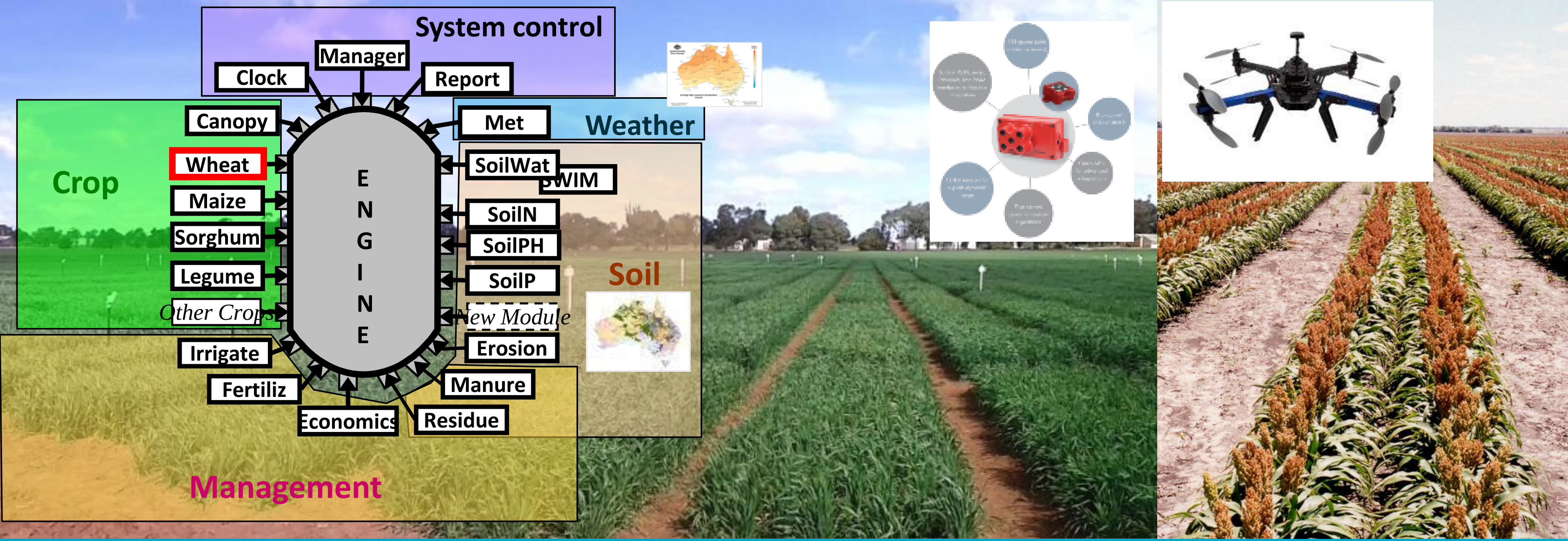


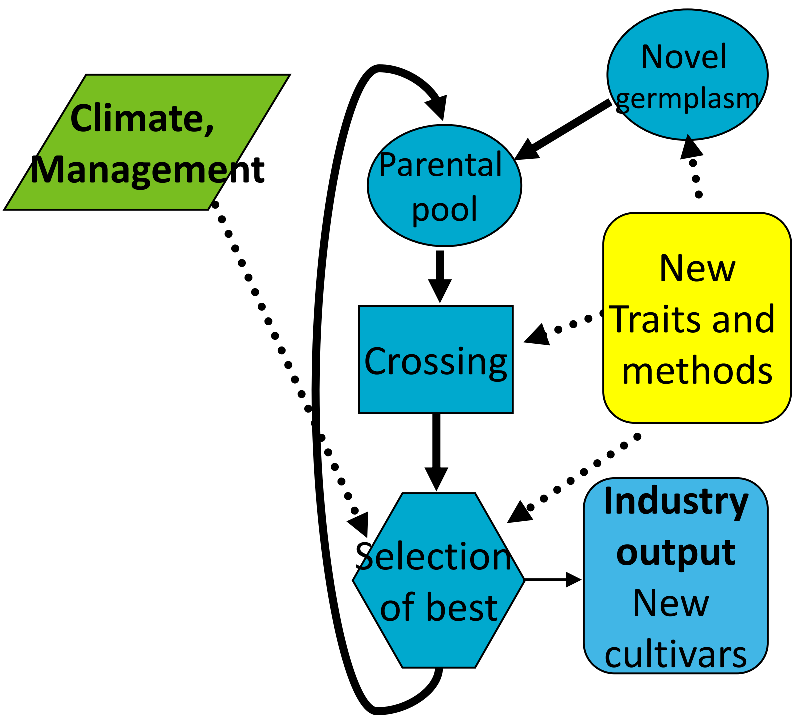
Challenges and Opportunities for Statistical Applications in High-Throughput Phenomics

Scott Chapman
Senior Principal Research Scientist, CSIRO Agriculture and Food
Professor in Crop Physiology, The University of Queensland

GRDC Grains Research & Development Corporation
Your GRDC working with you
Australian Plant Phenomics Facility
CSIRO
The University of Queensland Australia

What do breeders do?

Cycles of selection and evaluation in breeding



Chapman et al 2012
Crop & Pasture Science 63:251-268

Genetic Gain – the breeder’s equation

$$R = \overbrace{\underbrace{i}_{\text{Selection Intensity}}\underbrace{h^2}_{\text{Heritability}}\underbrace{\sigma_p}_{\text{Phenotypic variance}}}^{\text{Response per cycle}} / \text{ years per cycle}$$

Phenotypic prediction

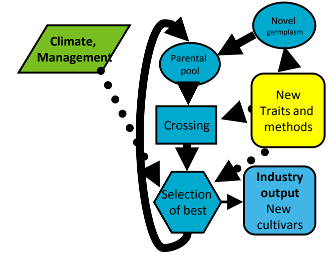
$$\mathbf{P_{ij} = \mu + G_i + E_j + (GE)_{ij}.}$$

- Increase intensity (more throughput)
- Increase heritability (more precision)

Correlated vs Direct Response

- A challenge for physiologists !

Over stages of selection: Small population size, reduced selection intensity and reduced σ^2_G



Crossing | Glasshouse | Nursery | Stage 1 & 2 | Stage 3 & 4





Population size and genetic variance

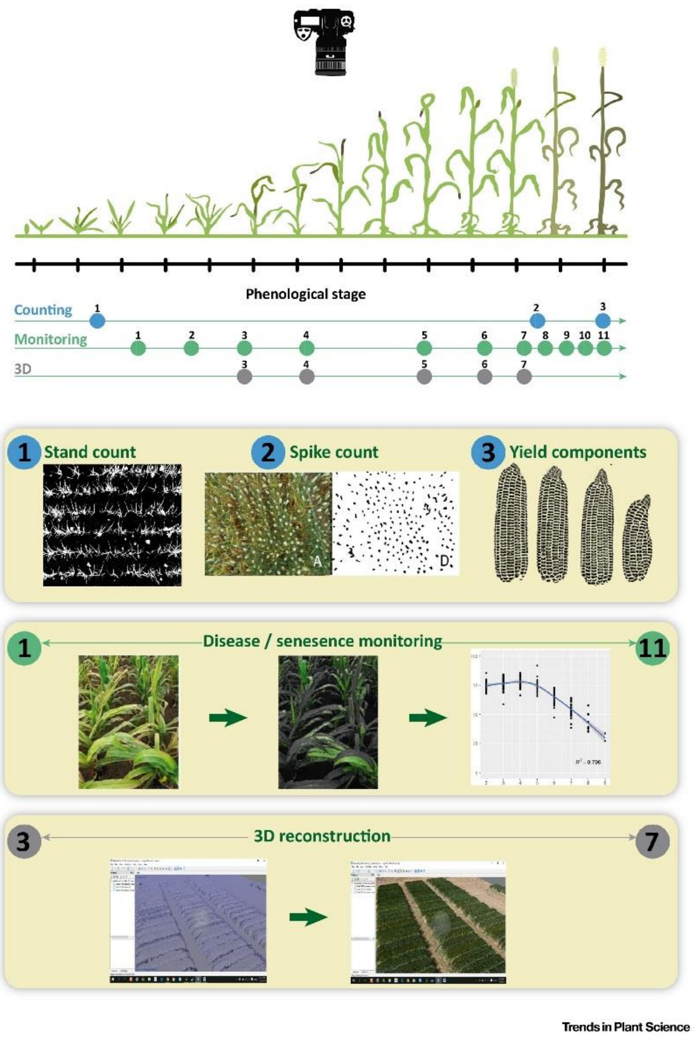
Field nursery is the bottleneck (and limit) – it sets the bar on the upper limit on the mean, potential line extremes/transgressives, and broader genetic variance

Breeders select while trying to maintain genetic variance from which selection (and progress) is made later with replicated plots. Heritability is small and so selection pressure (intensity) is relaxed

Types of traits

- Agronomic
  - Establishment scores
  - Plant stand counts
  - Canopy height
  - Heading/anthesis/flowering timing and patterns
  - Ear counts
  - Disease monitoring

- Physiological/dynamic
  - Leaf and root system characteristics
  - Estimation of derived parameters, e.g. light extinction, radiation use efficiency
  - Water productivity, stress indices
  - Predicting adaptation across environments



Statistical and dynamic modelling of phenotypes

Outline

ELSEVIER

Plant Science

Available online 30 June 2018

In Press, Accepted Manuscript ?

Modelling strategies for assessing and increasing the effectiveness of new phenotyping techniques in plant breeding

Fred van Eeuwijk [a], Daniela Bustos-Korts [a], Emilie J. Millet [a], Martin Boer [a], Willem Kruijer [a], Addie Thompson [b], Marcos Malosetti [a], Hiroyoshi Iwata [c], Roberto Quiroz [d], Christian Kuppe [e], Onno Muller [e], Konstantinos N. Blazakis [f], Kang Yu [g, h], Francois Tardieu [i], Scott Chapman [j, k]

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https://doi.org/10.1016/j.plantsci.2018.06.018 Get rights and content

Open Access funded by VSNU

van Eeuwijk et al 2018 Plant Science
Modelling strategies for assessing and increasing the effectiveness of new phenotyping techniques in plant breeding
https://doi.org/10.1016/j.plantsci.2018.06.018

CSIRO

Size input data	Modelling step	Input	Model / strategy	Output	Model dependence output
	Feature extraction	Multiple pixels/wavelengths for each plot, genotype, time point and environment (low level traits)	Segmentation Organ Tracking Indices (e.g. NDVI) Machine learning	Trait value for each plot, genotype, time point and environment	
	Correcting for design factors and spatial trends	Trait value for each plot, genotype, time point and environment	Mixed models: - AR⊗ AR - SpATS	Adjusted trait value for each genotype, time point and environment	
	Dynamical modelling	Trait value for each genotype, time point and environment	Standard mathematical functions (Logistic, Gompertz) Repeated measures Splines Crop growth models	Genotype specific parameters for each environment (intermediate level traits)	
	Modelling dependence on environmental gradients	Genotype specific parameters for each environment	Standard mathematical functions Splines Crop growth models	Genotypic sensitivities to environmental covariables (higher level traits)	
	Target trait prediction	Higher order traits and environmental characterizations	Mixed models (multi-trait, factorial regression) Crop growth models	Genotype specific yield predictions per environment	

- Feature extraction
- Design/spatial trends
- Dynamic modelling
- Modelling Env effects
- Target Trait Prediction

van Eeuwijk et al 2018 Plant Science (online)

https://doi.org/10.1016/j.plantsci.2018.06.018

Size input data	Modelling step	Input	Model / strategy	Output	Model dependence output
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van Eeuwijk et al 2018 Plant Science (online)

https://doi.org/10.1016/j.plantsci.2018.06.018

What is APSIM?

A highly advanced **agricultural systems model** created:

- To model system performance over time
- With an equal emphasis on crop and soil dimensions
- With a capability to deal comprehensively with management matters

An open and transparent 'APSIM Community Source Framework' (modified open source)

Free public good licensing (R&D and education)

Development and maintenance is underpinned by rigorous science and software engineering standards

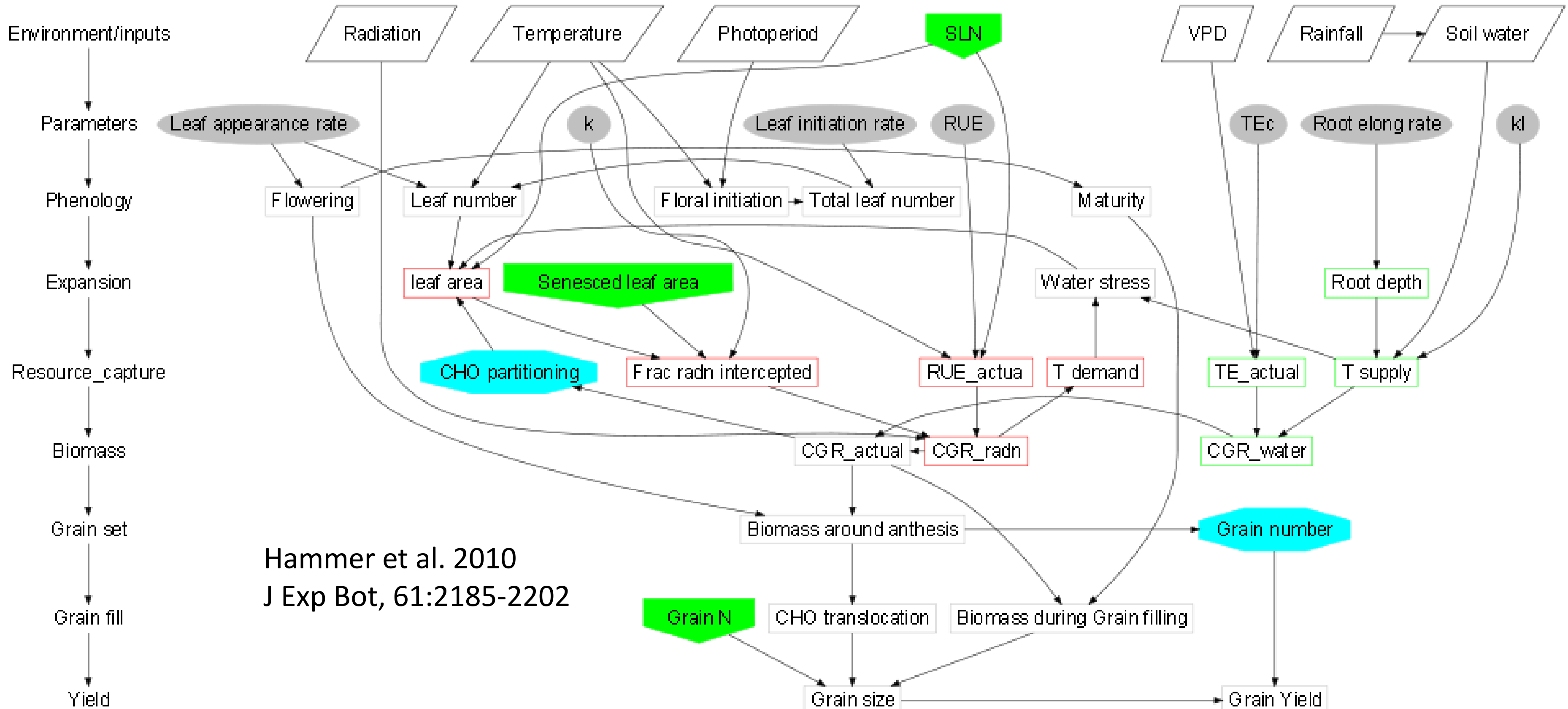
Owned by ***The APSIM Initiative***
www.apsim.info

The APSIM Initiative (AI)

- Established in the early 1990's (then as APSRU) to promote the development and use of the science modules and infrastructure software of APSIM
- APSIM development, maintenance and commercialisation are the responsibility of the AI
- Foundation Members of the AI:
  - CSIRO
  - the State of Queensland (Dept of Agric, F & F)
  - the University of Queensland
  - AgResearch (New Zealand)
- Strategic direction guided by the *Steering Committee*
- Science quality control maintained by the *Reference Panel*

No equations to see here:
Crop models to organise phenotyping across scales…

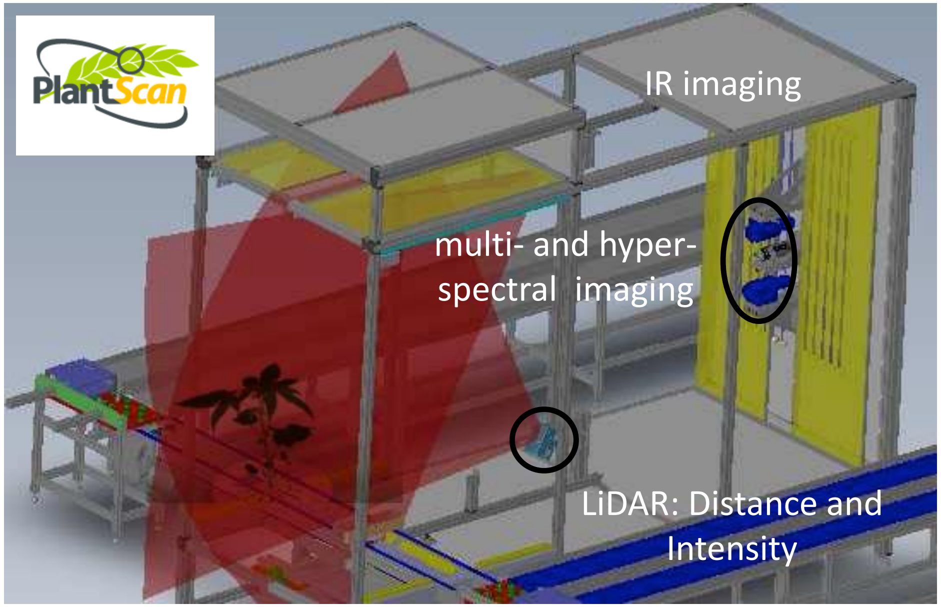
- Captures tangled web of dynamic interactions & feedbacks
- Understanding can be built up from multiple platforms



Hammer et al. 2010
J Exp Bot, 61:2185-2202

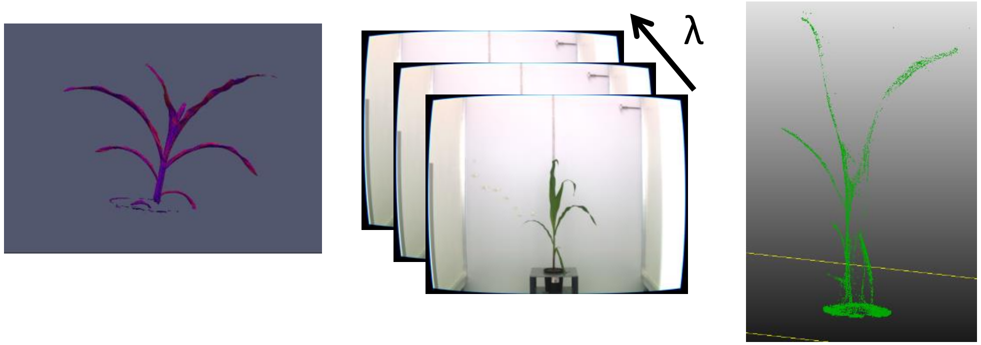
Variability in space and time
- examples with indoor platforms

