed yield, oil content, and GLS

    → Under greenhouse and field conditions

    → Using a **range** of GLS producing genotypes

2. Correlate seed characteristics to leaf chemical composition (i.e. elemental sulfur)

3. Determine if aliphatic-GLS can be reduced to a minimum without affecting aromatic-GLS or oil production

One-Year Greenhouse Study

Design: RCBD (split) with 6 replications

Treatments: 1. Sulfur Rate***:** 0, 33, 66, & 100%

2. Genotype: *B. carinata* (x3) & *B. napus*

Fertilization: *Modified Hoagland Nutrient Solution (S = Epsom salt, $MgSO_4$)

Label	**Species**	**Variety**	**GLS (µmol g^{-1})**
Control	B. napus	Canterra 1918	10 - 15
Low	B. carinata	Avanza	10 – 25 **+ 65**
Medium	B. carinata	A120	60 - 80
High	B. carinata	AGR 137	100 - 140

Results


30 DAP

0-S in Bolting Stage – 75 DAP



100-S in Flowering Stage – 75DAP


Red leaf margins (napus)


Leaf Cupping

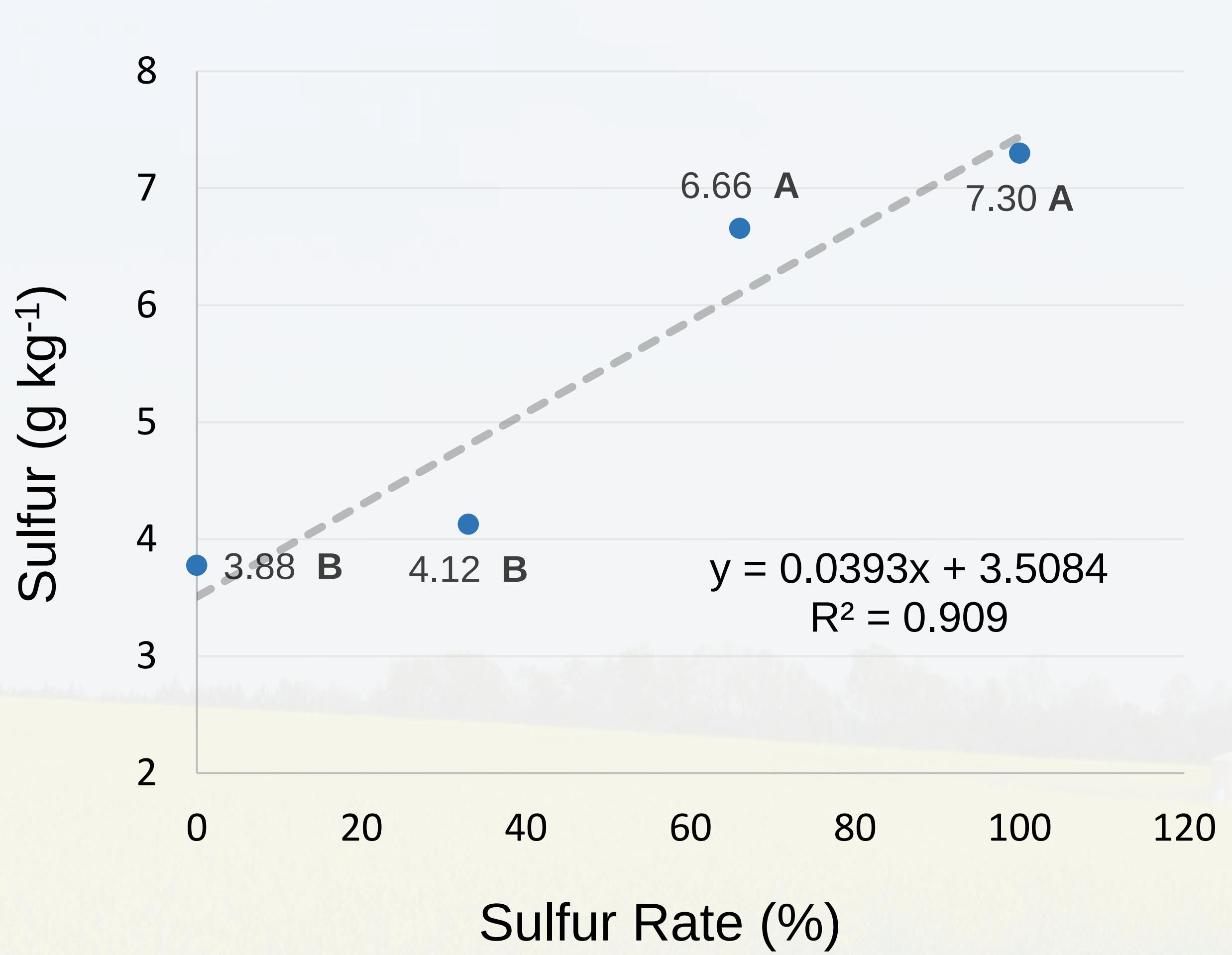
Sulfur in Leaves

Response to sulfur inputs

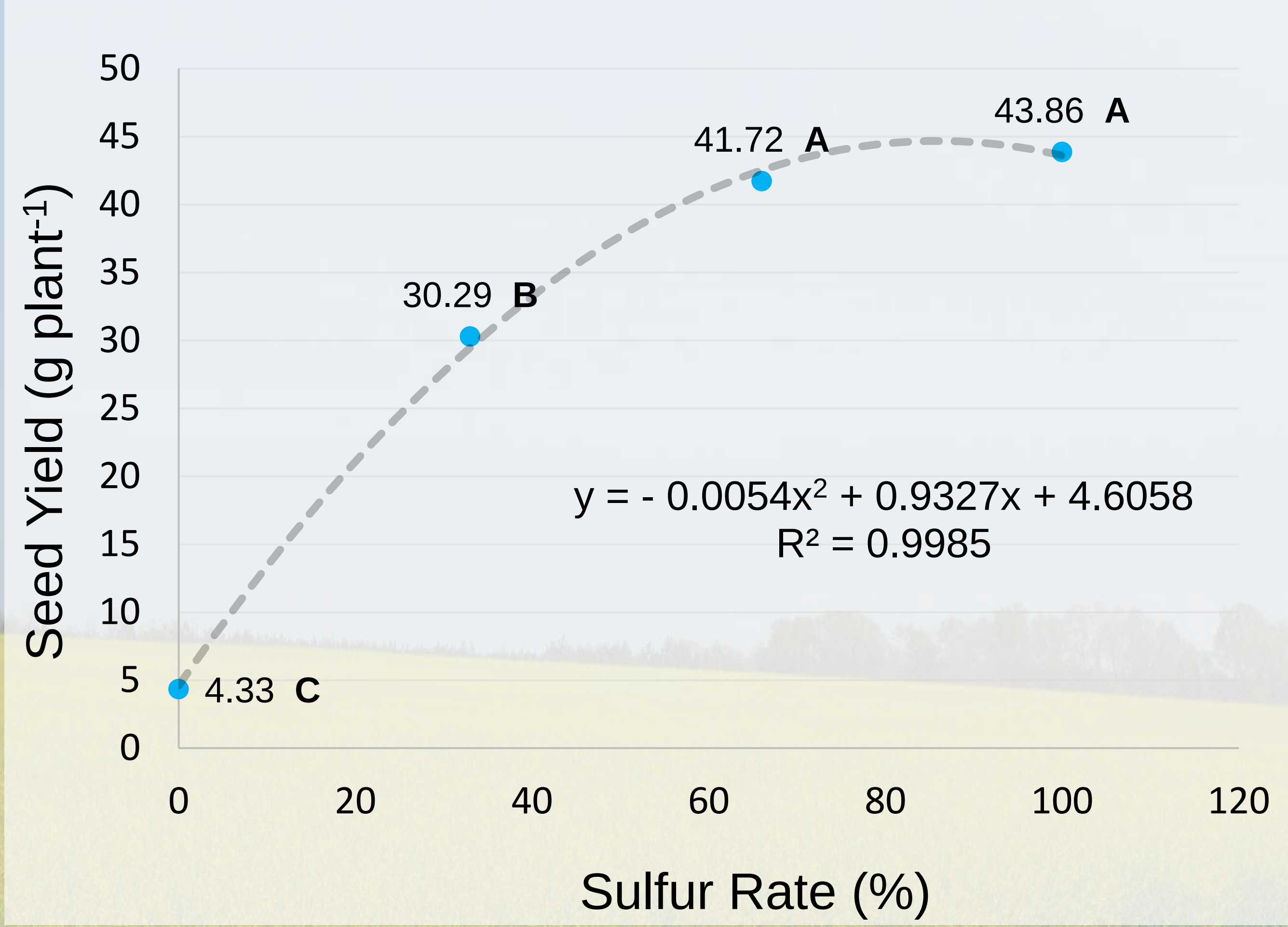
Linear relationship

Sulfur increase with availability

(p <0.001)



Seed Yield and Sulfur Rate

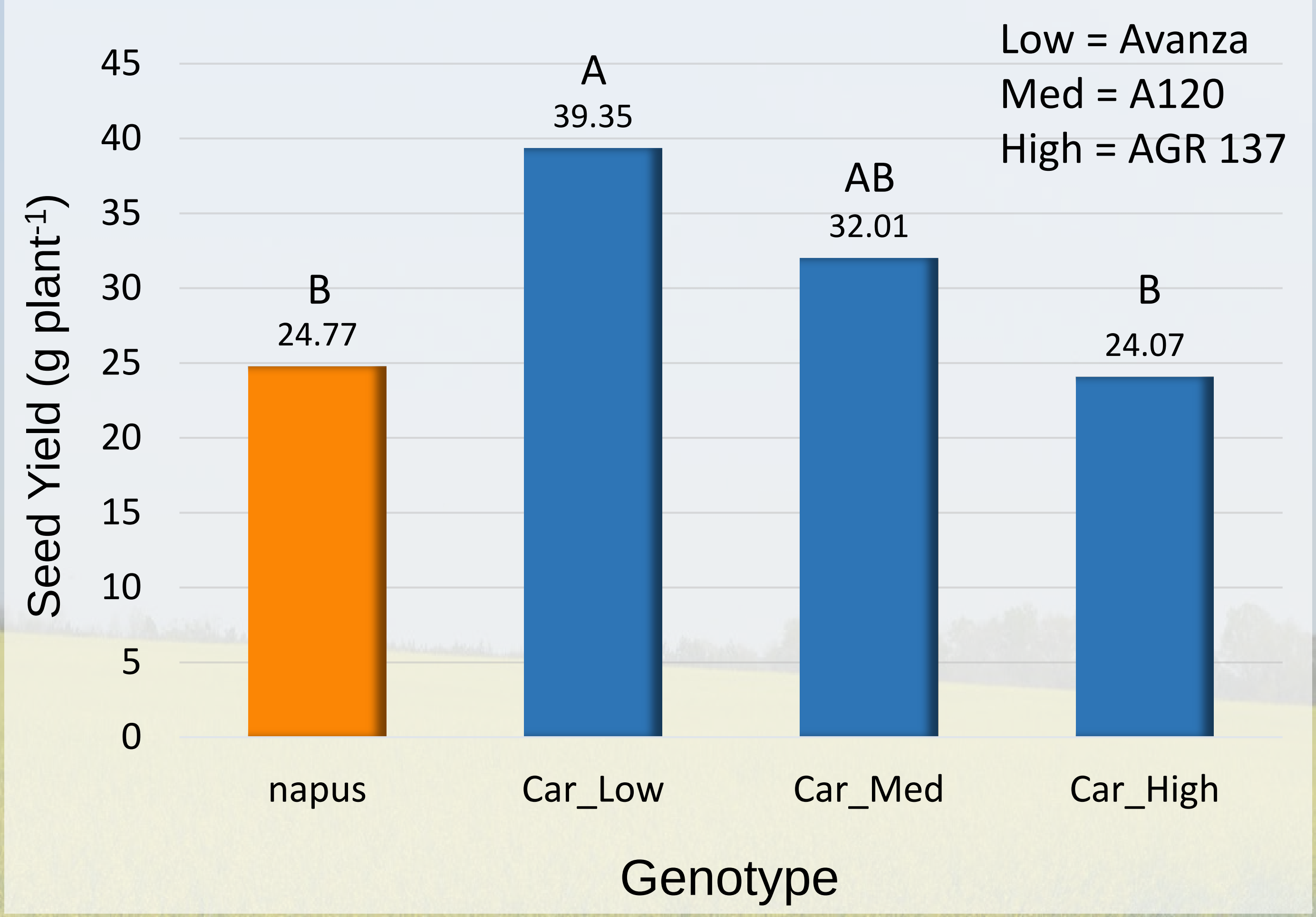


Response to sulfur rate ($p < 0.001$)

