



The CSIRO breeding program

September 2016

WARWICK STILLER
www.csiro.au



The Australian cotton industry

The average Australian cotton farm:

- is family owned and operated
- is run by growers with an average age of 39
- grows 495 hectares (1200 acres) of cotton, comprising 17% of the total farm area
- supplements cotton with other crops including wheat, chickpeas and sorghum, and many Australian cotton farmers also graze sheep and cattle
- 30% of cotton is produced on corporate farms, the largest has 22,000 ha of irrigated cotton (54,000 acres).



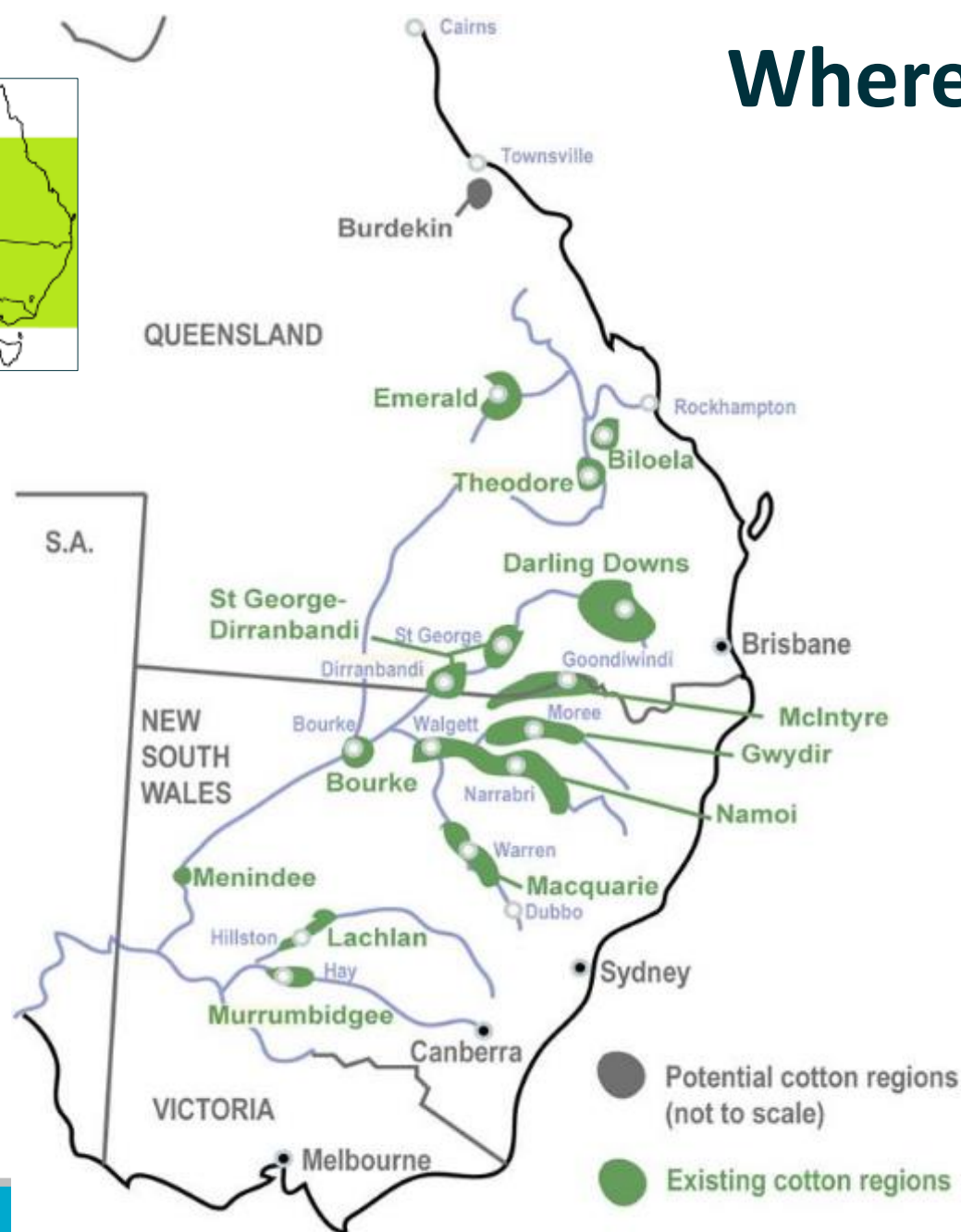
The Australian cotton industry

- There are only around 1500 cotton farms
- Total area between 200K and 600K ha (500K and 1.5M acres)
- 80% fully irrigated, 20% rainfed (dynamic)
- Average yield 2014/15 was 2610 kg/ha (2330 lb/ac)