PlantScan – leaf area dynamics and spectral imaging



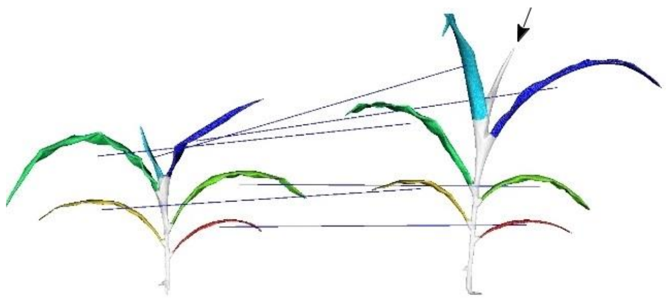
Integrated pipeline

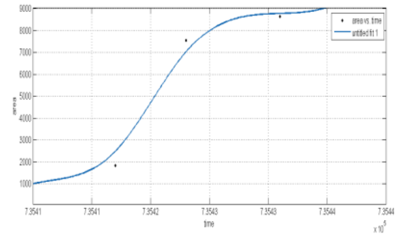
Real vs. in-silico



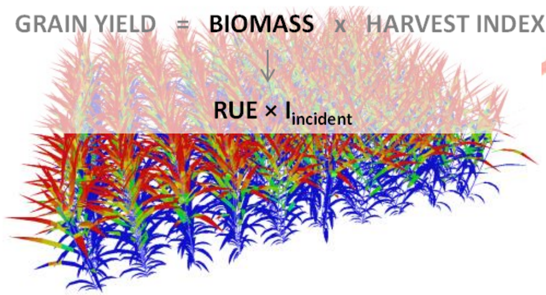
Paproki et al. (2012) BMC Plant Biology 12:63
Sirault *et al.* (2013) FSPM

Data assimilation: fusion of a mathematical models with observed data





Automated mathematical modelling -> derived data
(free form cubic spline (constrained by Ontology))



ScanAlea:

- Middleware (MTG)
- Model-assisted segmentation (Adel Model – model inversion (phenology)

Xavier Sirault, CSIRO

Paproki *et al.* 2012 BMC Plant Biology
Sirault *et al. In prep*

Root geometry and plant transpiration platforms (The University of Queensland)

- L-PAD Lysimetry platform
  - Automated watering system
  - Estimation of water use per unit leaf area

- Root angle
  - Moderate-throughput seedling screening
  - Selection for narrow or wide-angle roots

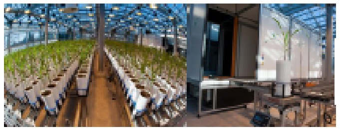
-

sorghum and wheat
e.g. Singh et al 2010, 2012; Manschadi et al 2006

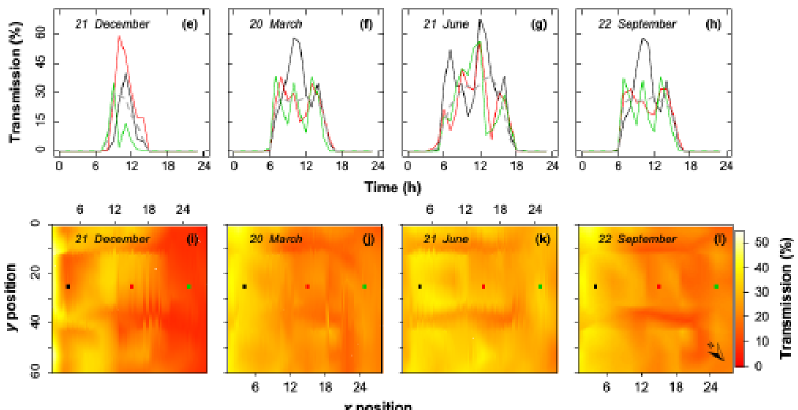
Graeme Hammer, UQ

Variability in platform experiments

Spatial and temporal variability also in inside platform (greenhouse and growth chamber)

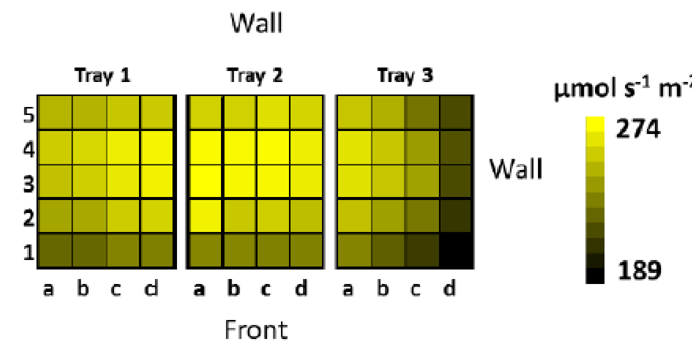


M3P Montpellier Plant Phenotyping Platforms



Cabrera-Bosquet et al. 2016

NaPPI Finnish National Plant Phenotyping Infrastructure



Mirko Pavicic, Kristiina Himanen

Need to be separated from the genetic signal: How to take into account this variability ?

1. Experimental design
2. **Correct for spatial trend**

Emilie Millet, María Xosé Rodríguez-Álvarez, Martin Boer, Fred van Eeuwijk

Spatial trends over time: two examples

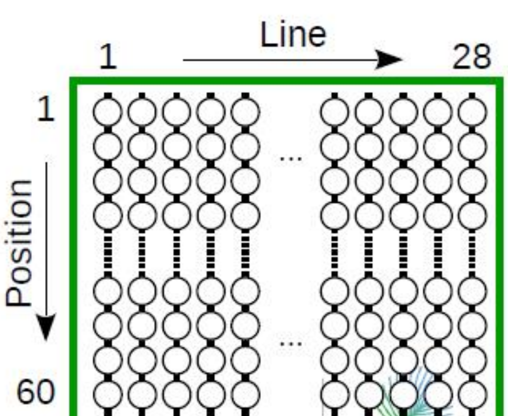
Phenoarch in Montpellier (Fr)

M3P Montpellier Plant Phenotyping Platforms

lepse

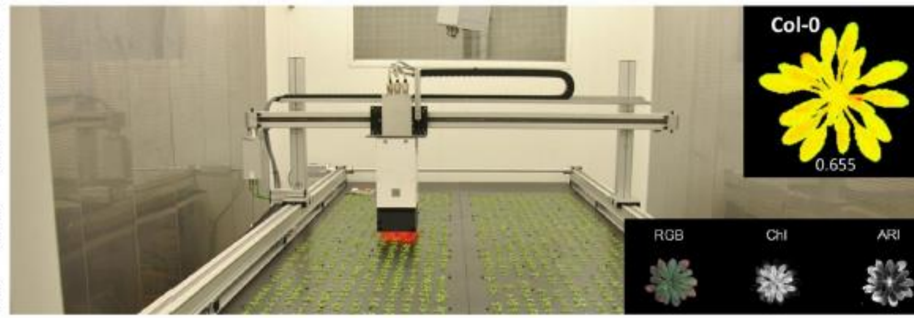
Llorenç Cabrera-Bosquet

Capacity : 1680 plants



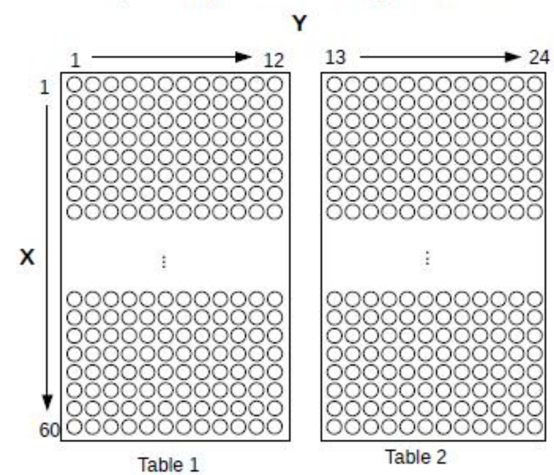
Phenovator in Wageningen (NL)

WAGENINGEN UNIVERSITY & RESEARCH

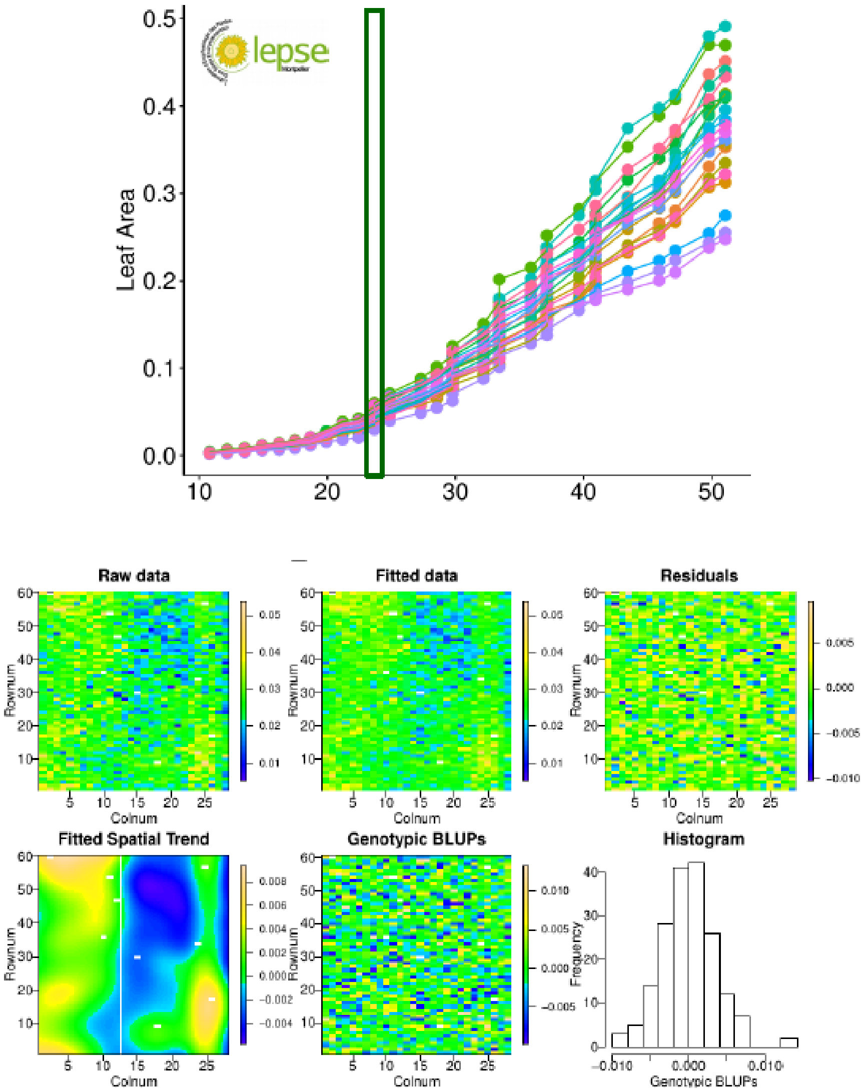


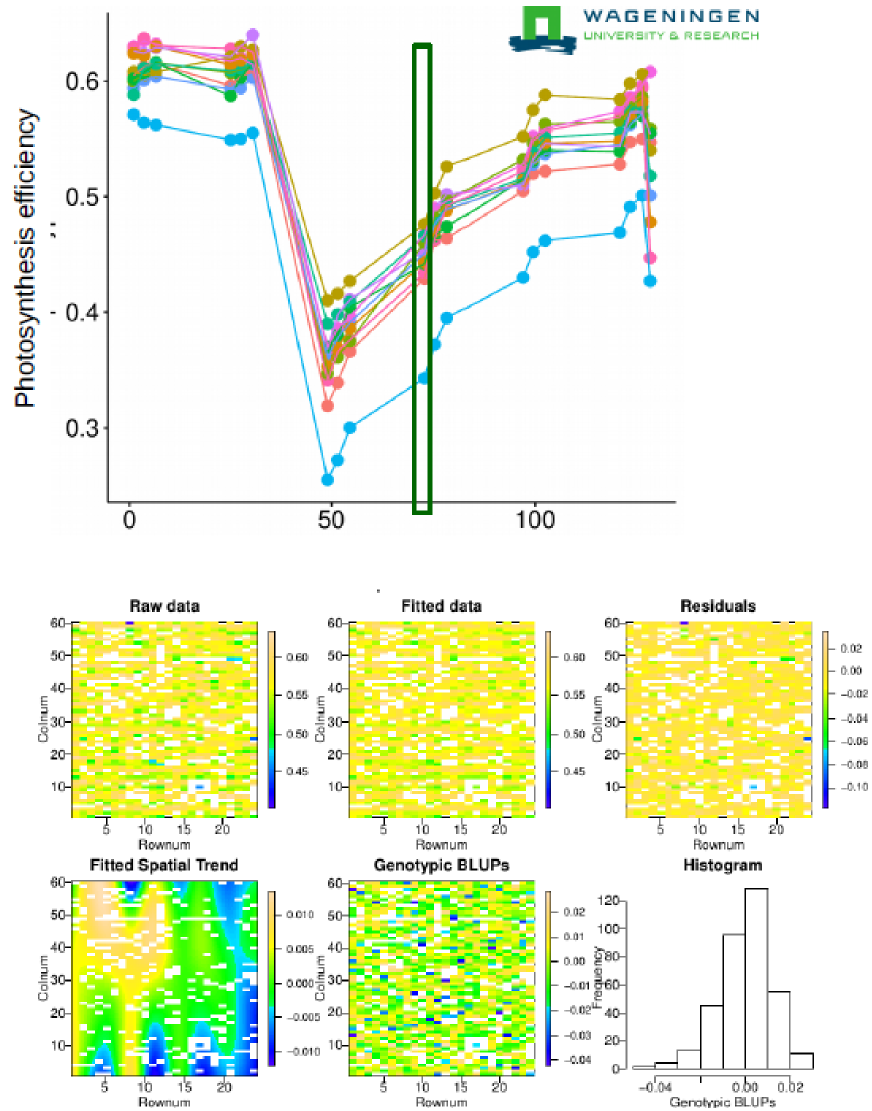
Mark Aarts, Roel van Bezouw

Capacity : 1440 plants



Using SpATS at each time point

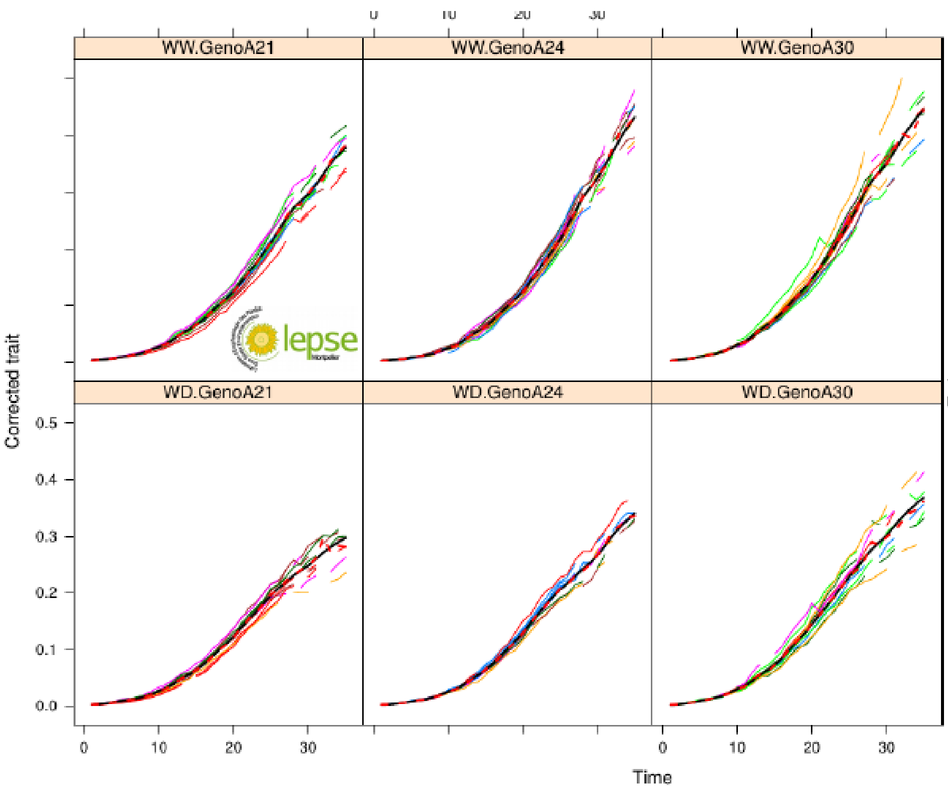


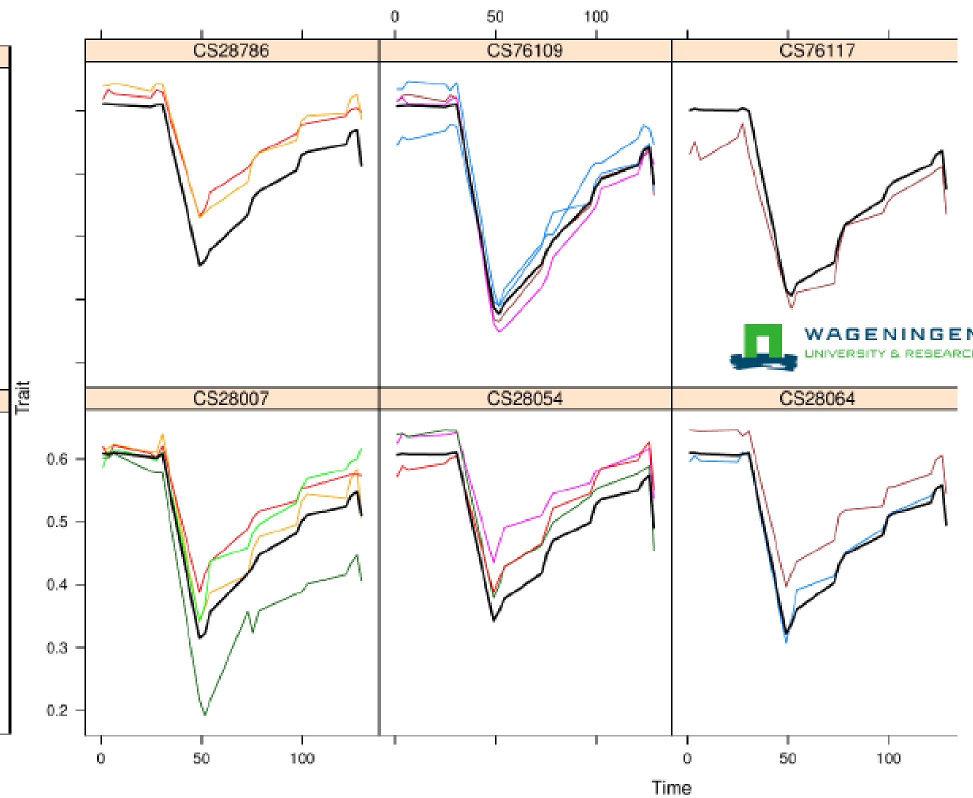


Emilie Millet, María Xosé Rodríguez-Álvarez,
Martin Boer, Fred van Eeuwijk

Estimating the genotypic means (BLUEs or BLUPs)

Using the `predict()` function, we can calculate the genotypic mean per time point and use the new time courses





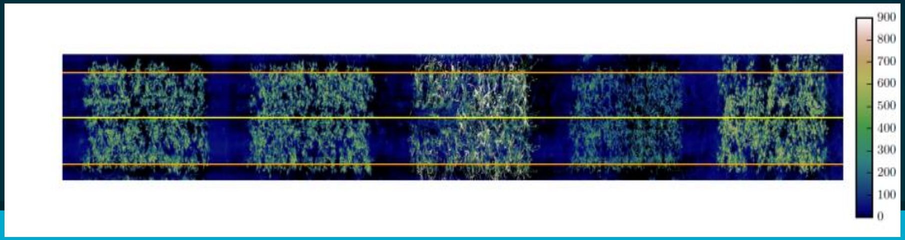
We can also remove the spatial trends and keep the information at the plant level