Quadratic relationship

Max uptake ≈86.4%

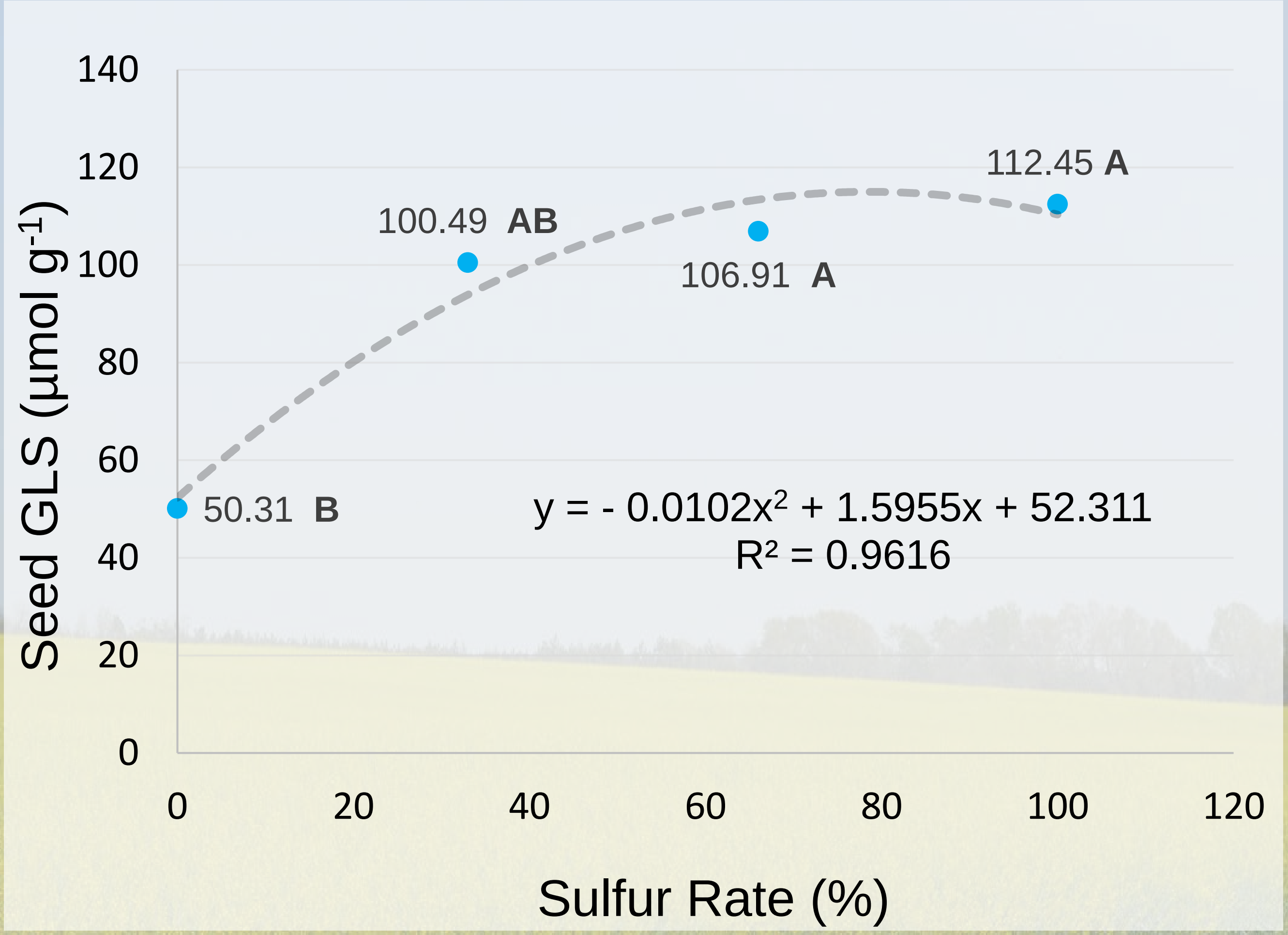
Seed Yield and Genotype



Rate response to Genotype ($p < 0.001$)

Carinata low yielded 37% more then napus and carinata high

Seed GLS and Sulfur Rates



Total GLS responds to sulfur rate ($p < 0.001$)

Quadratic relationship

Max total GLS ≈78.2%

Comparing Seed Characteristics

Variable 1	Variable 2	Spearman ρ	P-value	
GLS	Oil	-0.8138	<0.0001	↓
Erucic acid	Oil	-0.4784	0.0007	↓
Protein	Oil	-0.5849	<0.0001	↓
Protein	GLS	0.7932	<0.0001	↑
Protein	Erucic acid	0.7288	<0.0001	↑
Erucic acid	GLS	0.7536	<0.0001	↑

These results are typically found with oilseed brassicas

One-year Field study

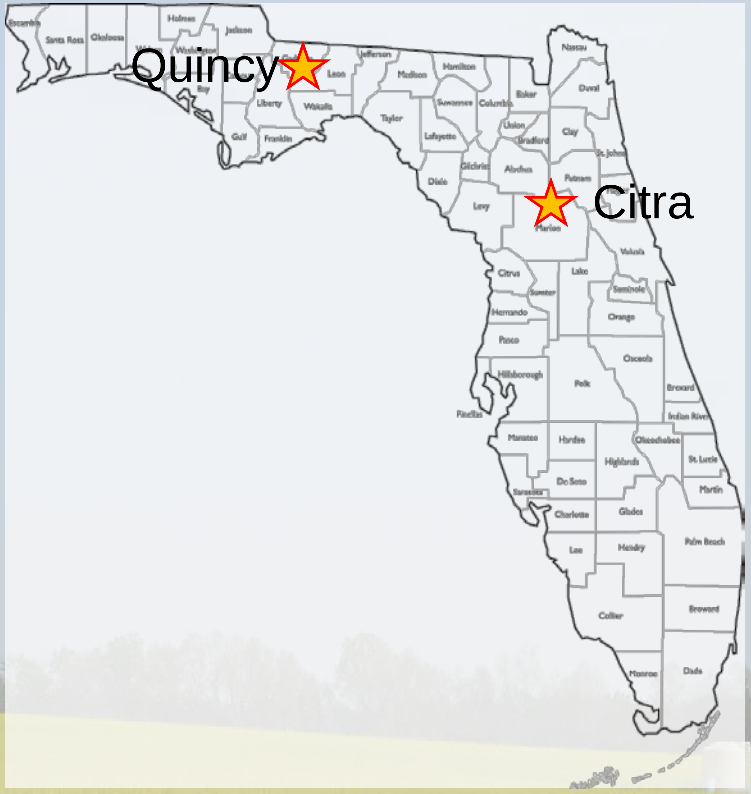
Two Locations:

1. NFREC, **Quincy**, FL
   (30.53963°, -84.58422°)
2. PSREU, **Citra**, FL
   (29.40784°, -82.17596°)

Soil type:

1. Norfolk loamy fine sand
2. Gainesville loamy sand

Source: USDA Web Soil survey(WSS)



Experimental Design

Design:	RCBD with 4 replications	
Treatments:	1. Quincy:	i. Species (*B. carinata and B. napus*)
		ii. Sulfur rate (0, 11, 22, 34, 45 kg ha^{-1})
	2. Citra:	i. Sulfur rate (0, 17, 34, 50 kg ha^{-1})
		ii. Application time (Split 2 and 3-ways)