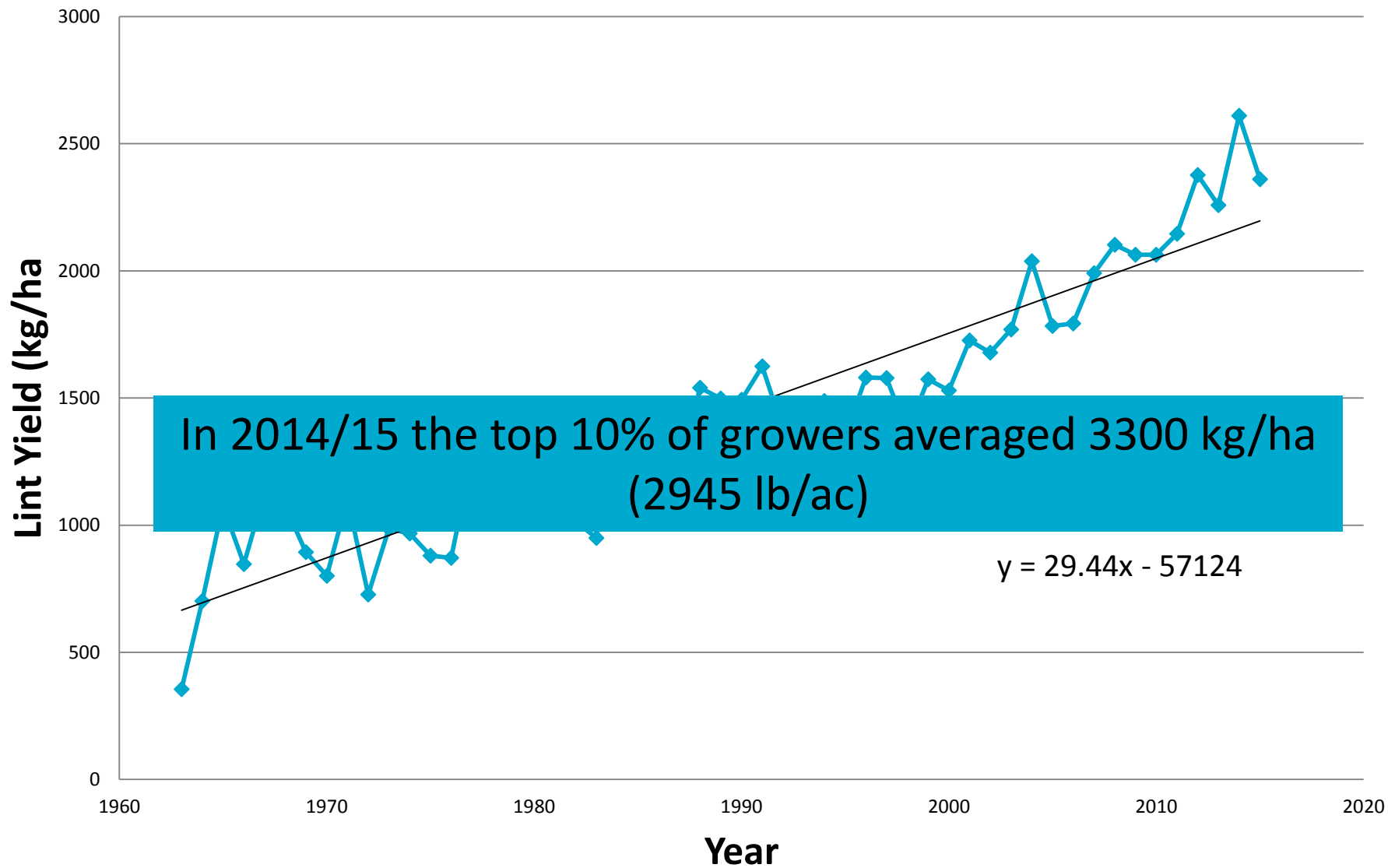
Characterised by:

- Young industry (growers are second or third generation)
- High proportion of tertiary education
- Professional farmers that rapidly adopt new technology

Where is cotton grown?



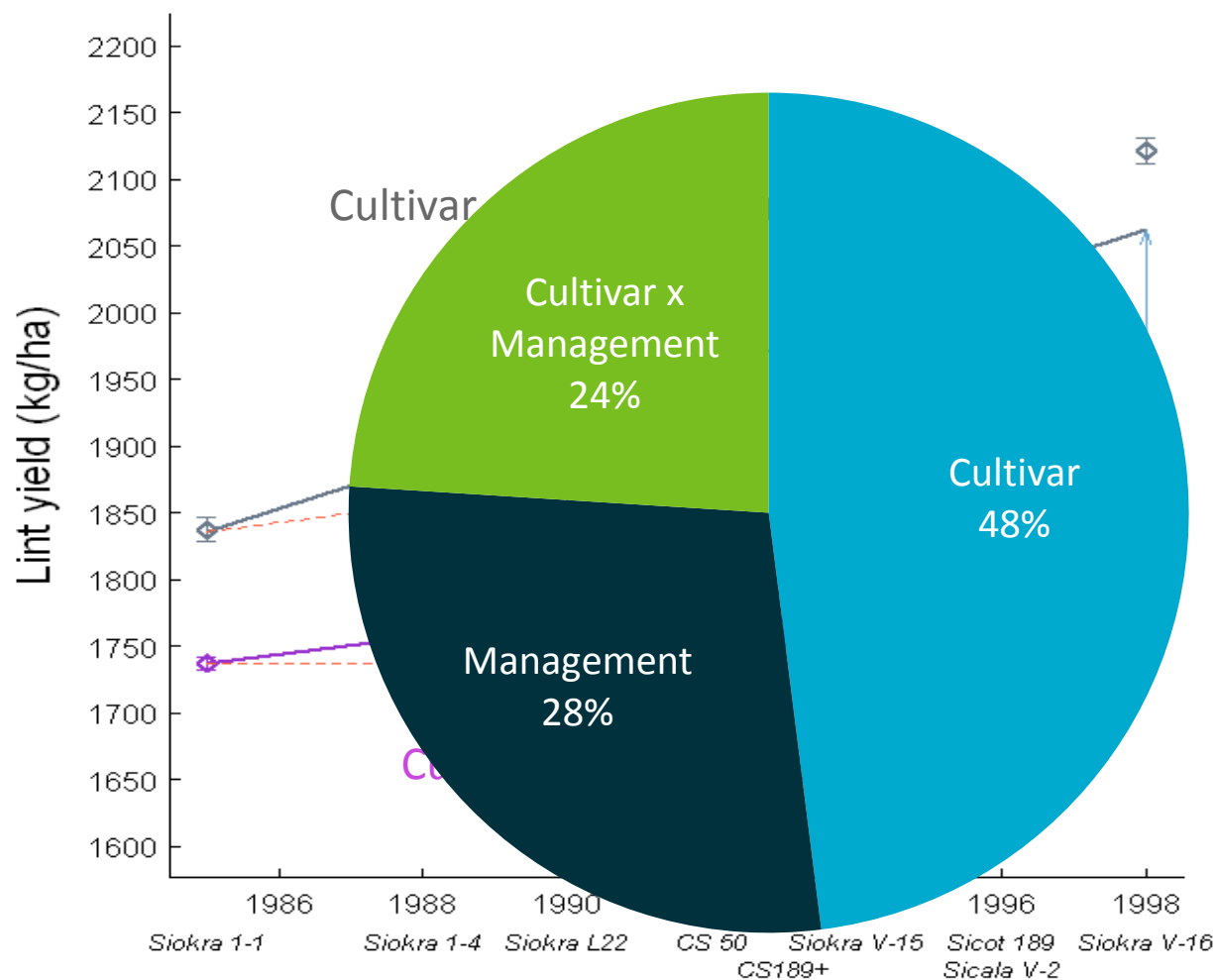
Average Australian cotton yield



Other facts

- Since the mid 2000's, all cotton grown in Australia is GMO
 - ~95% was Bollgard 3/RRF in 2016/17
- Since 2009 all cotton grown in Australia has been bred by CSIRO

Yield gain due to variety, management & their interaction



Cotton Breeding – Objectives

Through novel research and applied plant breeding develop Australian cotton varieties with the package of:

- increased yield
- fibre quality preferred by international spinners
- resistance to all important diseases
- widely adapted
- with GM traits of importance.