Emilie Millet, María Xosé Rodríguez-Álvarez,
Martin Boer, Fred van Eeuwijk

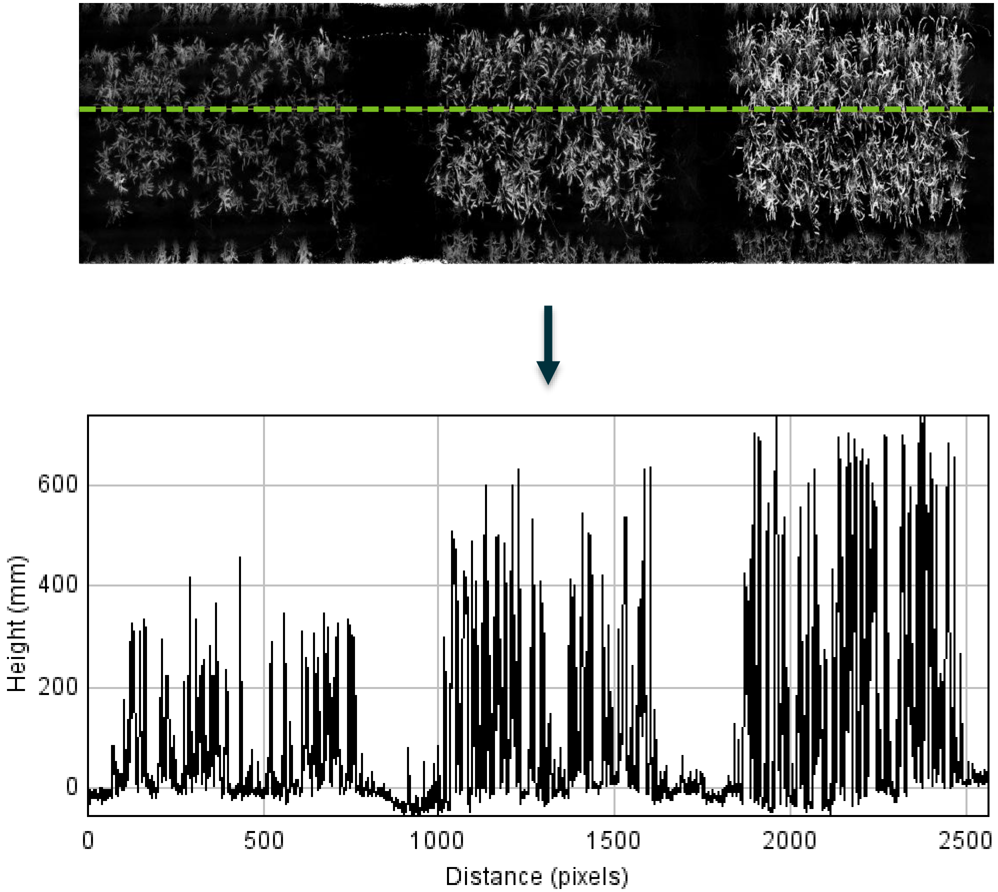
WAGENINGEN UNIVERSITY & RESEARCH

100 years 1918 — 2018

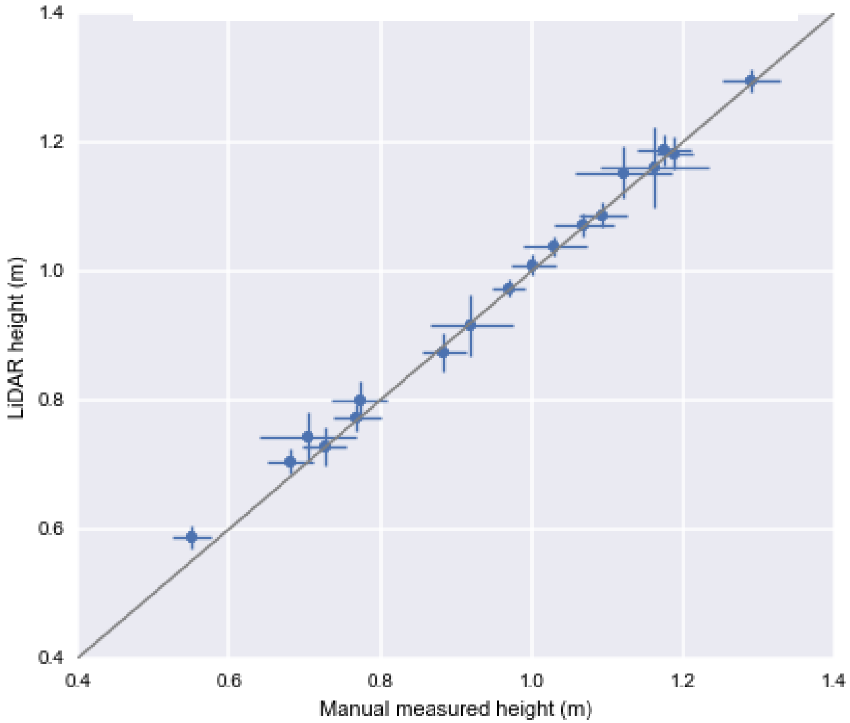
Variability in space and time
- Feature Extraction in the field



Canopy height
[LiDAR on Phenomobile Lite]

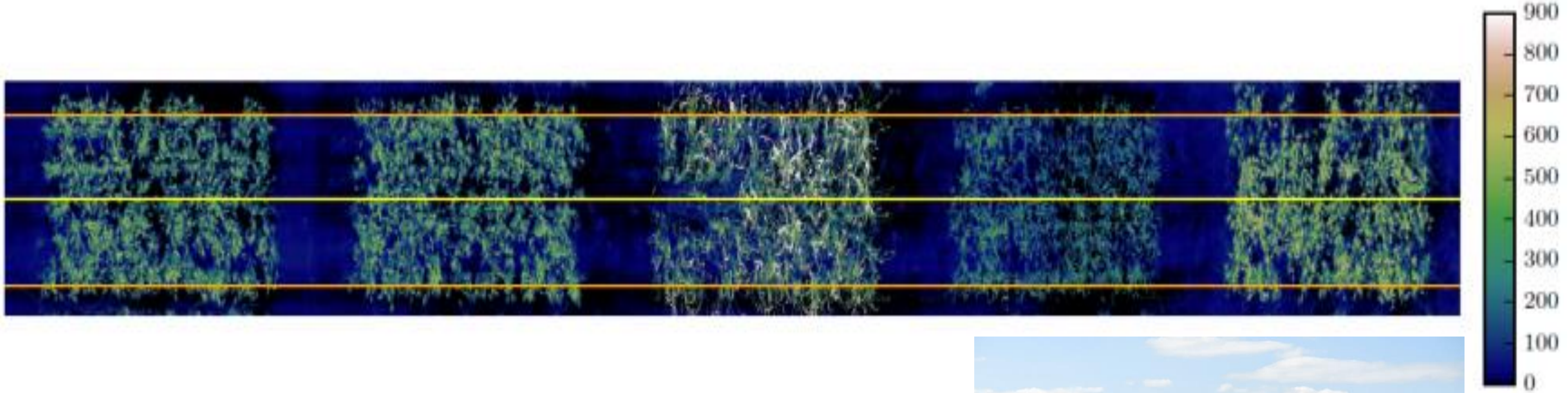


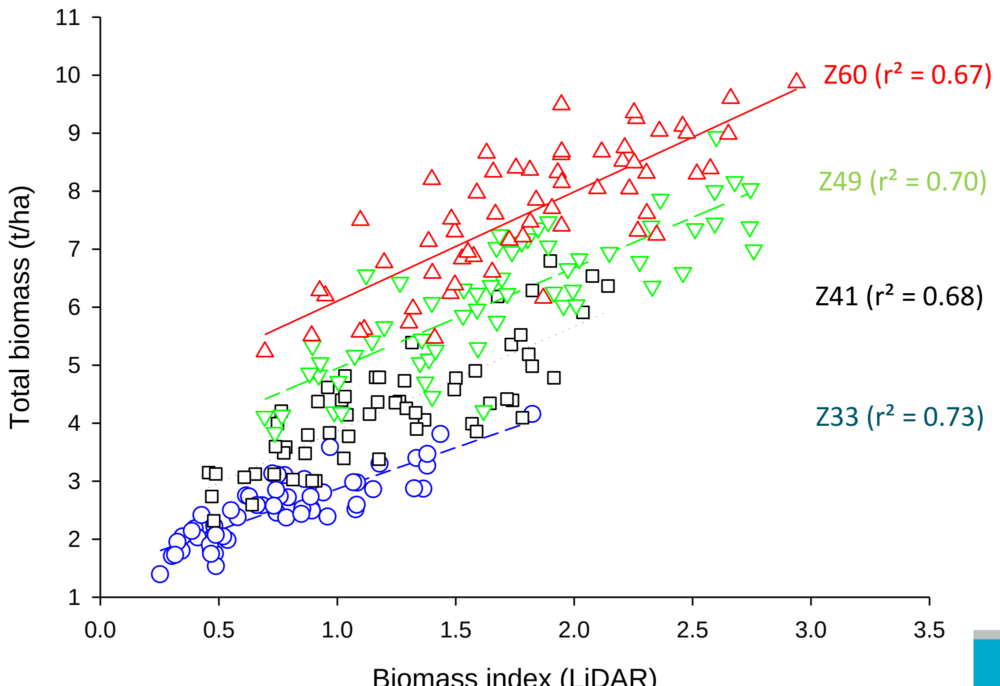
Canopy height from LiDAR validated with manual measurements



Greg Rebetzke, CSIRO

Validation of LiDAR: biomass



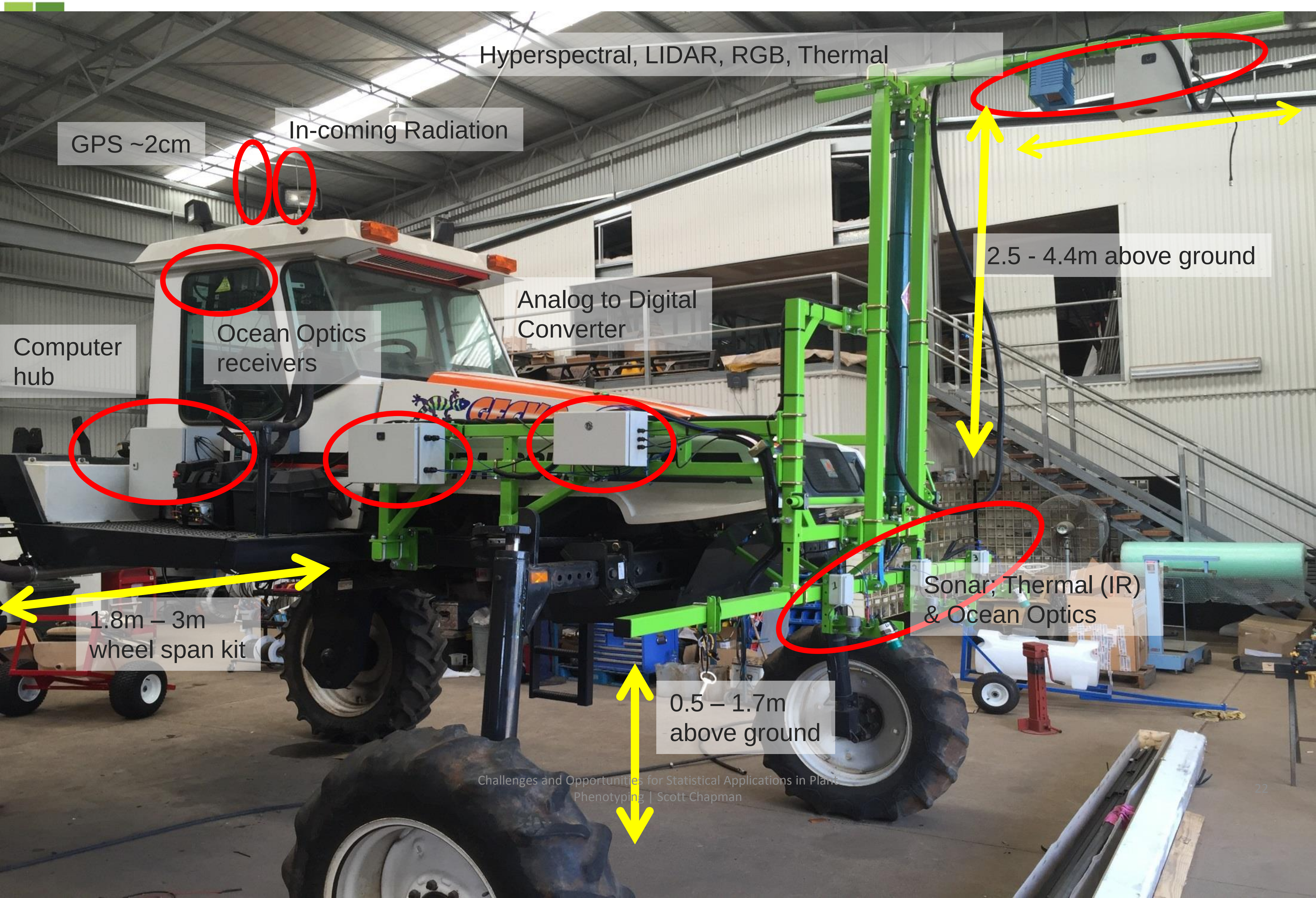


Trait	h^2
Biomass (field)	0.42
'Chlorophyll'	0.64
LiDAR Index	0.88

Greg Rebetzke, CSIRO

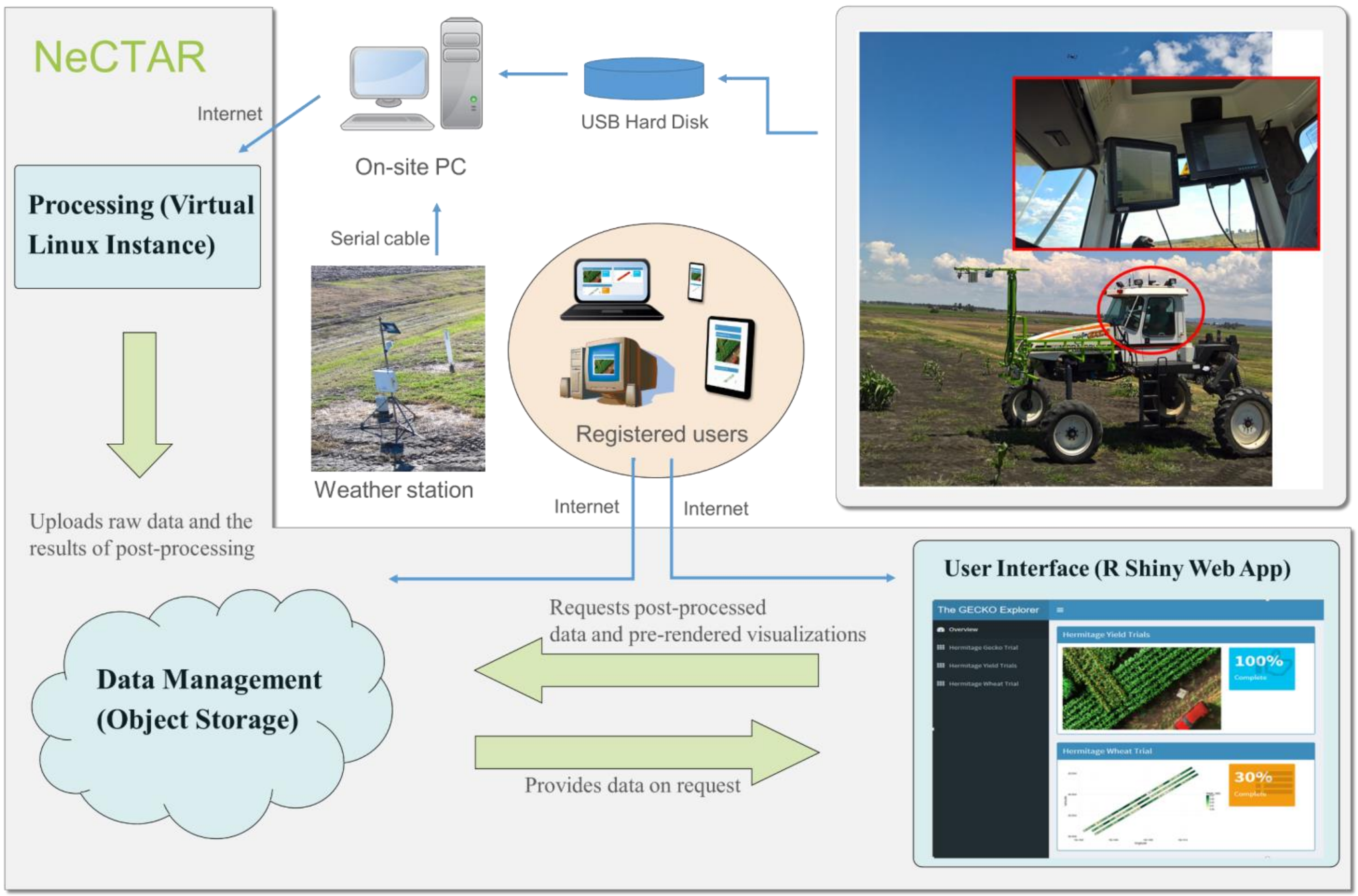
Approximately 5 seconds per plot

GECKO

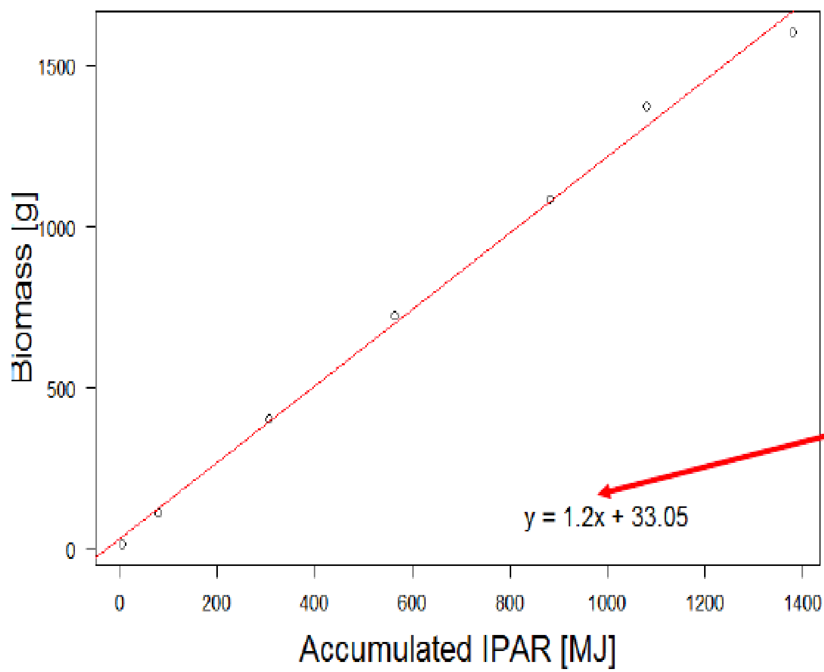


Hyperspectral, LIDAR, RGB, Thermal
GPS ~2cm
In-coming Radiation
2.5 - 4.4m above ground
Analog to Digital Converter
Ocean Optics receivers
Computer hub
Sonar, Thermal (IR) & Ocean Optics
1.8m – 3m wheel span kit
0.5 – 1.7m above ground

