

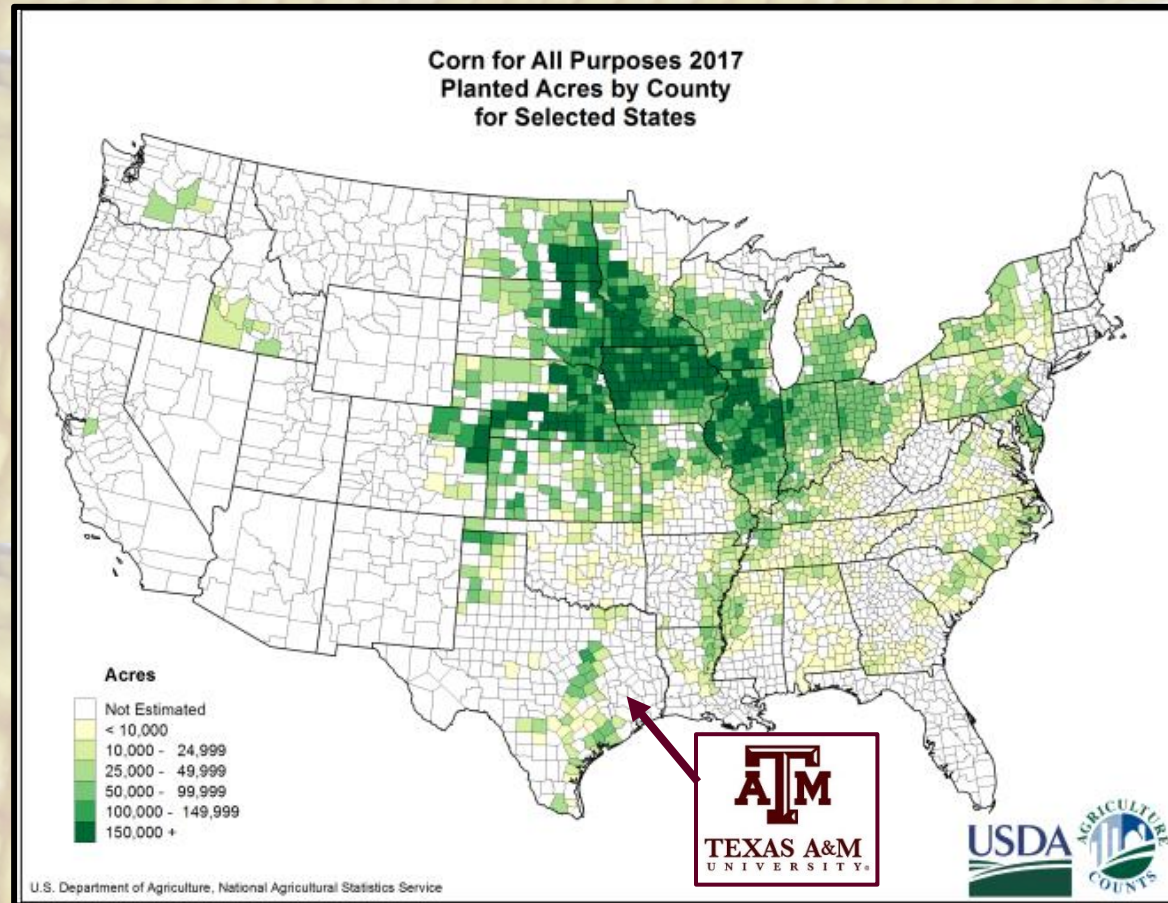
# Capturing nature AND nurture with temporal field phenomics to breed better crops

Seth C. Murray, **et al.**

Professor and Eugene Butler Endowed Chair  
Department of Soil and Crop Sciences  
Texas A&M University

AGBIODATA  
AUGUST 7, 2024

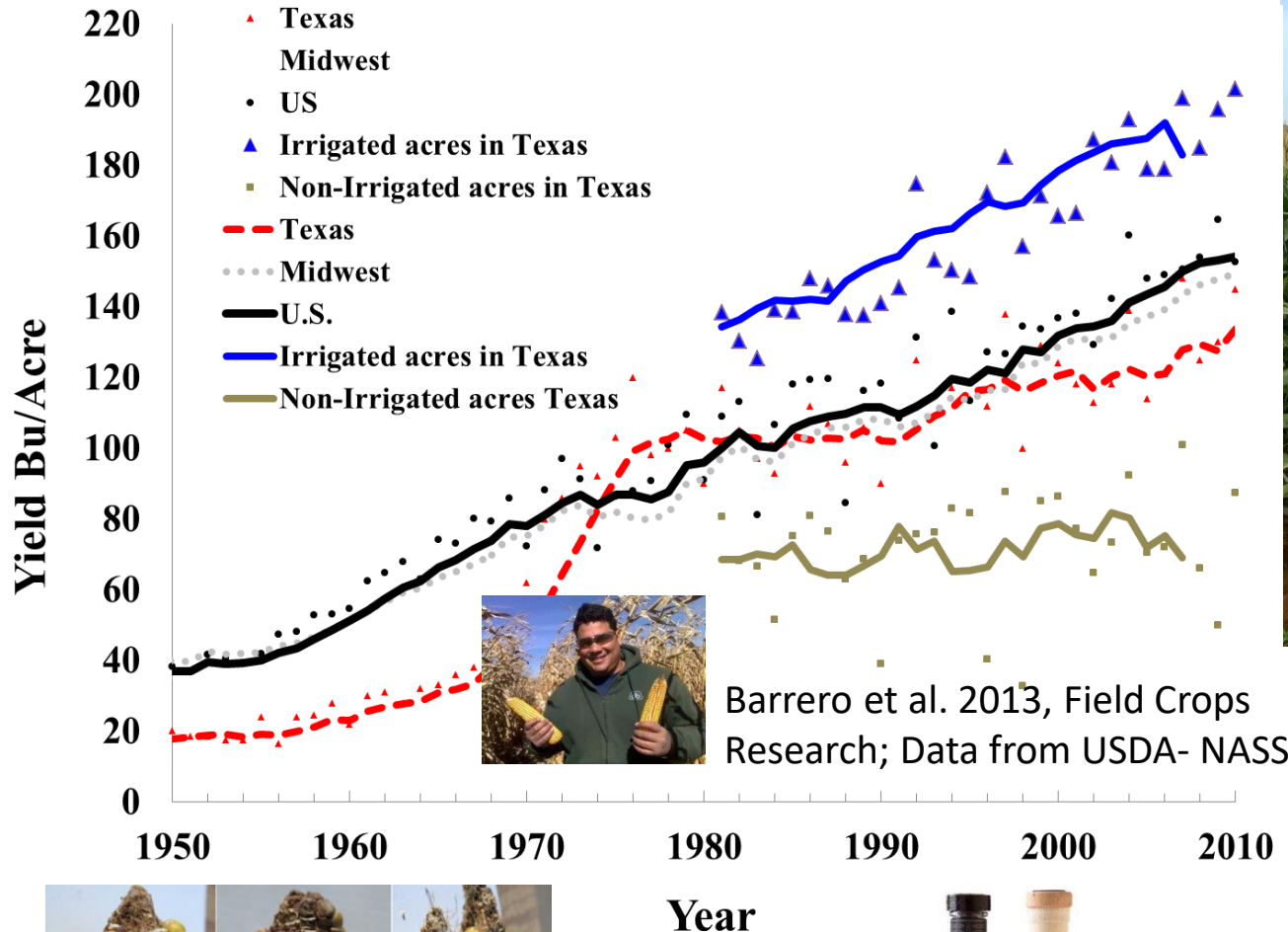
**Most maize (corn, *Zea mays* L.) is not sweet corn**  
**Corn - \$51.5 billion, 89 million acres, 404 million tons**  
**Sweet corn - \$860 million, 495,000 acres, 4 million tons**



*Zea mays*  
(Corn)  
Commercial  
Hybrid

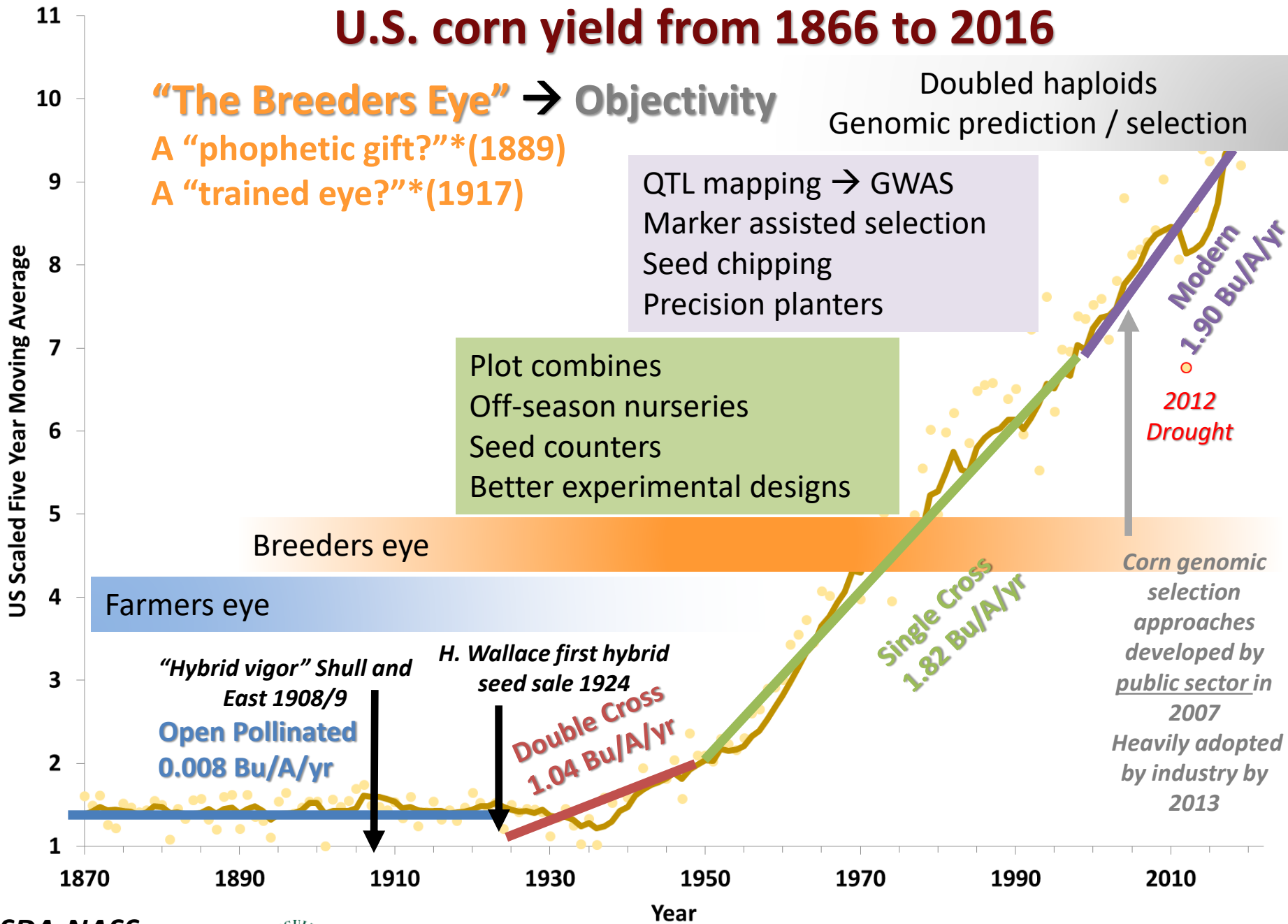
*Zea mays*  
(Corn)  
Commercial  
Hybrid

# Why is a public corn breeding program in Texas needed?



Murray et al. 2019, Journal of Plant Registrations

# U.S. corn yield from 1866 to 2016



# Improvements other than yield are needed

Reduced input use

Human health & nutrition

Farmer profitability

Ecosystem service provision

Climate change resilience

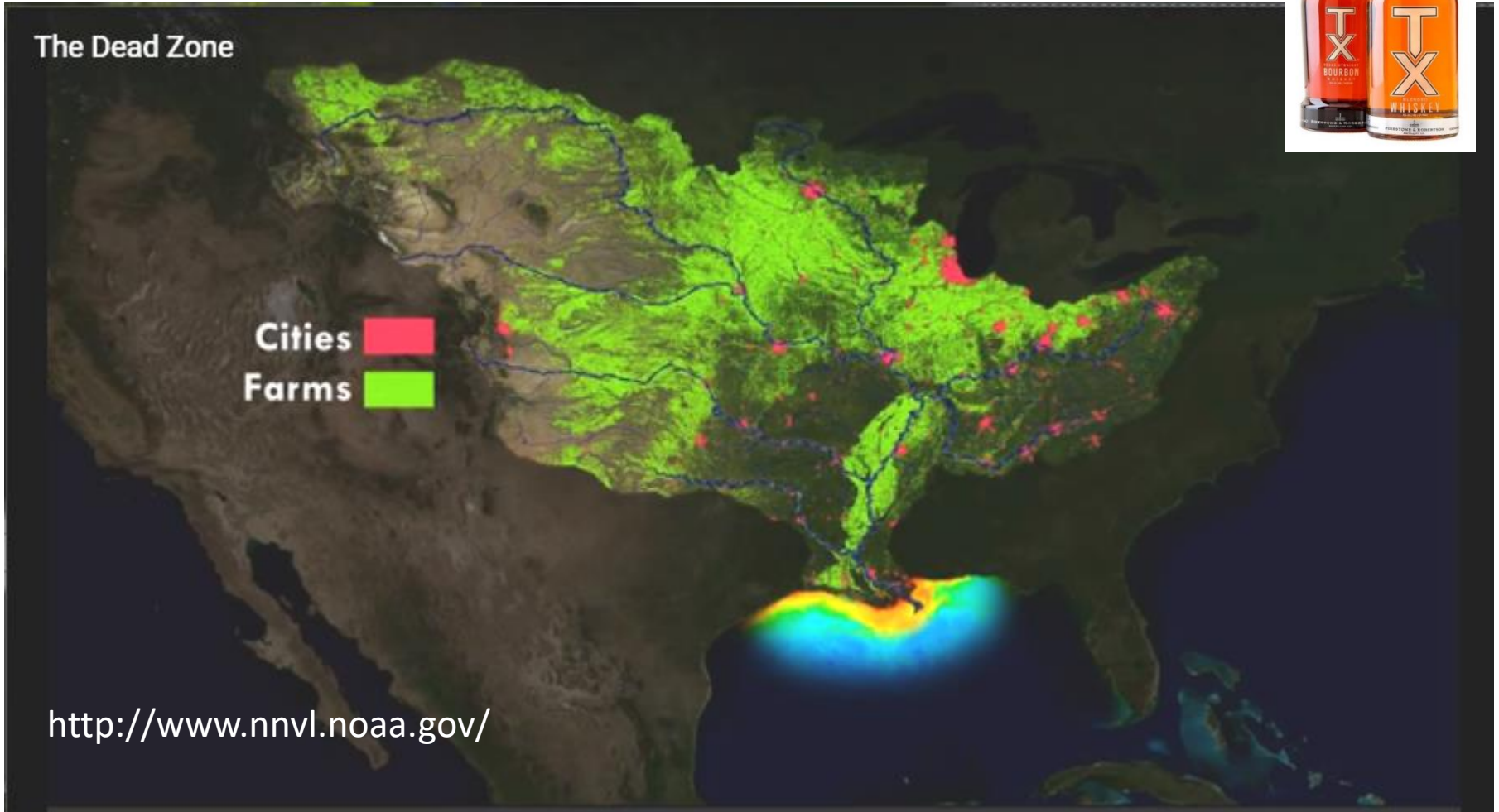
Flavor



The Dead Zone

Cities   
Farms 

<http://www.nnvl.noaa.gov/>



# Importance of language

EXPERT VIEW

**Functional phenomics: an emerging field integrating high-throughput phenotyping, physiology, and bioinformatics**

Larry M. York\*

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Correspondence: [lmeyork@noble.org](mailto:lmeyork@noble.org)

**Phenotype** – “...the totality of a plant’s observable characteristics”  
*Measured because believed to be of interest (the dependent trait)*

**Phene** – “...a more elemental component of phenotype”

**Phenome** – Totality of a plant’s measurable and estimable physical characteristics (measured because it can be used for prediction)

**Phenomic selection is NOT the same as phenotypic selection**

- \* Unmanned Aerial Systems (UAS)
- \* Unoccupied Aerial Systems (UAS)
- \* Uncrewed Aerial System (UAS)
- \* Unmanned Aerial Vehicles (UAV)
- \* Drones

**Multi-discipline language issues are not new**

- \* Multidimensional scaling (MDS)
- \* Principal Component Analyses (PCA)

# Three genomic methodological waves and generalizations

1986	2005	2007
QTL Linkage Mapping	Association Mapping (GWAS)	Genomic Selection / Genomewide Selection (GS)
Find loci Map based cloning → find genes	Find loci → find genes	Select the best individual
Bi-parental families	Diverse populations	Varied, mostly narrow breeding populations
Inference to specific populations – epistasis issues	Alleles work across populations structure	Predictive across training germplasm
Knowledge, publications, <b>?crop improvement?</b>	Knowledge, publications, <b>?crop improvement?</b>	Crop improvement, knowledge <b>?Publication?</b>

Discovery / Estimation

Prediction



Simple trait measurements  
(e.g. heights)

Value indices  
(yield, sustainability,  
etc.)



# High throughput field phenotyping

## 1) Automate routine measurements

- **Plant height**
- Estimate grain yield (sorghum, wheat)
- **Estimate disease**

## 2) Find new signatures of “eliteness”

- **Temporal growth patterns / biomass**
- **Senescence and grain filling period**

## 3) Phenomic selection

## 4) Deep learning and AI tools to directly use images

## 5) Identify stress signatures for farmers management

## 6) Identify new phenotypes and mechanisms of biological importance



Dr. Dale Cope



Dr. Sorin Popescu



Dr. John Valasek



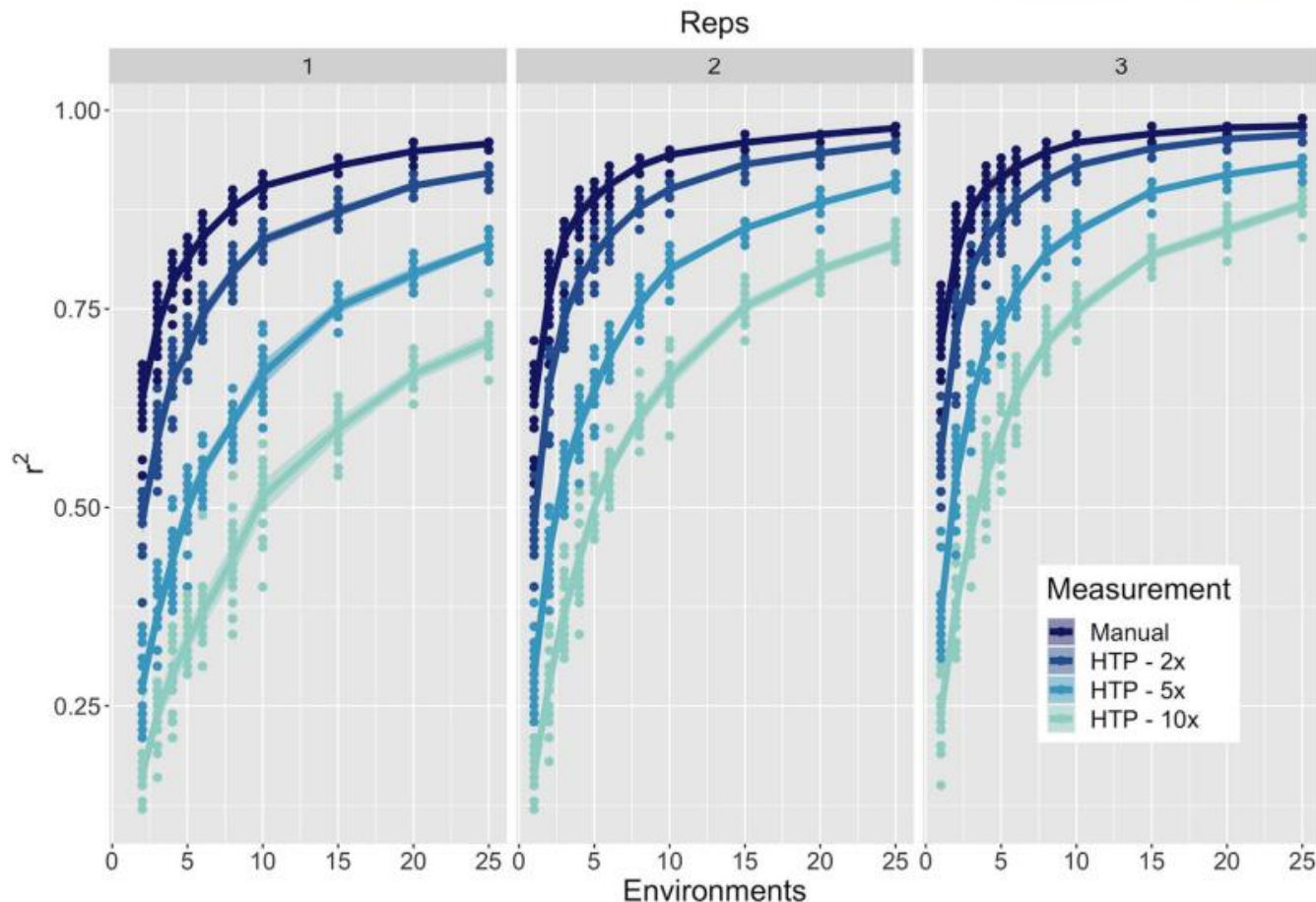
$i$  = Selection intensity = **Grow more plants**

Crop	Nursery	Yield trials
Corn (Murray)	10,000 plots	17,000 plots
Sorghum (Rooney)	15,000 plots total	20,000 plots +
Wheat (Ibrahim)	5,000 plots	5,000 plots x reps



Private sector is at a much larger scale for a few major crops but working with less diversity to develop “mega-varieties”

# High Throughput can produce better decisions than high accuracy when phenotyping plant populations



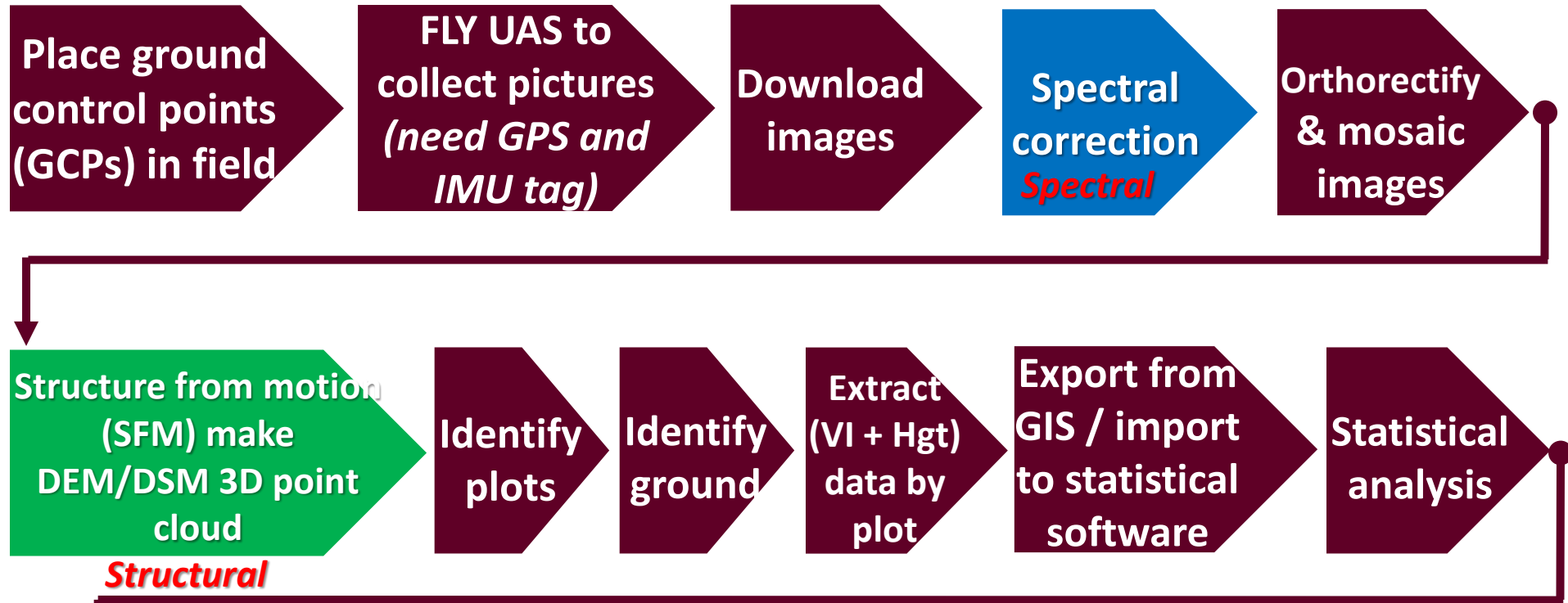
**FIGURE 1** Effect of increasing environments and replicates on best linear unbiased estimation (BLUE) accuracy in a simulated  $F_3$  population: Correlations ( $r^2$ ) between true simulated and statistically estimated genotypic entry values (BLUEs) under four measurement error rates and differing number of environments, divided along the x-axis by number of replicates. Four different error rates of measurement [manual, high throughput (HTP) with 2 $\times$  error, HTP with 5 $\times$  error, and HTP with 10 $\times$  error] were used on populations of 300 genotypes from an  $F_3$  population. Each simulated scenario was repeated 20 times. Shaded areas show a 95% confidence interval for the mean; the lines show the mean  $r^2$  for each scenario for the predicted and simulated true genetic heights for each line



Holly Lane, M.S.