Achieved through the integration of traditional breeding and modern tools



Trial sites, area and technology

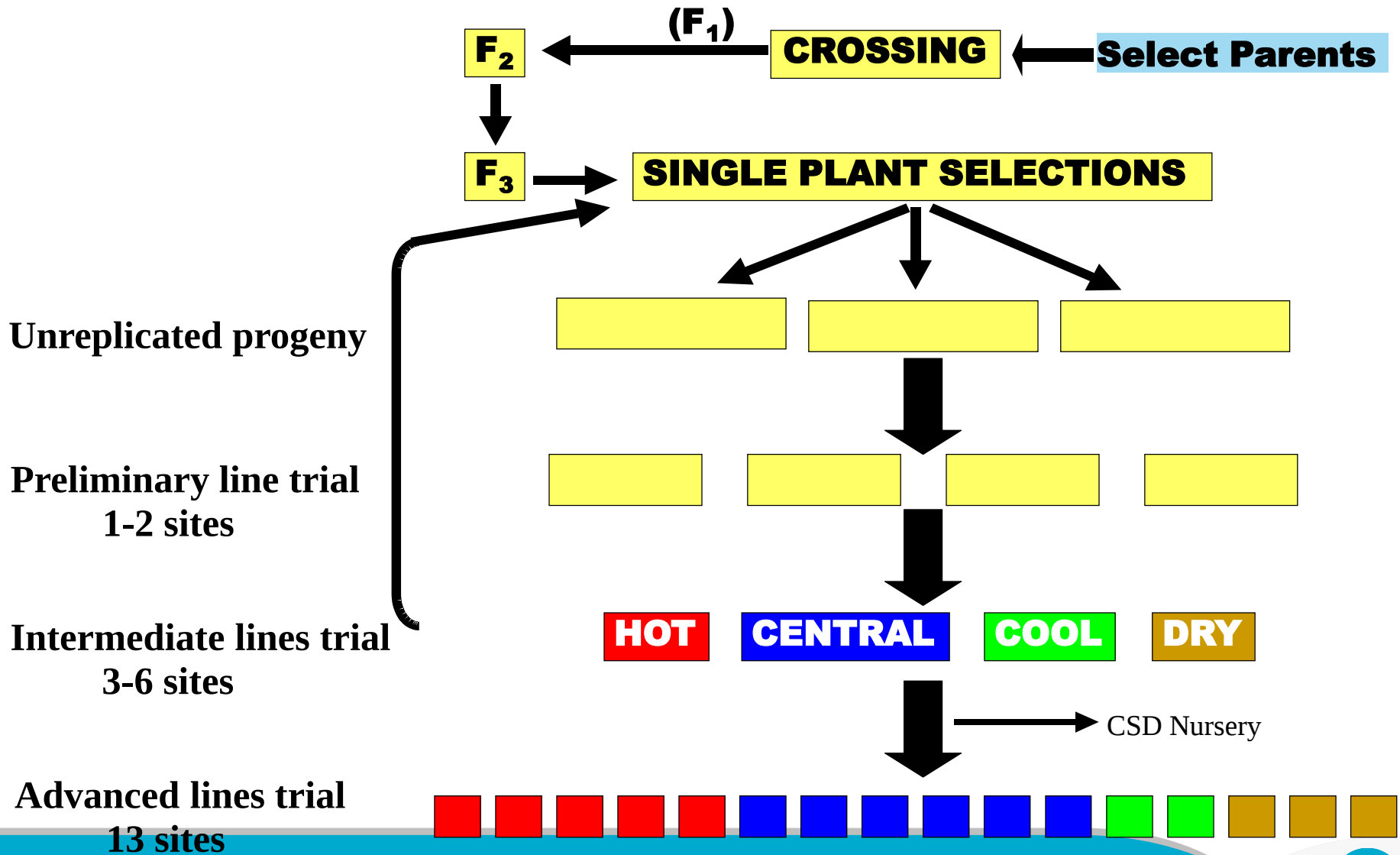


Site	B3XF	B3F	RRF	Conv	Total
Coleambally		1.7		1.2	2.9
Hillston		2.7	1	7.2	10.9
Warren		2.7			2.7
Moree Norwood		3.6	0.4	4.4	8.4
Goondiwindi	1.1	2.6	1	7.5	12.2