Solution: data pipeline



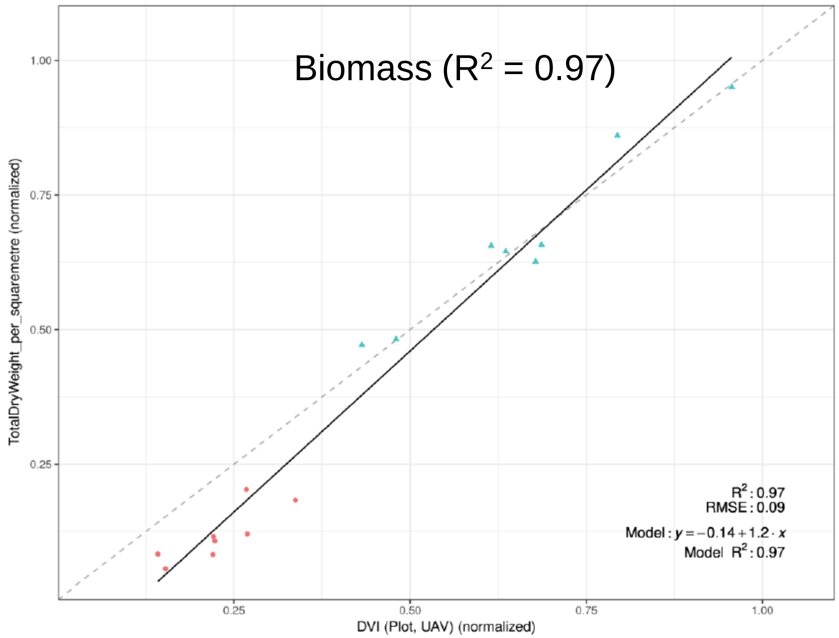
Trait: Radiation Use Efficiency (RUE) is best indicator of photosynthetic capacity



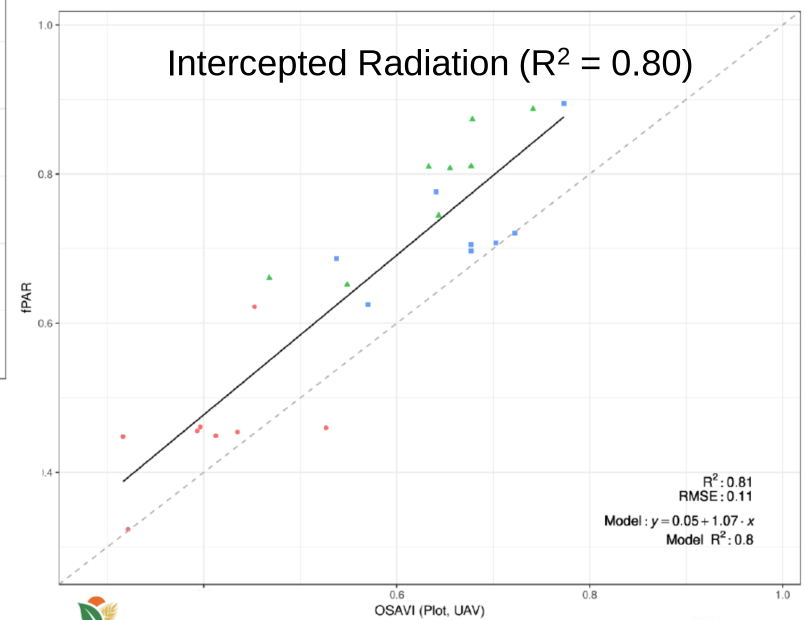
RUE [g/MJ]= Biomass/ Intercepted Radiation

Slope of the relationship between accumulated biomass versus accumulated IPAR over time.

Predicting of Biomass & Intercepted radiation



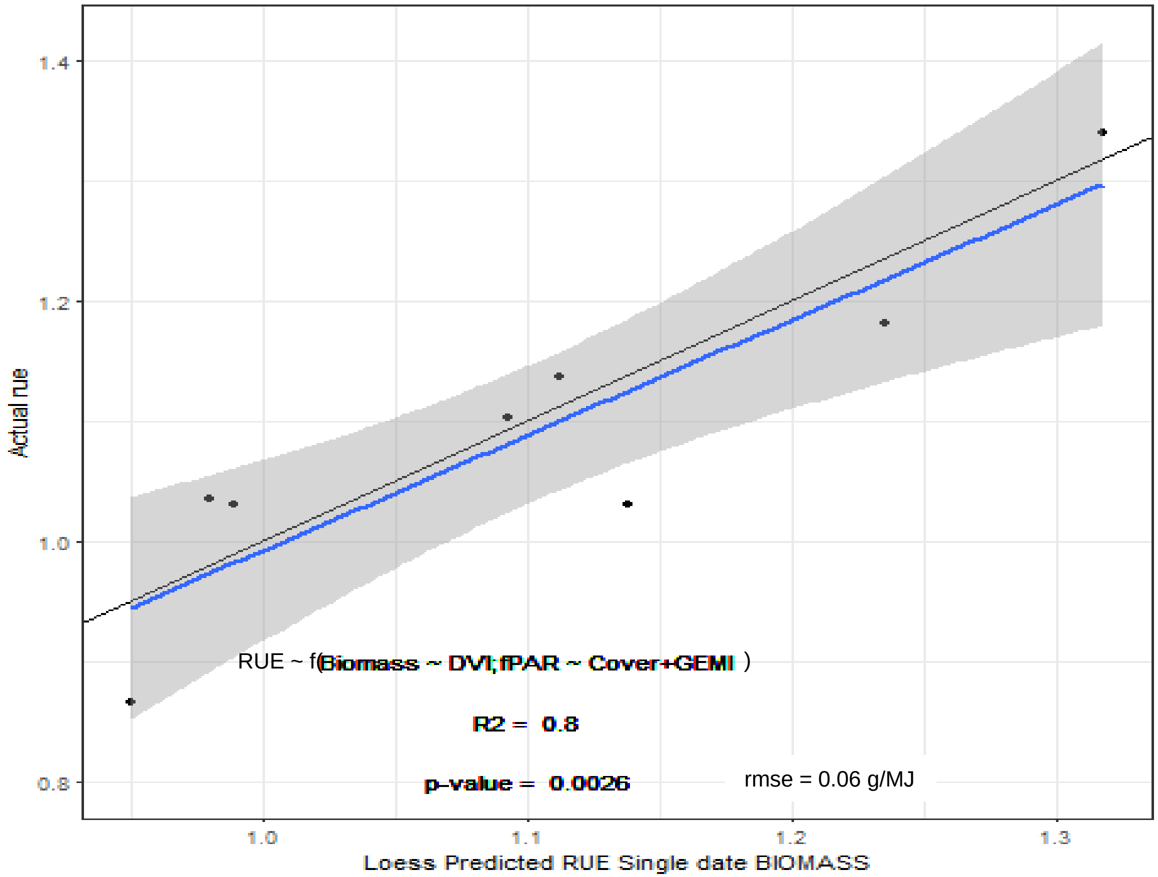
Significantly high predictability of Biomass & IPAR from sensing metrics



translational photosynthesis

The University of Queensland Australia
Queensland Alliance for Agriculture & Food Innovation
Queensland Government

Predicting of RUE (Biomass/FPAR)

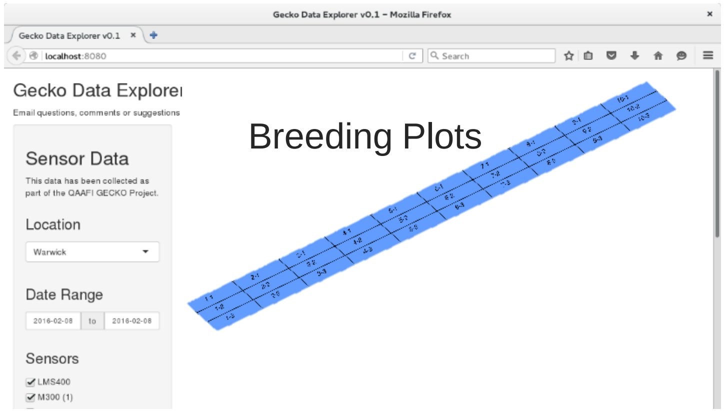


- Significantly high predictability using sensing technologies in predicting RUE
- Next: out scale to other genotypes and environments

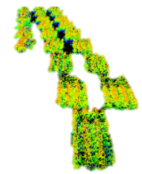
ARC CENTRE OF EXCELLENCE FOR translational photosynthesis

The University of Queensland Australia | Queensland Alliance for Agriculture & Food Innovation | Queensland Government

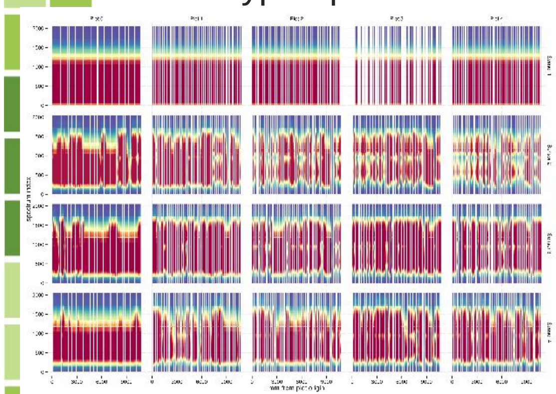
GECKO Explorer

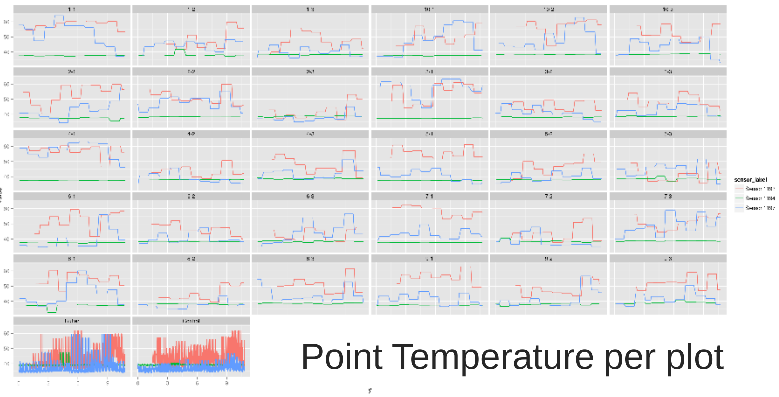


3D Canopy

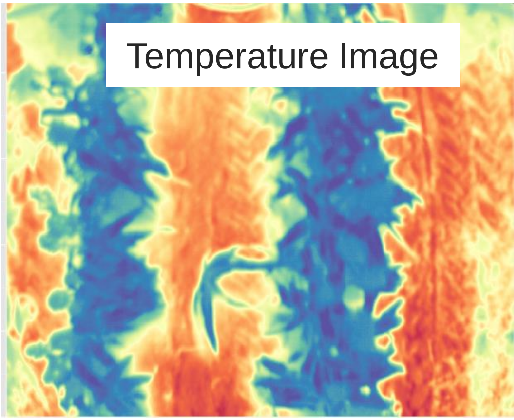


Point hyperspectral

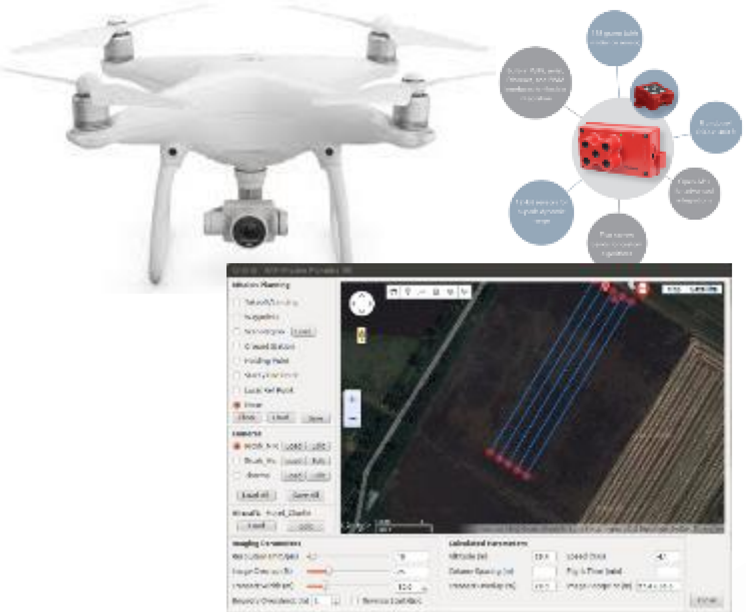




Point Temperature per plot

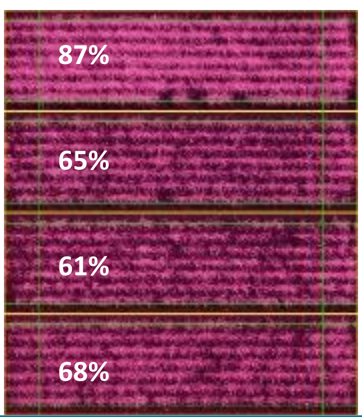


phenocopter.csiro.au: Aerial Imaging Platform

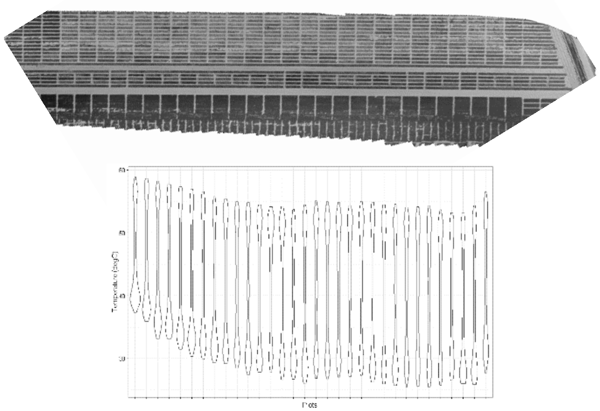


- Equipment specification – camera lens/speed, aircraft flight specs
- Mission planning
- Flights
- Image collation and geo-reference
- Post-processing to generate mosaics and 3D
- Identification of trial images and plots
- Extraction of plot images, straightening and trimming
- Image spectral extraction and analysis
- Experiment analysis of plot-level data