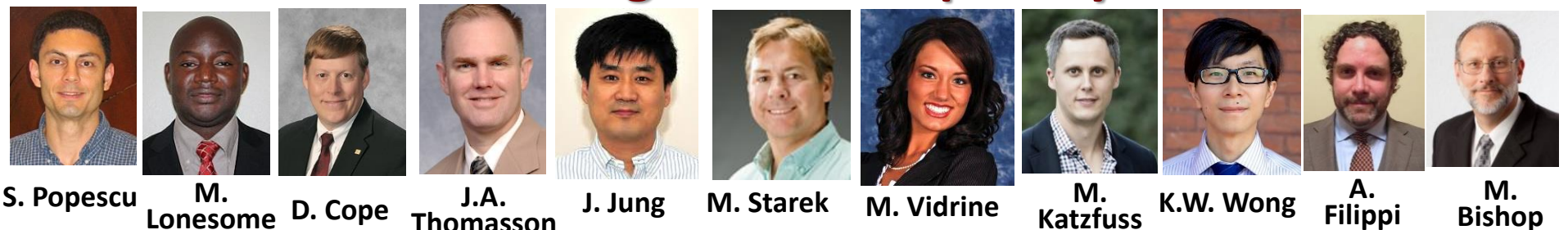
<https://access.onlinelibrary.wiley.com/doi/10.1002/csc2.20514>

# Steps to obtain and analyze UAS phenotyping data



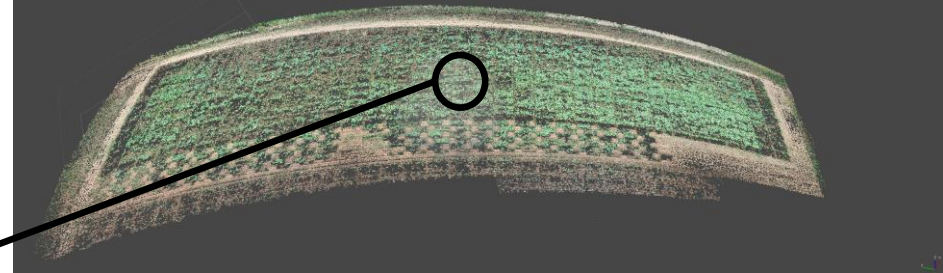
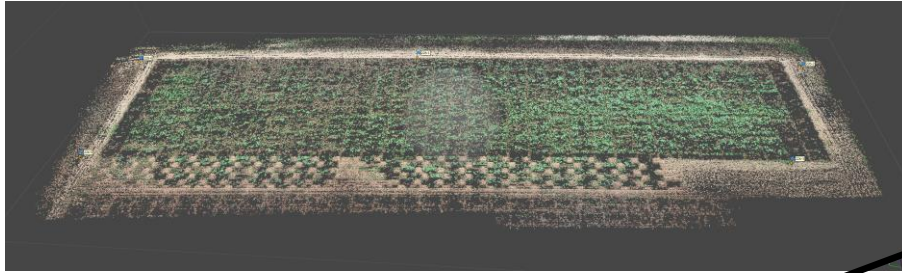
Estimate genotypic values → Determine measurements repeatability & value

**Took a huge transdisciplinary team**

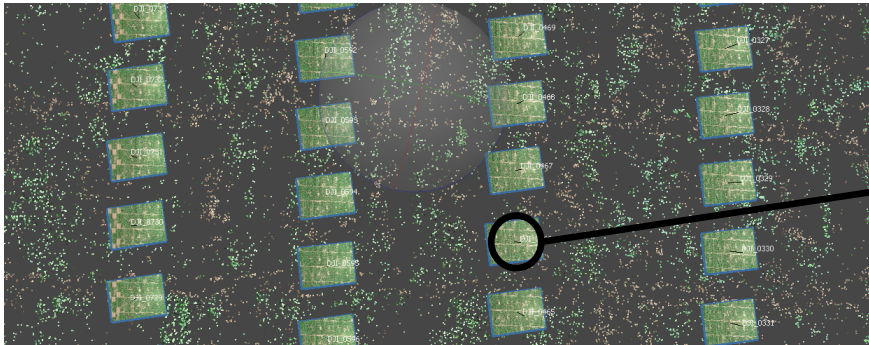


# Interdisciplinary challenges posed by agricultural photogrammetry

A)



C)



D)



“Hot spots” result in distortions and curvature in orthomosaics

Require corrections using ground control points or RTK UAVs, or obtaining oblique images

# Evolution of our UAS phenotyping data pipeline

Plant and place ground control points



FLY UAS to collect pictures (need GPS and IMU tag, plus RTK)



Download images



Orthomosaic



Shape File



Fact 4 Process



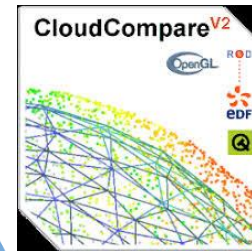
Mask Soil



Extract Vegetative Indices



Analyze Data

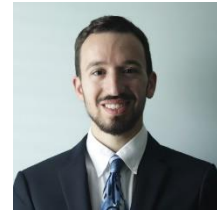


United States Department of Agriculture

National Institute of Food and Agriculture



Dr. Alper Adak  
Postdoc



Aaron DeSalvio  
PhD student



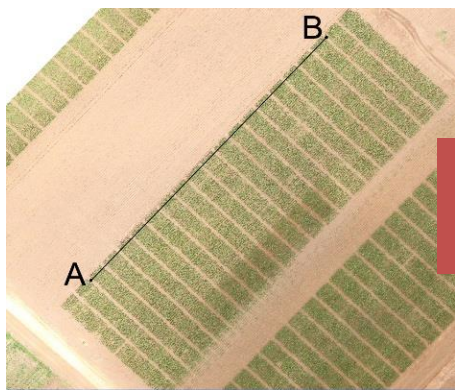
Mustafa Arik  
Research Assistant



# R/UASTools



<https://github.com/andersst91/UASTools>



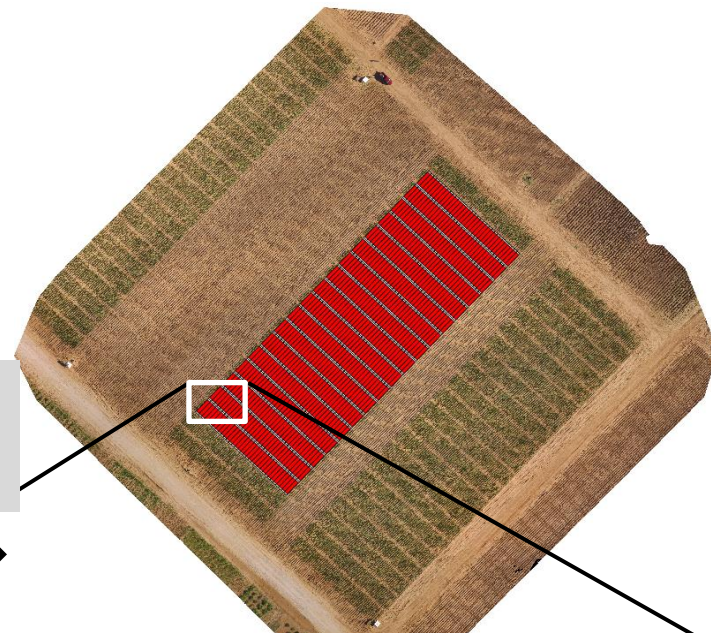
**AB-line UTM Coordinates**



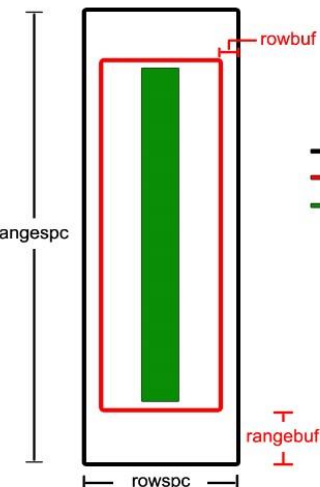
Stock	Pedigree	Rep	Range	Row	Loc	Test	Plot	Barcode
16.2.19716.04067.0000000	NILASQ4G311153/LH123HT	1	4	255	CS17	G2FE	001	CS17-G2FE-001
16.2.19716.04067.0000000	NILASQ4G311153/LH123HT	1	4	256	CS17	G2FE	001	CS17-G2FE-001
16.2.19716.04555.0000000.m1	2369/LH123HT	1	4	257	CS17	G2FE	002	CS17-G2FE-002
16.2.19716.04555.0000000.m1	2369/LH123HT	1	4	258	CS17	G2FE	002	CS17-G2FE-002
16.2.19716.04052.0000000	NILASQ4G310652/LH123HT	1	4	259	CS17	G2FE	003	CS17-G2FE-003
16.2.19716.04052.0000000	NILASQ4G310652/LH123HT	1	4	260	CS17	G2FE	003	CS17-G2FE-003
16.2.19716.04060.0000000	NILASQ4G310852/LH123HT	1	4	261	CS17	G2FE	004	CS17-G2FE-004
16.2.19716.04060.0000000	NILASQ4G310852/LH123HT	1	4	262	CS17	G2FE	004	CS17-G2FE-004
16.2.19716.04043.0000000.m1	NILASQ4G310352/LH123HT	1	4	263	CS17	G2FE	005	CS17-G2FE-005
16.2.19716.04043.0000000.m1	NILASQ4G310352/LH123HT	1	4	264	CS17	G2FE	005	CS17-G2FE-005
16.2.19716.04055.0000000.m1	NILASQ4G310753/LH123HT	1	4	265	CS17	G2FE	006	CS17-G2FE-006
16.2.19716.04055.0000000.m1	NILASQ4G310753/LH123HT	1	4	266	CS17	G2FE	006	CS17-G2FE-006
16.2.19716.04070.0000000	NILASQ4G311252/LH123HT	1	4	267	CS17	G2FE	007	CS17-G2FE-007
16.2.19716.04070.0000000	NILASQ4G311252/LH123HT	1	4	268	CS17	G2FE	007	CS17-G2FE-007
16.2.19716.04049.0000000	NILASQ4G310553/LH123HT	1	4	269	CS17	G2FE	008	CS17-G2FE-008
16.2.19716.04049.0000000	NILASQ4G310553/LH123HT	1	4	270	CS17	G2FE	008	CS17-G2FE-008
16.2.19716.04061.0000000	NILASQ4G310952/LH123HT	1	4	271	CS17	G2FE	009	CS17-G2FE-009
16.2.19716.04061.0000000	NILASQ4G310952/LH123HT	1	4	272	CS17	G2FE	009	CS17-G2FE-009
16.2.19716.04048.0000000	NILASQ4G310452/LH123HT	1	4	273	CS17	G2FE	010	CS17-G2FE-010
16.2.19716.04048.0000000	NILASQ4G310452/LH123HT	1	4	274	CS17	G2FE	010	CS17-G2FE-010
16.2.19716.04040.0000000	NILASQ4G310253/LH123HT	1	4	275	CS17	G2FE	011	CS17-G2FE-011
16.2.19716.04040.0000000	NILASQ4G310253/LH123HT	1	4	276	CS17	G2FE	011	CS17-G2FE-011
16.2.19716.04555.0000000.m1	2369/LH123HT	1	4	277	CS17	G2FE	012	CS17-G2FE-012
16.2.19716.04555.0000000.m1	2369/LH123HT	1	4	278	CS17	G2FE	012	CS17-G2FE-012

**Experimental Field Design**

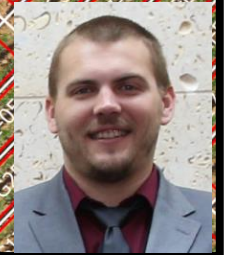
**R/UASTools::plotspcreate**



**Plot Dimensions**



- Plot ploygon
- Buffer polygon
- Plant material





# Temporal plant growth curves segregate in a population

Original Research

## Prediction of Maize Grain Yield before Maturity Using Improved Temporal Height Estimates of Unmanned Aerial Systems

Steven L. Anderson II, Seth C. Murray,\* Lonesome Malambo, Colby Ratcliff, Sorin Popescu, Dale Cope, Anjin Chang, Jinha Jung, and J. Alex Thomasson



S. Anderson  
PhD student



D. Cope



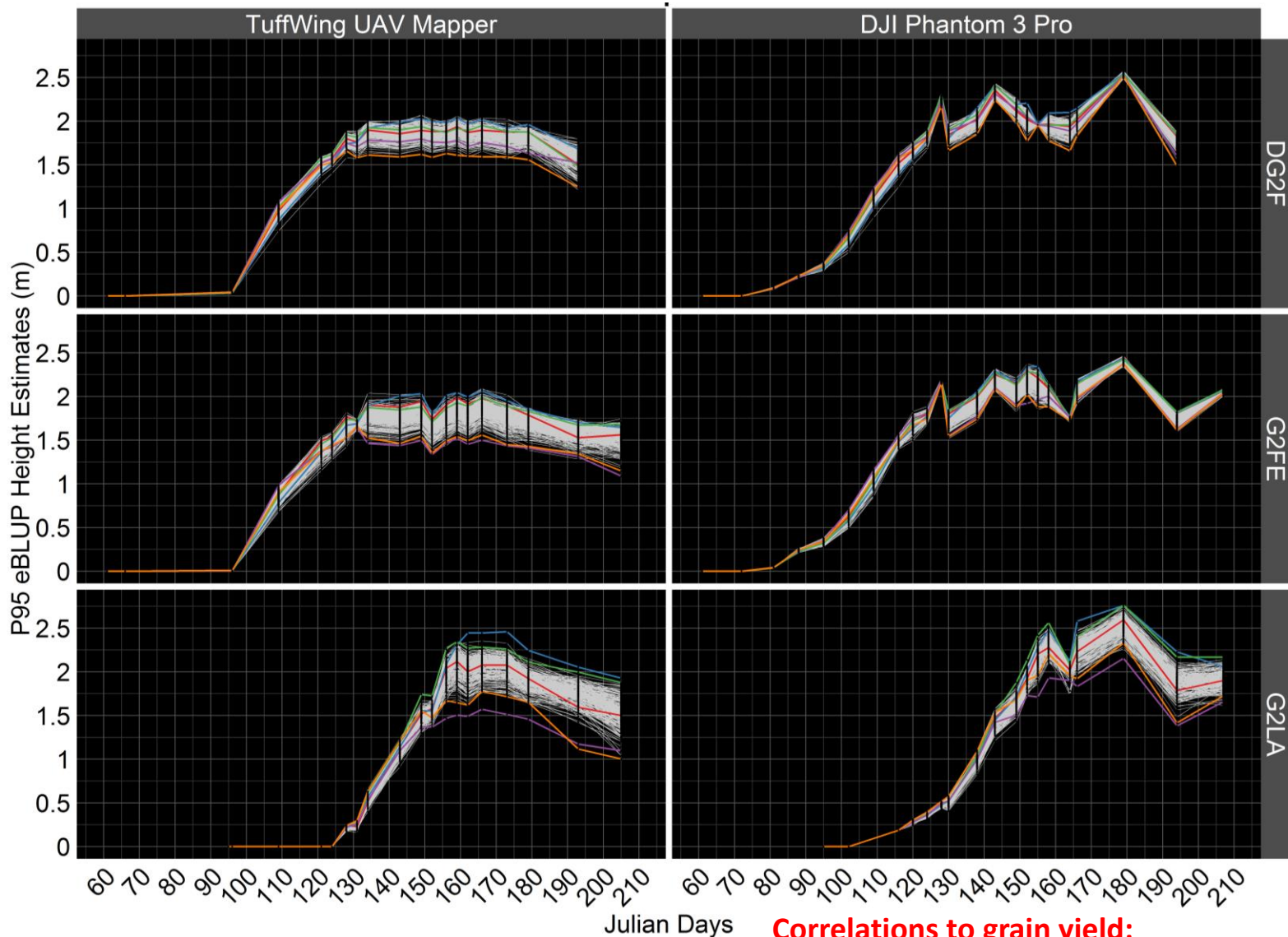
S. Popescu



M. Lonesome

Genotype  
 ■ B73/MO17  
 ■ CG44/CGR01  
 ■ CG60/HH162  
 ■ PHV63/NK787  
 ■ TX303/TX777  
 ■ Other Hybrids

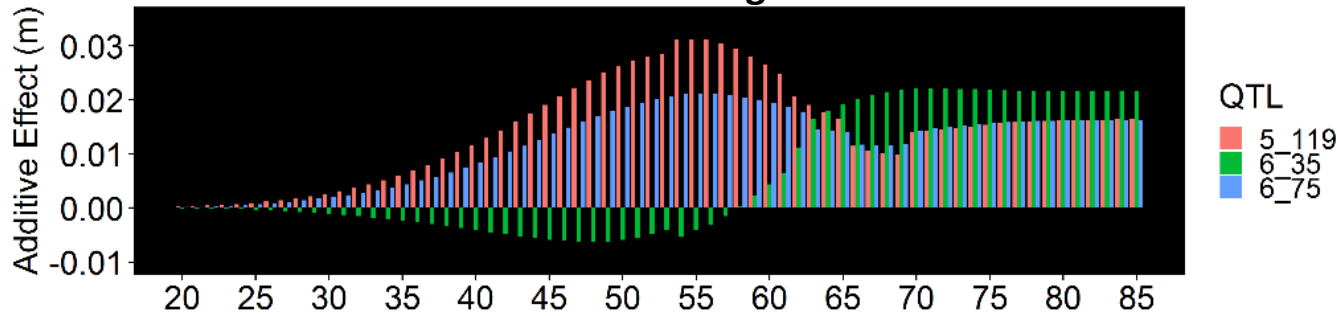
Estimates  
 ◆ P95 eBLUP Estimates



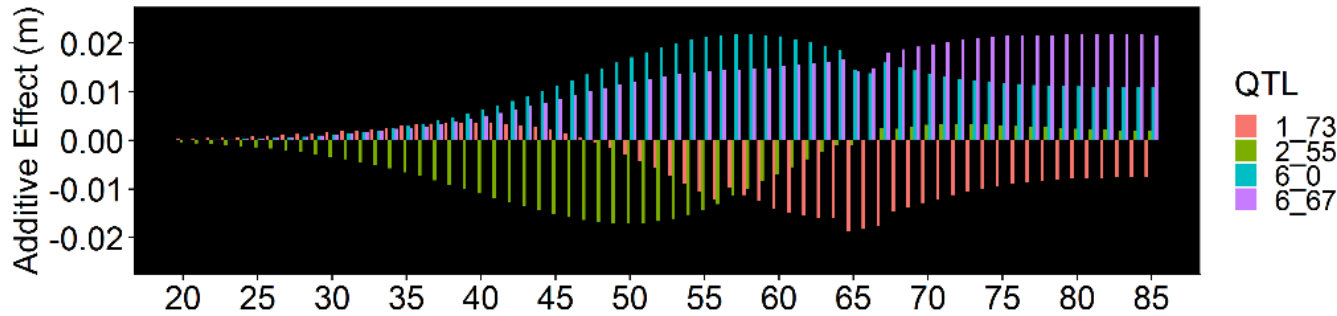
**Correlations to grain yield:**  
 UAS ( $r = 0.36-0.48$ ), manual ( $r = 0.23-0.28$ )