**40,000 plots plus
25,000 single plants
each season**

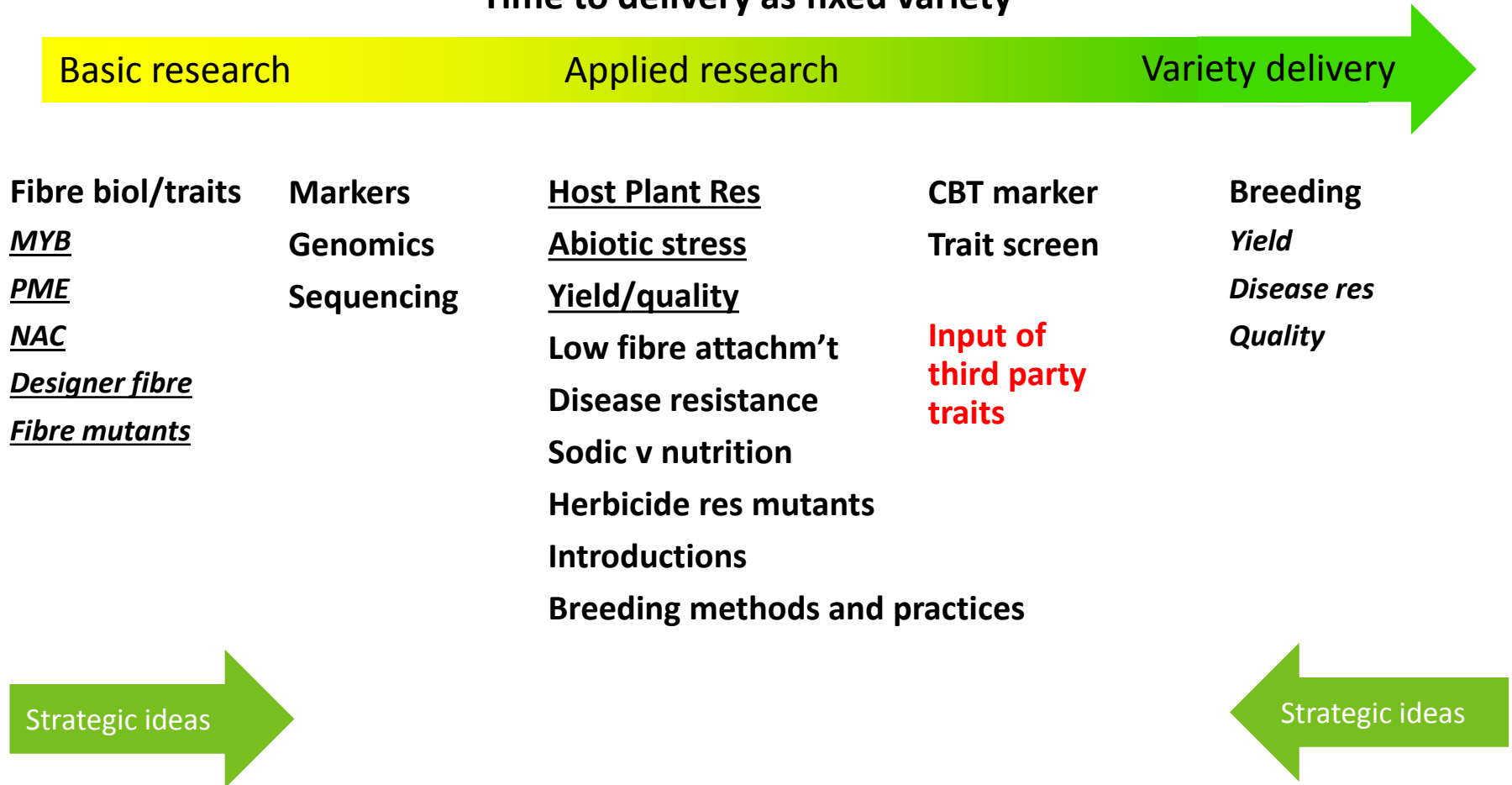
Maules Ck Verticillium					
Locharba	0.2				0.2
ACRI		7	1	19.5	27.5
Field L2 (Leitch)	4.2	2.7	0.7	20.9	28.5
Total	6.6	36.1	6.4	77.9	127

Breeding method (Pedigree)