Crop cover

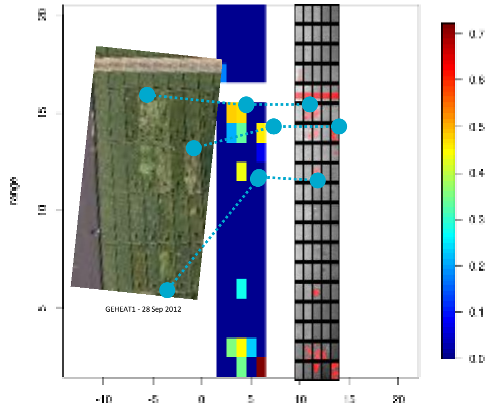


Canopy Temperature



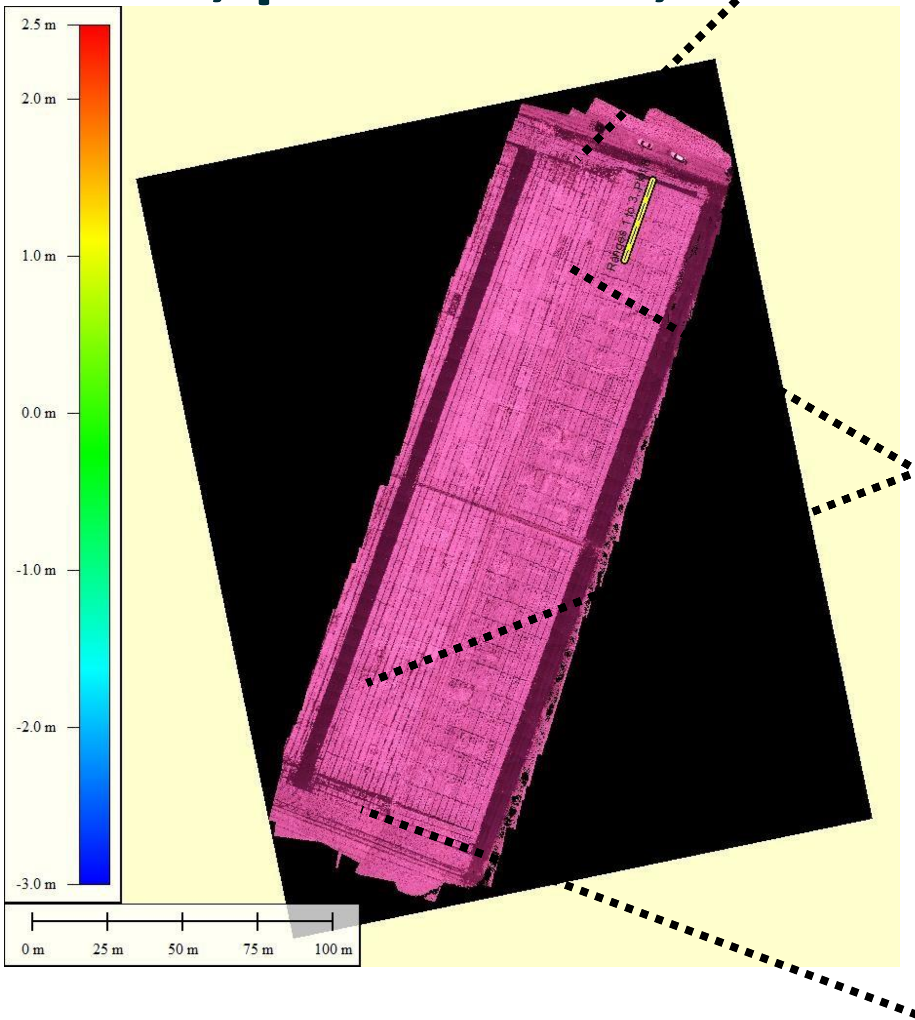
Head counting

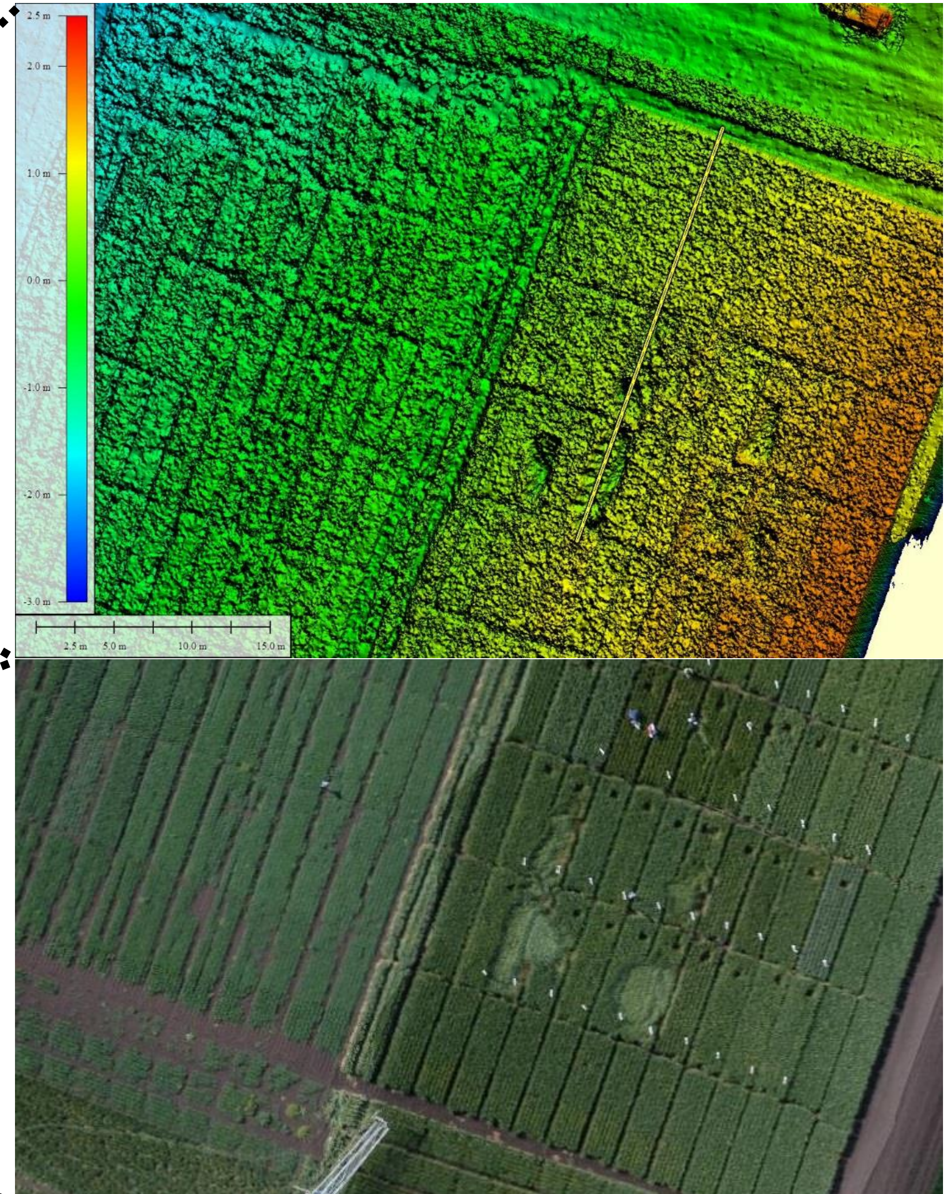
3-D lodging estimates



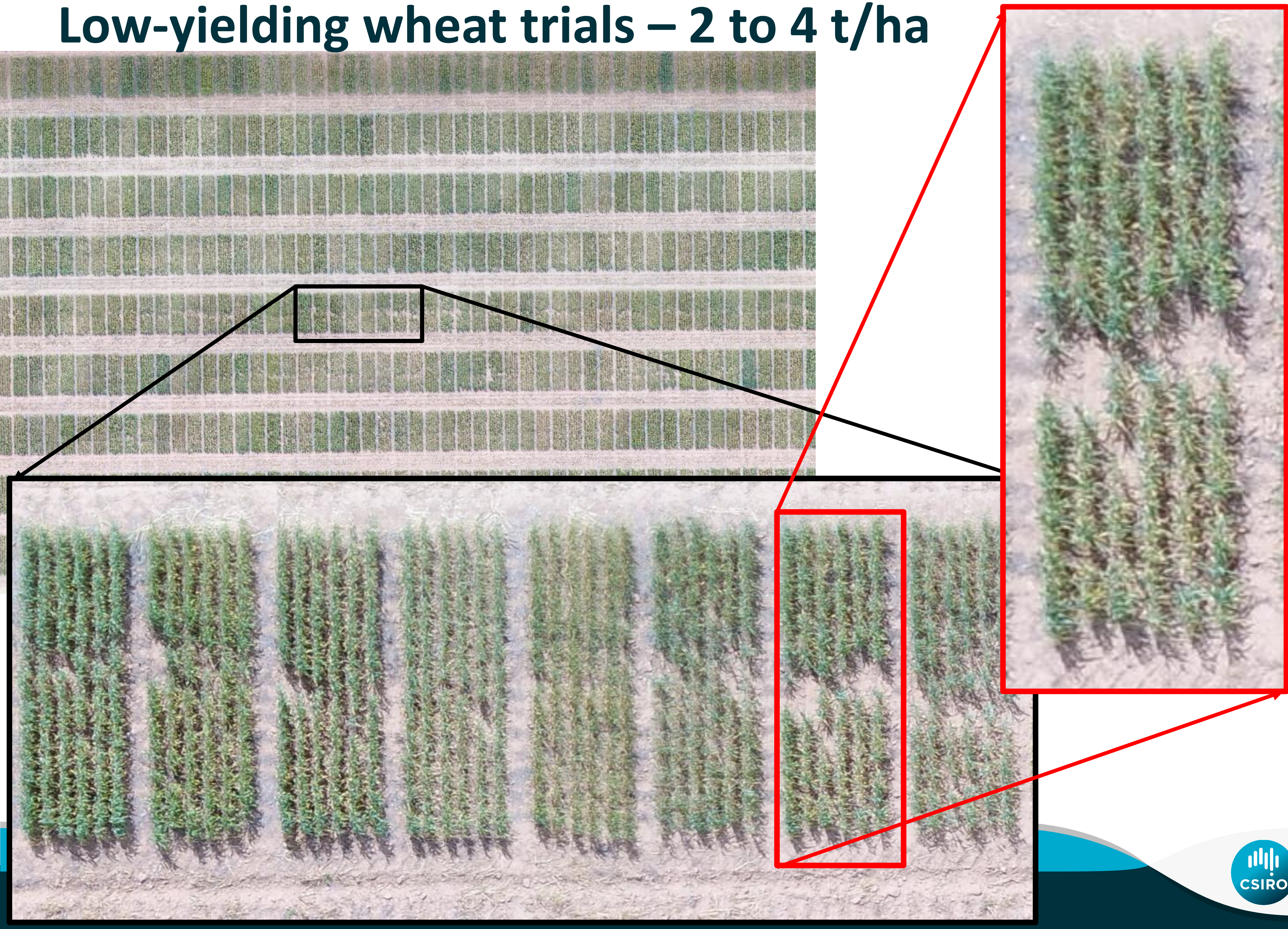
Chapman et al. 2014 MDPI Agronomy **phenocopter.csiro.au**

Mosaic processing to RGB, point cloud, DEM





Between and within-plot variability in Low-yielding wheat trials – 2 to 4 t/ha



Moderate yielding canola – 3-4 t/ha

Within-plot variability - ‘Gappiness’

- Calculate a ‘Gappiness’ index to use as a covariate for yield
- Four ‘environment populations’ within plots
  - Bordered plants – the ones we want to estimate
  - Unbordered plants – usually with higher yield than bordered
  - Compromised plants – those in proximity to a negative soil impact
  - Gaps – no plants
- Can we apply a pixel based analysis to compute row length of plants in these environments and adjust the harvester result?

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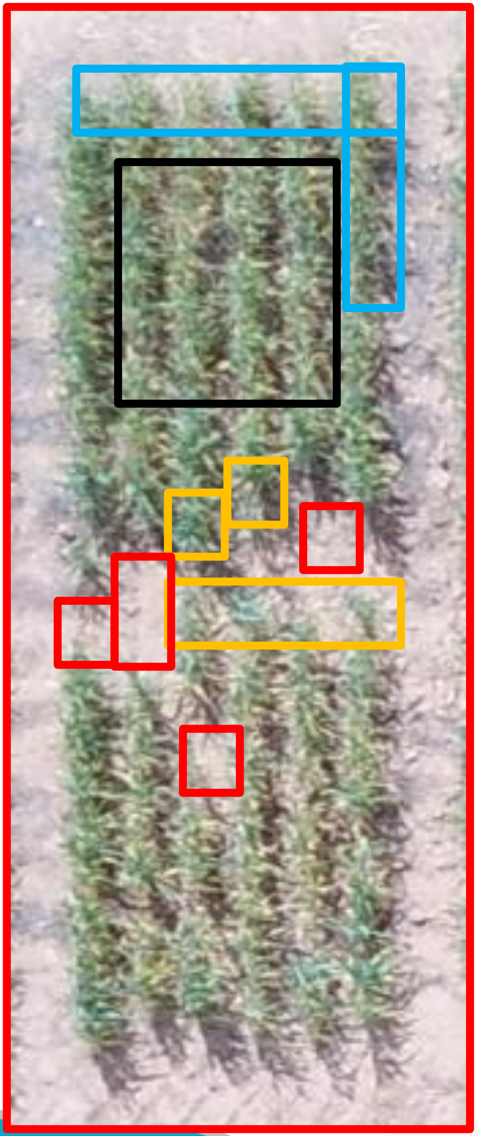
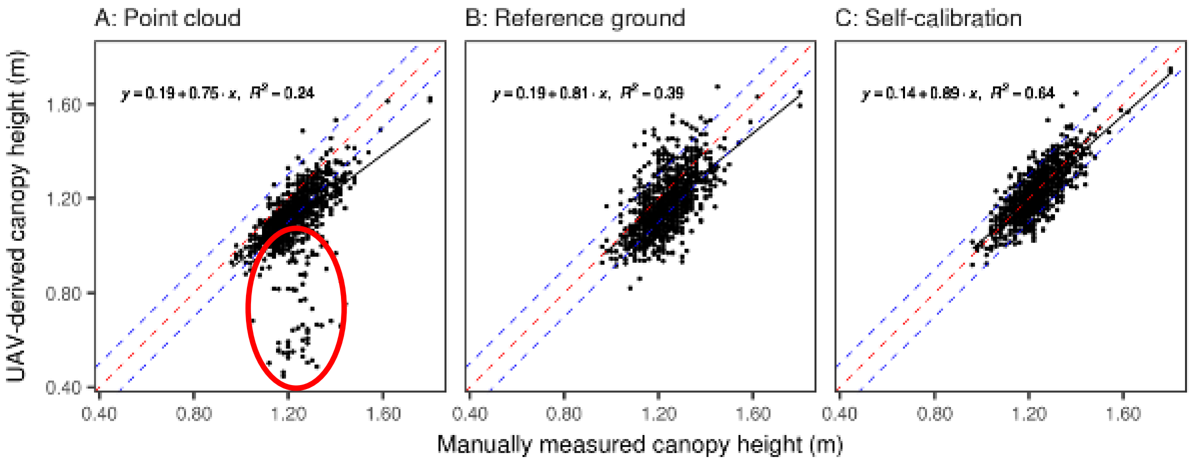


Estimating plant height in sorghum - a reliable self-calibrating method



Challenge

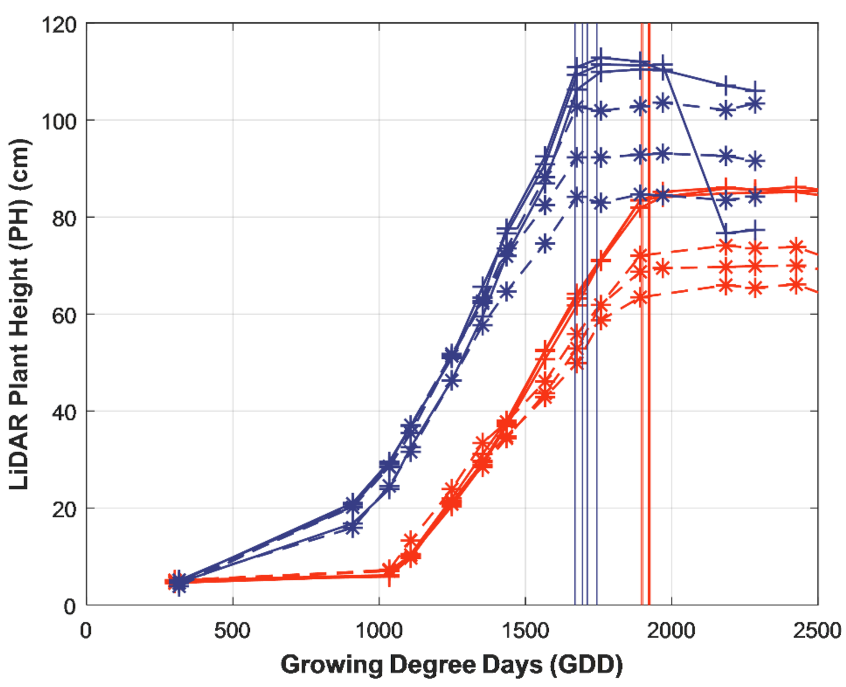
- measure 1200 plots (1 person day by conventional means)
- Ground-referenced point cloud is not precise (left figure)

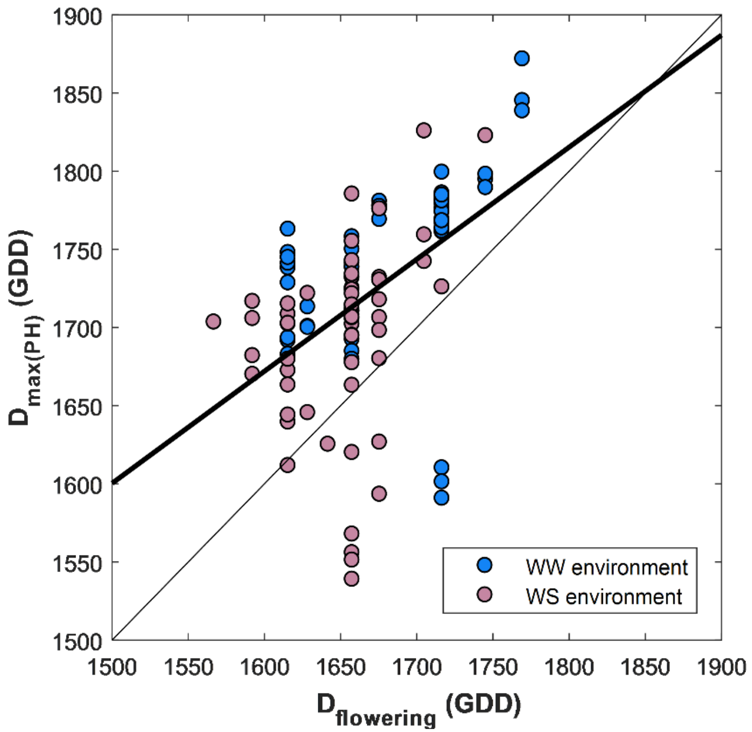
Solution

- 20 min flight + 20 min ground measurement (measuring the red plots in the field)
- <1h software processing
- **Repeatability = as good as ground measurement = ca. 0.74**

Hu et al 2018 European J Agronomy

Indirect traits - Estimating flowering time from plant height





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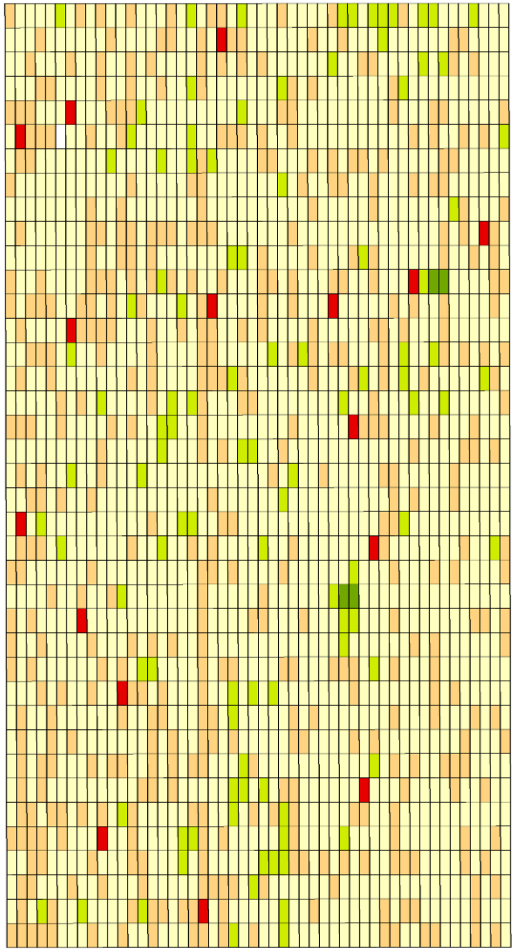
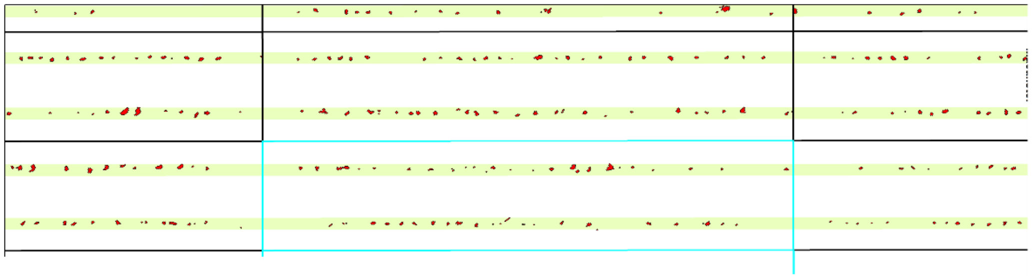
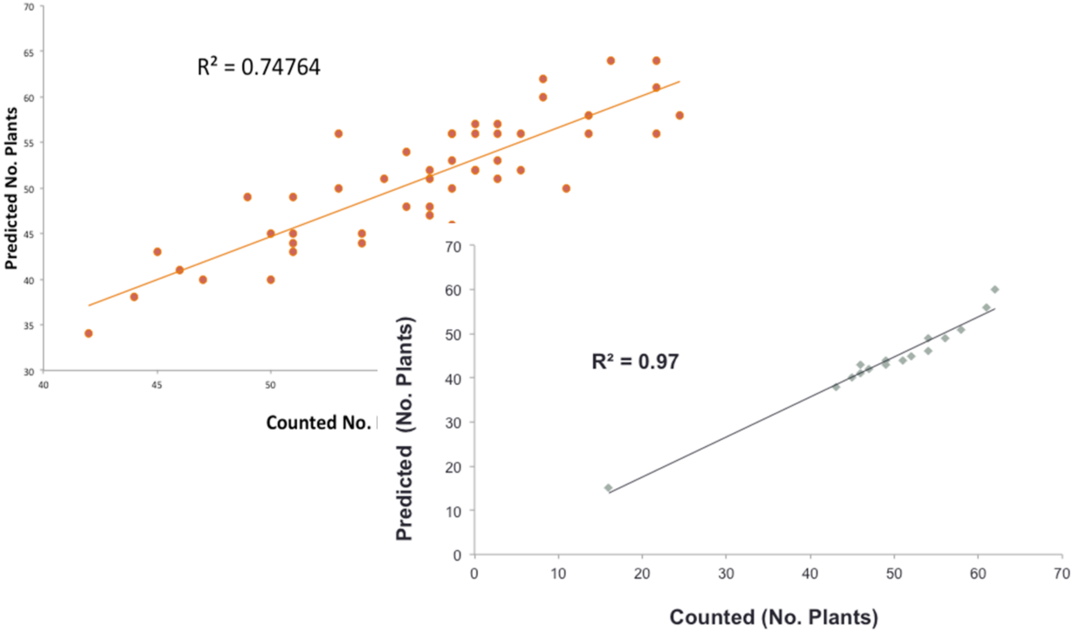
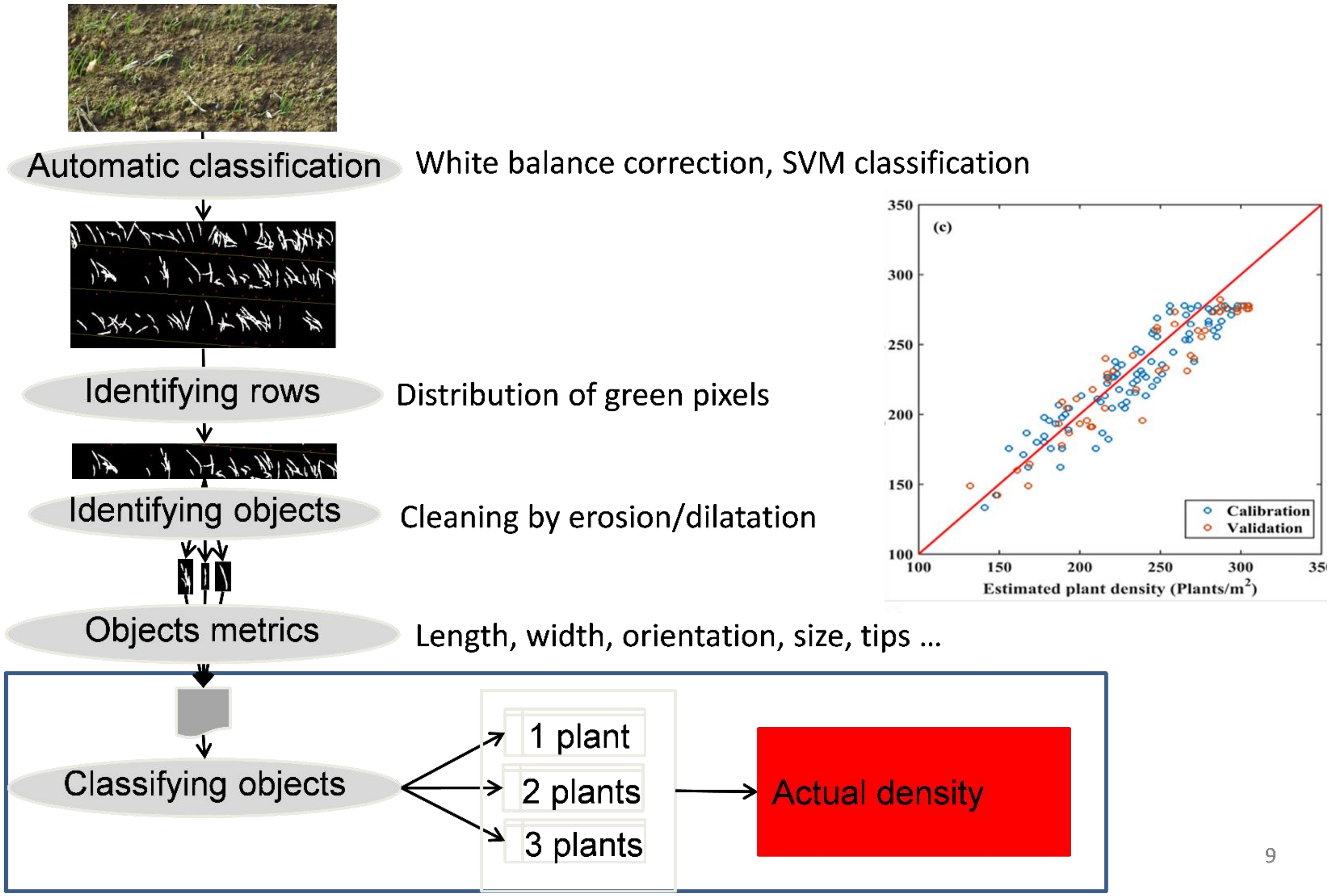
Counting plants – correlation method