# Genetic effects from individual loci change over plant growth

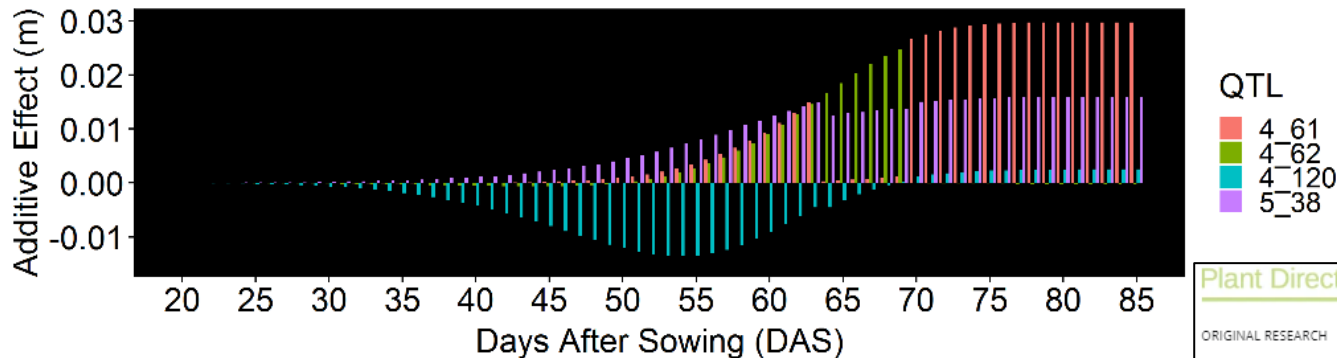
## Tx740 x NC356 irrigated trials



## Tx740 x NC356 non- irrigated trials



## Ki3 x NC356 non- irrigated trials



**Dr. Steven  
Anderson**

Plant Direct

Open Access

ORIGINAL RESEARCH | Open Access |

Unoccupied aerial system enabled functional modeling of maize height reveals dynamic expression of loci

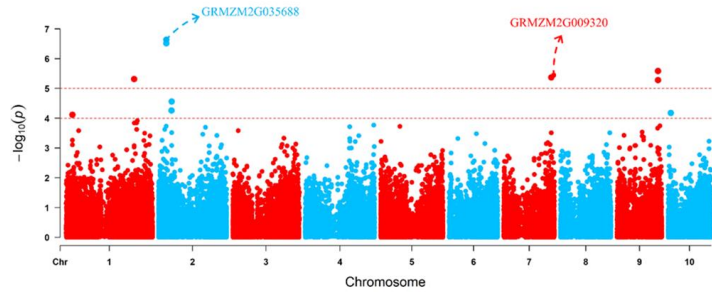
Steven L. Anderson II, Seth C. Murray , Yuanyuan Chen, Lonesome Malambo, Anjin Chang, Sorin Popescu, Dale Cope, Jinha Jung

First published: 10 May 2020 | <https://doi.org/10.1002/pld3.223>

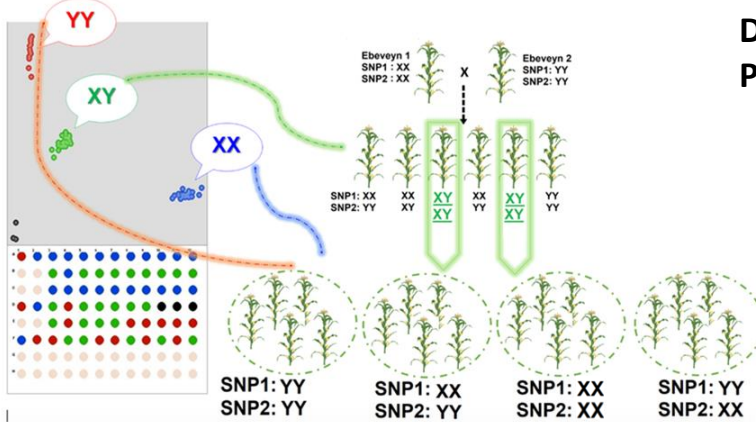


# UAS validated loci for plant height that terminal manual measures could not

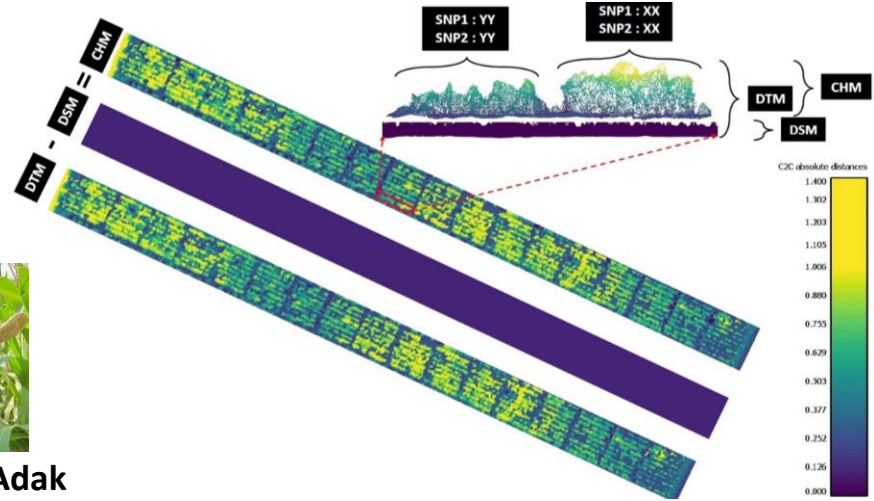
1) Early study on discovering candidate gene for PHT and GY



2) KASP marker development to advance the NILs

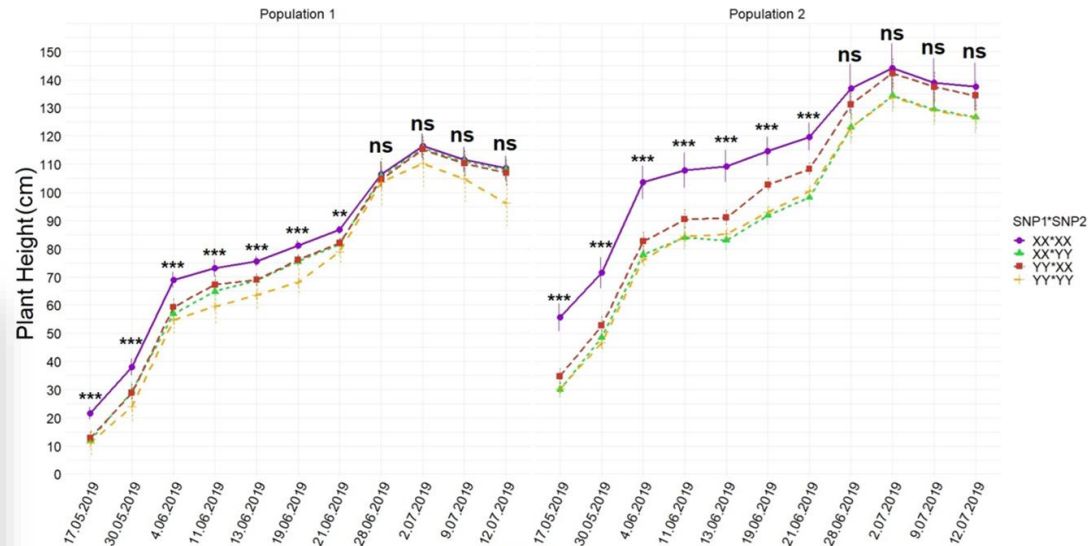


3) Conduct the FHTP to get temporal PHT



Dr. Alper Adak  
Postdoc

4) Compare temporal PHT between NILs temporally



OXFORD **G3** Genes | Genomes | Genetics

G3, 2021, 11(6), jkab075  
DOI: 10.1093/g3journal/jkab075  
Advance Access Publication Date: 5 April 2021  
Investigation

**Validation of functional polymorphisms affecting maize plant height by unoccupied aerial systems discovers novel temporal phenotypes**

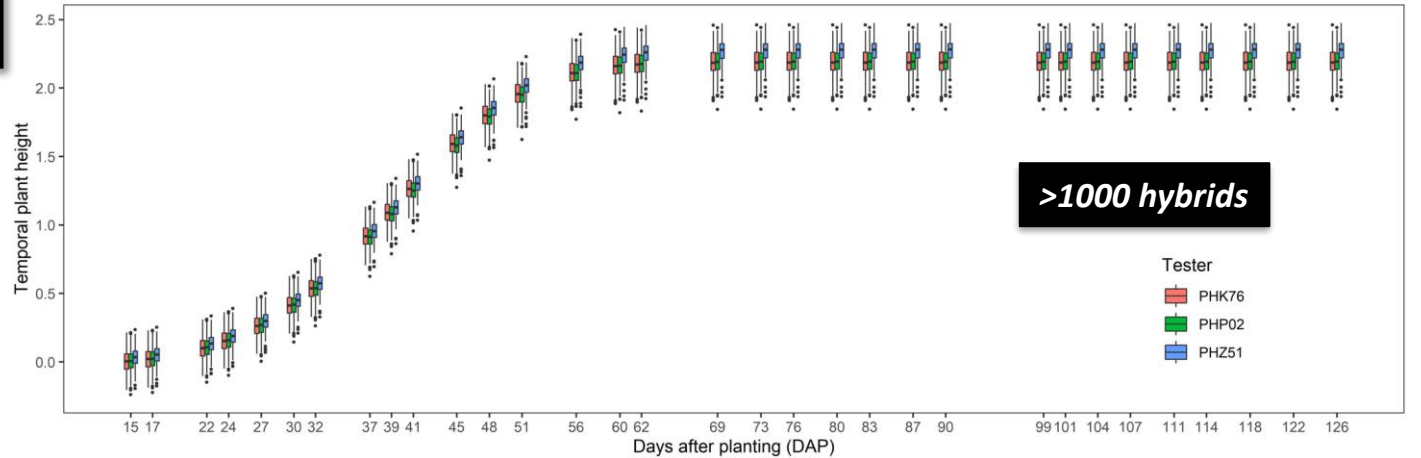
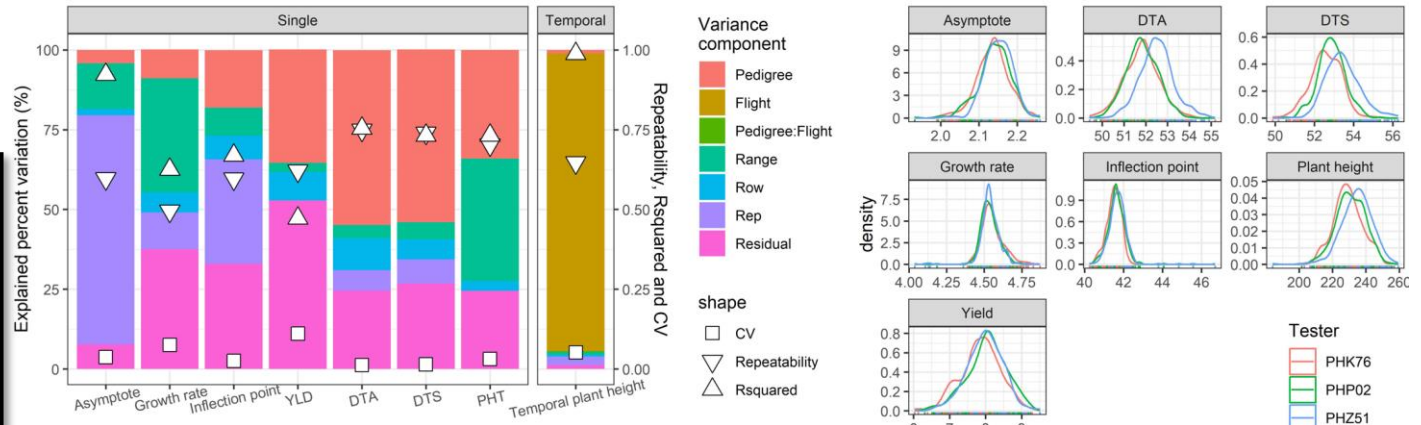
Alper Adak<sup>1</sup>, Clarissa Conrad<sup>1</sup>, Yuanyuan Chen<sup>1,2</sup>, Scott C. Wilde<sup>1</sup>, Seth C. Murray<sup>1,\*</sup>, Steven L. Anderson II<sup>1,3</sup> and Nithya K. Subramanian<sup>1</sup>

# A large phenomic dataset showing excessive temporal variations in plant height

## Weibull fit

$$a \cdot \left( 1 - \exp \left( - \left( \frac{DAP}{b} \right)^c \right) \right)$$

a = Asymptote  
b = Inflection Point  
c = Growth Rate



 University of Missouri



**Dr. Jacob Washburn**  
USDA-ARS / U of Missouri



**Dr. Alper Adak**  
Postdoc

The Plant Phenome Journal

OPEN ACCESS



ORIGINAL ARTICLE |  Open Access |  

**High temporal resolution unoccupied aerial systems phenotyping provides unique information between flight dates**

Jacob D. Washburn | Alper Adak, Aaron J. DeSalvio, Mustafa A. Arik, Seth C. Murray



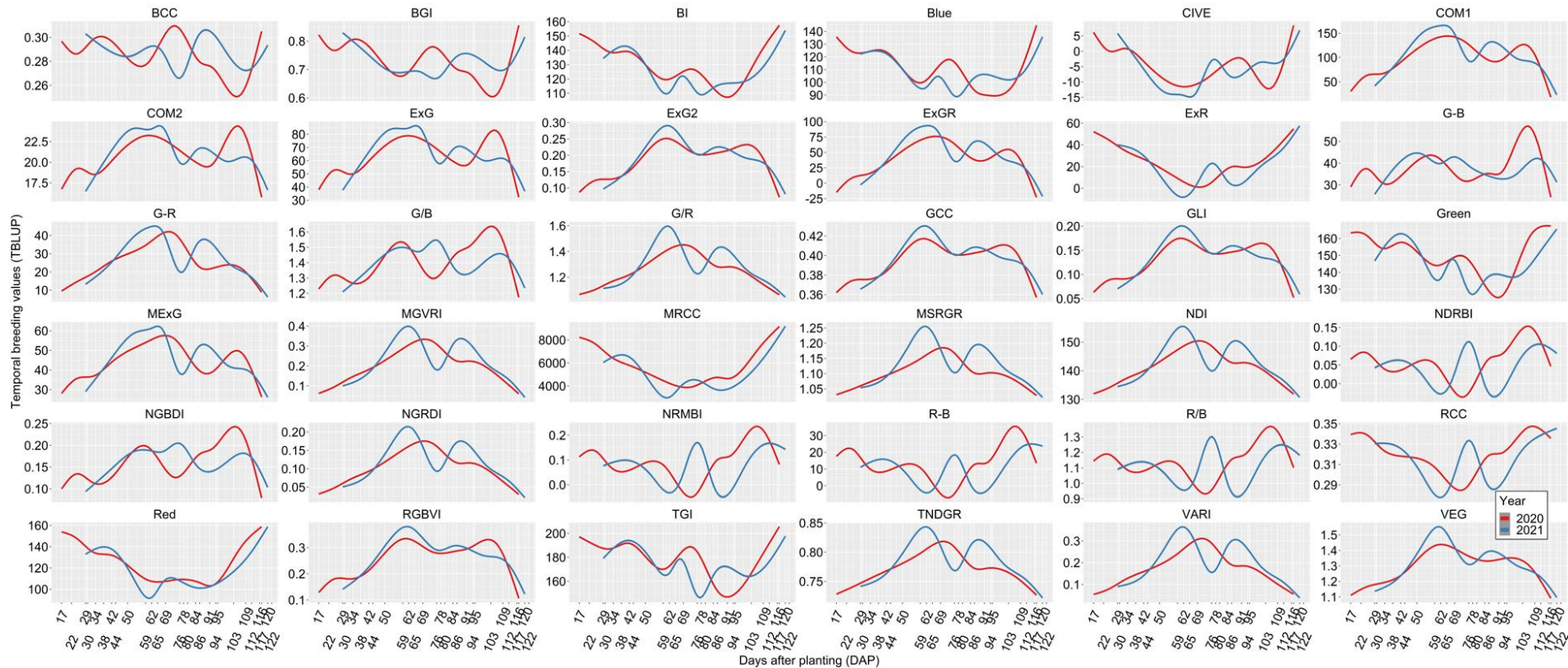
## Data processing

**Table.** Available indices in *FIELDimageR*. Any other index can be implemented using the option *myIndex* and the new formula (*FIELDimageR::fieldIndex*).

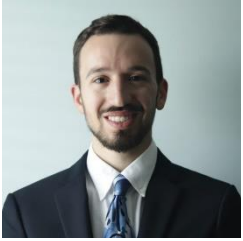
Description	Index	Formula	Related traits	References
Brightness Index	BI	$\sqrt{(R^2+G^2+B^2)/3)}$	Vegetation coverage, water content	Richardson and Wiegand (1977)
Soil Color Index	SCI	$(R-G)/(R+G)$	Soil color	Mathieu et al. (1998)
Green Leaf Index	GLI	$(2*G-R-B)/(2*G+R+B)$	Chlorophyll	Louhaichi et al. (2001)
Primary Colors Hue Index	HI	$(2*R-G-B)/(G-B)$	Soil color	Escadafal et al. (1994)
Normalized Green Red Difference Index	NGRDI	$(G-R)/(G+R)$	Chlorophyll, biomass, water content	Tucker (1979)
Spectral Slope Saturation Index	SI	$(R-B)/(R+B)$	Soil color	Escadafal et al. (1994)
Visible Atmospherically Resistant Index	VARI	$(G-R)/(G+R-B)$	Canopy, biomass, chlorophyll	Gitelson et al. (2002)
Overall Hue Index <sup>#</sup>	HUE	$\text{atan}(2*(B-G-R)/30.5*(G-R))$	Soil color	Escadafal et al. (1994)
Blue Green Pigment Index	BGI	B/G	Chlorophyll, LAI	Zarco-Tejada et al. (2005)
Plant Senescence Reflectance Index	PSRI	$(R-G)/(RE)$	Chlorophyll, nitrogen, maturity	Merzlyak et al. (1999)
Normalized Difference Vegetation Index	NDVI	$(NIR-R)/(NIR+R)$	Chlorophyll, LAI, biomass, yield	Rouse et al. (1974)
Green Normalized Difference Vegetation Index	GNDVI	$(NIR-G)/(NIR+G)$	Chlorophyll, LAI, nitrogen, protein content, water content	Gitelson et al. (1996)

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
Flight_date	Plot_ID	ID	Red	Green	Blue	BI	GLI	NGRDI	VARI	BGI	BCC	CIVE	COM1	COM2	ExG	ExG2	ExGR	ExR	GmB	GmR	GdB	GdR	GCC	MExG
20200529	3016763	1	0.30257	0.337805	0.267822	0.304442	0.085687	0.054633	0.092774	0.789688	0.293178	18.75004	20.04575	9.049477	0.105218	0.118398	0.019425	0.085793	0.069983	0.035235	1.277261	1.120272	0.372799	0.075545
20200529	3016764	2	0.314704	0.340968	0.273255	0.311232	0.076491	0.040289	0.06774	0.795389	0.29185	18.75491	19.9883	9.043298	0.093978	0.105284	-0.00564	0.099617	0.067713	0.026265	1.268985	1.08671	0.368428	0.067122
20200529	3016765	3	0.276143	0.317359	0.249631	0.28279	0.094419	0.067862	0.116042	0.783413	0.294134	18.74796	20.09213	9.053999	0.108945	0.130815	0.039704	0.06924	0.067728	0.041217	1.286186	1.151421	0.376938	0.078762
20200529	3016766	4	0.27901	0.312365	0.251604	0.282412	0.083406	0.056013	0.096537	0.799465	0.296116	18.75403	20.0319	9.046817	0.094116	0.115079	0.015867	0.078249	0.060761	0.033355	1.259988	1.123283	0.371693	0.069311
20200529	3016767	5	0.298039	0.334019	0.247147	0.295612	0.102138	0.055501	0.089443	0.737011	0.279069	18.74315	20.10582	9.057541	0.122853	0.141826	0.039618	0.083235	0.086872	0.03598	1.372513	1.121224	0.380609	0.081203
20200529	3016768	6	0.268459	0.305387	0.239563	0.272837	0.091359	0.061936	0.105612	0.783194	0.292905	18.7504	20.07178	9.051356	0.102751	0.126468	0.032295	0.070456	0.065823	0.036928	1.287334	1.137827	0.375489	0.073576
20200529	3016769	7	0.28294	0.315348	0.248568	0.283937	0.086013	0.052473	0.089065	0.784144	0.29141	18.75218	20.03959	9.048098	0.09919	0.118748	0.018423	0.080767	0.066781	0.032409	1.284671	1.115227	0.372916	0.070547
20200529	3016770	8	0.280534	0.306217	0.242964	0.278135	0.08168	0.04374	0.073061	0.783843	0.289479	18.75637	20.00442	9.044149	0.088935	0.112671	0.002404	0.086531	0.063252	0.025683	1.287967	1.095007	0.37089	0.062891
20200529	3016771	9	0.277682	0.31262	0.235076	0.277416	0.10056	0.057419	0.093479	0.745828	0.281695	18.74688	20.09556	9.055502	0.112481	0.139725	0.036345	0.076136	0.077543	0.034937	1.358502	1.126756	0.379908	0.075946
20200529	3016772	10	0.264718	0.301495	0.226427	0.266454	0.1036	0.062603	0.102369	0.744925	0.282415	18.74685	20.1107	9.056861	0.111844	0.144146	0.042733	0.069111	0.075067	0.036777	1.360076	1.139384	0.381382	0.076057
20200529	3016773	11	0.265673	0.298013	0.226555	0.265407	0.096076	0.055153	0.090697	0.756086	0.284212	18.75015	20.07369	9.052215	0.103798	0.133217	0.02987	0.073929	0.071458	0.03234	1.336736	1.121431	0.377739	0.070779
20200529	3016774	12	0.264956	0.297998	0.228458	0.265689	0.095265	0.056509	0.094077	0.760577	0.285819	18.75058	20.07169	9.051812	0.102582	0.131942	0.029641	0.07294	0.06954	0.033042	1.326839	1.124414	0.377314	0.070802
20200529	3016775	13	0.310076	0.329267	0.276202	0.306344	0.064067	0.032107	0.054703	0.823951	0.297434	18.7635	19.92316	9.035253	0.072255	0.087988	-0.03258	0.10484	0.053065	0.019191	1.226599	1.06934	0.362663	0.055528