CSIRO cotton: a fully integrated continuum

Time to delivery as fixed variety

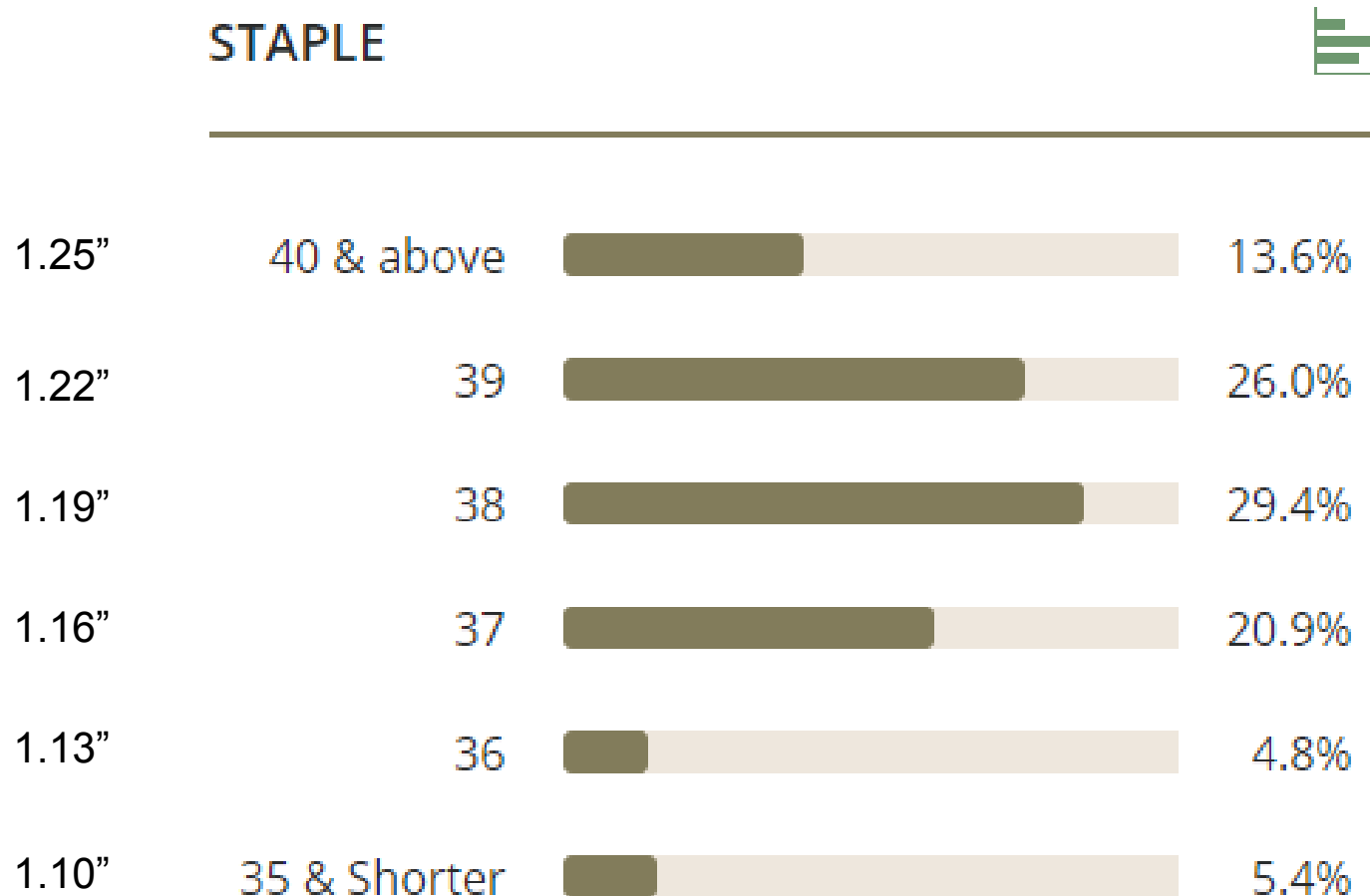


Collaboration with crop management and post harvest research groups

Current activities

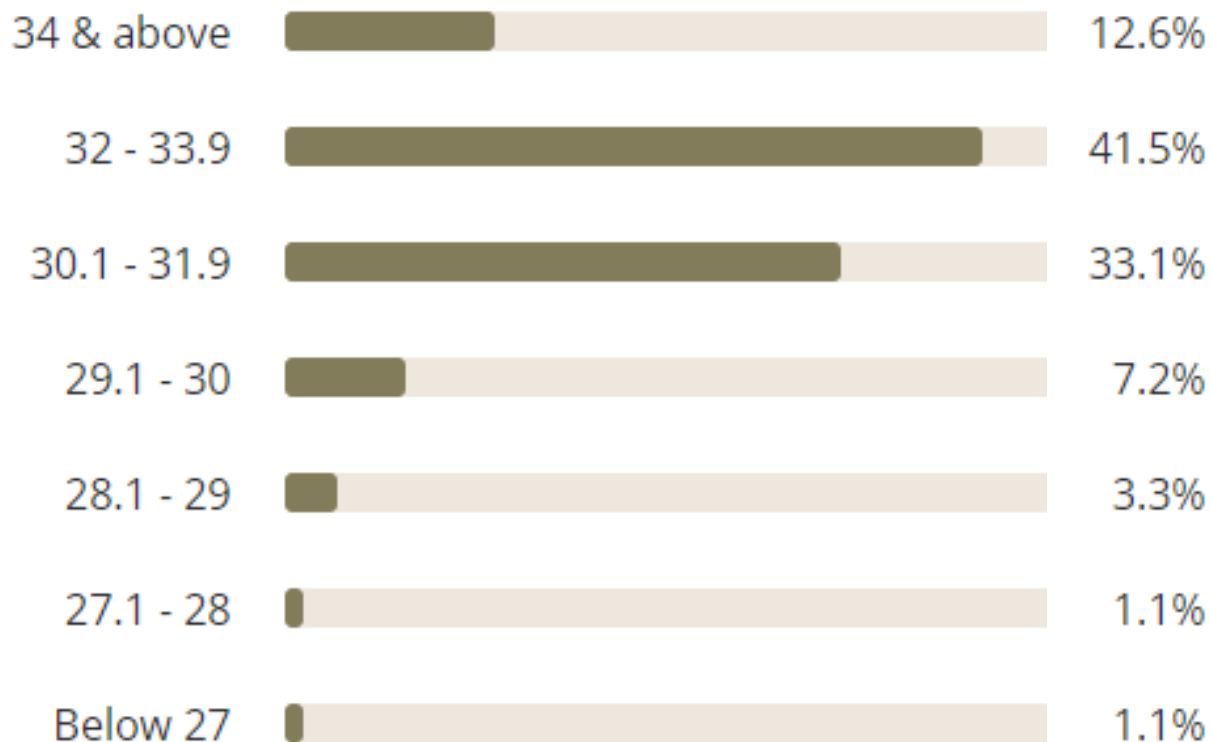
- Yield improvement
- Fibre quality
- Host Plant resistance
 - Disease resistance (Fusarium wilt, Verticillium wilt, Cotton Bunchy Top, Black Root Rot)
 - Two spotted spider mite, Silverleaf Whitefly
- Abiotic stress tolerance