Fertilization: N source: ammonium nitrate (34-0-0); S source: sulfate of potash (0-0-50-17). K levels were corrected with muriate of potash (0-0-60)

Quincy: N and S applied at planting (20%), bolting (40%) and flowering (40%)

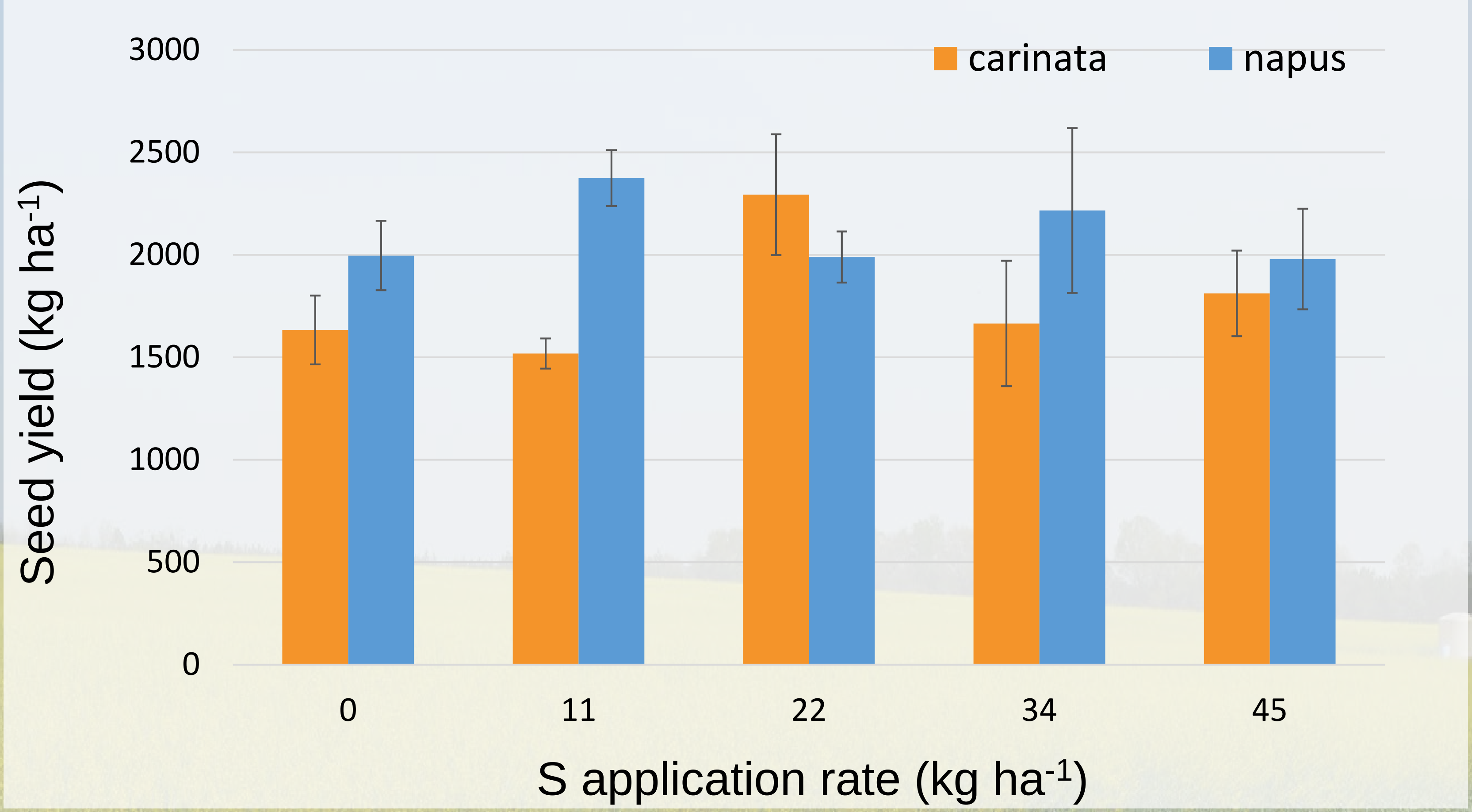
Citra: 2-way split: at planting (50%) and bolting (50%)

3-way split: at planting (20%), bolting (30%) and flowering (50%)