# Temporal phenomic data reveals patterns for vegetative indices (VIs) across the growing season



- Different temporal patterns for different VIs across flight times revealed extreme VI values at plant emergence, flowering times, and conclusion of reproductive stage



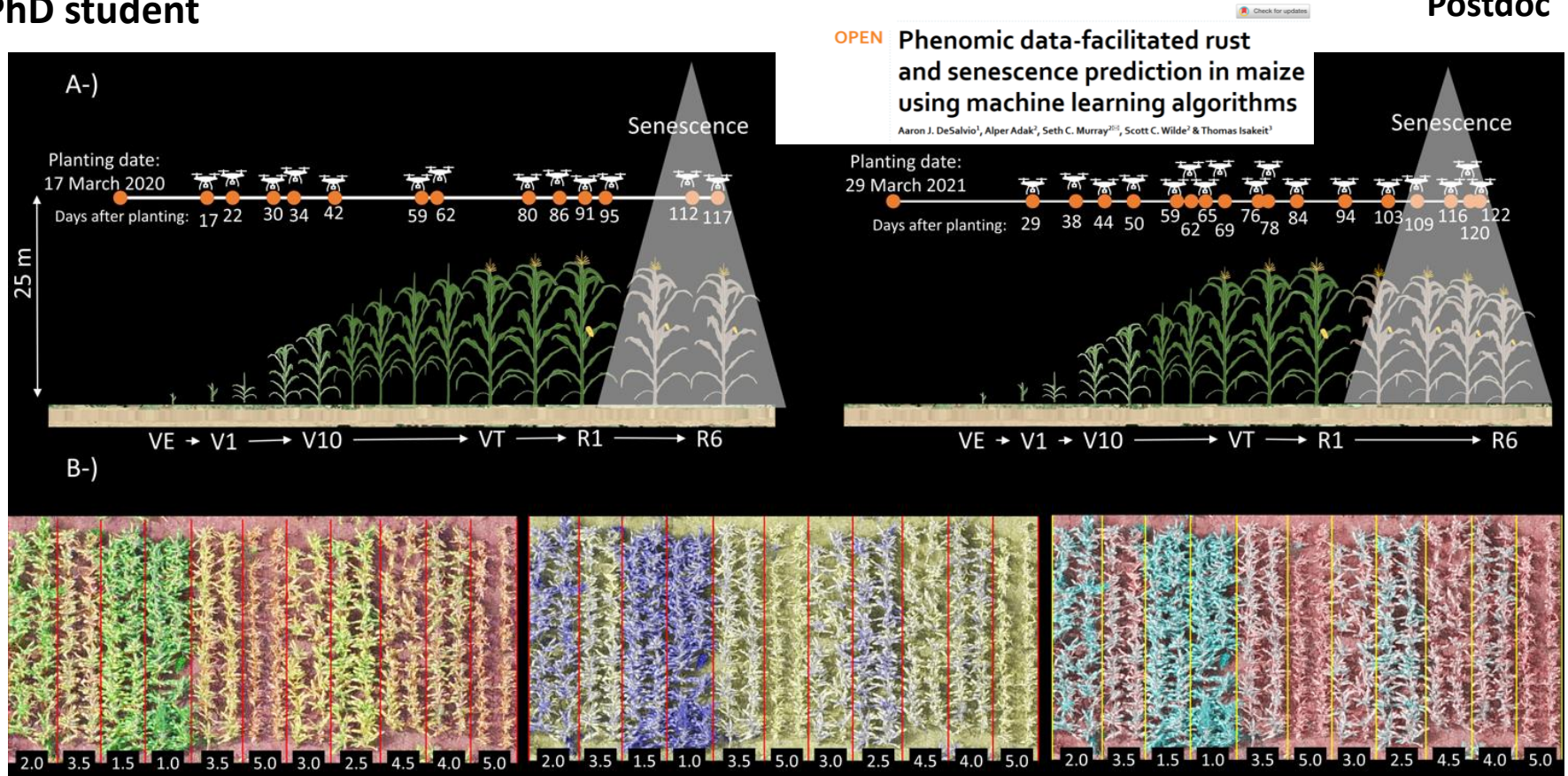
Aaron DeSalvio  
PhD student

# Novel approach to Southern Rust and senescence scoring

scientific reports



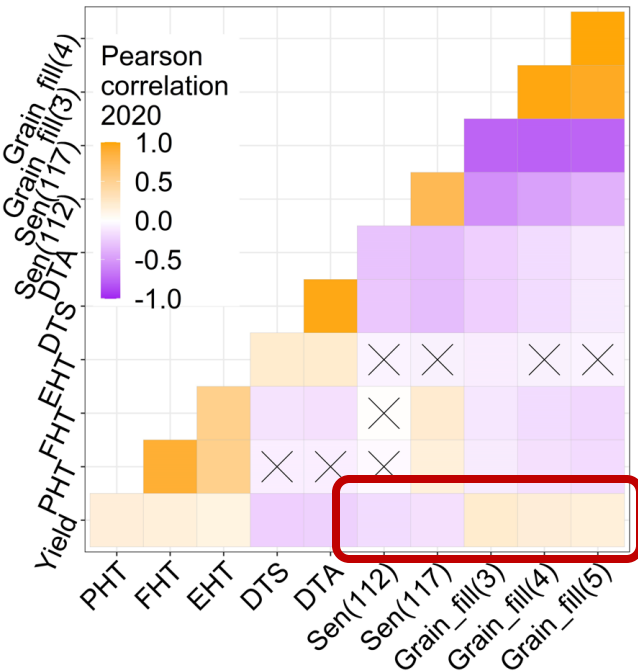
Alper Adak  
Postdoc



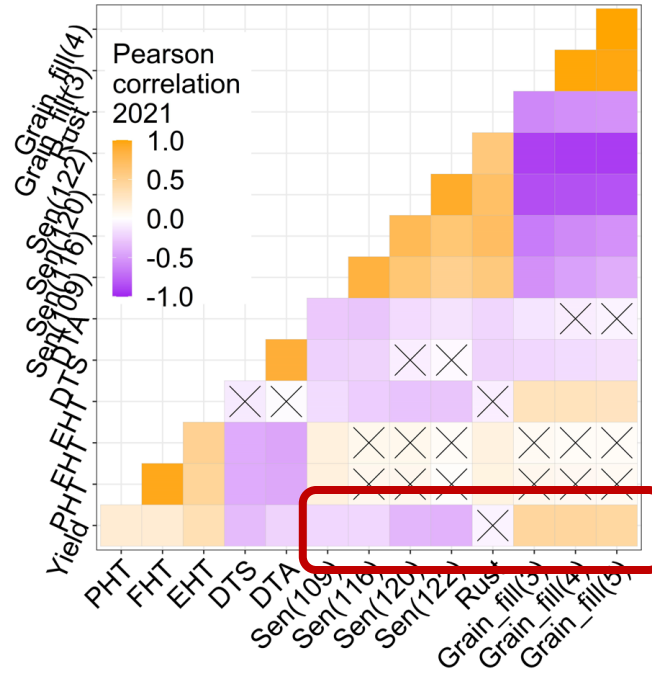
- Senescence annotated using drone images (orthomosaics) vs. hand-scoring in the field

# A catalyst for future studies: grain filling and yield

## F-) Correlation



2020

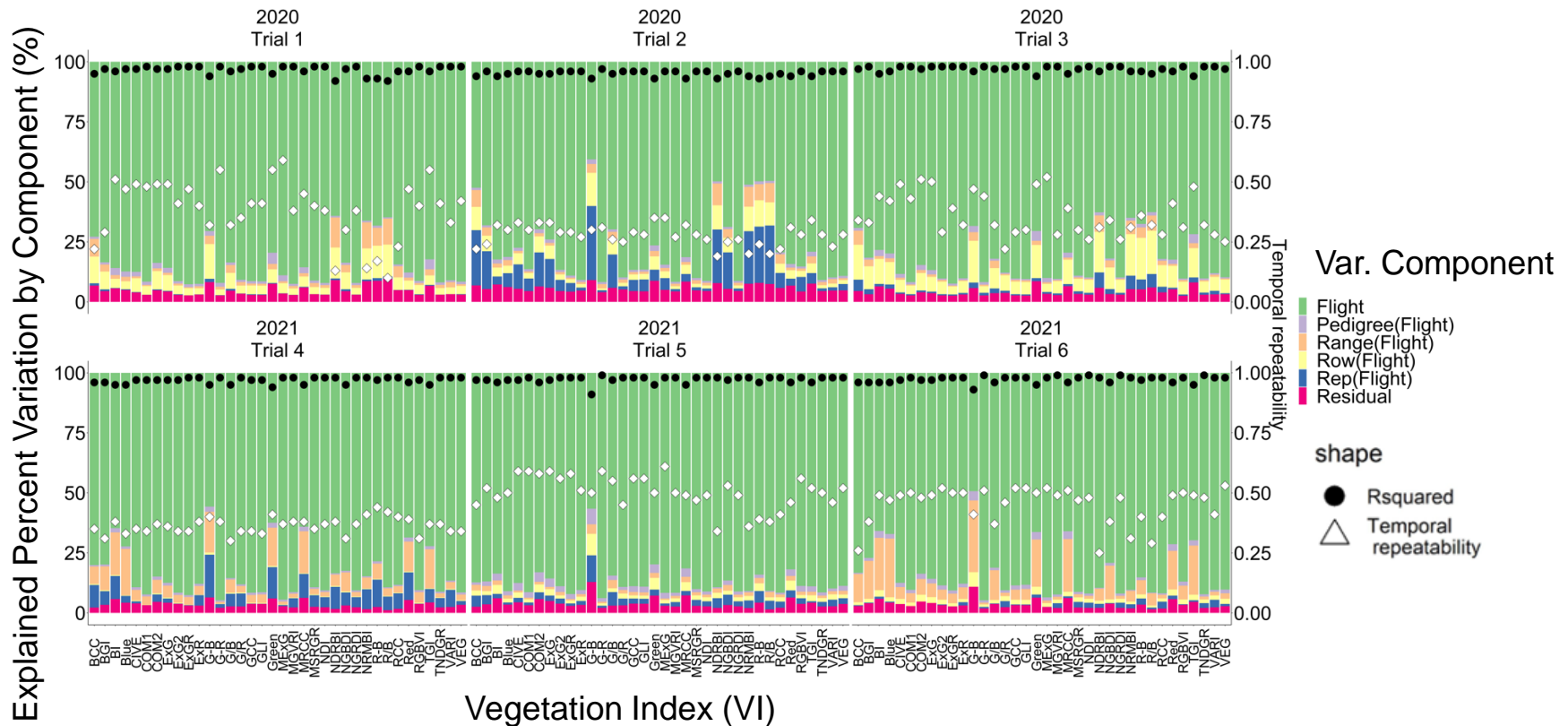


2021

Grain filling period had highest correlations with yield versus other traits (0.22 in 2020; 0.44 in 2021)

Grain filling period calculated as days between DTA and days to senescence as estimated by linear model

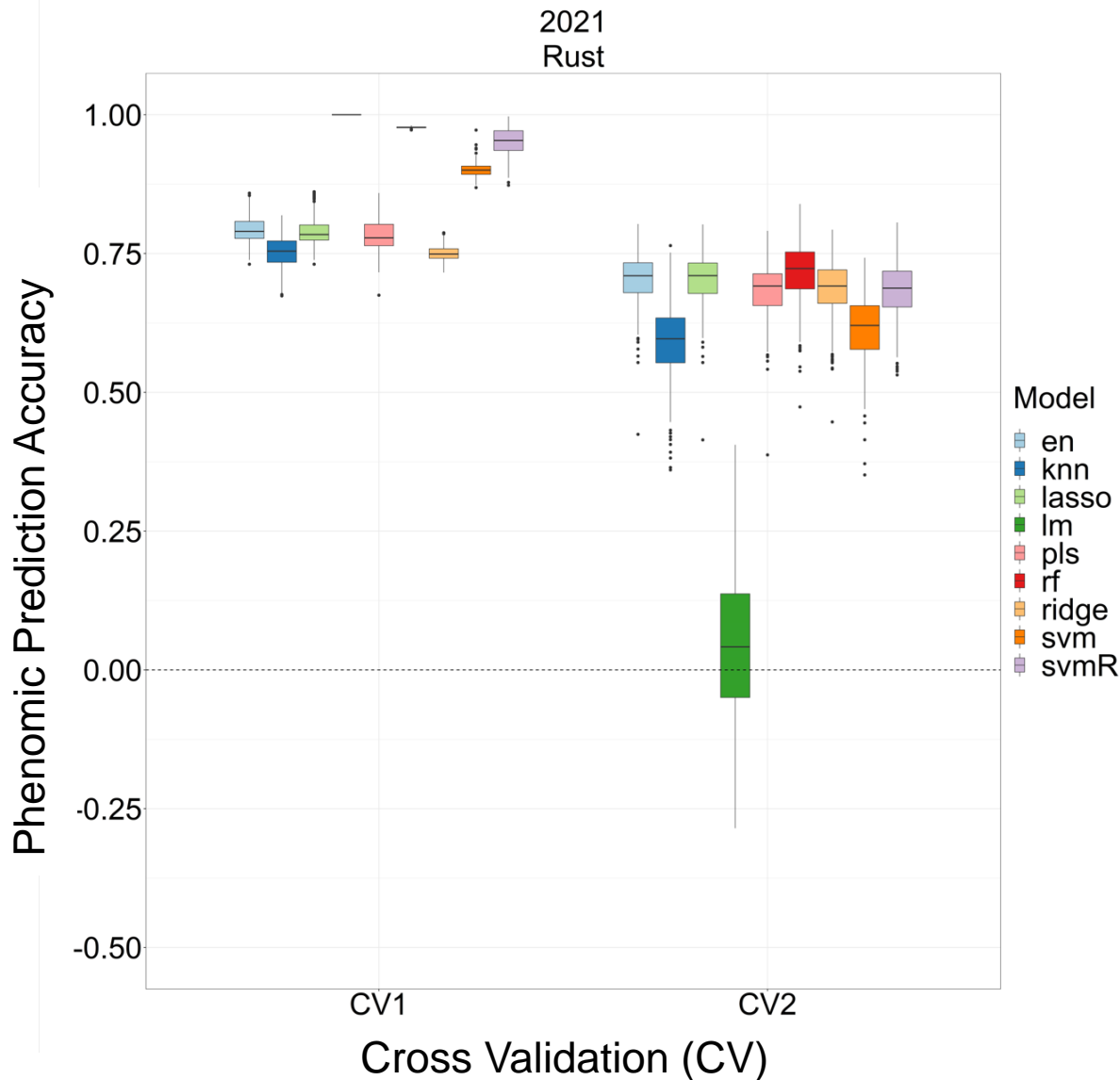
# Variance explained by nested design



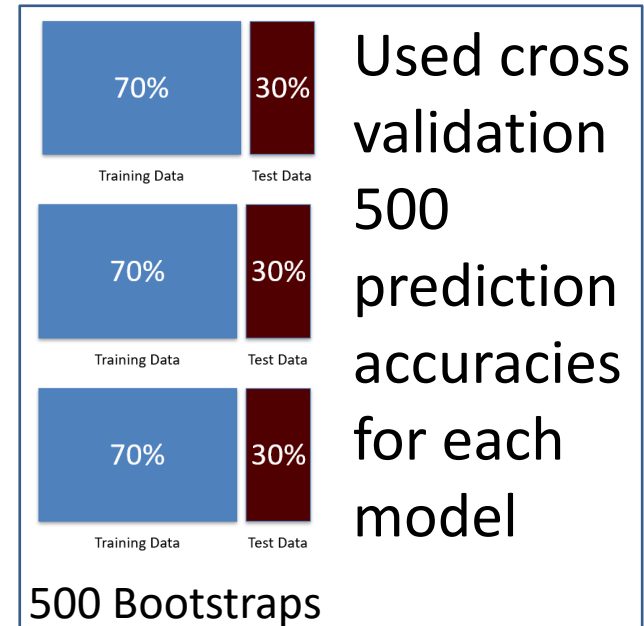
- Flight component in nested design explained highest percent of experimental variation for all VIs in both years

# Phenomic prediction model assessment

## Phenomic Prediction in Rust



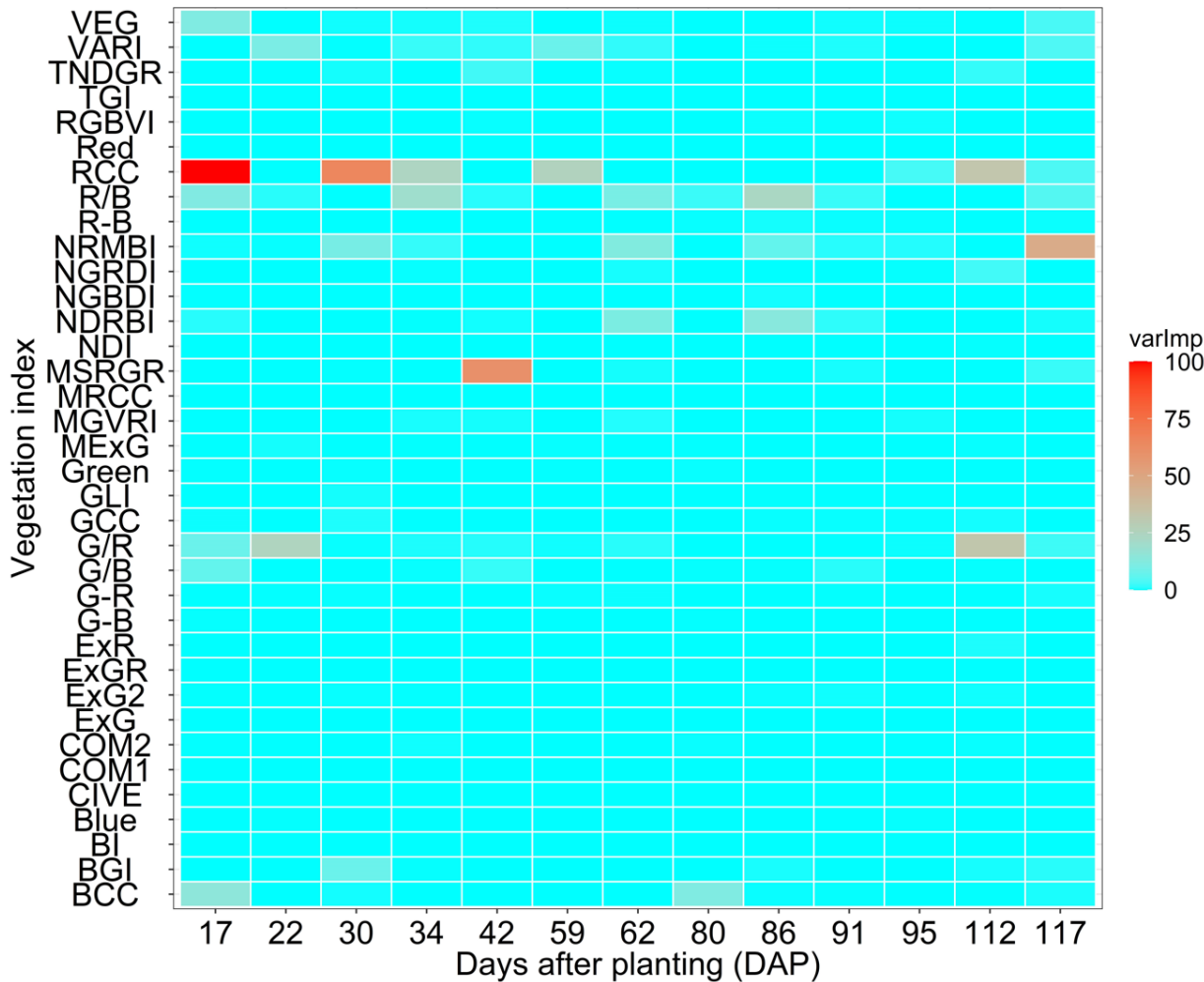
- Prediction accuracies: machine learning models outperformed linear model when predicting performance of untested genotypes





# Variable Importance Scores

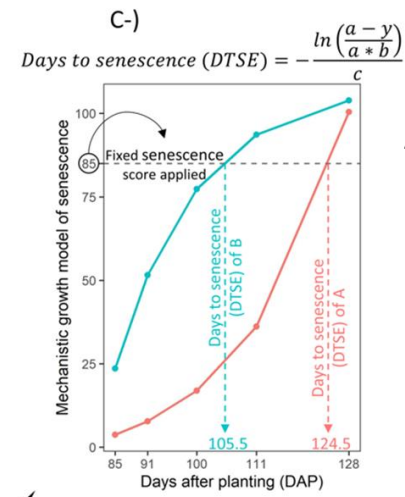
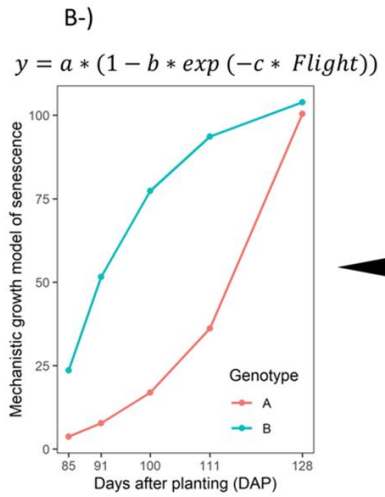
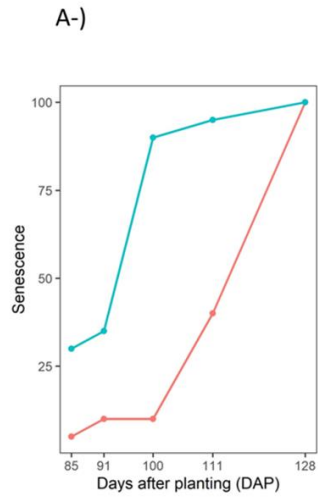
A-) Variable importance score of Sen(112) in 2020