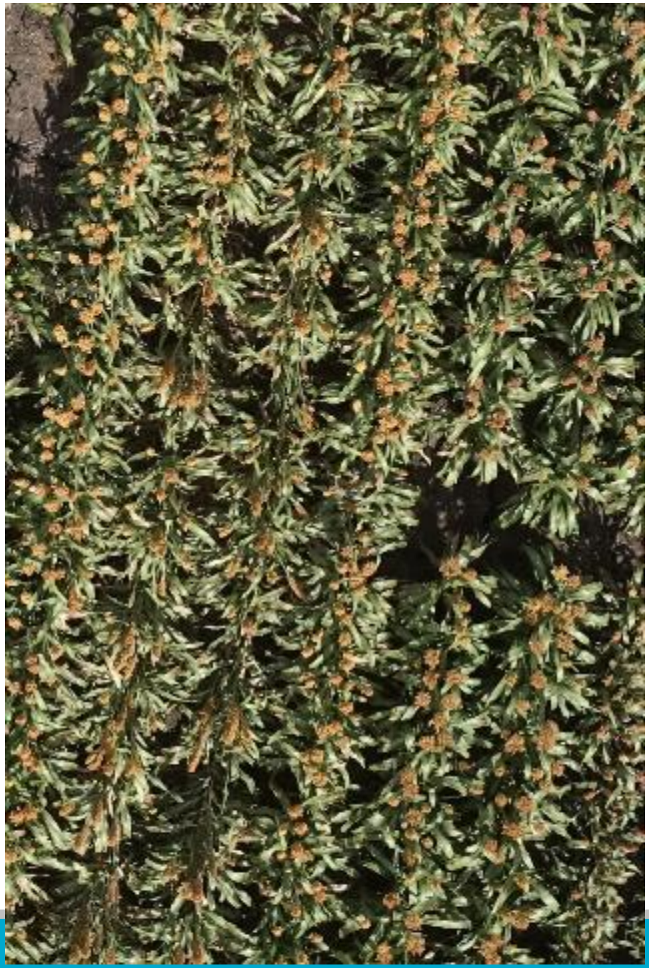


Counting plants – direct object estimation



Counting heads in sorghum to estimate tiller number

- Decision tree approach plus weighting for **head size**
- Counting accuracy ~ 90%

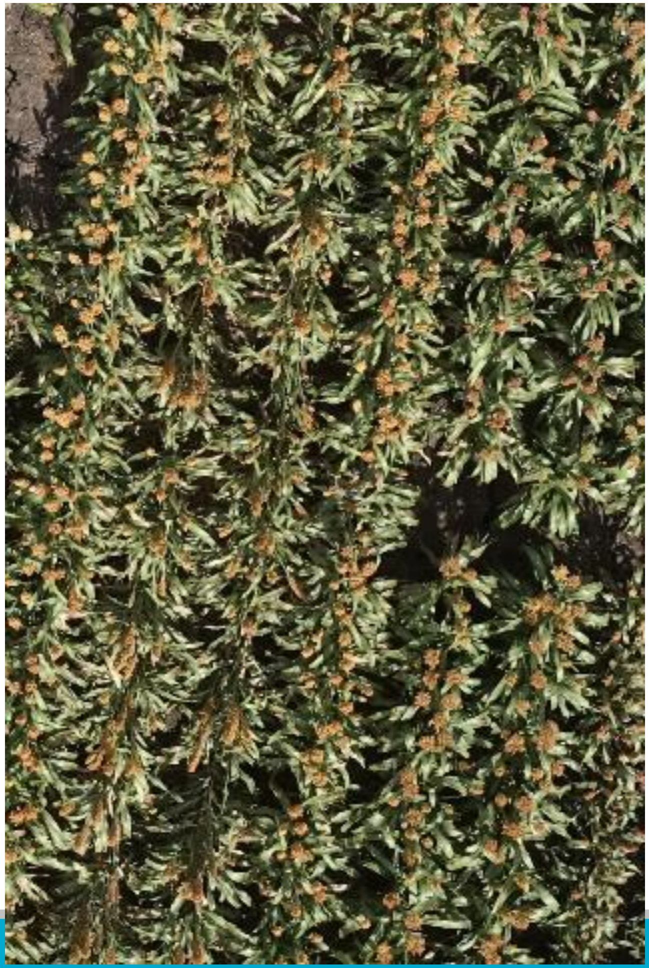


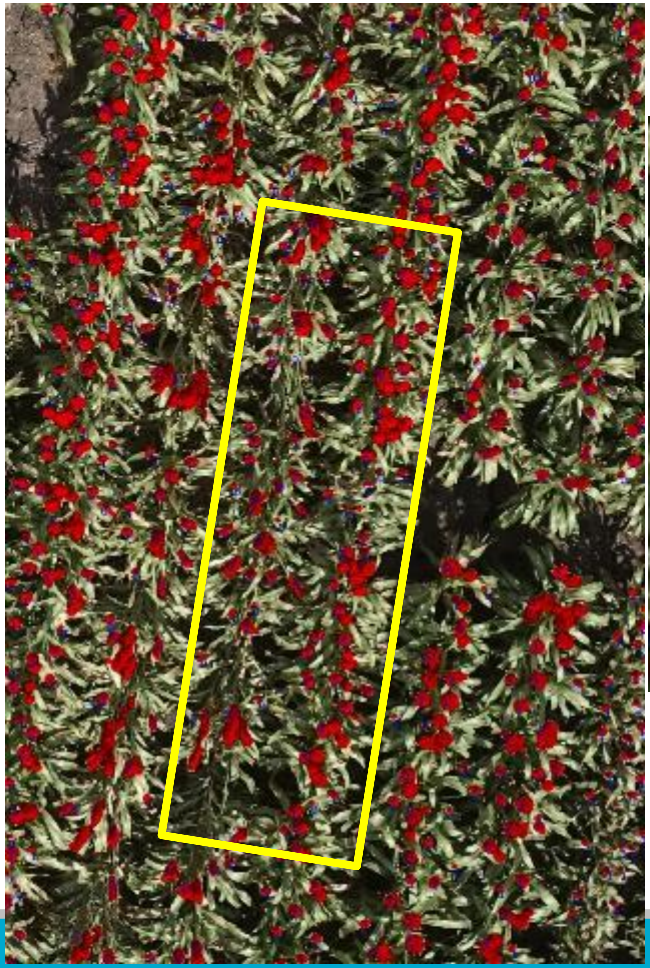
UQ, CSIRO and U Tokyo

(submitted)

Head counting in sorghum to estimate tiller number

- Decision tree approach plus weighting for **head size**
- Counting accuracy ~ 90% **BUT plot-based accuracy ~ 60%**





UQ, CSIRO and U Tokyo

(submitted)

Size input data	Modelling step	Input	Model / strategy	Output	Model dependence output
	Feature extraction	Multiple pixels/wavelengths for each plot, genotype, time point and environment (low level traits)	Segmentation Organ Tracking Indices (e.g. NDVI) Machine learning	Trait value for each plot, genotype, time point and environment	
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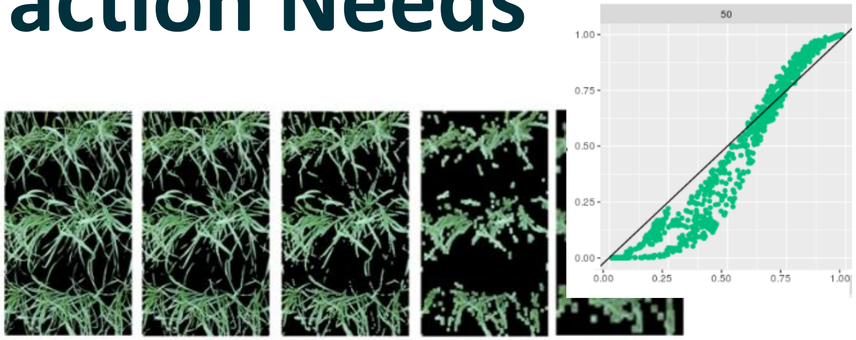
- **Feature extraction**
- Design/spatial trends
- Dynamic modelling
- Modelling Env effects
- Target Trait Prediction

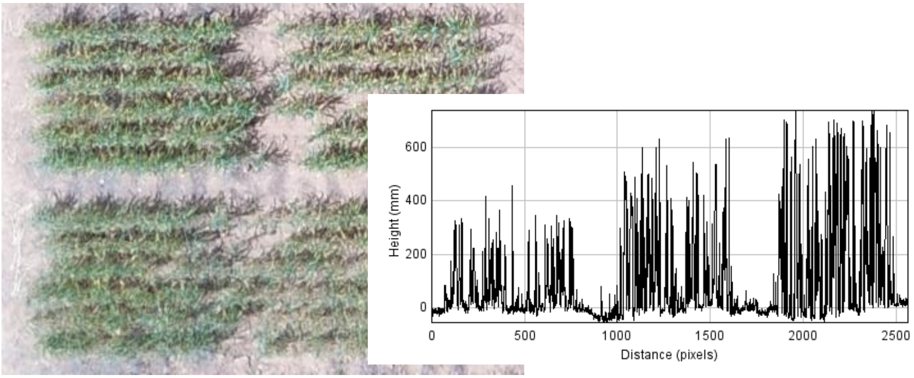
van Eeuwijk et al 2018 Plant Science (online)
https://doi.org/10.1016/j.plantsci.2018.06.018

Summary of Feature Extraction Needs

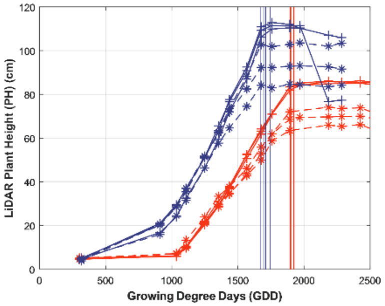
- Ground cover - correction of pixel-scaling
  - During segmentation, adjusting for dimensions of scene elements and pixel size, e.g. leaf width at different scales



- Utilise high-density within-plot data
  - Estimates of co-variates or yield adjustments from within-plot pixel data
  - Sub-sampling of areas, counts
  - Extraction of traits from distributions



- Longitudinal models to
  – Extract phenotypes as statistical model parameters
  – Estimate inflections, rates and maxima



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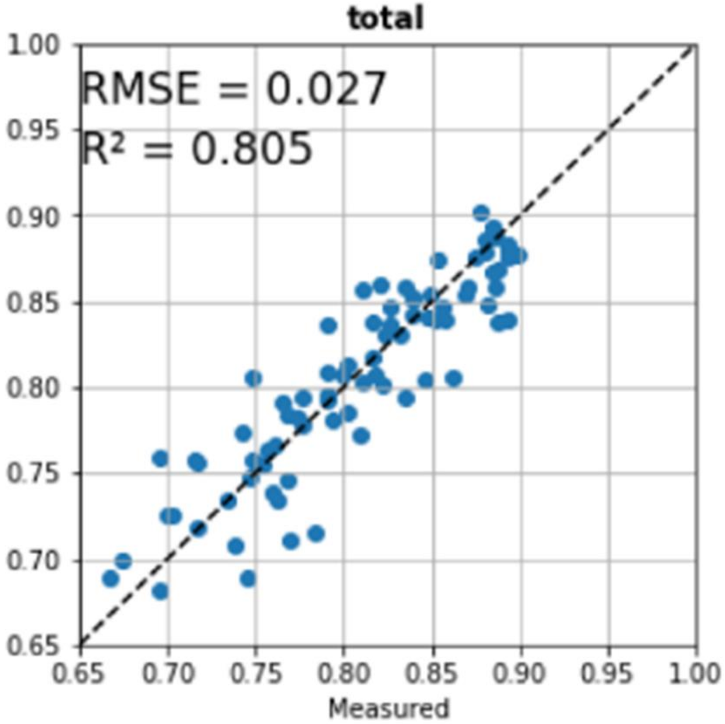
Indirect traits – Fractional light interception



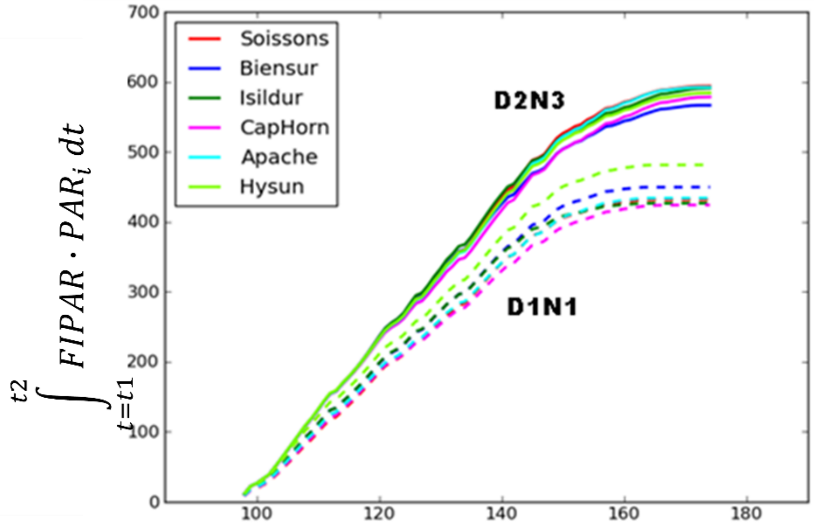
- Measurement of FIPAR over a set of microplots
  From DHP measurements
- Calibration of a regression for each flight:

$$FIPAR = \alpha_0 \sum_{i=1}^{6} \alpha_i \cdot R_i \,/\, R_{ref}$$

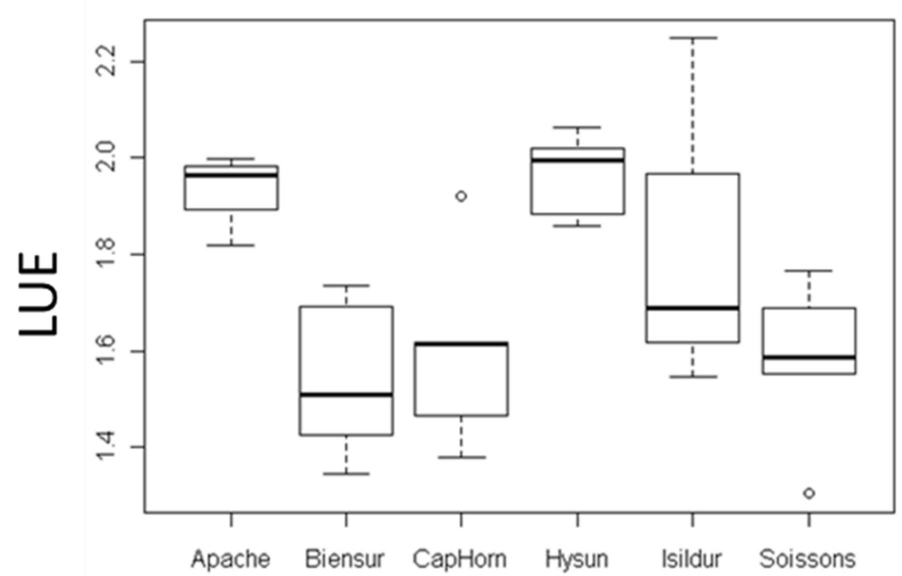
Fred Baret et al

Indirect traits – estimating RUE

$$\Delta_{t1}^{T2} Biomass = LUE \int_{t1}^{t2} FIPAR \cdot PAR_i \cdot dt$$



Time since sowing



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Integrated crop reaction: Green Area Index