Staple length 2016/17



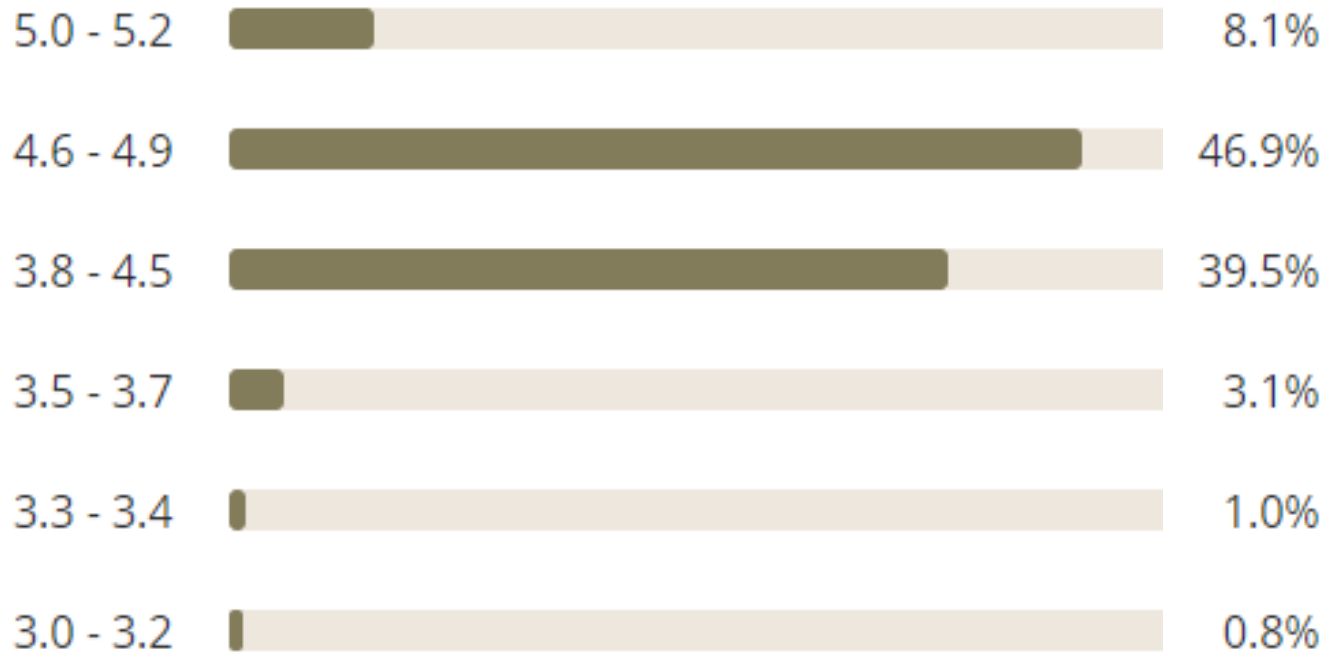
Strength 2016/17

STRENGTH



Micronaire 2016/17

MICRONAIRE



Fusarium wilt



Verticillium wilt



Black Root Rot

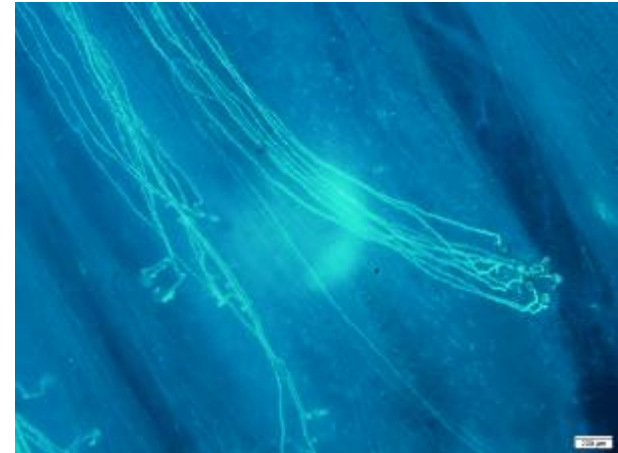


Abiotic Stress

- Assays developed for pollen viability, fruit set & retention under heat stress
- Validate and refine the use of the canopy temperature for assessing crop water use
- Phenotype diverse cotton collection for heat tolerance

Pollen viability studies

- Few studies have focused on cotton reproductive characteristics as a mechanism for stress tolerance, despite direct impact on fruit.
- *In vivo* study- method
 - Sicot 746B3F
 - Irrigated and rainfed water treatment
 - Elevated and ambient temperature
 - 0 DPA flowers (on four occasions)
 - Collected for microscopic analysis
 - Tagged to assess boll components



Canopy temperature phenotyping



- Water-use efficiency measurements
 - Physiological: slow, laborious, low heritability (G x E interactions)
 - Crop level: inferred by soil water or weather data.
- No suitable indirect screening method has been developed for assessing the response of large populations of breeding material to water limited conditions.
- On an agronomic level, canopy temperature has been shown to relate to crop water use

Heat tolerance assay

- Biochemical assay developed based on cell viability following an *in vitro* heat stress
- When live cells come in contact with a triphenyl tetrazolium chloride (TTC) solution, the TTC undergoes a chemical reduction producing a red product
- A higher absorbance value reflects more functional cells, thus the higher the tolerance of the plant to the stress.



Rainfed breeding protocol

- Variable rainfall environment, opportunistic rainfed industry
 - Disconnect between target and testing environments, plus trial variability.
- Developed a Managed Stress Environment (MSE) protocol where a furrow irrigation is applied in years that testing environment does not match target environment
 - Based on crop simulation modelling and field validation
- Since 2015, selection under MSE at one location combined with offsite multi-location testing



Keys to success

- Characterise the target environment
- Understand the marketplace
 - What do growers want/need
 - Academic studies are limited to those that contribute to variety development
- Long term view
 - Ensure what you are doing is important, then focus on it.
- Collection of accurate and reliable data
- Always look for efficiencies – smarter often beats bigger
- Nothing can substitute for hard work
- Engage with industry

Thank you

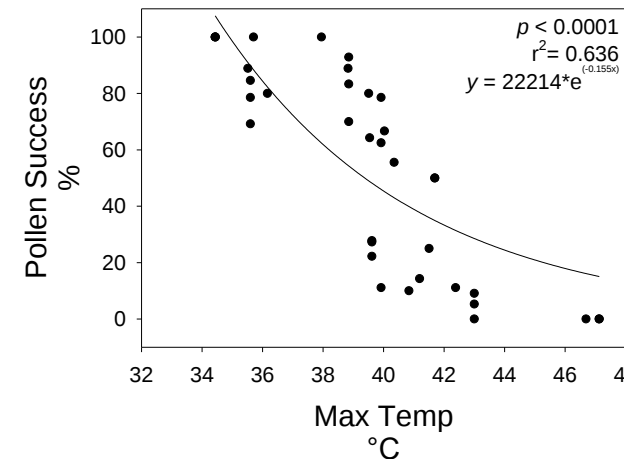
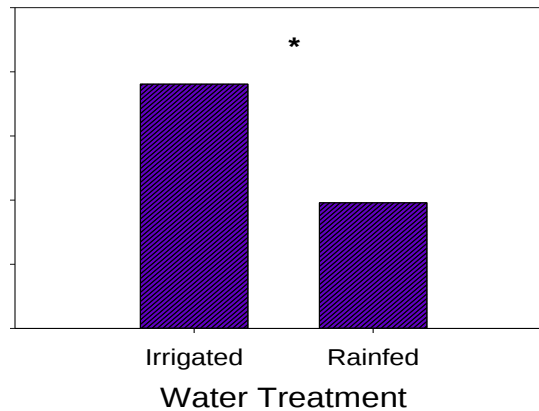
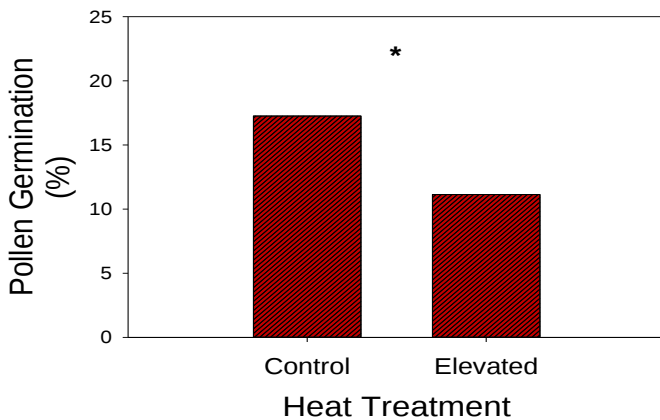
Dr Warwick Stiller