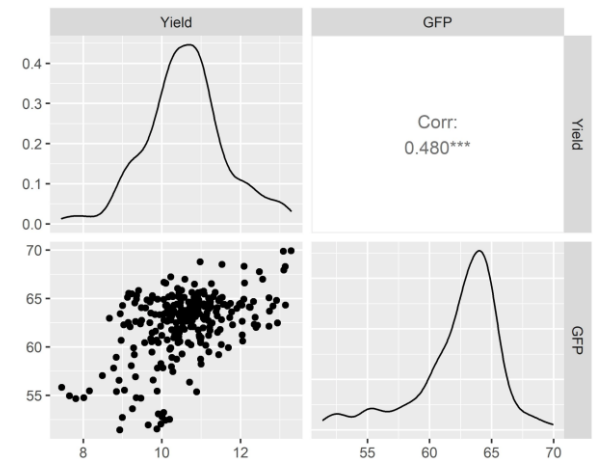
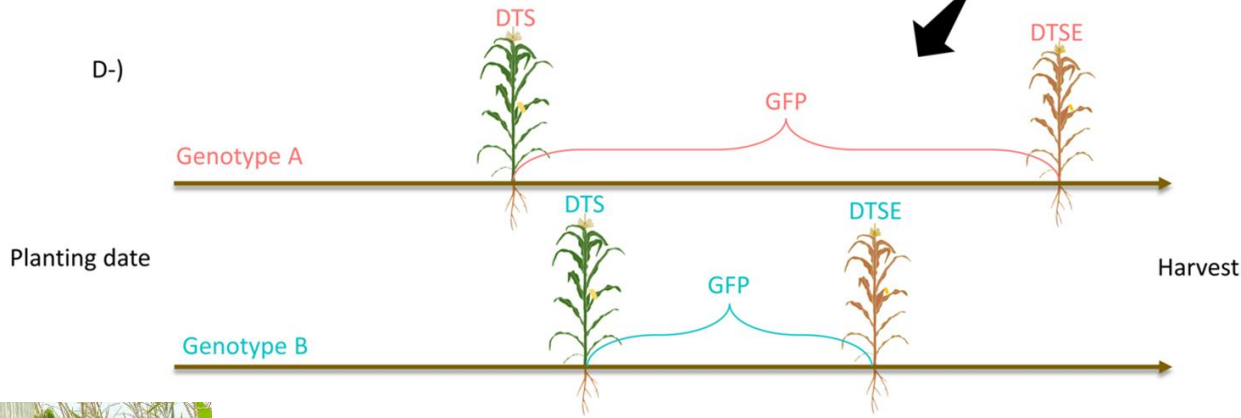
- Early, mid, and late-season phenotypic indicators revealed by variable importance scores
- Brighter red indicates VI was more important for model accuracy (had more predictive power)

May enable early predictions of senescence times for different genetic backgrounds; early warning for rust

# Calculation of the days to senescence and grain filling period using the *temporal senescence progression data*



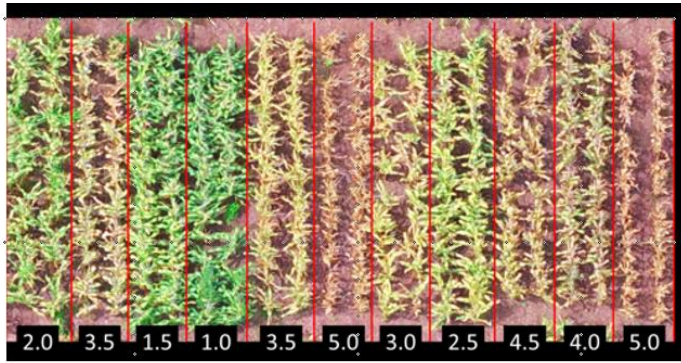
- A) Plot based senescence scores
- B) Mechanistic growth model
- C) Days to senescence (DTSE)
- D) Grain filling period



**Dr. Alper Adak**  
Postdoc

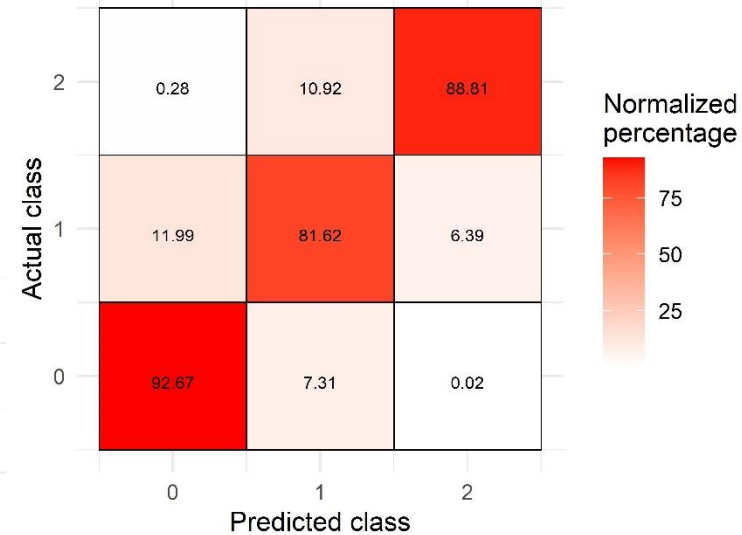
# Deep learning for senescence scoring 22,000 plots!

## TX1, TX2, TX3, WI1, WI2, WI3, MO

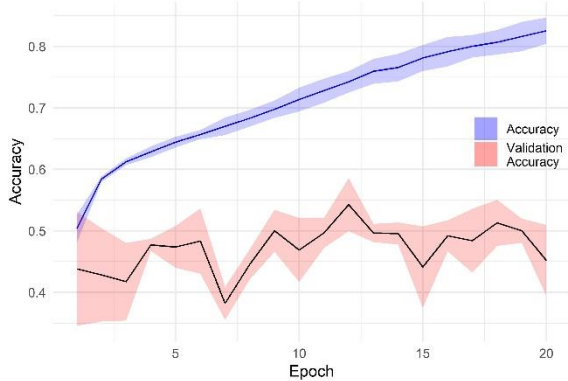


50 train - 50 test  
3 classes

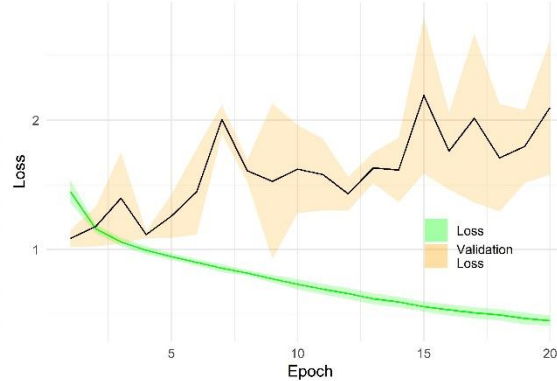
C) Confusion Matrix



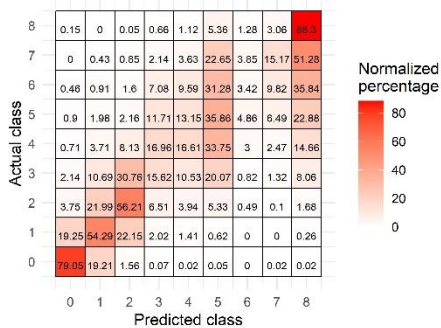
A) 80/20 Accuracy



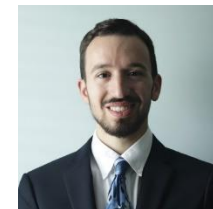
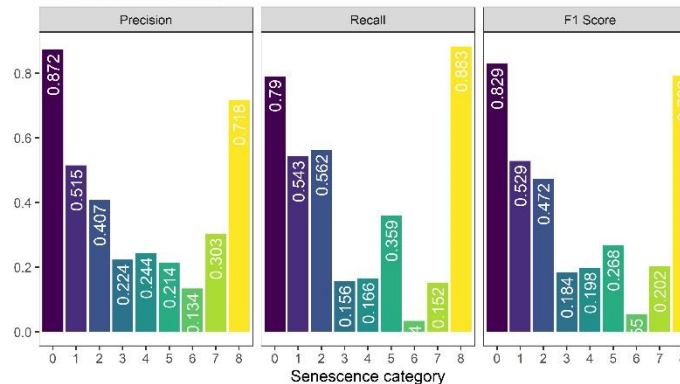
B) 80/20 Loss



C) Confusion Matrix



D) Evaluation Metrics



Aaron DeSalvio  
PhD student



Cody Kettler  
PhD student



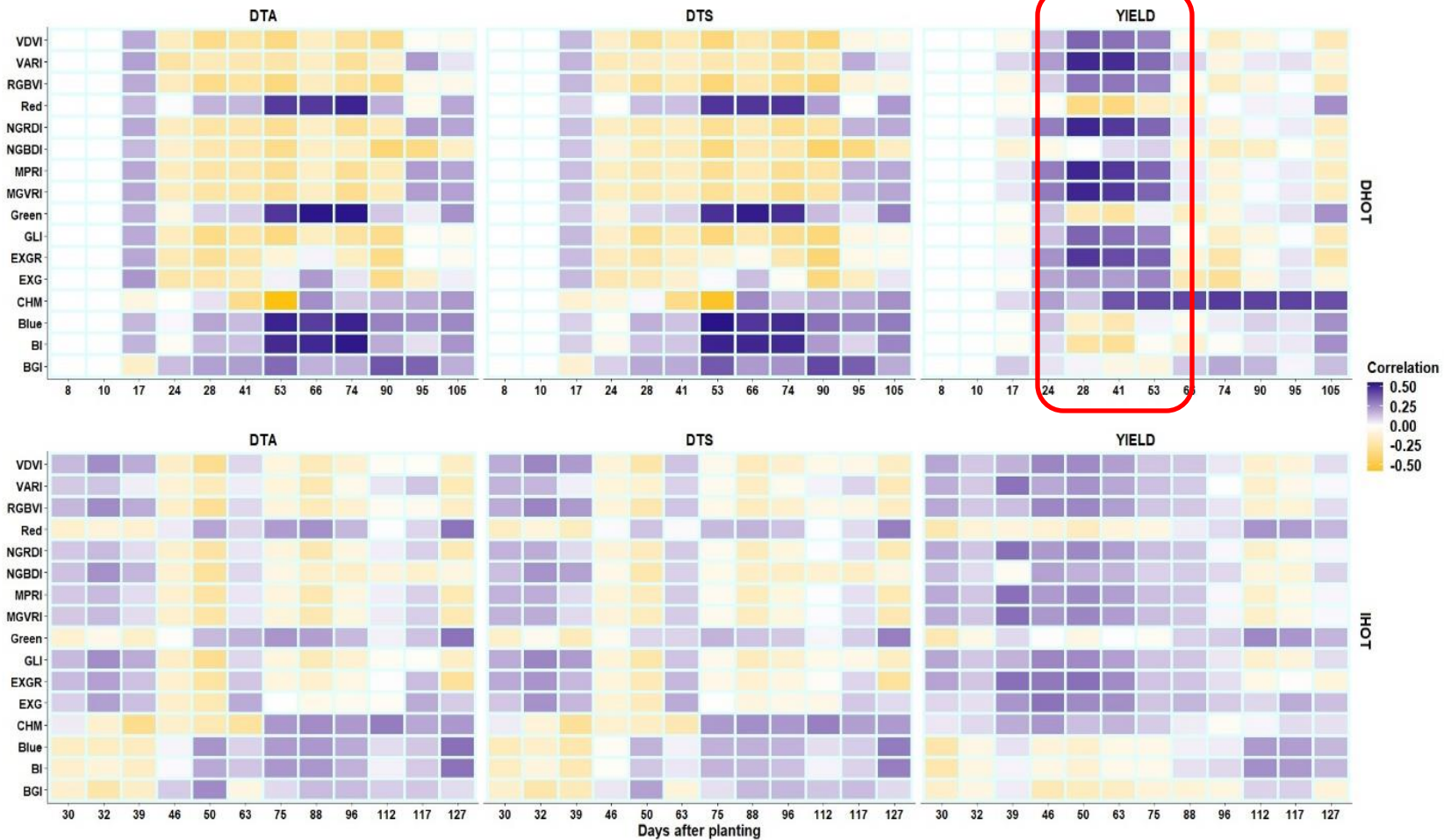
Luke Gray  
Undergraduate

Nick Shephard  
PhD student

# Correlation between VI's and Agronomics



Alper Adak



In late (stress) planting, the best predictors of yield were BEFORE flowering, possibly because this is where unselected variation is?

[alperadak@tamu.edu](mailto:alperadak@tamu.edu) for further questions



Article

**Temporal Vegetation Indices and Plant Height from Remotely Sensed Imagery Can Predict Grain Yield and Flowering Time Breeding Value in Maize via Machine Learning Regression**

Alper Adak <sup>1</sup>, Seth C Murray <sup>1,\*</sup>, Sofija Božinović <sup>2</sup>, Regan Lindsey <sup>1</sup>, Shakirah Nakasagga <sup>1</sup>, Sumantra Chatterjee <sup>1</sup>, Steven L. Anderson II <sup>3</sup> and Scott Wilde <sup>1</sup>

# Basics of Near Infrared Reflectance Spectroscopy (NIRS)



Scan whole kernel corn

Grind and scan ground corn



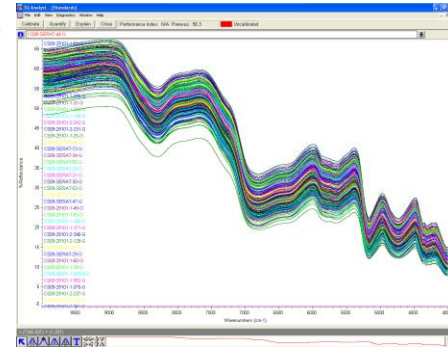
www.udyo.com



Send for chemical analysis



Partial least squares (PLS) to calibrate between reflectance and wet-chemistry

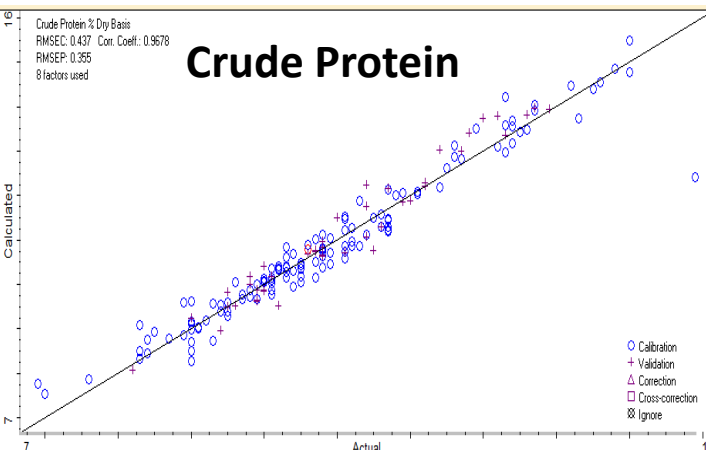


Smpl.	1	... n
Strch.		
Oil		
Prtn.		
Phos.		
Oleic.		

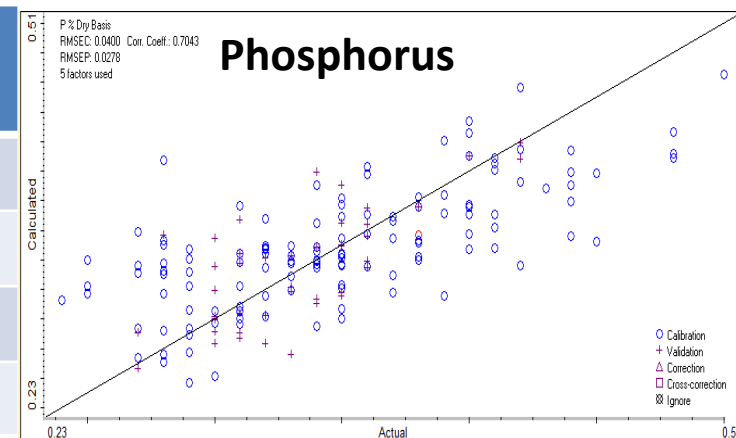
Find predictive equation using independent validation samples



Apply to breeding or genetics research



	Crude Protein	Phosphorus
$\sigma_G^2$	36 %	35 %
$\sigma_E^2$	17 %	21 %
$\sigma_{Row}^2$	4 %	5 %
$\sigma_{error}^2$	43 %	38 %



# Phenomic selection (Rincent et al. 2018 G3)



# GWAS study data (Barrera et al. 2014 PLOS One)

- Used NIRS to predict complex traits in wheat and poplar
- **PS was as accurate as genomic selection but cheaper**
- 346 Hybrids ( x Tx714)
- 4000 samples total
  - 1700 Ground and 2300 Whole
- Dryland and irrigate



GENOMIC PREDICTION



## Phenomic Selection Is a Low-Cost and High-Throughput Method Based on Indirect Predictions: Proof of Concept on Wheat and Poplar

Renaud Rincent,\* Jean-Paul Charpentier,<sup>1,2</sup> Patricia Falvo-Rampant,<sup>3</sup> Etienne Paux,\*

Jacques Le Gouis,\* Catherine Bastien,<sup>1</sup> and Vincent Segura<sup>1,2</sup>

<sup>1</sup>GDRC, INRA, UCA, 63000 Clermont-Ferrand, France, <sup>2</sup>BioForA, INRA, ONF, 45075 Orléans, France, <sup>3</sup>GenoTis analytical platform, INRA, 45075 Orléans, France, and <sup>4</sup>EPGV, INRA, CEA-IG/CNG, 91057 Evry, France

ORCID ID: 0000-0003-0885-0969 (R.R.); 0000-0002-6029-0498 (J.-P.C.); 0000-0002-3094-7129 (E.P.); 0000-0001-5726-4902 (J.L.G.); 0000-0002-9391-6637 (C.B.); 0000-0003-1860-2256 (V.S.)

**ABSTRACT** Genomic selection - the prediction of breeding values using DNA polymorphisms - is a disruptive method that has widely been adopted by animal and plant breeders to increase productivity. It was recently shown that other sources of molecular variations such as those resulting from transcripts or metabolites could be used to accurately predict complex traits. These endophenotypes have the advantage of capturing the expressed genotypes and consequently the complex regulatory networks that occur in the different layers between the genome and the phenotype. However, obtaining such omics data at very large scales, such as those typically experienced in breeding, remains challenging. As an alternative, we proposed using near-infrared spectroscopy (NIRS) as a high-throughput, low cost and non-destructive tool to indirectly capture endophenotypic variants and compute relationship matrices for predicting complex traits, and coined this new approach "phenomic selection" (PS). We tested PS on two species of economic interest (*Triticum aestivum* L. and *Populus nigra* L.) using NIRS on various tissues (grains, leaves, wood). We showed that one could reach conditions as accurate as with molecular markers for development

### KEYWORDS

Poplar  
Wheat  
breeding  
endophenotypes  
Near InfraRed  
Spectroscopy  
(NIRS)  
Genomic  
Prediction  
GenPred  
Ground Truth



### OPEN ACCESS

**Citation:** Farfan IDB, De La Fuente GN, Murray SC, Isakiet T, Huang P-C, Warburton M, et al. (2015) Genome Wide Association Study for Drought, Aflatoxin Resistance, and Important Agronomic Traits of Maize Hybrids in the Sub-Tropics. PLoS ONE 10 (2): e0117737. doi:10.1371/journal.pone.0117737

**Academic Editor:** Lewis Lukens, University of Guelph, CANADA

### RESEARCH ARTICLE

## Genome Wide Association Study for Drought, Aflatoxin Resistance, and Important Agronomic Traits of Maize Hybrids in the Sub-Tropics

Ivan D. Barrero Farfan<sup>1</sup>, Gerald N. De La Fuente<sup>1,2</sup>, Seth C. Murray<sup>\*1</sup>, Thomas Isakiet<sup>2</sup>, Pei-Cheng Huang<sup>2</sup>, Marilyn Warburton<sup>3</sup>, Paul Williams<sup>3</sup>, Gary L. Windham<sup>3</sup>, Mike Kolomiets<sup>2</sup>

<sup>1</sup> Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas, United States of America, <sup>2</sup> Department of Plant Pathology, Texas A&M University, College Station, Texas, United States of America, <sup>3</sup> USDA ARS Corn Host Plant Resistance Research Unit, Mississippi State, Mississippi, United States of America

\* Current address: Department of Agronomy, Iowa State University, Ames, Iowa, United States of America  
\* sethmurray@tamu.edu

### Abstract

The primary maize (*Zea mays* L.) production areas are in temperate regions throughout the world and this is where most maize breeding is focused. Important but lower yielding maize growing regions such as the sub-tropics experience unique challenges, the greatest of which are drought stress and aflatoxin contamination. Here we used a diversity panel consisting of 346 maize inbred lines originating in temperate, sub-tropical and tropical areas testcrossed to stiff-stalk line Tx714 to investigate these traits. Testcross hybrids were evaluated under irrigated and non-irrigated trials for yield, plant height, ear height, days to anthesis, days to silking and other agronomic traits. Irrigated trials were also inoculated with

# NIRS results: whole kernel spectral yield train & predict with 2011 & 2012

## Partial Least Squares Results:

Yield (NIRS vs. actual)

$R^2 = 0.19$  to  $0.84$

RMSEP < 26 bu/ac

Protein (vs. actual yield)

$R^2 = 0.58$

Protein + starch + oil

$R^2 = 0.64$

## Functional Regression Results:

Yield (NIRS vs. actual)

$R^2 = 0.13$  to  $0.75$



Holly Lane, M.S.