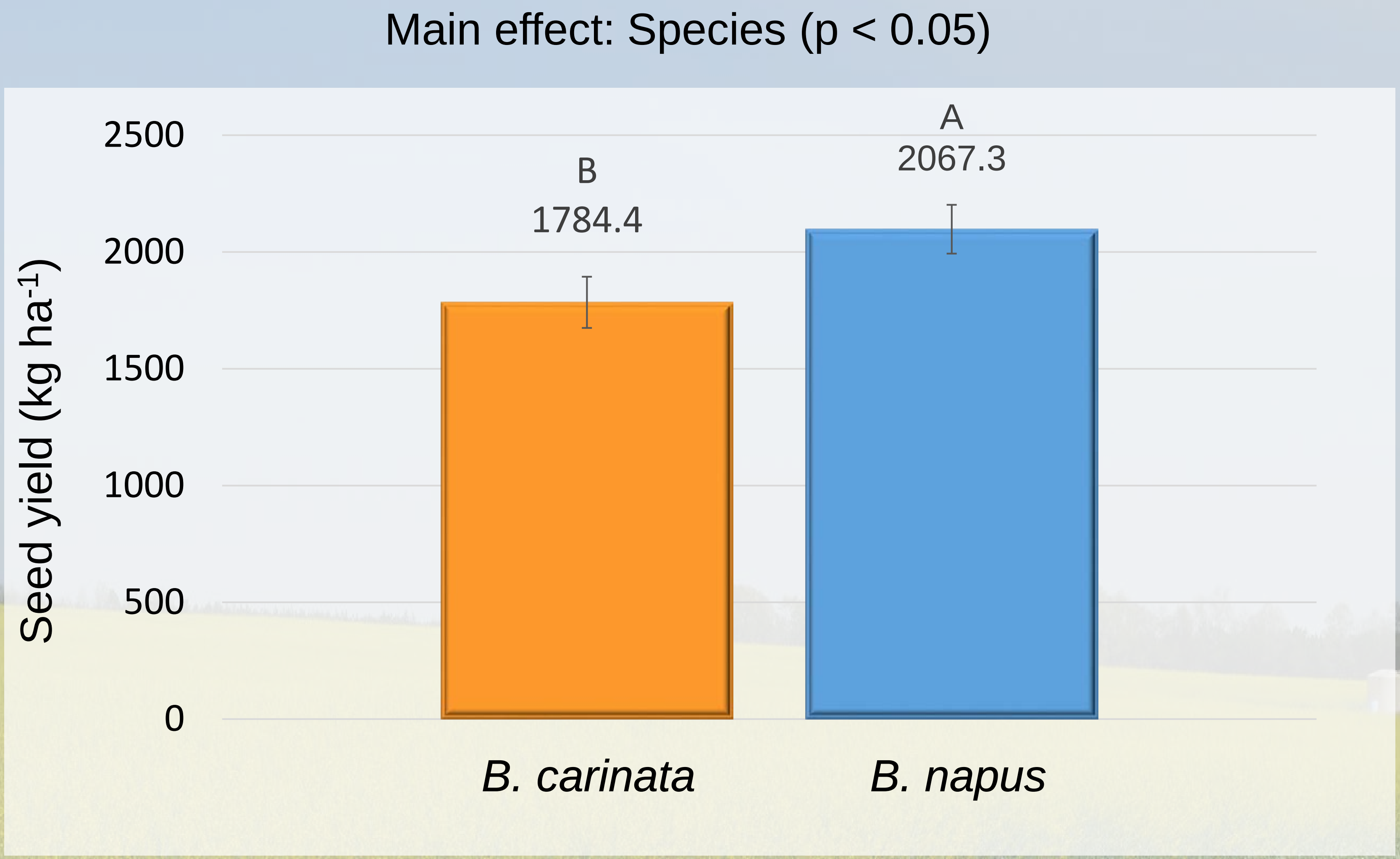
Results

Seed Yield Response to S-rate
Quincy, FL

($p < 0.05$)