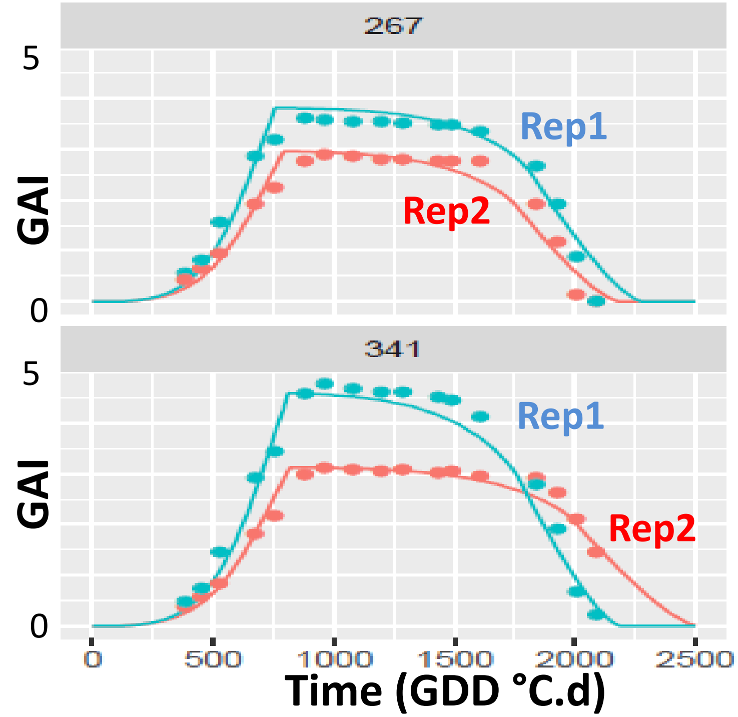
Modelling the GAI time course

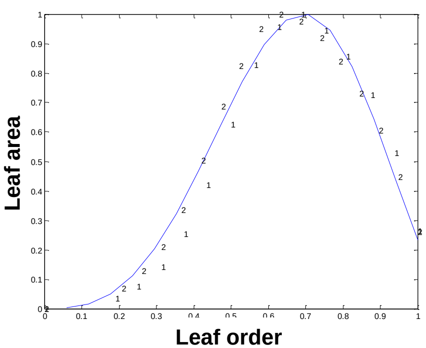
Assumptions:

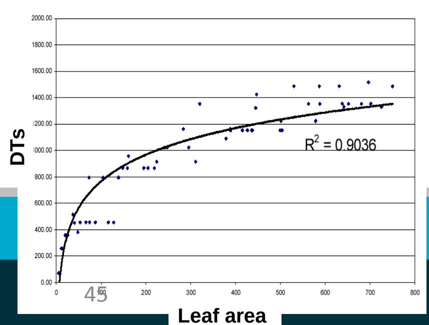
- Leaf are emitted at regular intervals (phyllochrone)
- Leaf size depends on leaf order
- Senescence depends on leaf area

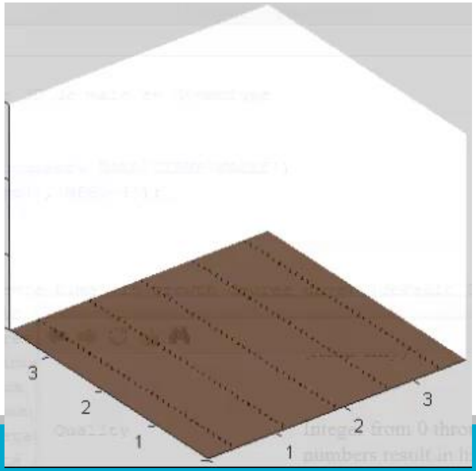
6 parameters:

- Plant density
- Total number of leaves
- Area of largest leaf
- Emergence date
- Phyllochrone
- Senescence





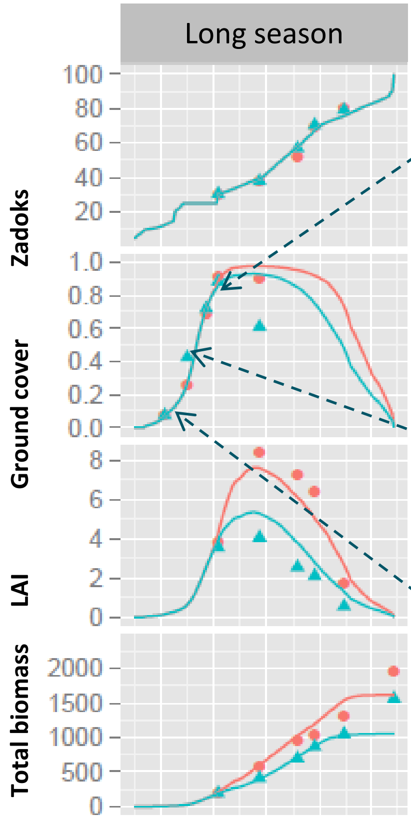




Robust model Allows to:

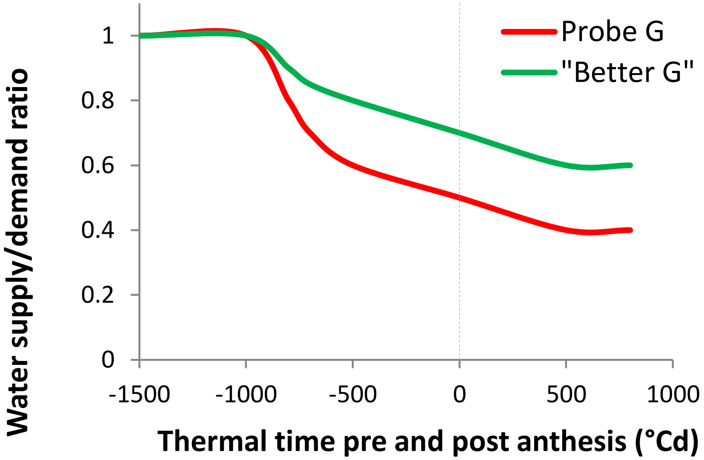
- **Use the parameters as traits**
- **Compute metrics derived from the GAI time course:**
  - **Slopes (growth, senescence)**
  - **Specific dates**
  - **integrated values**

Integration of UAV and crop simulation models e.g. using GC/canopy condition + model in order to estimate seasonal water use



1. UAV - monitor ground cover
2. Calibrate simulation model with UAV ground cover
3. Compute seasonal changes in biomass and water use pattern.

This is VERY hard to measure directly



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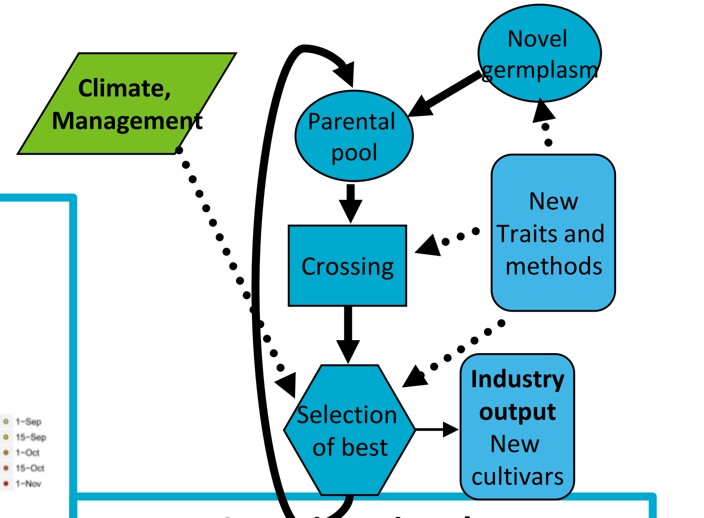
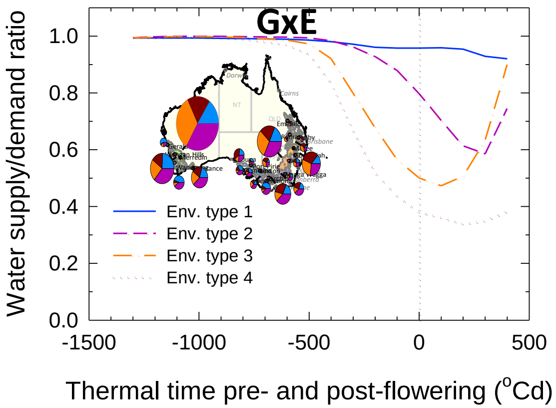
Roles for Models in breeding

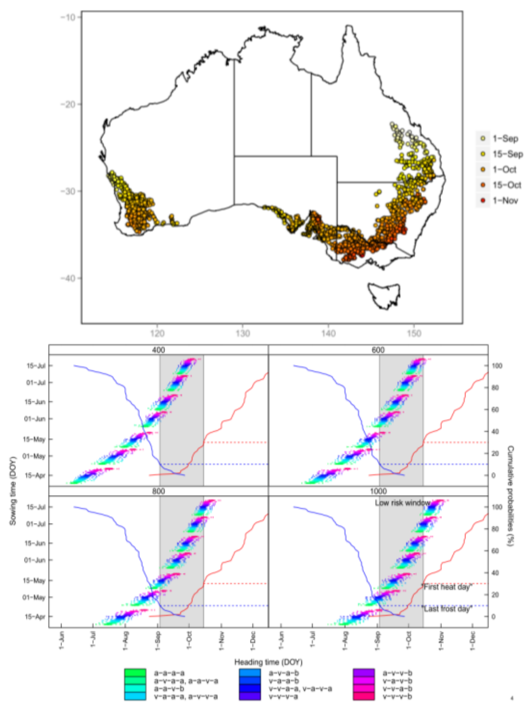


Environment characterisation - Drought patterns to interpret GxE



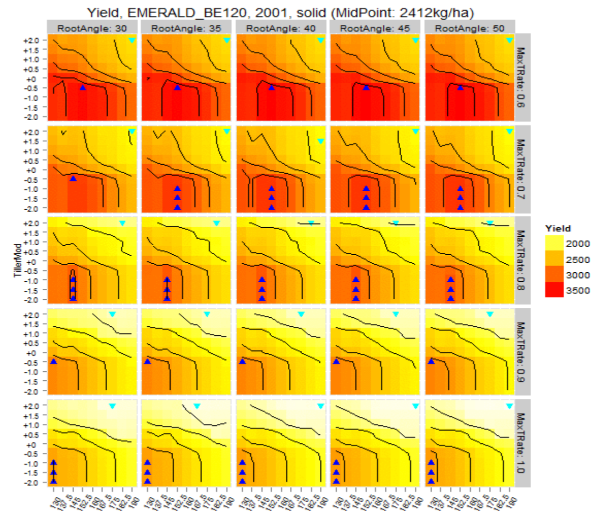
Muchow et al 1996
Chapman et al 2000 AJAR
Chenu et al 2013 New Phyto
'Envirotyping' Cooper et al 2014

Prediction of Adaptation



'Breeding for the future'
Chapman et al 2012 CPS
Zheng et al 2012 GCB, 2013 JXB, 2015 GCB, 2015 JXB

Genetic trait value



Chapman et al 2002, Hammer et al 2006, Chenu et al 2009
Hammer et al 2010, 2015
Cooper et al 2014, 2016,
Messina et al 2015

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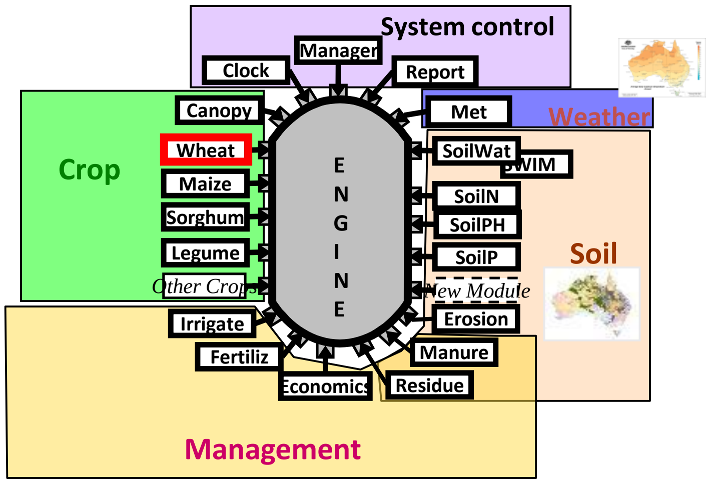
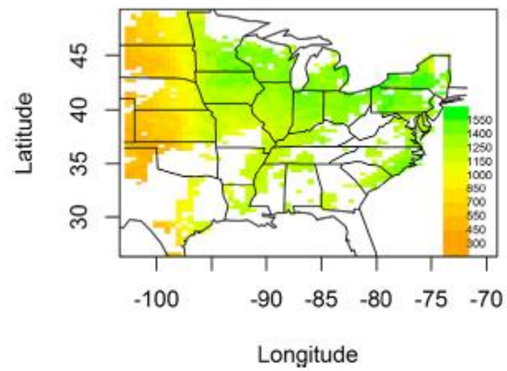
APSIM Simulation – Data Analytics

M3.20	Develop and validate physically based models to predict plant response and biomass	**Calibrate APSIM sorghum growth model for biomass sorghums.**	18/02/2018	75
M3.21	Develop and validate physically based models to predict plant response and biomass	**Use scenario analysis tool** to identify trait combinations that maximize biomass productivity in a range to production environments and **create phenotyping analysis workflow** for comparisons of measured and predicted crop performance.	23/08/2018	5
M3.23	Predict terminal biomass yield of individual lines from field data	Evaluate sensor capability to predict biomass yield using ground-based and aerial platforms using precision and recall metrics.	18/02/2018	2



- Calibrate APSIM using field observations
  - Propose how remote-sensing can do this
- Project trait value effects across corn belt
  - Determine mean and variability of performance for different traits



Messina et al (2015) Agron J 107:1978–1986

IBM

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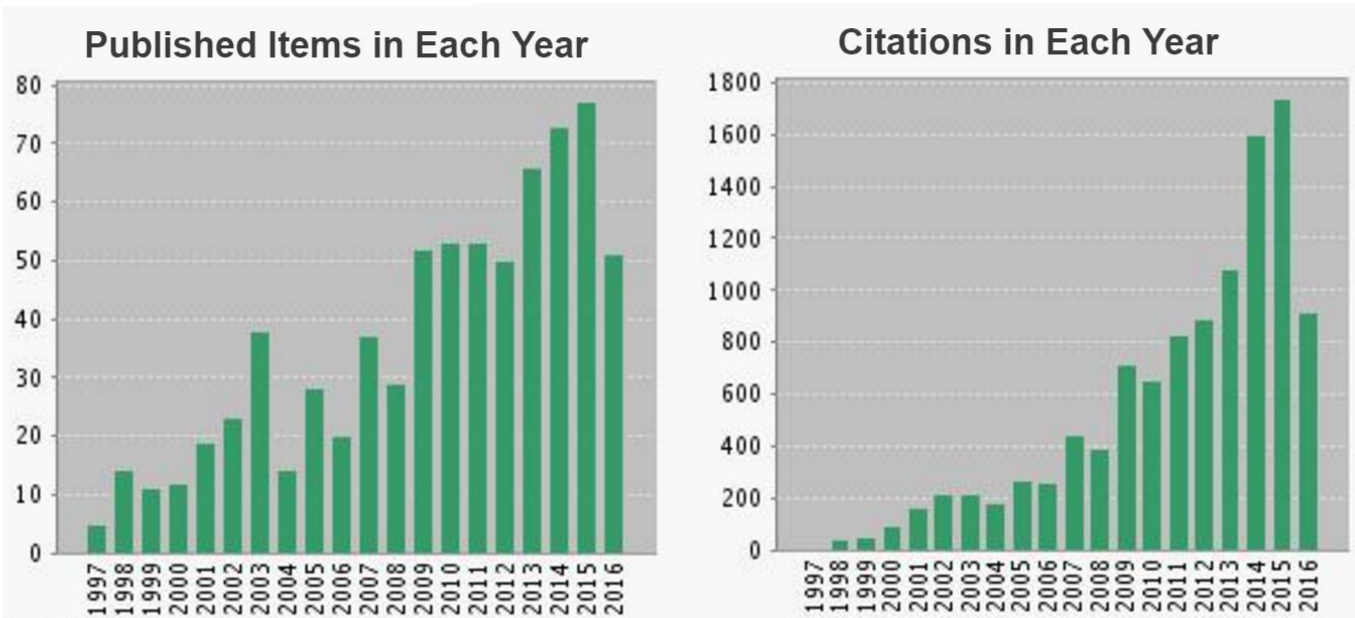
APSIM – entrenched in the literature



Results found:	744
Sum of the Times Cited [?] :	10778
Sum of Times Cited without self-citations [?] :	7291
Citing Articles [?] :	5402
Citing Articles without self-citations [?] :	4837
Average Citations per Item [?] :	14.49
h-index [?] :	48

ISI Web of Knowledge, 30/6/2016

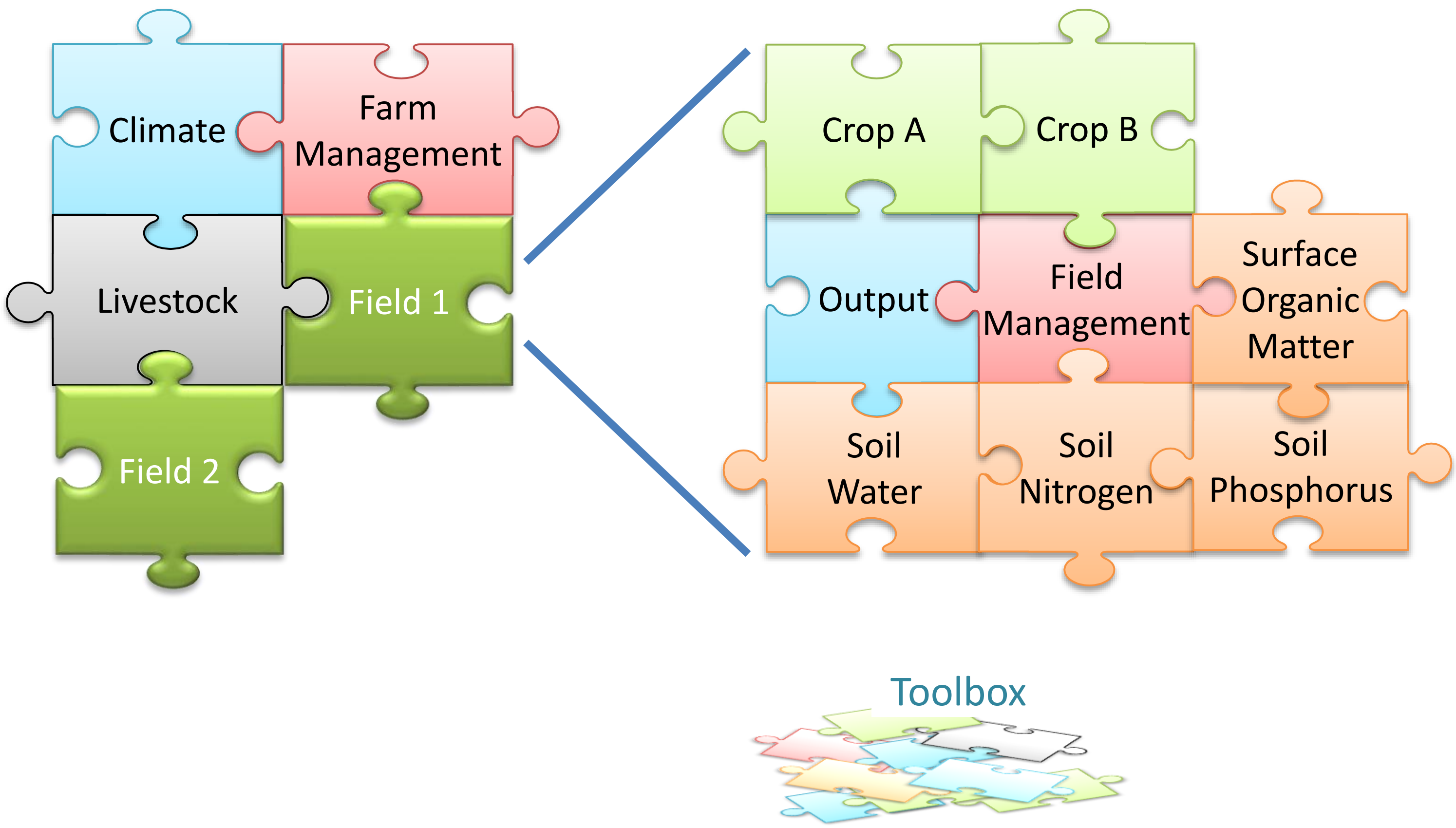
APSIM - Guiding philosophy

The soil provides a central focus, crops, seasons and managers come and go, finding the soil in one state and leaving it in another

