What are the Aussies Up to Down Under?

December 8, 2015

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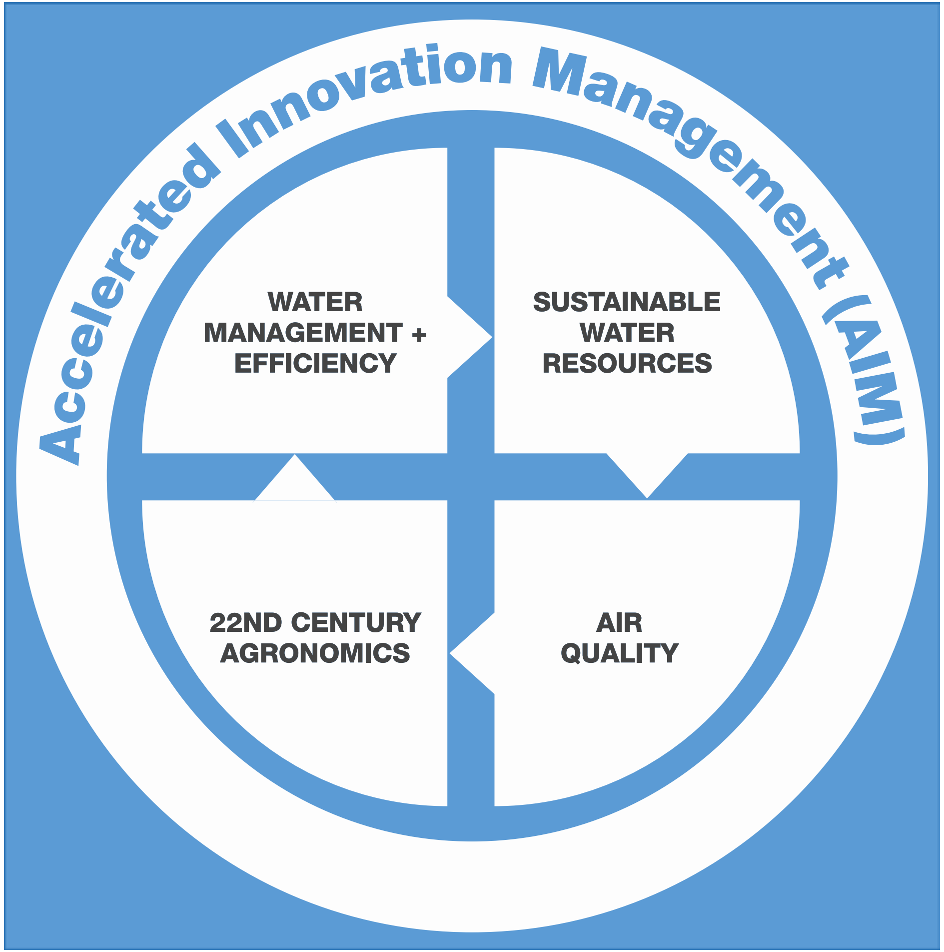
The Almond Conference



Accelerated Innovation Management (AIM)
WATER MANAGEMENT + EFFICIENCY
SUSTAINABLE WATER RESOURCES
22ND CENTURY AGRONOMICS
AIR QUALITY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Speakers

Karen Lapsley, Almond Board (Moderator)

Brenton Woolston, Almondco Australia Ltd. (Moderator)

Michelle Wirthensohn, University of Adelaide

Michael Coates, University of South Australia

Karen Lapsley, Almond Board

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Almond Breeding and Research in Australia

Michelle Wirthensohn

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Almond Board of California

The Australian almond industry

- Regions - Riverina (NSW), Adelaide Plains & Riverland (SA), Sunraysia (VIC)
- Production is 6% of world, California 85%
- 28,967 ha today

  4% non-bearing
- 80,500 t kernel in 2015 = $925m
- 83,000 t by 2018 = $953 million
- Global consumption inc.

  to 1,200,000 tonnes

Only 3 main cultivars:

Nonpareil, Carmel, Price

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Levy funded projects since late 1990's

- Performance evaluation of local selections and imports
- Breeding - hybridisations
- Virology
- Micropropagation/Cryopreservation
- Genetic fingerprinting
- Molecular markers/tools for almond breeding
- Almond transformation for self fertility
- Understanding flavour of almond kernels
- Physical mapping of almond/quantitative genetics
- Evaluation of water use efficiency
- Biochemical testing - fatty acid & Vitamin E analysis

Industry requirements

Currently based on one main cultivar ‘Nonpareil’

- 50.5% plantings
- self-incompatible
- papershell
- small to medium size
- good appearance

NIBF in Carmel

Industry want new improved cultivars……

Breeding

- Objectives: to breed improved cultivars with high productivity, self fertility and superior kernel quality
- Classical breeding using local and imported material
- Waite almond germplasm collection
- Primary evaluation based on nut & kernel characteristics
- Secondary evaluation on productivity, disease tolerance