CSIRO Agriculture and Food

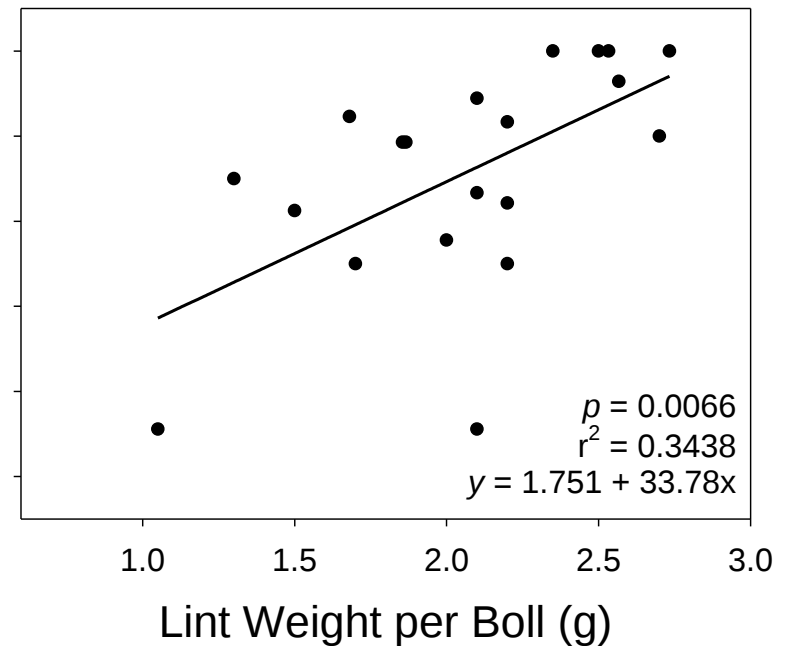
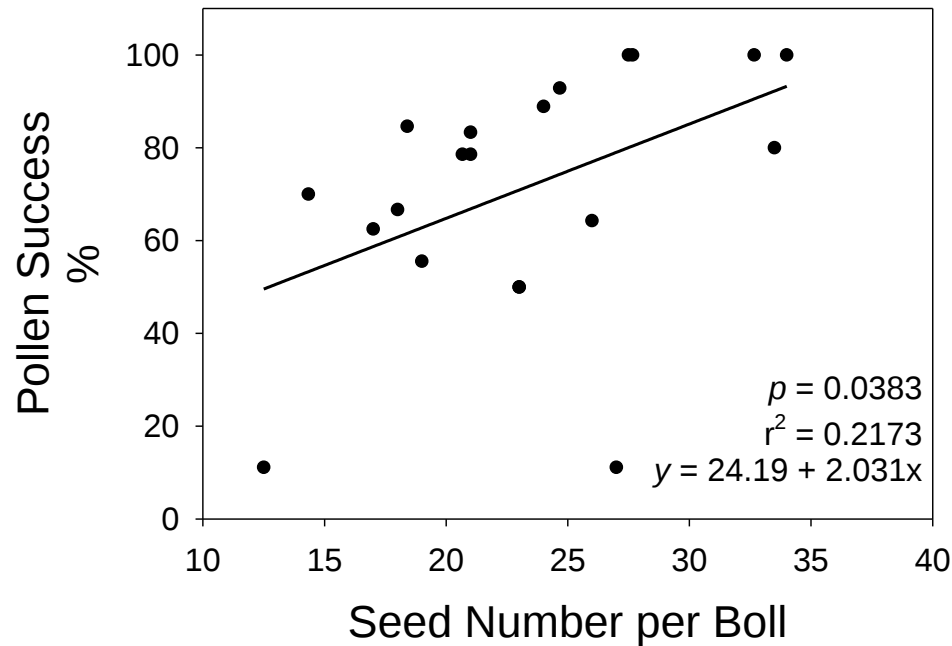


Pollen viability studies

- Results (3 measurement dates)
- Temperature and water treatment significant
 - Elevated temperature reduced pollen germination ($p=0.021$) and water stress reduced pollen germination ($p<0.001$)
 - No temperature x water interaction
- Relationship observed between $T_{\max}$ and VPD and likelihood floral stigma has germinated pollen
- Relationship between pollen viability and seed number / lint weight per boll

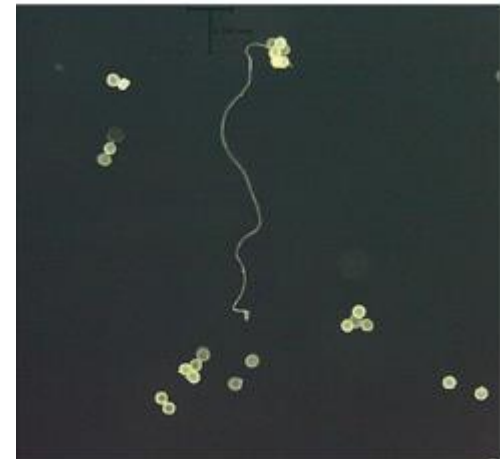
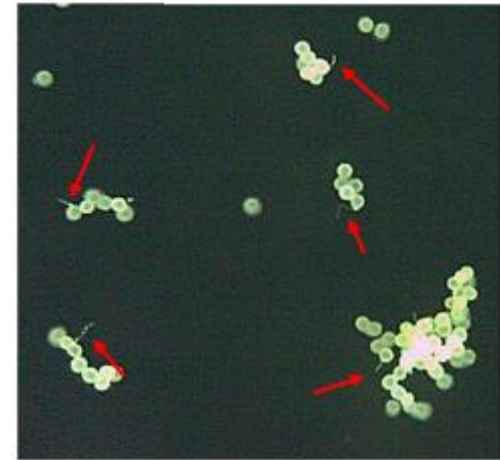


Pollen vs. yield components



Pollen viability studies

- *In vitro* study
- Refine media cocktail for pollen germination and growth, based on Kakani *et al.*, 2005.
 - Examples of a pollen tubes and differences in growth due to media cocktail.
 - Short pollen tubes (highlighted by red arrows; top)
 - Long pollen tube (bottom)
- Experimental phase will expose pollen on artificial media from a range of germplasm to evaluate the effect of heat and humidity.