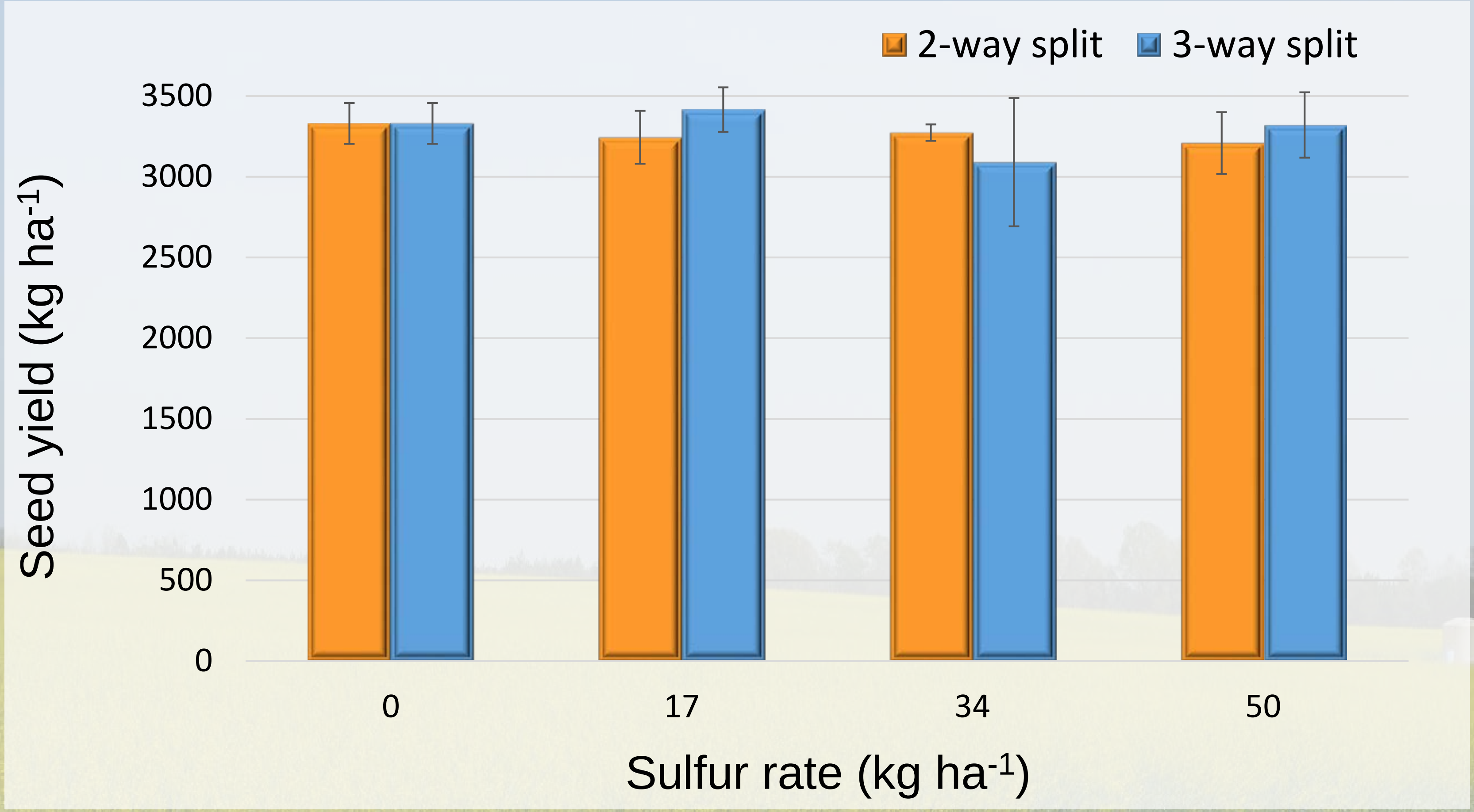


Seed Yield vs Species
Quincy, FL



Seed Yield vs Sulfur Rate
Citra, FL

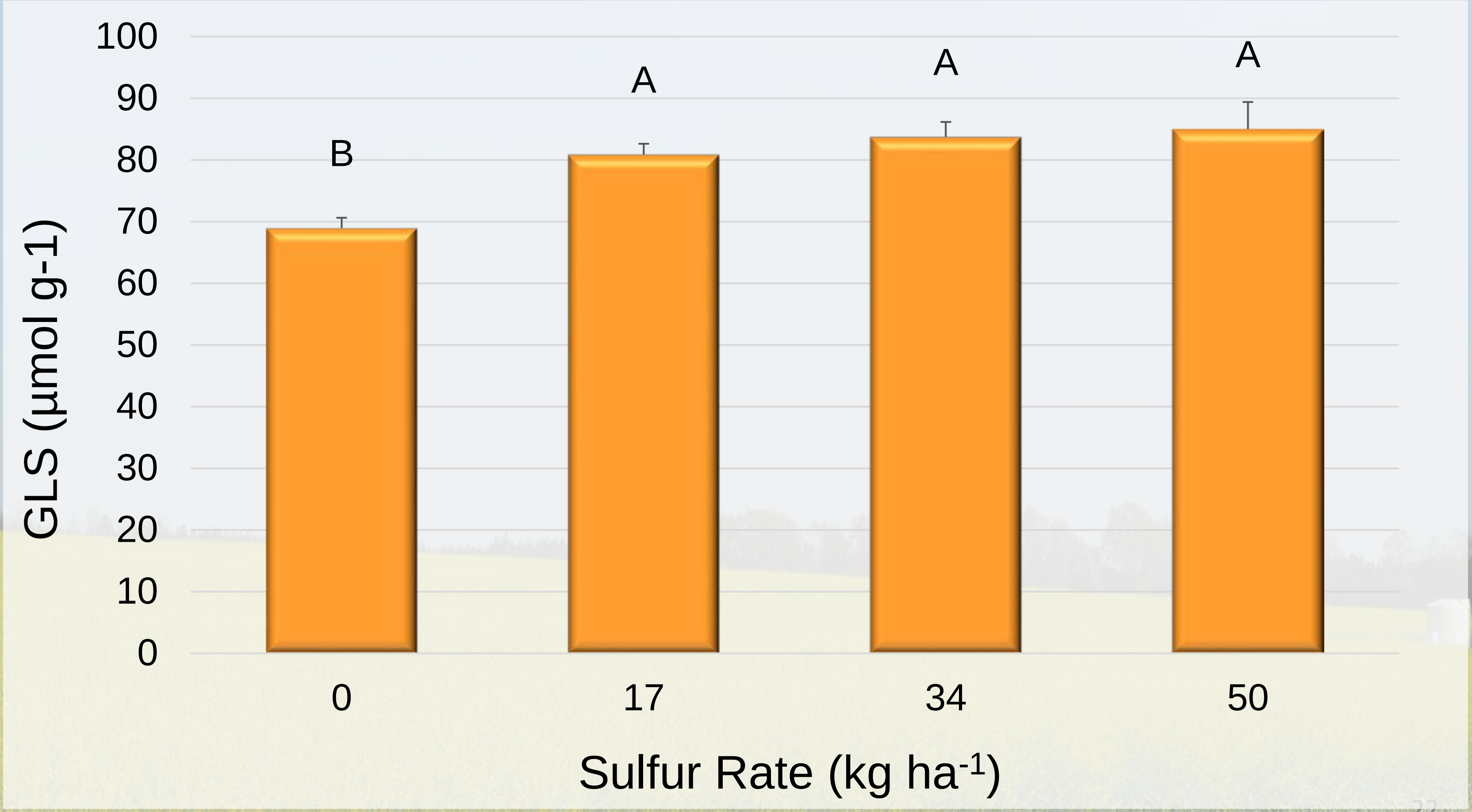
Main effect: None ($p < 0.05$)



Avg = 3270 kg ha^{-1}

GLS vs Sulfur rate
Citra, FL

Main effect: Sulfur Rate ($p < 0.001$)



Planting and Harvesting

	Quincy	Citra
Planting Date	1/5/2017	12/8/2017
Harvest Date	6/9/2017	5/18/2017
Seeding Rate	6.73 kg/ha (6 lbs/A)	6.73 kg/ha (6 lbs/A)
Row Spacing	30.5 cm (12in)	35.56 cm (14 in)
Plot Size	2.4m x 6.1m	2.4m x 6.1m
Plot Area	14.6 m^2	14.6 m^2

Nutrient Analysis for Quincy, FL

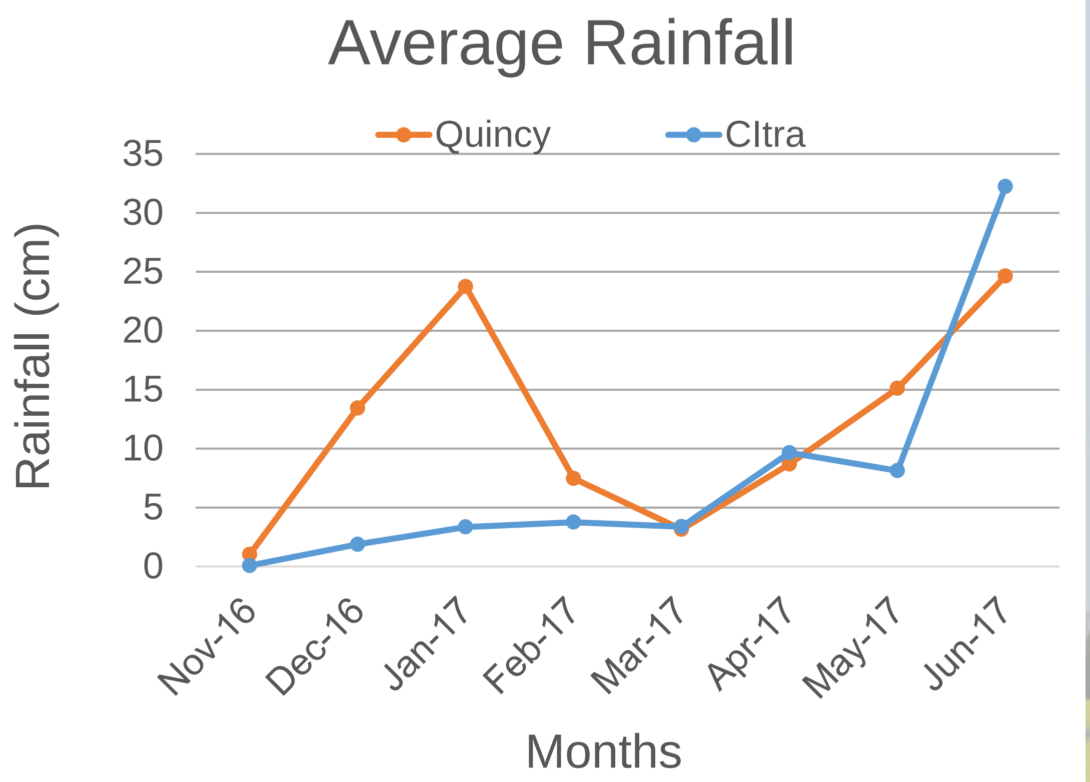
kg ha^{-1}										meg $100g^{-1}$	pH	
P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu	CEC	Soil pH	Buffer
29	178	171	687	41	0.2	3.4	9	24	0.5	5.1	5.8	7.7

Weather Conditions

Temperature (range):
13.9 – 24.6 °C at Quincy
16.9 – 24.4 °C at Citra

Average Rain (range):
1.02 – 24.6 cm at Quincy
0.08 – 32.2 cm at Citra

Solar Radiation (range):
98.0 – 238.6 cm in Quincy
109.4 – 250.1 cm in Citra



Summary and Conclusion

- Both seed yield and GLS concentrations respond to sulfur rates producing similar quadratic response curves

- *B. carinata* can produce more glucosinolates (67.7%), erucic acid (33.2%), and protein (10.7%) then *B. napus*

- Leaf sulfur concentrations could be used as an indicator for seed GLS concentrations and possibly yield at harvest

Next Steps (now underway)

- Run similar sulfur trials, but under **nutrient deficient** field conditions and variable soil types (i.e. locations)
- Identify the classes of glucosinolates within plant tissue and their responses to sulfur availability
- Biotic stress response (e.g. RKN, *Sclerotinia*) to glucosinolate production
- Identify classes of glucosinolate that increase resistance to specific plant pathogens in various tissue organs → **resistant genotypes**

Thank You!