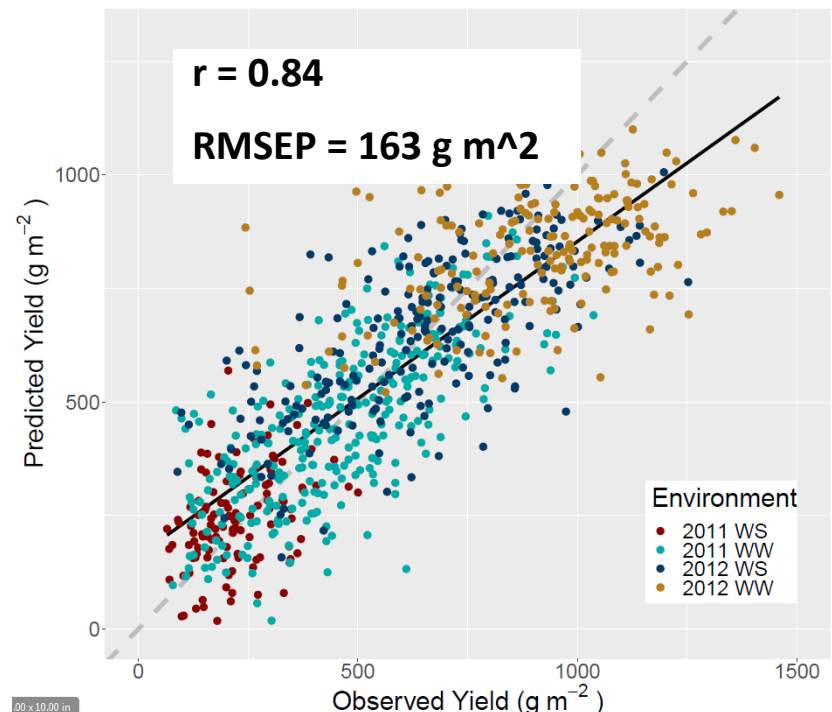


Dr. José Crossa

## Global PLSR

Model trained on 1573 samples

Tested on remaining 848 samples



The Plant Phenome Journal

OPEN ACCESS



ORIGINAL RESEARCH | [Open Access](#) | [CC](#) | [BY](#) | [NC](#) | [SA](#)

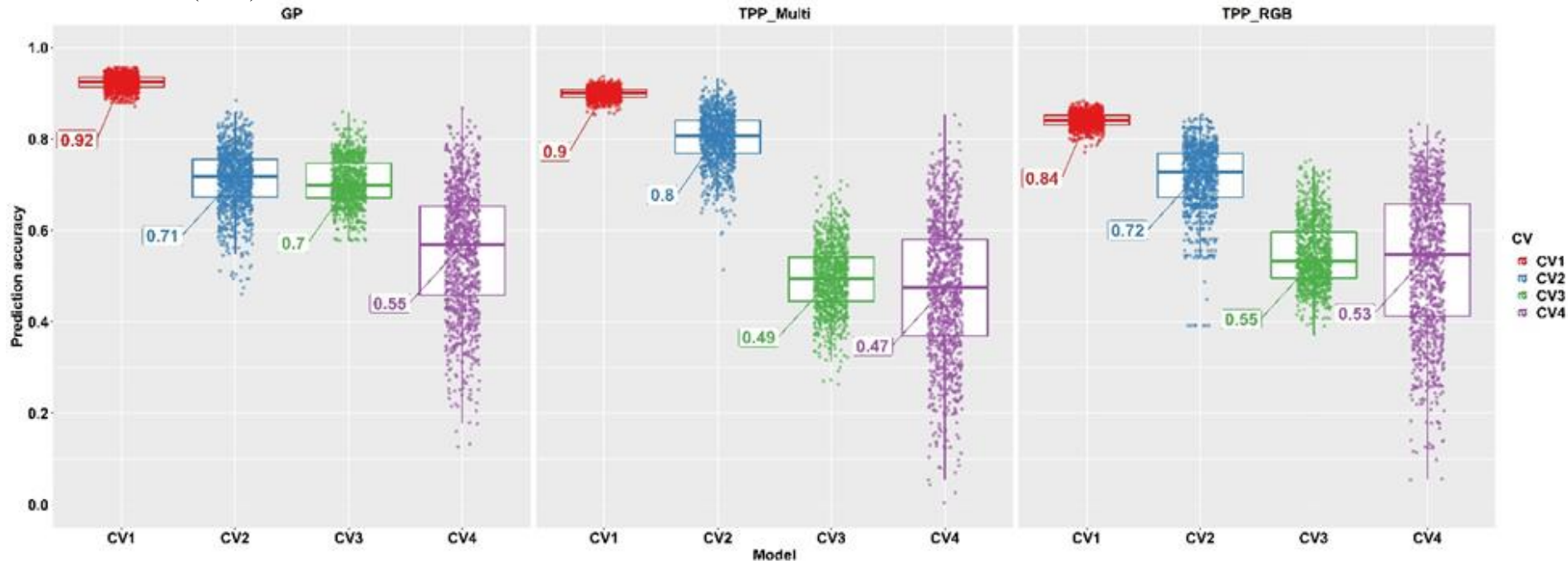
Phenomic selection and prediction of maize grain yield from near-infrared reflectance spectroscopy of kernels

Holly M. Lane, Seth C. Murray, Osval A. Montesinos-López, Abelardo Montesinos-López, José Crossa, David K. Rooney, Ivan D. Barrero-Farfan, Gerald N. De La Fuente, Cristine L. S. Morgan

First published: 06 April 2020 | <https://doi.org/10.1002/ppj2.20002>

# UAS temporal phenomic prediction $\approx$ genomic prediction

**Fig. 3** shows the prediction accuracy results of yield belonging to the three models. GP represents the prediction accuracy of genomic prediction, TPP\_Multi represents the prediction accuracy of phenomic prediction using the VIs derived from the multispectral images with low resolution, TPP\_RGB represents the prediction accuracy of phenomic prediction using the VIs derived from the RGB images with high resolution. Four cross validation schemes were used: predicting tested genotypes in tested environments (CV1), predicting untested genotypes in tested environments (CV2), tested genotypes in untested environments (CV3), and untested genotypes in untested environments (CV4).



**Alper Adak**  
PhD Student

**Genomes to Fields population: 1188 plots, 280 hybrids, 2 TX environments**

**Only 158 hybrids with genomic data: 70% training, 30% testing**

**GP = 153,252 markers for genomic prediction**

**TPP\_Multi = 1068 phenomic features (89 VI's x 12 time points)**

**TPP\_RGB = 525 phenomic features (35 VI's x 15 time points)**

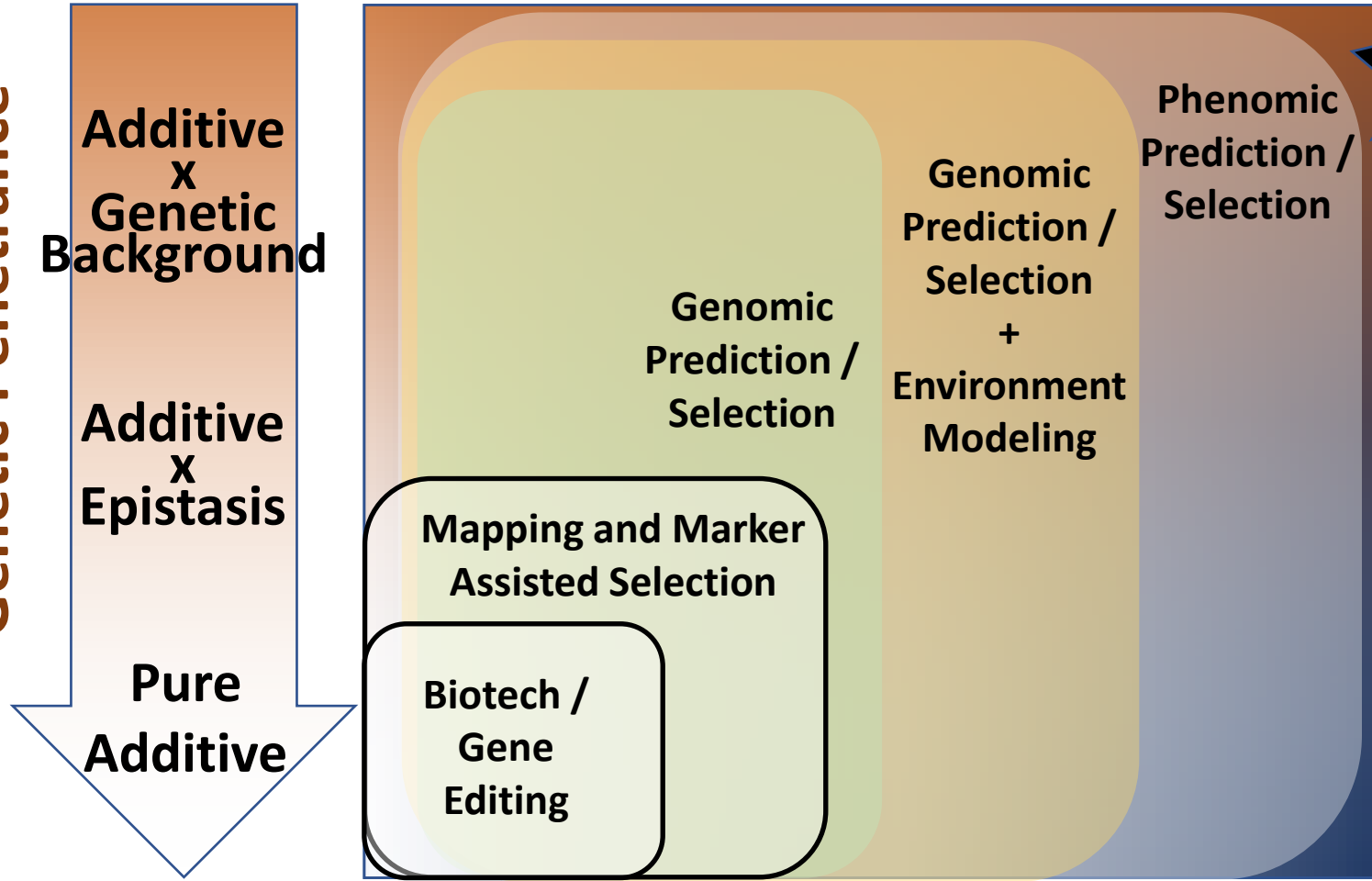
Adak, A., Murray, S. C., & Anderson, S. L. (2021). Temporal phenomic predictions from unoccupied aerial systems can outperform genomic predictions. *bioRxiv*.





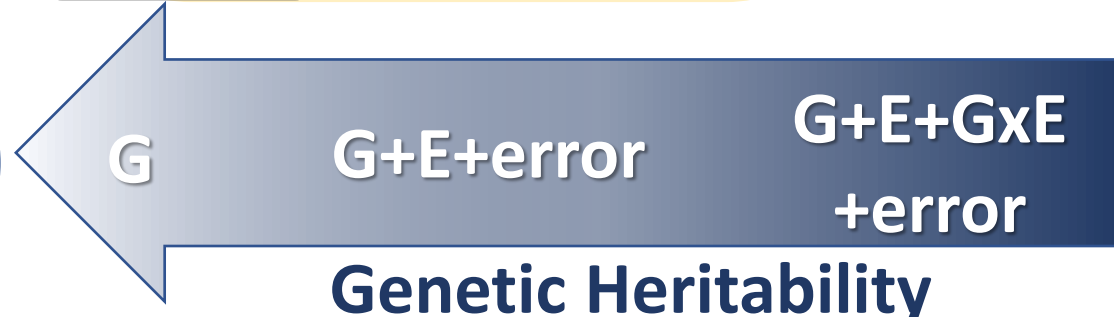
# Approaches based on complexity for genetics

Genetic Penetrance



Here reality is messy

Here reality equals theory

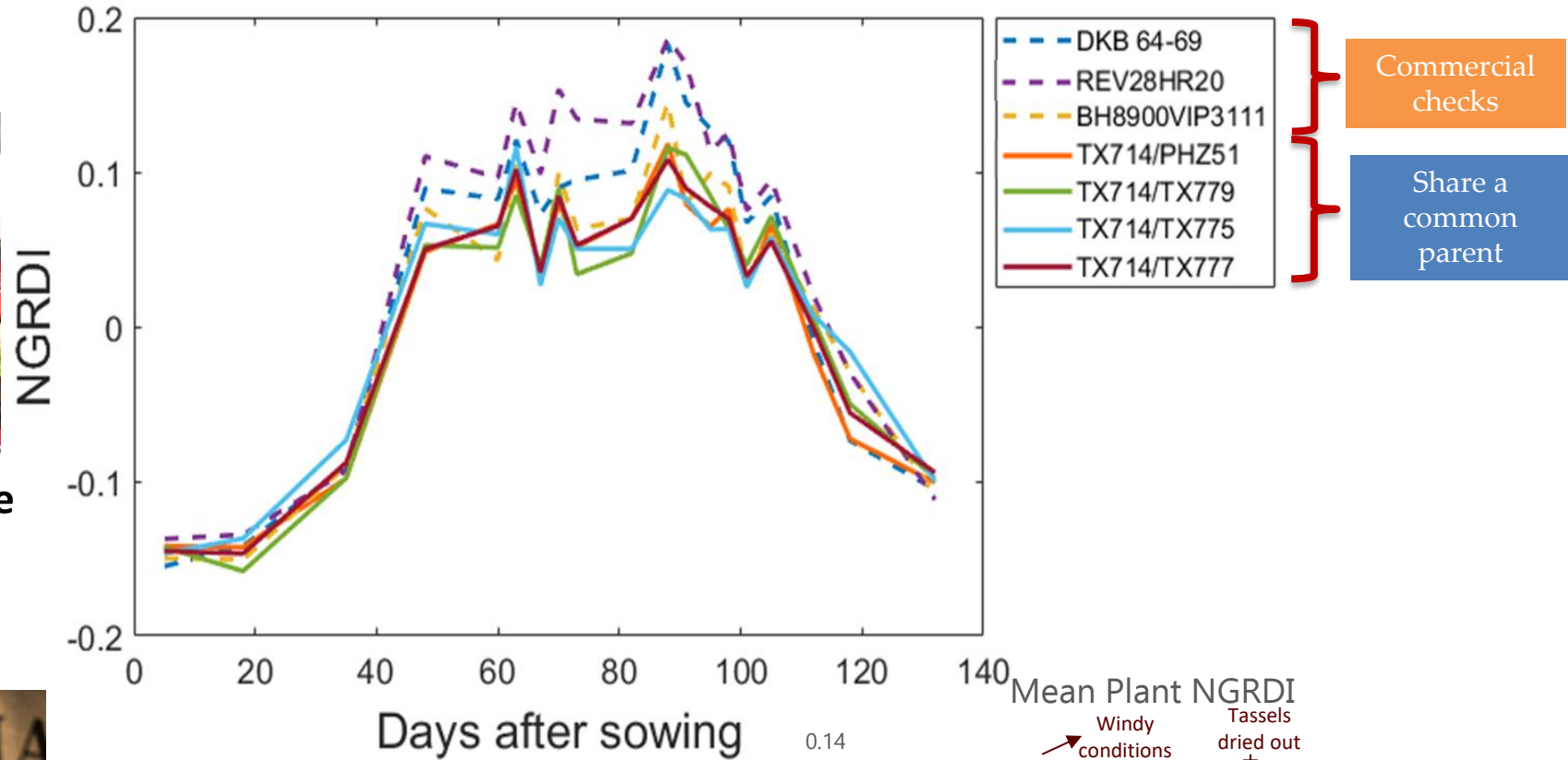


# Spectral (NGRDI) time series shows genetic patterns

NGRDI temporal-series: Mean plant NGRDI index versus days after sowing (DAS) by genotype.



Dale Cope



Nathália Cruzato  
PhD student

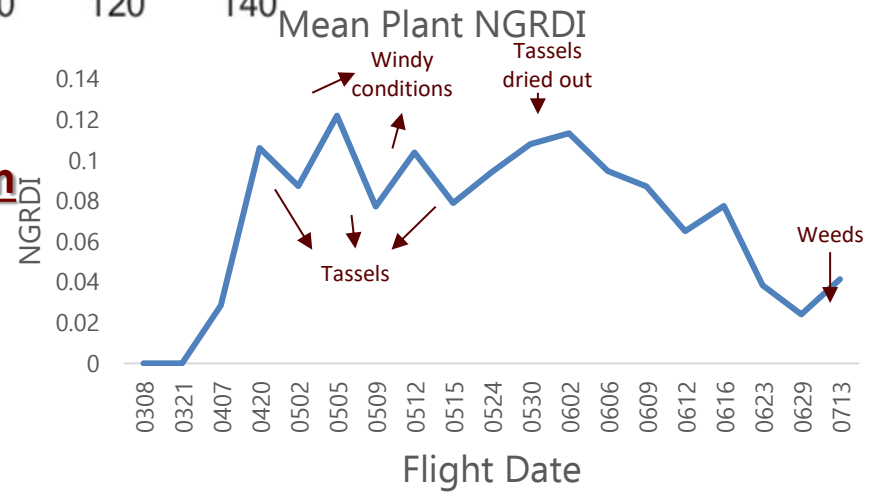
## Other sources of NGRDI variation

**Tassels**

**Tassels + wind**

**Tassels drying out**

**Weeds**

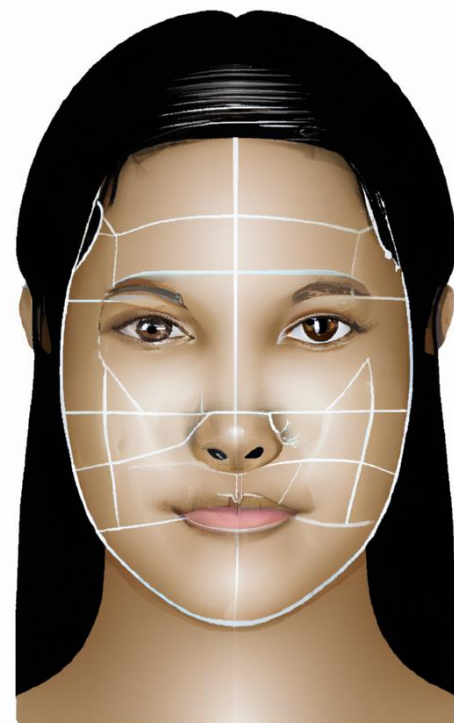
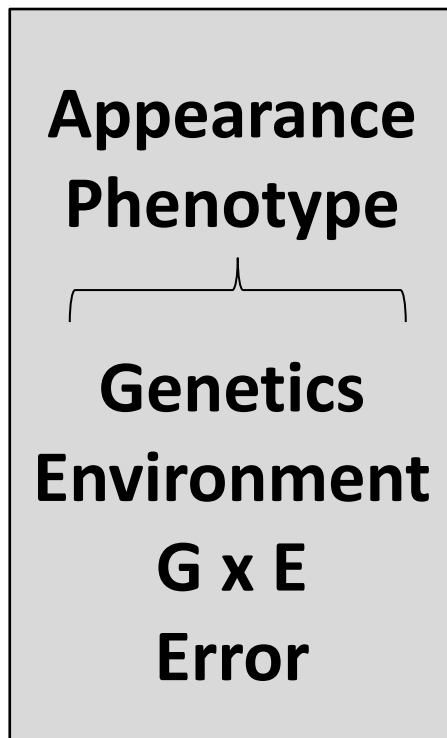


# Humans recognize resemblances in parents and siblings appearances and expect similarities in other traits

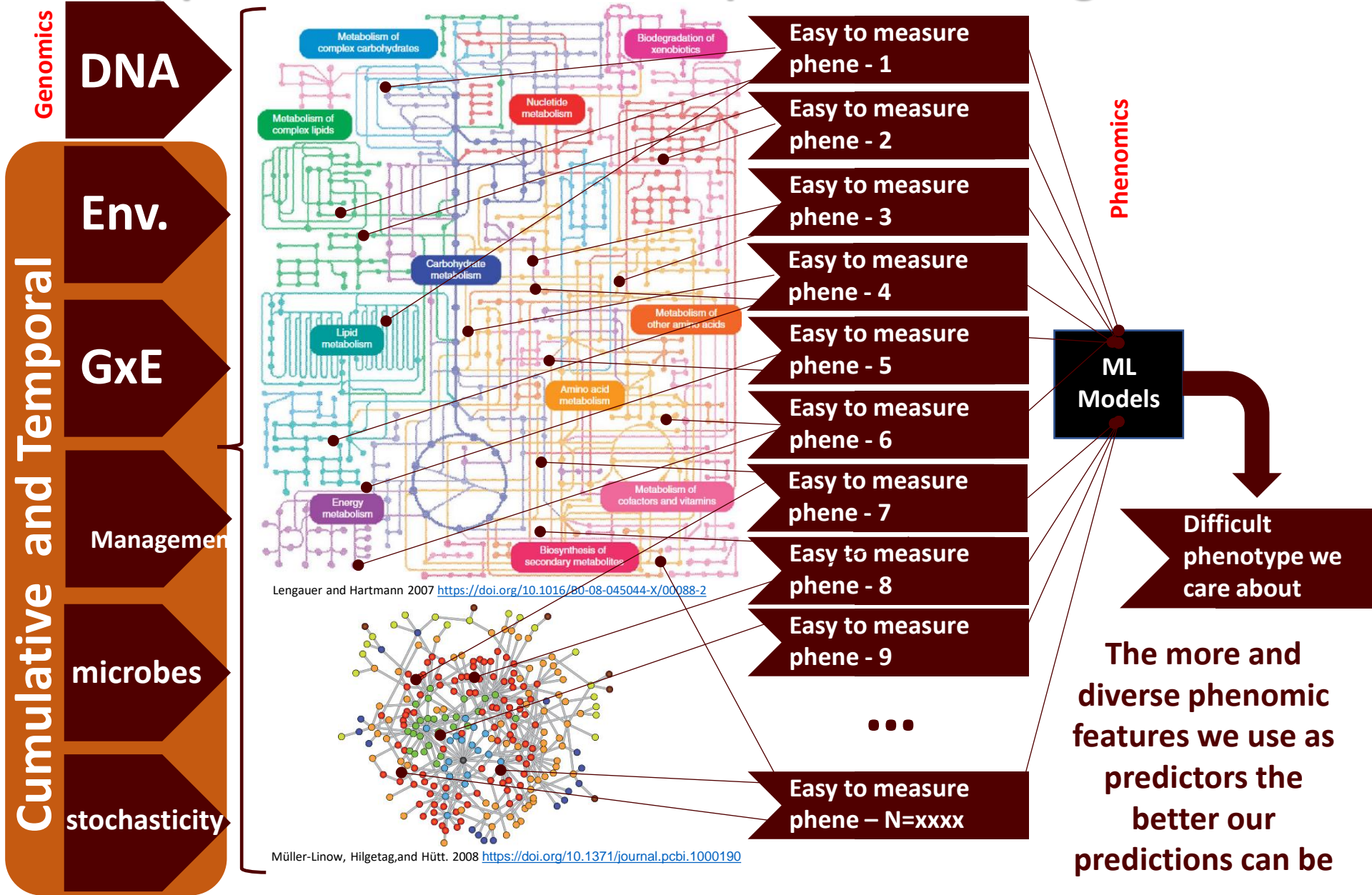


Humans recognize resemblances but hard to describe the quantitative similarities

Specific resemblance features change over time but maintain resemblance



# Cartoon of mechanism for why and how phenomics approaches could be more predictive than genomics



# Science has saturated the genome...

“...681,257 single-nucleotide polymorphism (SNP) markers...” – Romay et al. 2013

Research | [Open Access](#) | [Published: 11 June 2013](#)

Comprehensive genotyping of the USA national maize inbred seed bank

[Maria C Romay](#), [Mark J Millard](#), [Jeffrey C Glaubitz](#), [Jason A Peiffer](#), [Kelly L Swarts](#), [Terry M Casstevens](#), [Robert J Elshire](#), [Charlotte B Acharya](#), [Sharon E Mitchell](#), [Sherry A Flint-Garcia](#), [Michael D McMullen](#), [James B Holland](#), [Edward S Buckler](#) & [Candice A Gardner](#)

[Genome Biology](#) 14, Article number: R55 (2013) | [Cite this article](#)

40k Accesses | 341 Citations | 19 Altmetric | [Metrics](#)

“...Ames panel of ~1,500 inbred lines scored with 12.2 million single-nucleotide polymorphisms...”