Features:

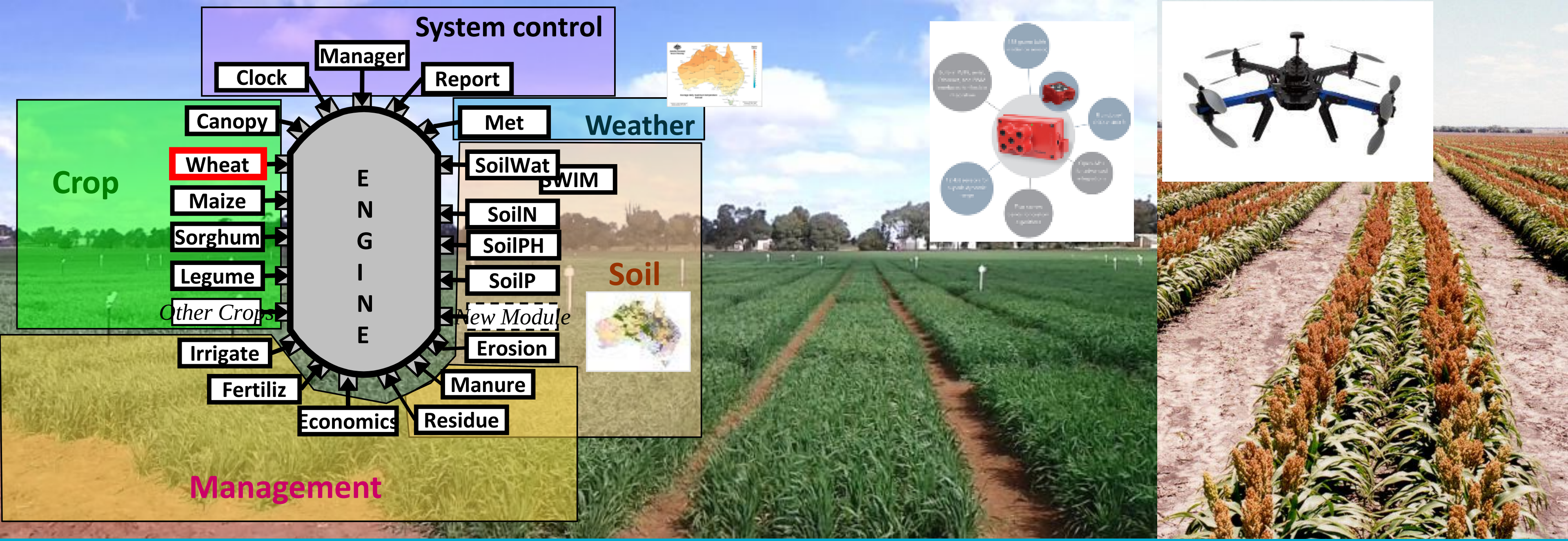
- ✓ Mechanistic growth of crops, pastures, trees, weeds ...
- ✓ Dynamics of populations (e.g. weed seedbank)
- ✓ Key soil processes (water, solutes, N, P, carbon, pH)
- ✓ Surface residue dynamics & erosion
- ✓ Rain fed or irrigated systems
- ✓ Range of management options
- ✓ Crop rotations + fallowing + mixtures
- ✓ Short or long term effects
- ✓ Multi-point simulations
- ✓ High software engineering standards
- ✓ Supports multiple languages
- ✓ Links to livestock modules

APSIM – Plug-in / Pull-out modularity



APSIM modules *(Holzworth et al 2014)*

APSIM model	Origin / references	APSIM model	Origin / references	APSIM model	Origin / references
Plants:		Mungbean	(Robertson et al., 2002)	***Soil:***	
AgPasture	(Li et al., 2011a)	Navybean	(Robertson et al., 2002)	DCD	(Cichota et al., 2010)
Bambatsi		Oats	(Peake et al., 2008)	Erosion	(Freebairn et al., 1989; Littleboy et al., 1992)
Barley	(Manschadi et al., 2006)	Oil Mallee		Nitrogen (SoilN)	(Probert et al., 1998a)
Broccoli	(Huth et al., 2009)	Oil Palm	(Huth et al., 2014)	Phosphorus	(Delve et al., 2009)
Butterfly pea		Pasture	(Moore et al., 1997)	Pond	(Gaydon et al., 2012b)
Canola	(Robertson et al., 1999)	Peanut	(Hammer et al., 1995)	Solute	(Paydar et al., 2005)
Centro			(Robertson et al., 2001c)		(Poulton et al., 2005)
Chickpea	(Robertson et al., 2002)	Pigeonpea	(Robertson et al., 2001b)	Surface	(Connolly et al., 2001)
Cotton	*OZCOT:*	Potato	(Brown et al., 2011a)	Surface OM	(Probert et al., 1998a)
	(Hearn, 1994)	Rice	*ORYZA:*		
Cowpea	(Adiku et al., 1993)		(Bouman and van Laar, 2006)	SWIM	(Huth et al., 2012)
Fababean	(Turpin et al., 2003)		(Gaydon et al., 2012a)		(Connolly et al., 2002)
Field pea	(Chen et al., 2008a)	Sorghum	(Hammer et al., 2009)		(Verberg et al., 1996)
	(Robertson et al., 2002)		(Whish et al., 2005)	Temperature	(Campbell, 1985)
French bean	(Henderson et al., 2011)	Soybean	(Robertson and Carberry, 1998)	Water (SoilWat)	(Probert et al., 1998a)
GRASP	(Bell et al., 2008)	Stylo	(Carberry et al., 1996c)		(Verberg and Bond, 2003)
	(Rickert et al., 2000)	Sugarcane	(Keating et al., 1999)	Water Supply	(Gaydon and Lisson, 2005)
Growth	*Eucalyptus species*	Sunflower	(Chapman et al., 1993)	***Animal:***	
	(Huth et al., 2002)	Sweet corn	(Henderson et al., 2011)	DDRules	
Lablab	(Hill et al., 2006)	Sweet Sorghum		Graz	(Owens et al., 2009)
Lucerne	(Dolling et al., 2005)	Vine		Stock	(Freer et al., 1997)
	(Probert et al., 1998b)	Weed		Supplement	
	(Verburg et al., 2007)	Wheat	(Brown et al., 2014)	***Climate:***	
Lupin	(Farré et al., 2004)		Wheat (Wang et al., 2003)	Canopy	(Carberry et al., 1996b)
Maize	*Origin: AUSIM-maize*		NWheat (Keating et al., 2001)	E0	(Meinke et al., 2002)
	(Carberry and Abrecht, 1991)		I_Wheat (Meinke et al., 1998)	MicroClimate	(Snow and Huth, 2004)
Millet	(van Oosterom et al., 2001)		Nwheats (Asseng et al., 1998)		
Mucuna	(Robertson et al., 2005)				



Challenges and Opportunities for Statistical Applications in High-Throughput Phenomics

CSIRO Scott Chapman/UQ, Bangyou Zheng, Greg Rebetzke, David Deery, Xavier Sirault
UQ David Jordan, Graeme Hammer, Andries Potgieter, Barbara George-Jaeggli
WUR/BCAM/INRA Emilie Millet, Daniela Bustos-Korts, María Xosé Rodríguez-Álvarez, Martin Boer, Fred van Eeuwijk, Fred Baret, Shouyang, Simon Madec, Alexis Comar

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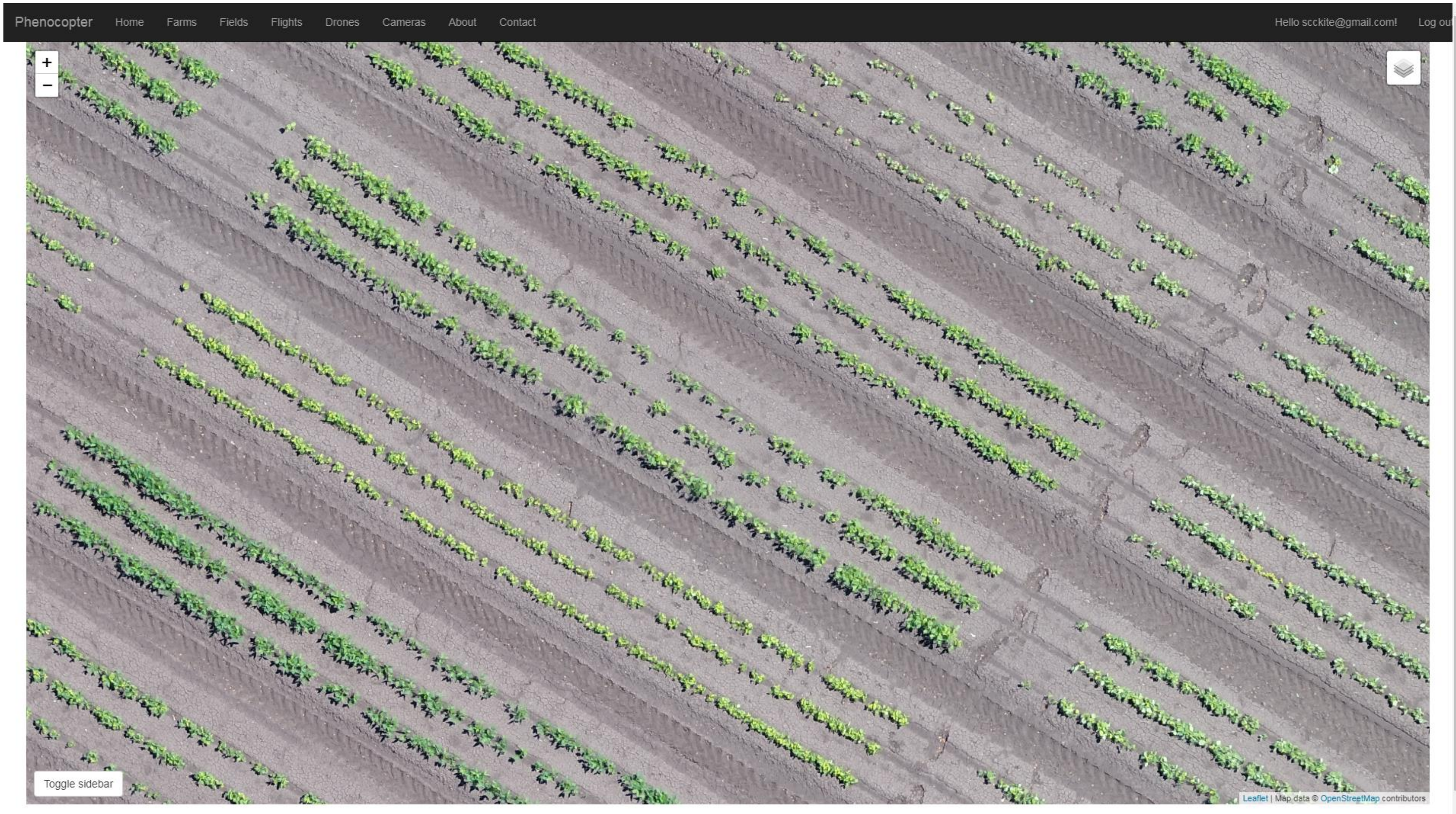
A shopping list for Statistical Issues in Plant Phenotyping

- Experiment type and design
  - Indoor/field experiments
  - Replication, sampling, statistical modelling
- Measurement protocols and processing
  - Sampling over space and time
    - Data cleaning/outliers
    - Spline fitting
    - Self-calibration
  - Image processing and analysis
    - Image quality and mosaicking
    - Segmentation of scenes and objects
    - Quantification of reflectance and indices
- Estimation of treatment effects
    - Accounting for design and sensor effects
    - Incorporation of genotypic data
- Modelling
    - Integrating statistical and dynamic models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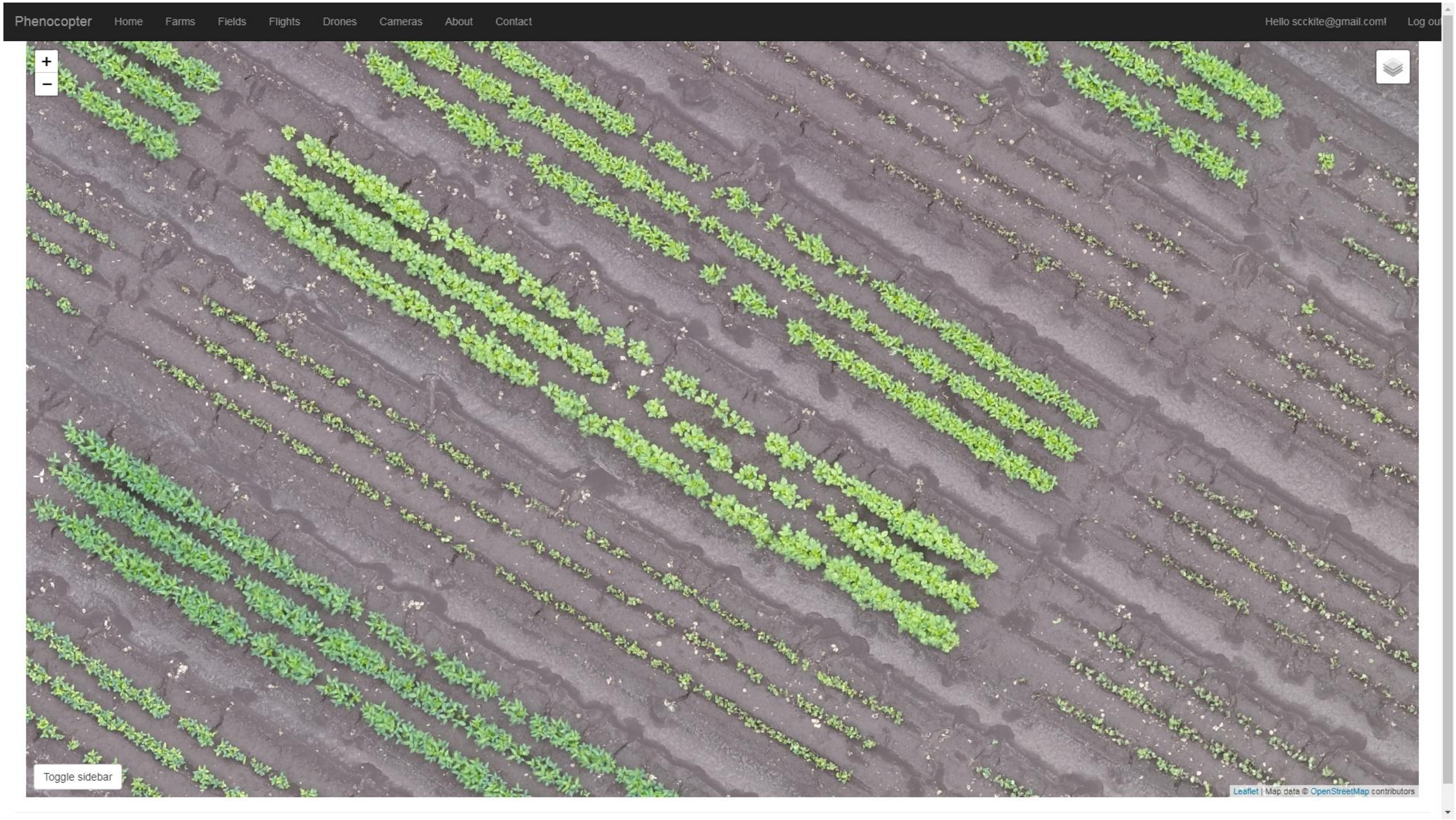
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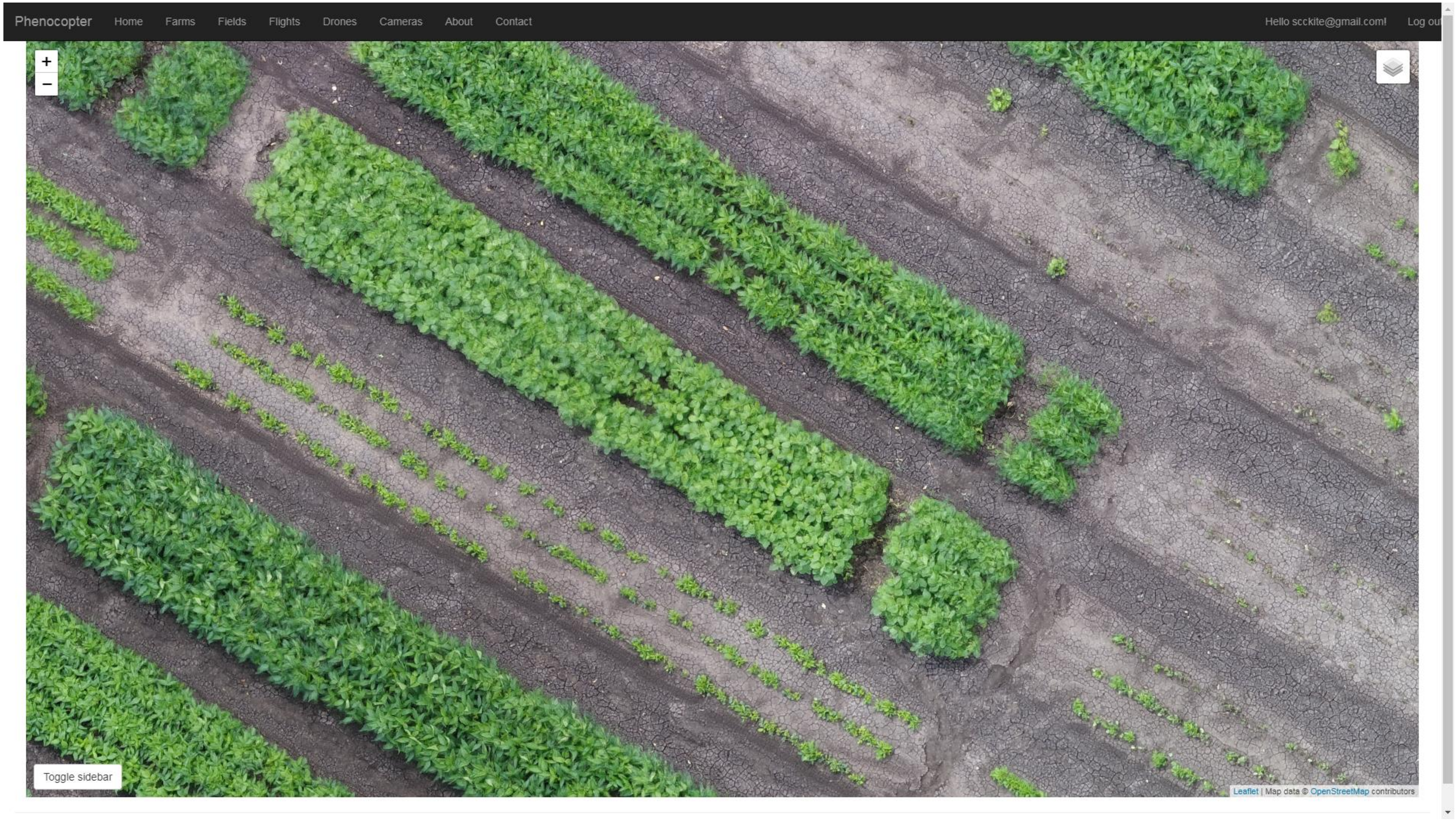
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