Heat tolerance assay

- Glasshouse study to assess assay:

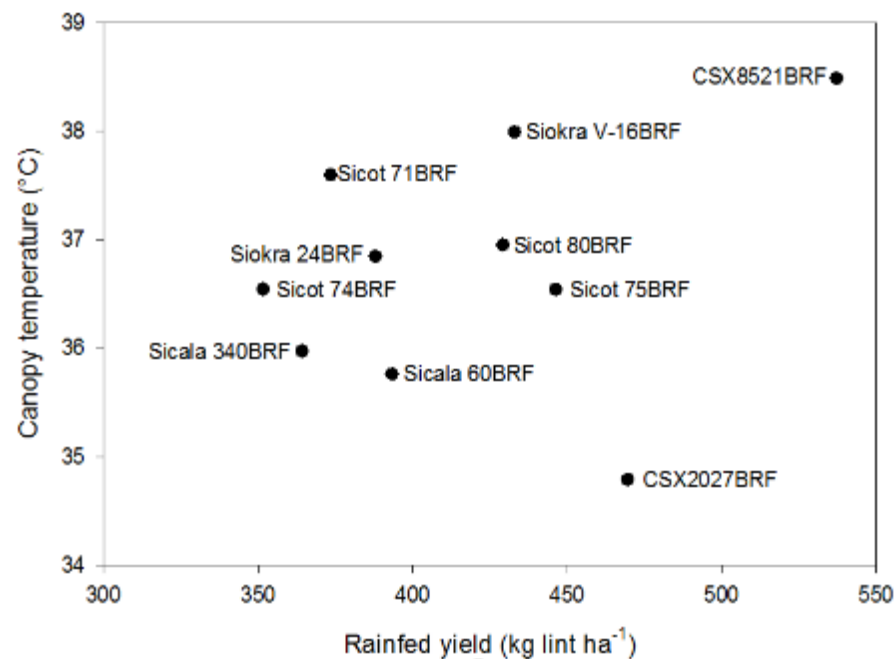
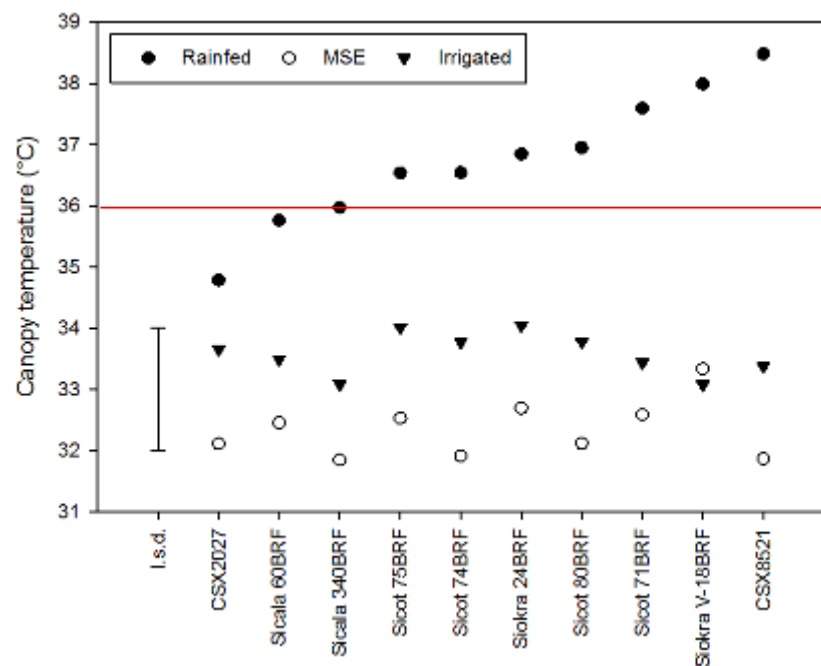
- 30 diverse genotypes of 4 species
- 32/18°C; 5d heat wave 42/28°C
- Study was replicated three times

	P value	var. explained
Genotype	<0.001	0.56
G x Experiment	<0.001	0.07
G x Measurement	<0.001	0.13
G x Temperature	<0.001	0.06

Genotype	Abs40	Rank Abs40	RelAbs4 0	Rank RelAbs40		80:20	70:30	60:40	50:50
Sicot 80	0.268	2	83.9	2		1	1	1	1
Acala Maxxa	0.247	3	82.3	3		2	2	2	2
VH8-4602 (<i>G. barb</i>)	0.213	10	91.4	1		7	5	4	3
CS 50	0.241	4	78.5	8		3	3	3	4
STV 825	0.219	9	80.4	5		8	6	6	5
DP 5415	0.240	5	78.1	10		5	4	5	6
Namcala	0.209	12	81.6	4		12	11	7	7
Sicala V-2	0.211	11	78.9	6		10	10	8	8
Siokra L23	0.233	7	76.5	13		9	9	9	9
Sicot 53	0.239	6	73.9	15		6	8	10	10
Siokra V-16	0.208	13	78.3	9		13	13	12	11
Sicot 730	0.203	14	77.9	11		14	14	14	12
BM13H (<i>G. arboreum</i>)	0.282	1	65.0	25		4	7	11	13
64224-212	0.225	8	71.0	19		11	12	13	14
Pima A-8 (<i>G. barb.</i>)	0.180	20	78.8	7		17	16	15	15
SG125	0.184	19	77.2	12		18	18	17	16
Sicala 40	0.203	15	71.7	18		15	15	16	17
CIM 496	0.202	16	72.1	17		16	17	18	18
Ersan92	0.175	21	74.7	14		20	20	20	19
CIM448	0.200	17	69.5	20		19	19	19	20
Sicala 45	0.149	27	72.4	16		25	24	22	21
SG248	0.174	22	68.5	22		22	22	21	22
ST474	0.157	24	66.7	23		24	23	24	23
YZ (<i>G. arboreum</i>)	0.198	18	56.5	30		21	21	23	24
DP5111	0.171	23	61.5	26		23	25	25	25
Simian 3	0.146	28	69.1	21		28	28	27	26
Sicot 71	0.150	26	65.4	24		27	26	26	27
STV468	0.156	25	60.7	27		26	27	28	28
BA119	0.132	29	59.1	28		29	29	29	29
<i>G. herbaceum</i>	0.125	30	58.7	29		30	30	30	30

Canopy temperature

- CT-Multiscan platform



Highlights and challenges

- An increased understanding of the effect of temperature and VPD on *in vivo* pollen viability, and its relationship to yield components. This provides a basis of the potential development of genotypes with thermotolerant pollen.
- Successful development of an *in vitro* heat tolerance pollen assay medium. Once optimisation of the media are completed, genotypes will be screened for pollen germination thermotolerance.
- Experimentation has validated the reproducibility and assisted in the interpretation of the heat tolerance assay data.

Challenges:

- Inconsistent results in measured canopy temperature phenotypes with the CT-multiscan. No genotype differences observed in UAV thermal imagery data from 2015.
- The high ambient temperatures experienced in the 2016/17 season affected the quality of experimental data collected, as non-stressed (control) comparisons cannot be made.
- The effect of early season glyphosate drift on the MSE and heat tolerance experiments has limited the results obtained in the 2016 season.