Combining GWAS and TWAS to identify candidate causal genes for tocochromanol levels in maize grain

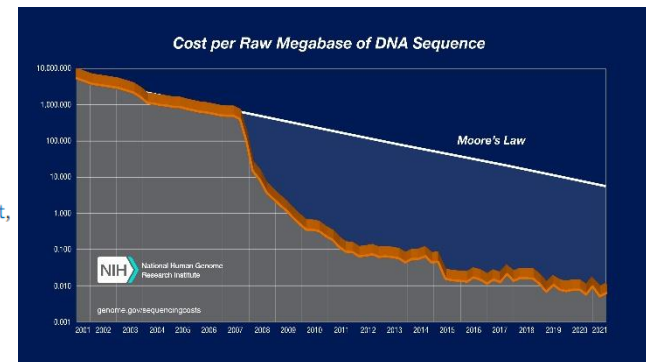
[Di Wu](#), [Xiaowei Li](#), [Ryokei Tanaka](#), [Joshua C Wood](#), [Laura E Tibbs-Cortes](#), [Maria Magallanes-Lundback](#), [Nolan Bornowski](#), [John P Hamilton](#), [Brieanne Vaillancourt](#), [Christine H Diepenbrock](#) ... [Show more](#)

[Author Notes](#)

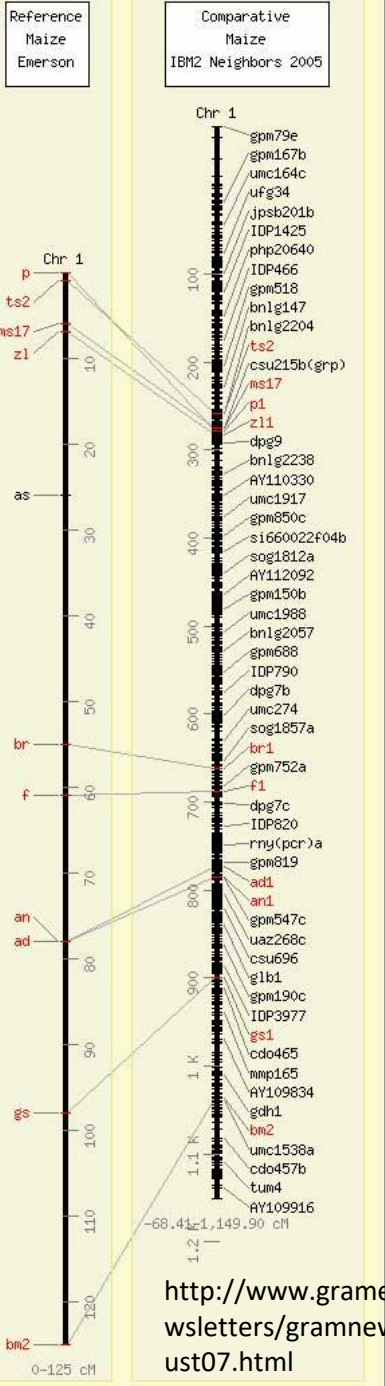
*Genetics*, Volume 221, Issue 4, August 2022, iyac091,

<https://doi.org/10.1093/genetics/iyac091>

Published: 06 June 2022 [Article history](#) ▾



<https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data>



<http://www.gramene.org/newsletters/gramnews/julyaugust07.html>

# Can we saturate the phenome?

# Maize genomes to fields (G2F) project

<https://www.genomes2fields.org/>

2023 = 9<sup>th</sup> year!

Plant morphology	Agronomic
Plant height	Stand count
Ear height	Lodging
	Days to silk
	Days to anthesis
Productivity	Weather station
Grain yield	Humidity
Test weight	Temperature
Grain moisture	Solar radiation
	Soil moisture
	Wind

## G + E + M + GxE + GxExM

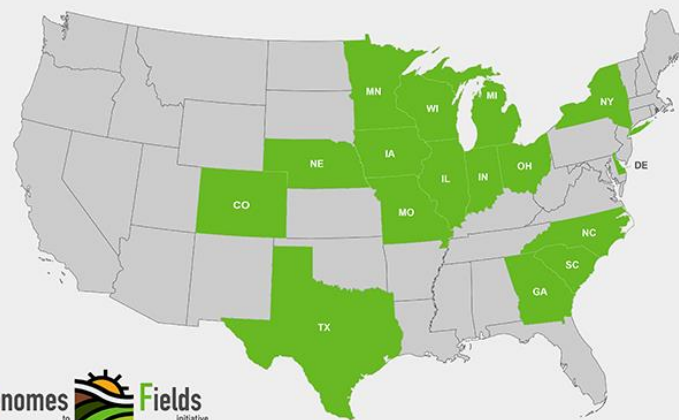
2022 Academic & Federal Institutions

Clemson University  
Colorado State University  
Cornell University  
Iowa State University  
Michigan State University  
North Carolina State University

Ohio State University  
Purdue University  
Texas A&M University  
University of Delaware  
University of Georgia  
University of Illinois

University of Minnesota  
University of Missouri  
University of Nebraska-Lincoln  
University of Wisconsin  
USDA-ARS

Georg-August-Universität Göttingen  
(Not shown on map)



**180,000 Field Plots**  
**2500+ corn varieties**  
**162 unique environments**  
*Some flew drones*



### Genomes to Fields (G2F) Genotype by Environment Prediction Competition

★ 59

Organized by: G2F\_2022\_GxE\_challenge\_host

Starts on: Nov 15, 2022 12:00:00 AM

Ends on: Jan 15, 2023 11:59:59 PM

Rank	Participant team	Mean_RMSE (L)
1	CLAC	2.328863
2	igorkf	2.345147
3	phenomaize	2.374471
4	UCD_MegaLMM	2.387404



United States Department of Agriculture

National Institute of Food and Agriculture



Year	Environment	Number of flights for plant height	RGB Vegetation Indices	Multispectral Vegetation Indices	Plant Height Elevation Models	File size: Orthomosaics and plant height point cloud files	File size: All files (including raw image and Agisoft Files)
2020	College Station, TX	12	Yes	na	Yes	566 GB	1.08 TB
2020	Arlington, WI	na	Yes	na	na	56.7 GB	481 GB
2020	Hancock, WI	na	Yes	na	na	38.2 GB	231 GB
2020	Madison, WI	na	Yes	na	na	63 GB	433 GB
2020	Missouri	87	Yes	na	Yes	707 GB	1.74 GB
2020	Michigan	12	Yes	Yes	Yes	73.9 GB	404 GB
2020	New York	na	Yes	Yes	na	39.4 GB	39.4 GB
2020	Minnesota	11	Yes	na	Yes	94 GB	451 GB
2020	Delaware	13	Yes	Yes	Yes	88.2 GB	465 GB
2020	Iowa	na	Yes	na	na	583 GB	722 GB
2020	Colorado	na	TBD	na	na	93.3 GB	564 GB
2021	College Station, TX	na	Yes	Yes	na	47.9 GB	na
2021	Hancock, WI	na	Yes	na	na	110 GB	~1 TB +
2021	Madison, WI	na	Yes	na	na	139 GB	~1 TB +
2021	Arlington, WI	na	Yes	na	na	80.6 GB	~1 TB +
2021	Minnesota	10	Yes	na	Yes	39.5	307 GB
2021	Nebraska	8	Yes	na	Yes	18.1 GB	41.2
2021	Iowa	na	Yes	na	na	92 GB	686 GB





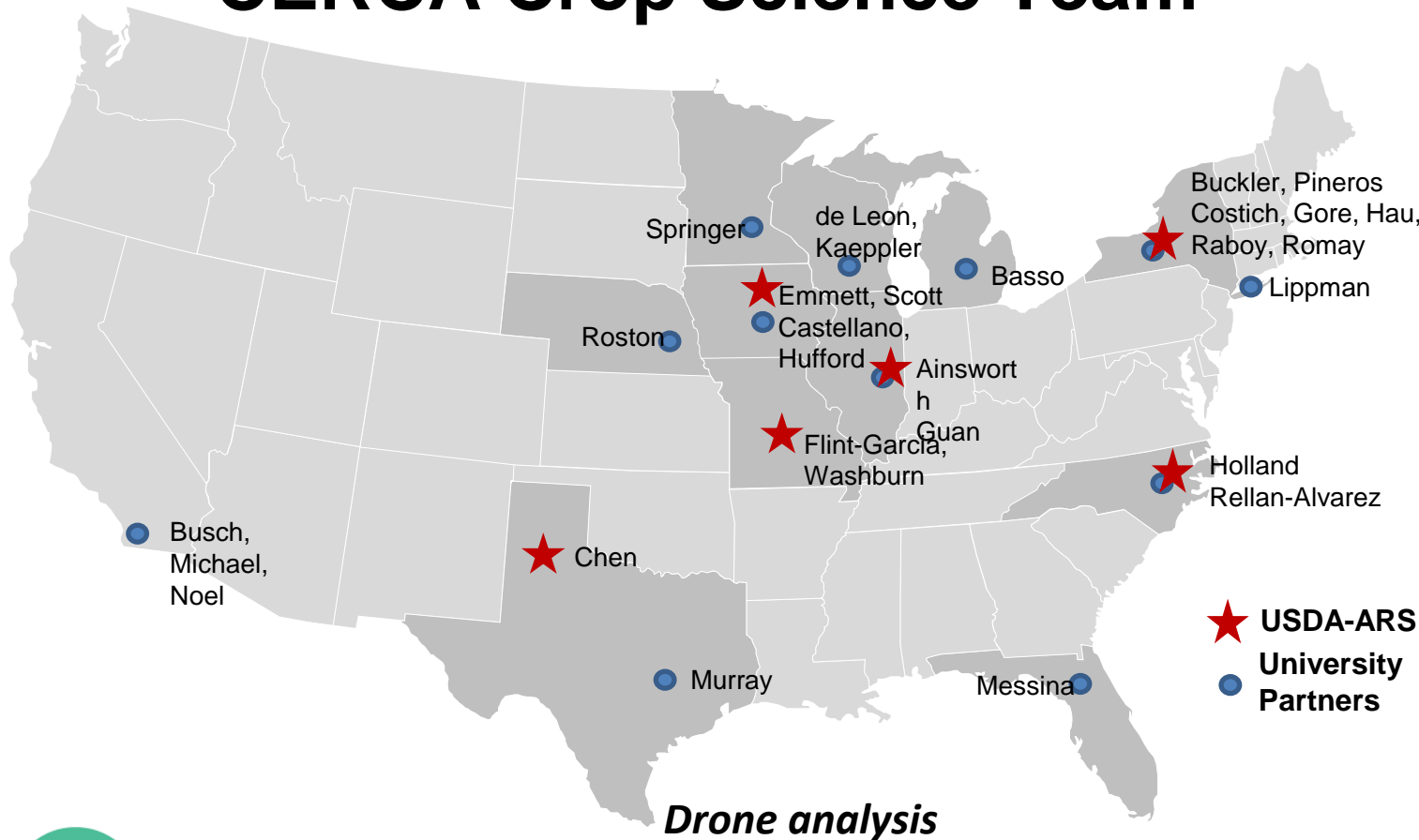
# CERCA

Circular Economy that Reimagines Corn Agriculture

## CERCA Crop Science Team



PI Dr. Edward Buckler  
USDA-ARS / Cornell

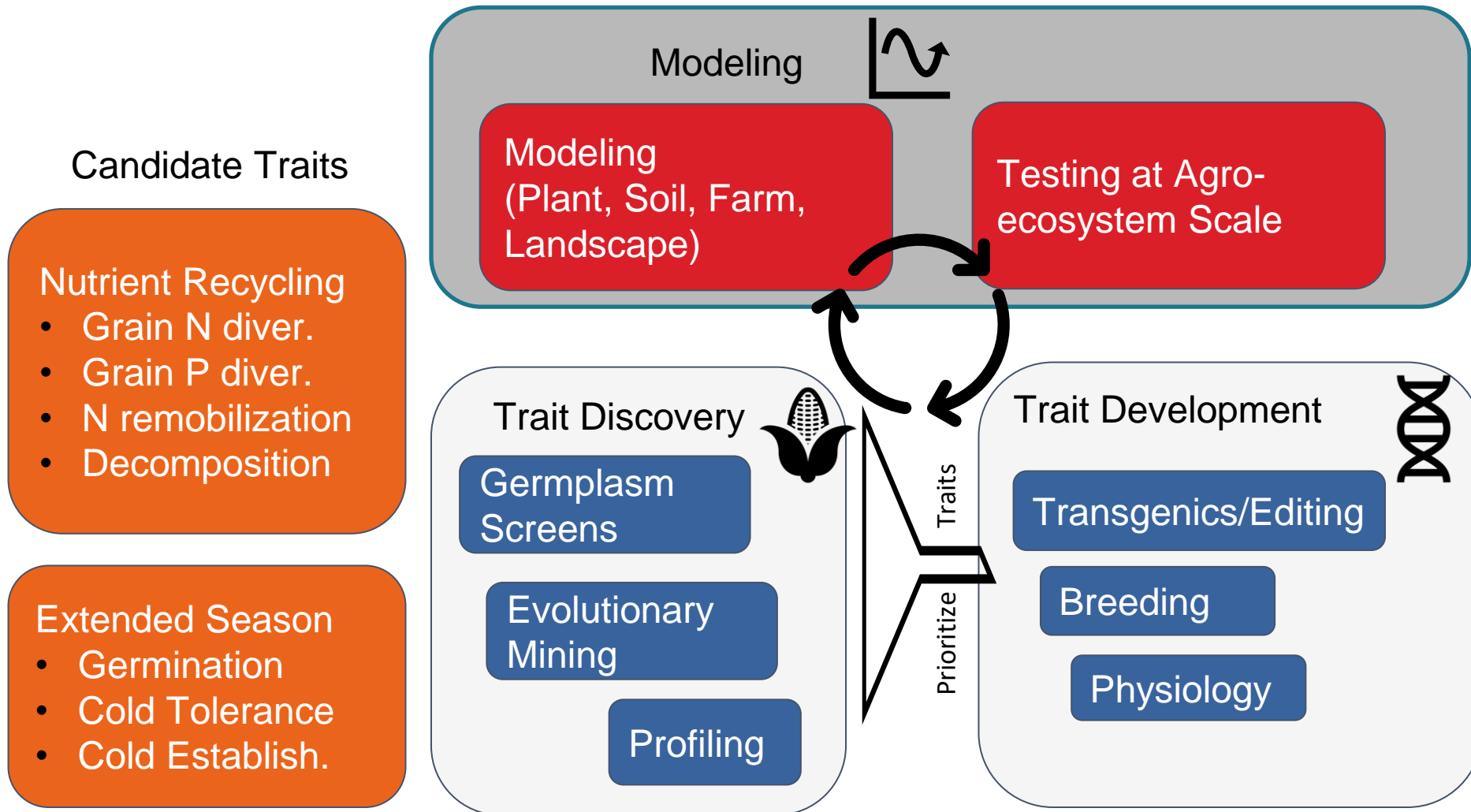


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RESEARCH

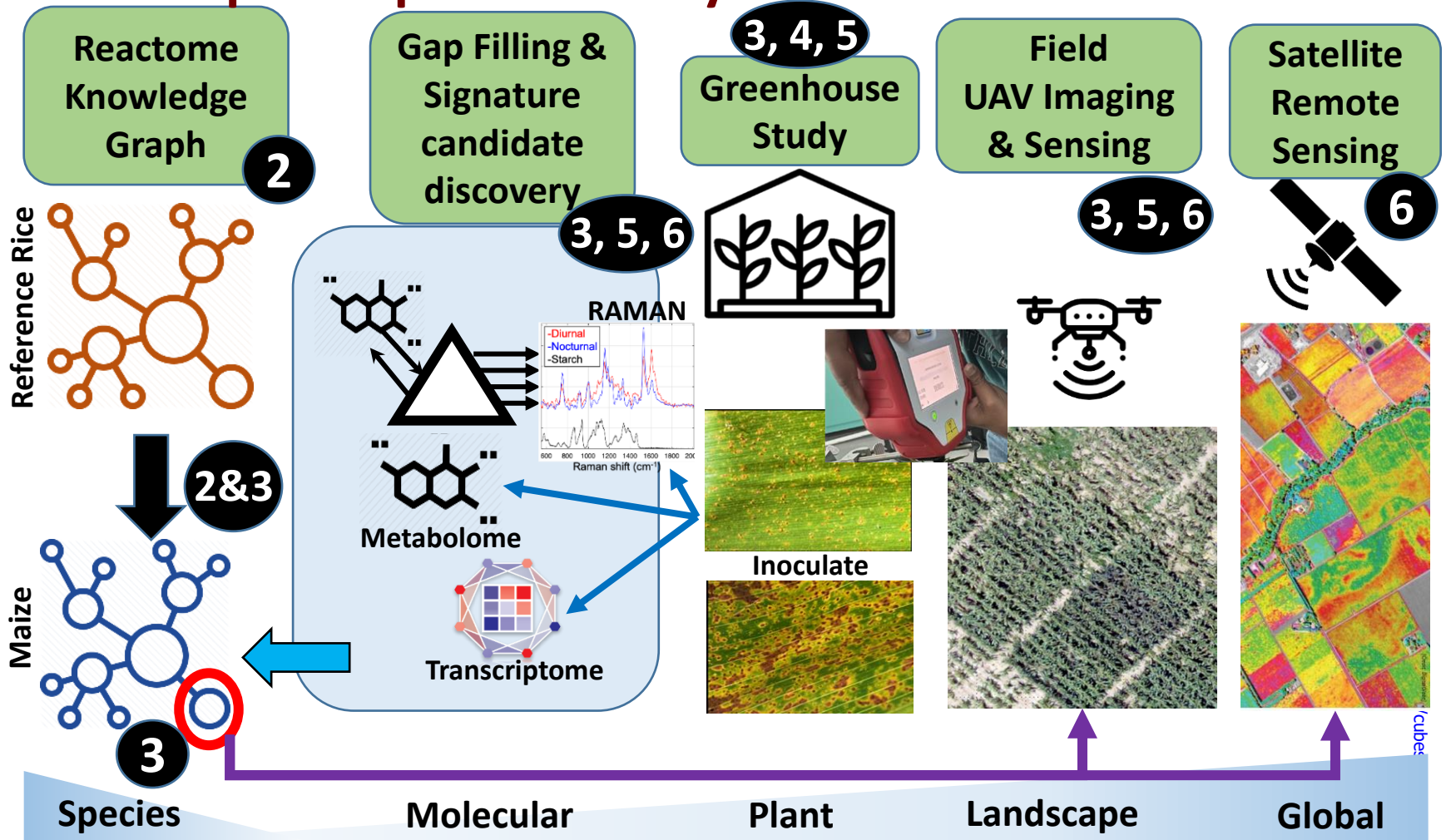
*Drone analysis*  
*Grow outs*  
*Phosphorus phenotyping*  
*Perennial germplasm*

# CERCA

Circular Economy that Reimagines Corn Agriculture  
FFAR (50%), Industry (50%), USDA-ARS



# FS2: Reactome knowledge graph to phenomes of major staple crops for food system threat detection



Pankaj Jaiswal

Sushma Naithani



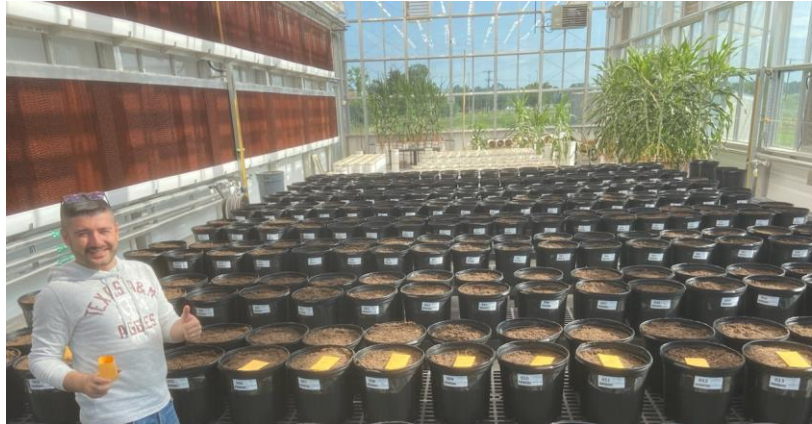
Aart Verhoef



Alper Adak

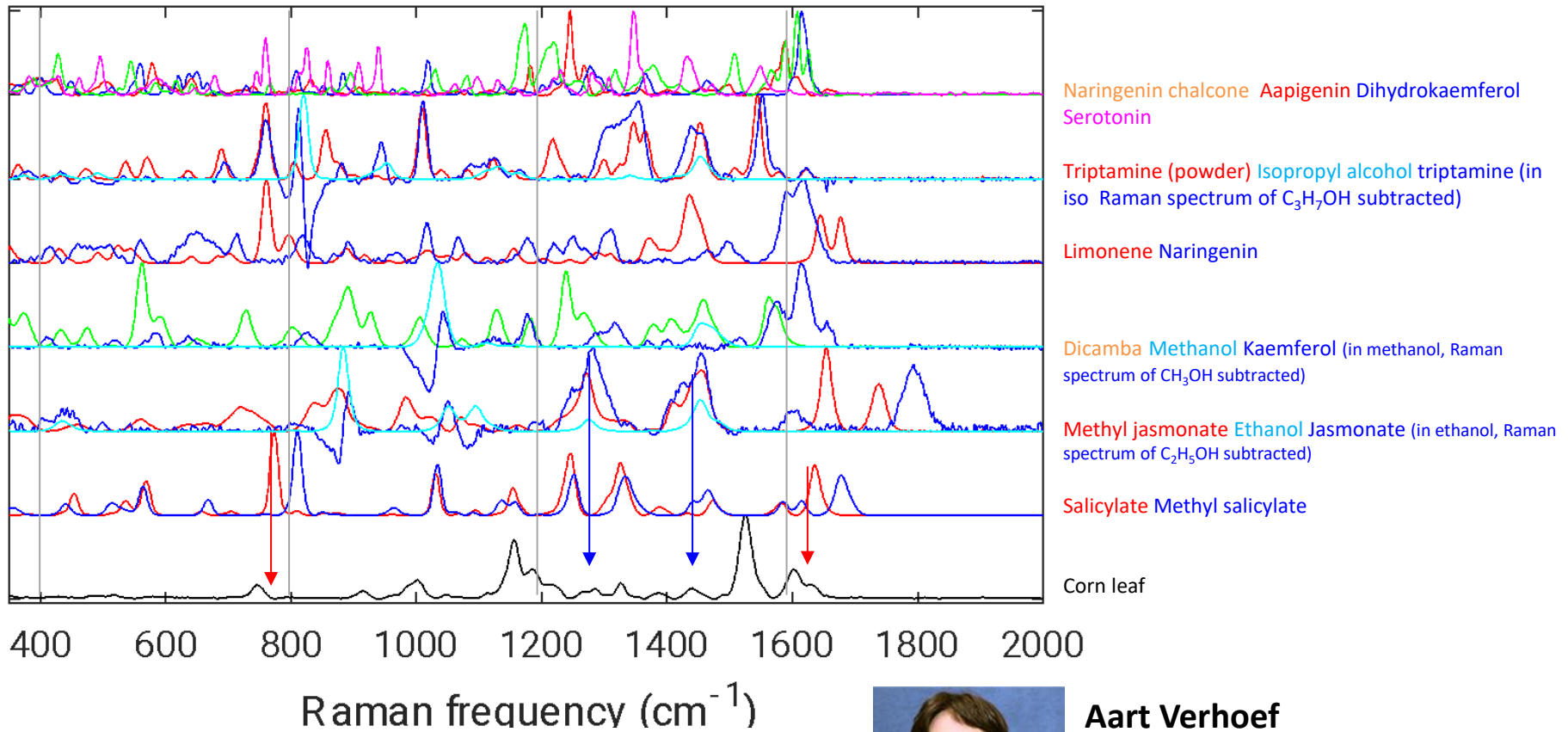
# 2023 greenhouse plantings

- Planted April 25 in College Station, TX
- ~27 hybrids, 10 inbreds,
- 2 treatments (inoculated vs. uninoculated) x 4 reps,  
(rep = 3 seeds / 1 plant per pot)