TEDTRIALS
IDEASWORTHEXPERIMENTING

UF IFAS UNIVERSITY of FLORIDA

AGRISOMA

ARA

FLORIDA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES

SPARC
Southeast Partnership for Advanced Renewables from Carinata

Qualitative and quantitative evaluation of glucosinolates in cruciferous plants during their life cycles

Natalia Bellostas, Jens C. Sørensen, Hilmer Sørensen
Department of Natural Sciences, Biochemistry and Natural Product Chemistry, The Royal Veterinary and Agricultural University, Frederiksberg (Denmark).

2013

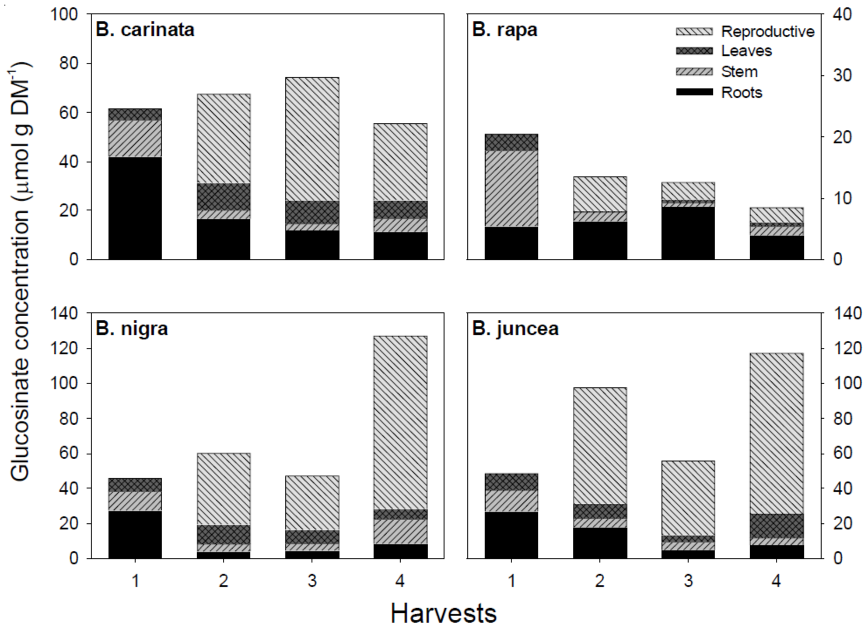


Figure 1. Glucosinolate concentration (imol g DM^{-1}) in the different plant parts at the four growth stages monitored for the four species.

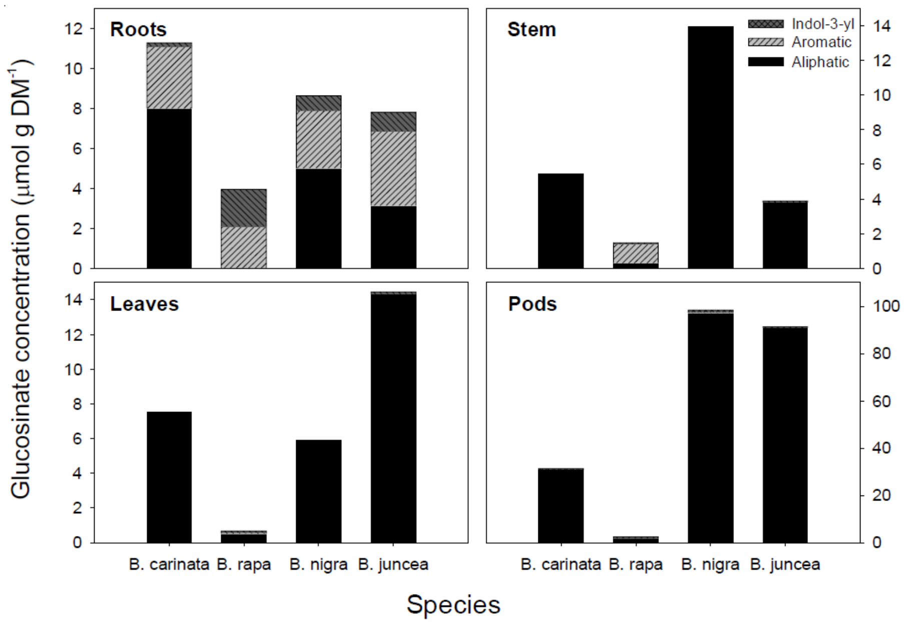


Figure 2. Concentrations (imol g DM^{-1}) of different glucosinolate types (aliphatic, aromatic and indol-3-yl) present in plant parts of each species at the last growth stage monitored (green seeds in pods).

Are GLS Bad for Business?

If GLS is bred out then:

- Reduce S inputs
- Reduce refinery steps
- Increase palatability

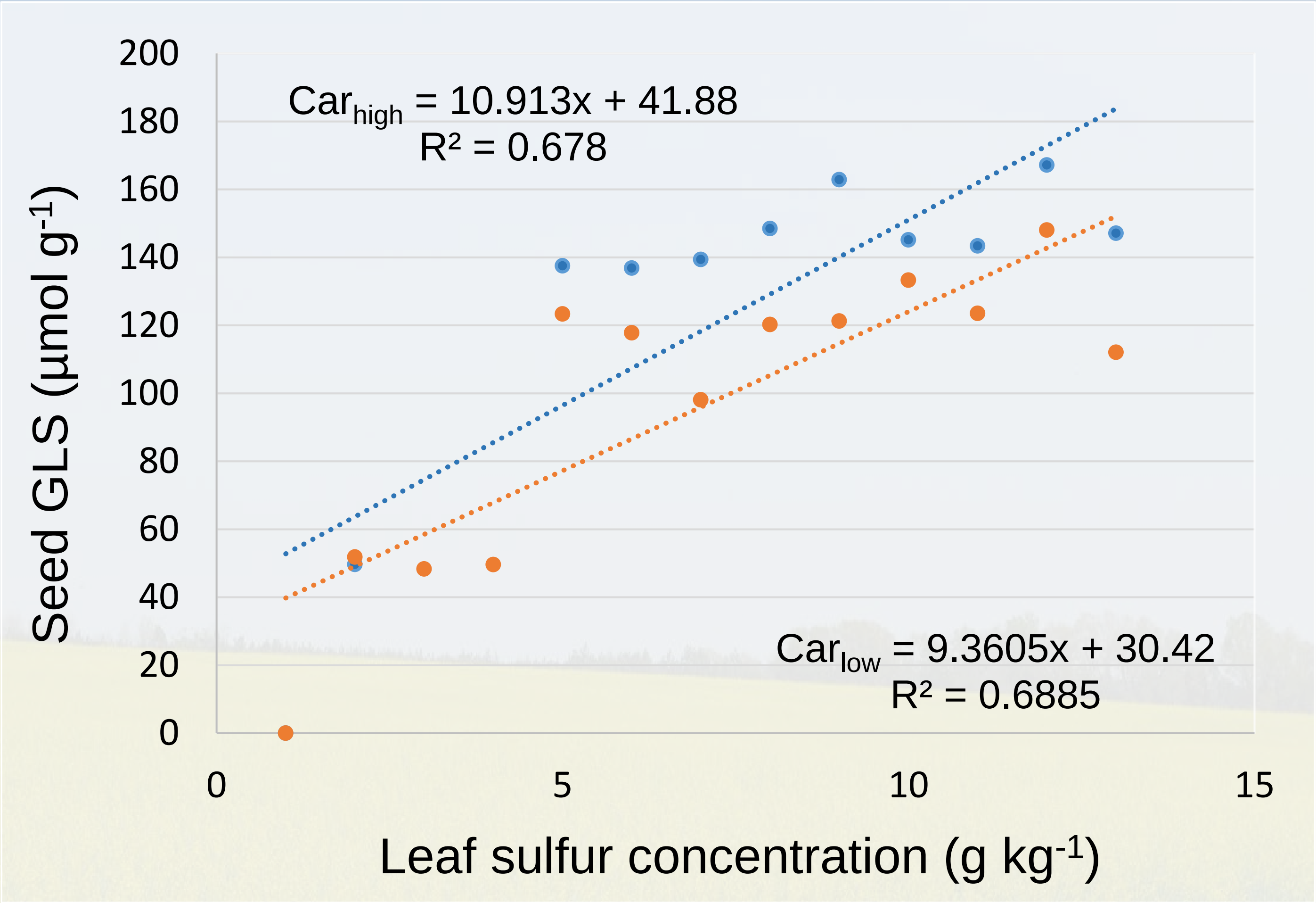
= $$$ (…not really)

Ex. *B. rapa* 'Harmoni'

- '00' (GLS, Erucic acid)
- Reduction in seed GLS + specifically all aliphatics
- Rest had high indole and aromatic-GLS

A polish spring rape was introduced in the 1960's with low GLS, possibly due to transporter gene knockout
(McGrath and Zhao, 1999)

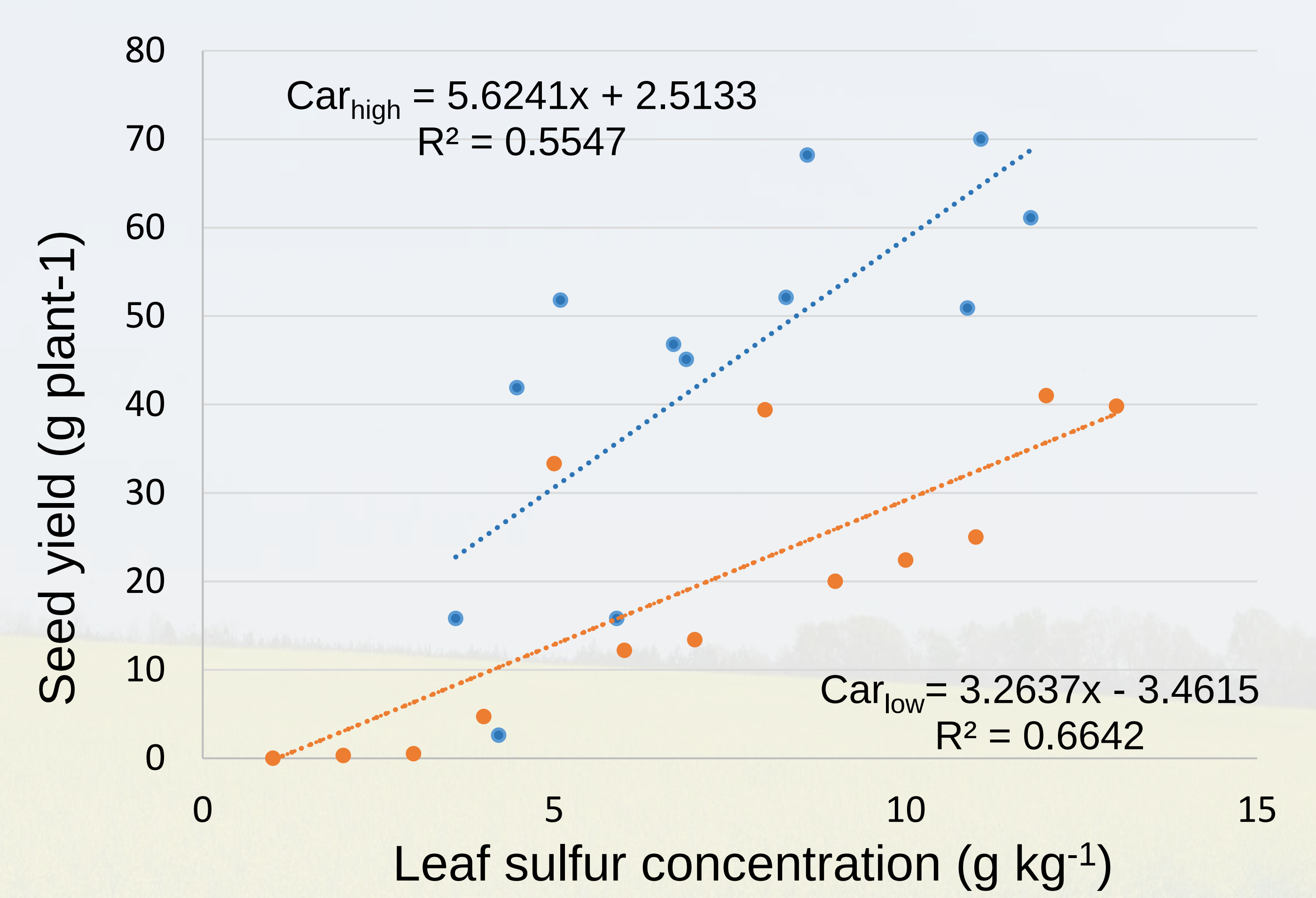
Seed GLS and Leaf Sulfur



Positive correlation in genotypes

Similar efficiency of Sulfur uptake to seed GLS

Seed Yield and Leaf Sulfur



Positive correlation of in genotypes

Stronger in carinata low

Carinata high more efficient at utilization for seed yield

High-throughput Nematode Assay

Stain
30 DAP

Erioglaucine (egg mass)

Acid Fuchsin (nematodes)

HPLC Data (so far) - Canola