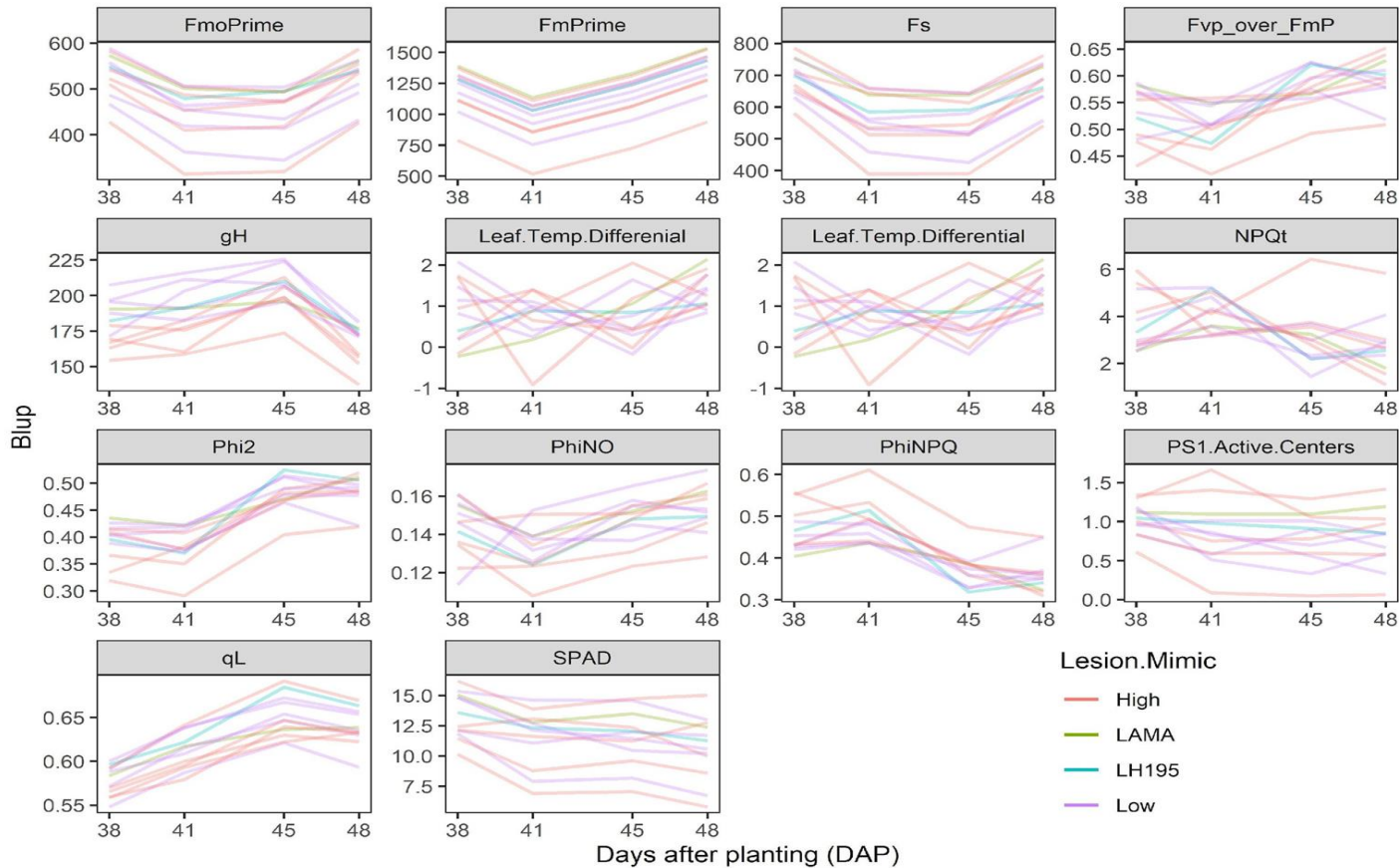
# Raman spectra of compounds nominated from reactome

Pure compounds possible to measure *in planta* if sufficient quantities present



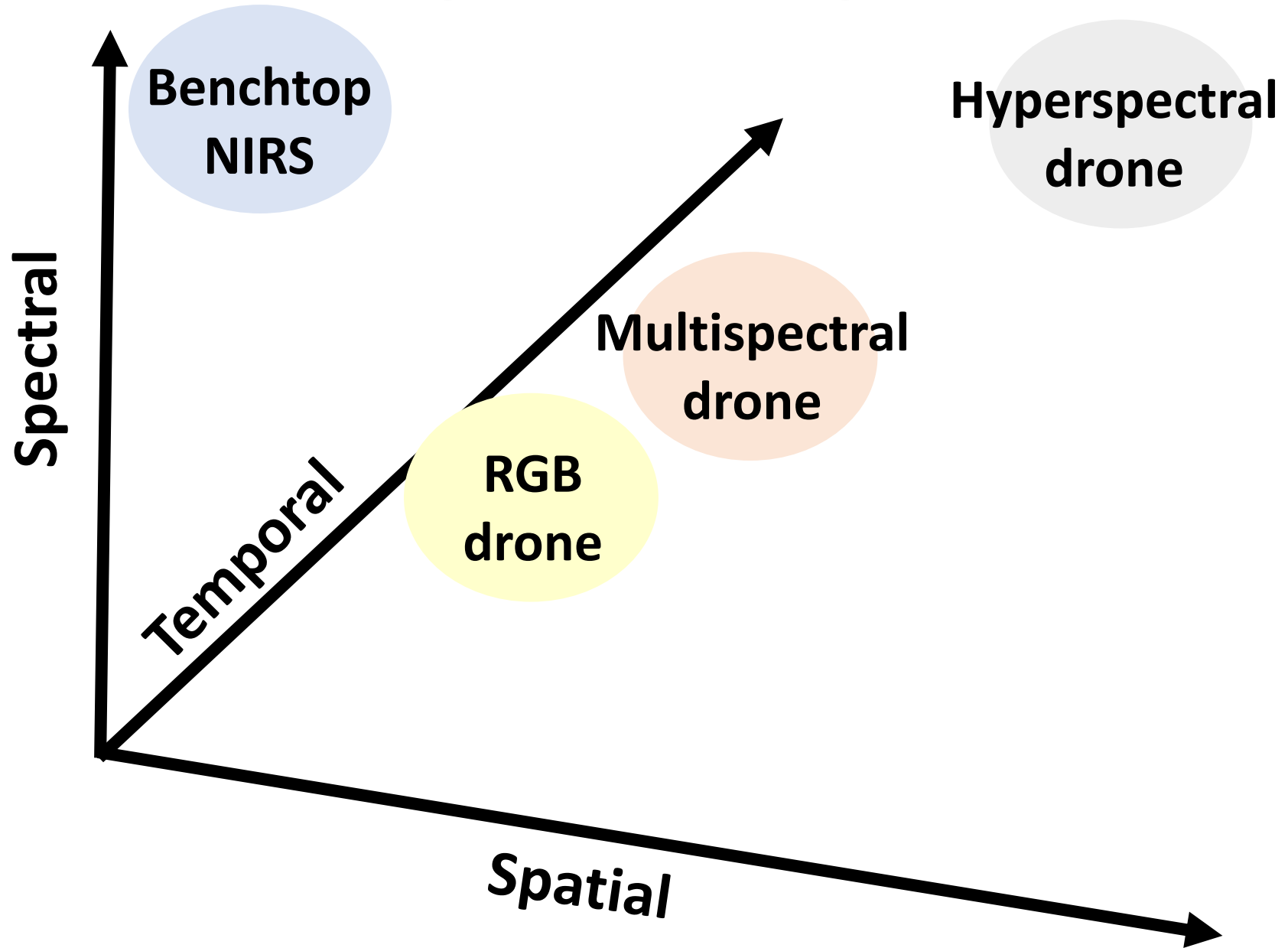
Aart Verhoef

# PhotosynQ traits

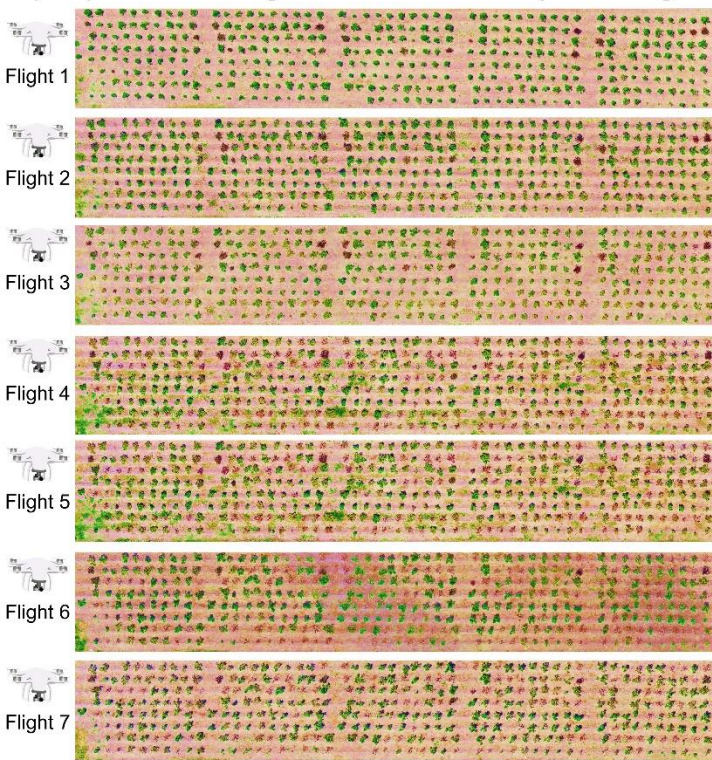


FmoPrime: Minimum Fluorescence  
 FmPrime: Maximum Fluorescence  
 Fs: Chlorophyll fluorescence  
 Fvp\_over\_FmP: Maximum quantum efficiency  
 gH+: Proton conductivity  
 Leaf temperature differential  
 PhiNPQ: Non photochemical quenching  
 Phi2: Quantum yield  
 PhiNO: Non-regulatory Energy Dissipation  
 PS1active centers  
 qL: PSII redox state (Lake model)  
 SPAD: Relative chlorophyll content

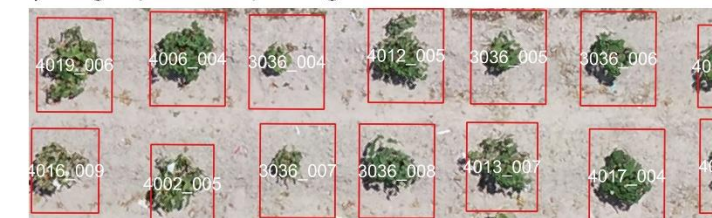
# Dimensionality to increase phenome data



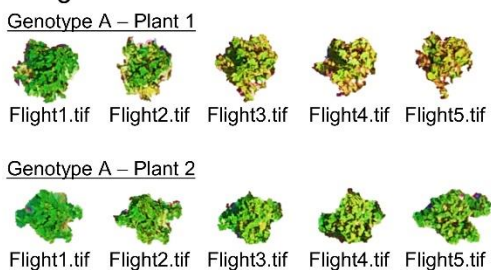
### A) Repeated UAS flights and orthomosaic processing



### B) Single-plant shapefile generation



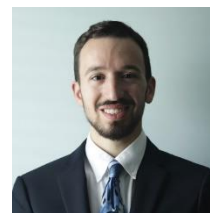
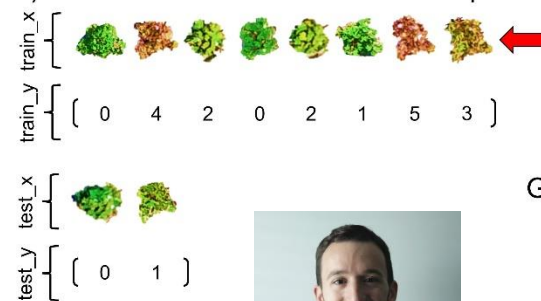
### C) Single-plant temporal image extraction



### D) Temporal senescence scoring

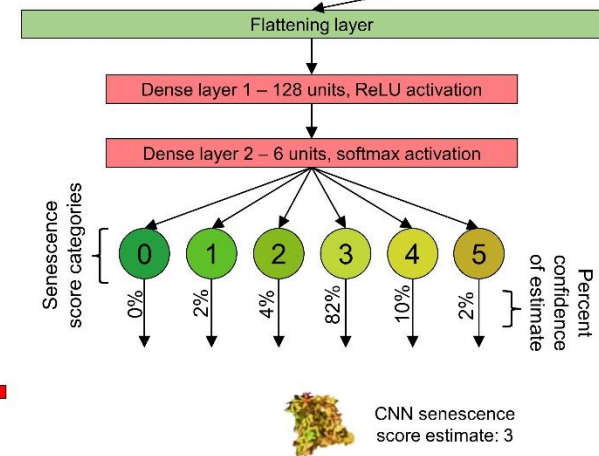
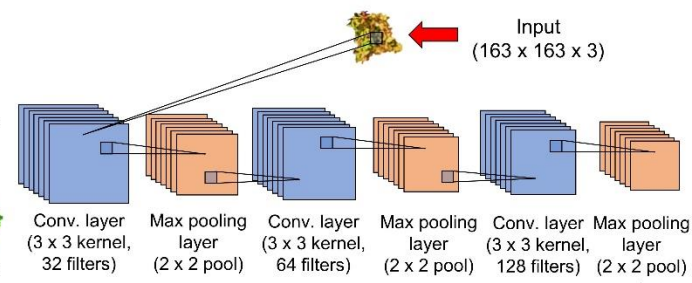
ID	Flight 1	Flight 2	Flight 3	Flight 4
GenoA-Plant1	0	1	2	3
GenoA-Plant2	0	2	2	2
GenoB-Plant1	0	1	1	2
GenoB-Plant2	0	2	3	4

### E) Partition data via 80/20 train/test split



**Aaron DeSalvio**  
PhD student

### F) Convolutional neural network (CNN)



### G) Apply CNN to unseen 20% of data

$$\text{Classification accuracy} = \frac{\text{True positives}}{\text{Total images in test data set}}$$



# What does the future look like in plant breeding?

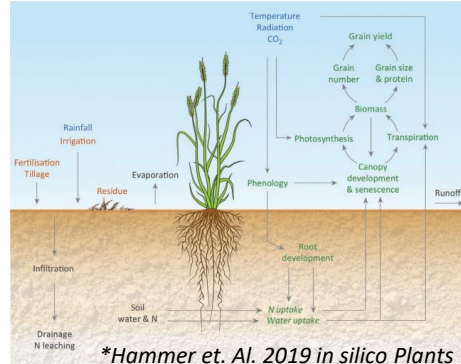
	Dependent "Traits" of interest			Physiological Measures			Temporal Phenomic "Trait" Measures			Segregating Remote Sensing Measures			Genomic Measures			
	Yield	Nutrition	Ecosystem	Flower	Leaf angle	Leaf #	Hgt 30 day	Hgt 60 day	Hgt 90 day	Phene X	Phene Y	Phene Z	SNP 1	SNP 2	SNP 3	SNP 4
Hybrid 1	100	9	3	70	35	16	0.02	0.30	0.61	0.542	0.179	0.449	AA	CC	CT	AG
Hybrid 2	150	10	9	65	32	15	0.04	0.30	0.62	0.068	0.505	0.744	AA	GG	TT	AA
Hybrid 3	125	2	9	68	30	17	0.05	0.33	0.63	0.523	0.949	0.271	AC	CC	CC	AA
Hybrid 4	175	3	2	66	37	16	0.07	0.40	0.70	0.823	0.814	0.537	CC	GG	TT	AA
Hybrid 5	180	7	3	73	40	16	0.10	0.42	0.72	0.637	0.815	0.703	CC	CG	TT	GG
Hybrid 6	75	6	10	67	32	13	0.11	0.54	0.75	0.874	0.368	0.785	CC	GG	TT	AA
Hybrid 7	90	3	10	66	33	19	0.12	0.55	0.81	0.984	0.406	0.867	CC	CC	TT	GG
Hybrid 8	170	5	6	65	32	19	0.16	0.56	0.84	0.491	0.418	0.176	AA	GG	TT	GG
Hybrid 9	160	10	5	70	31	17	0.24	0.58	0.85	0.693	0.287	0.013	CC	CC	TT	GG
Hybrid 10	155	9	7	72	39	15	0.26	0.59	0.99	0.130	0.624	0.566	AA	GG	CC	GG
	Multiple Replicates			Multiple Replicates			Multiple Replicates			Multiple Replicates			na	na	na	na
	Multiple Environments			Multiple Environments			Multiple Environments			Multiple Environments			na	na	na	na
Heritability	0.4	0.2	0.3	0.6	0.3	0.8	0.2	0.6	0.8	0.4	0.01	0.7	1	1	0.98	1

Statistical analysis  
(e.g. Functional Regression)

Growth models  
(w/ weather data)

Relationship matrices

Predict best untested hybrids for untested environments



	Hybrid 1	Hybrid 2	Hybrid 3	Hybrid 4	Hybrid 5	Hybrid 6	Hybrid 7	Hybrid 8	Hybrid 9	Hybrid 10
Hybrid 1	~1	0.906	0.331	0.02	0.864	0.373	0.715	0.416	0.248	0.836
Hybrid 2	0.906	~1	0.276	0.323	0.008	0.608	0.364	0.941	0.106	0.009
Hybrid 3	0.331	0.276	~1	0.24	0.163	0.339	0.47	0.172	0.315	0.832
Hybrid 4	0.02	0.323	0.24	~1	0.532	0.647	0.423	0.406	0.73	0.348
Hybrid 5	0.864	0.008	0.163	0.532	~1	0.173	0.363	0.715	0.334	0.397
Hybrid 6	0.373	0.608	0.339	0.647	0.173	~1	0.991	0.127	0.409	0.774
Hybrid 7	0.715	0.364	0.47	0.423	0.363	0.991	~1	0.231	0.219	0.008
Hybrid 8	0.416	0.941	0.172	0.406	0.715	0.127	0.231	~1	0.995	0.13
Hybrid 9	0.248	0.106	0.315	0.73	0.334	0.409	0.219	0.995	~1	0.613
Hybrid 10	0.836	0.009	0.832	0.348	0.397	0.774	0.008	0.13	0.613	~1

Figure 3. Crop model schematic showing the key processes involved in crop growth and development and their interactions with the crop system (Chenu et al. 2017; with permission from Elsevier).

# Multiple groups cover UAS phenotyping



Agricultural Genome to Phenome Initiative



S1069: Research and Extension for Unmanned Aircraft Systems (UAS) Applications in U.S. Agriculture and Natural Resources

(Multistate Research Project)

Status: Active



NC1212: Exploring the Plant Phenome in Controlled and Field Environments

(Multistate Research Project)

Status: Active

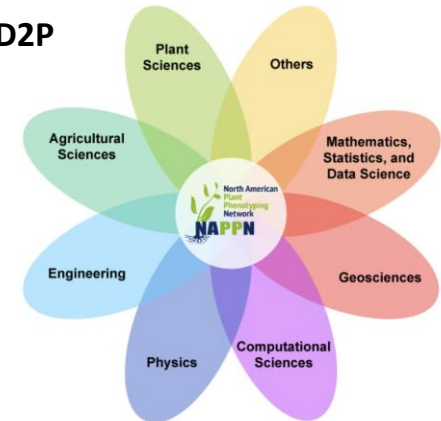
AI IN AGRICULTURE: INNOVATION AND DISCOVERY TO EQUITABLY MEET PRODUCER NEEDS AND PERCEPTIONS



ODD-PIGG

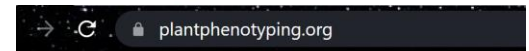
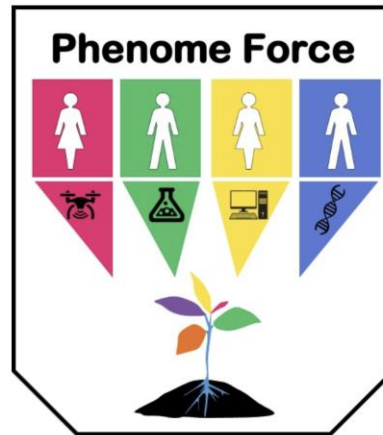
Livestock?

D2P



Conference 12539

Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping VIII



NAPPN

North American Plant Phenotyping Network



# Acknowledgements to what was presented

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