Desirable traits in almond

- Sweet kernel, large kernel, thin skin, light color
- Self-fertile, autogamous
- Hard shell, good seal
- High productivity
- High flower density
- Slightly erect tree habit
- Drought tolerance
- Disease tolerance

Primary evaluation of kernels

- Kernel taste – sweet
- Kernel size – >1.4 g (18/20 kernels per ounce)
- % double kernels – <5%
- Kernel colour – light
- Shell hardness – paper, soft, semihard, hard
- Shell seal – well sealed
- Kernel appearance – score /10

Variation in morphological traits

Kernel size & testa colour

Double kernels

Progress to date

- 84 parent cultivars used
- 315 different crosses achieved
- 34,000 progeny produced
- 37 cultivars imported since 1997
- 60+ superior selections to date
- Secondary and tertiary evaluations blocks established

Secondary evaluation phase

Secondary evaluation phase

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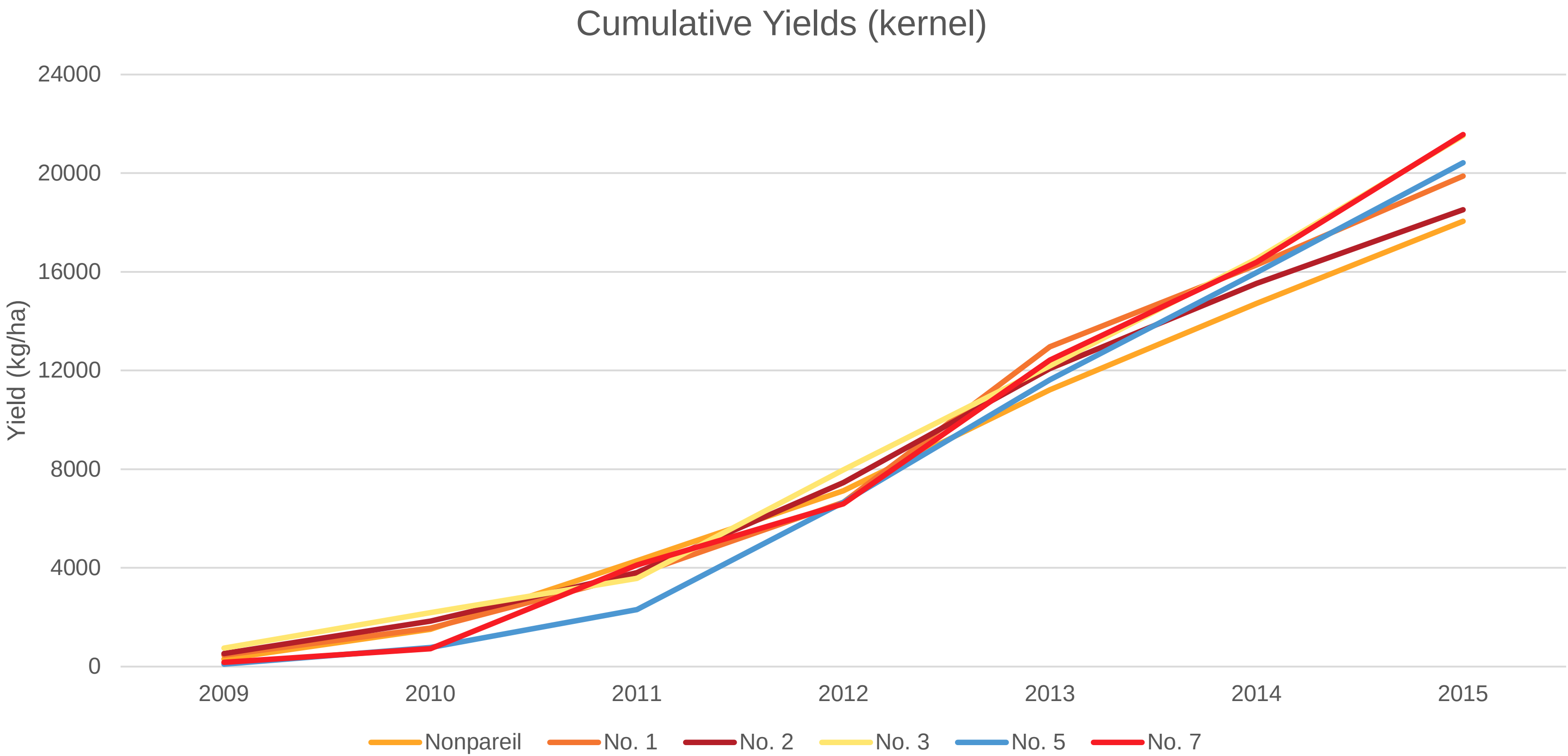
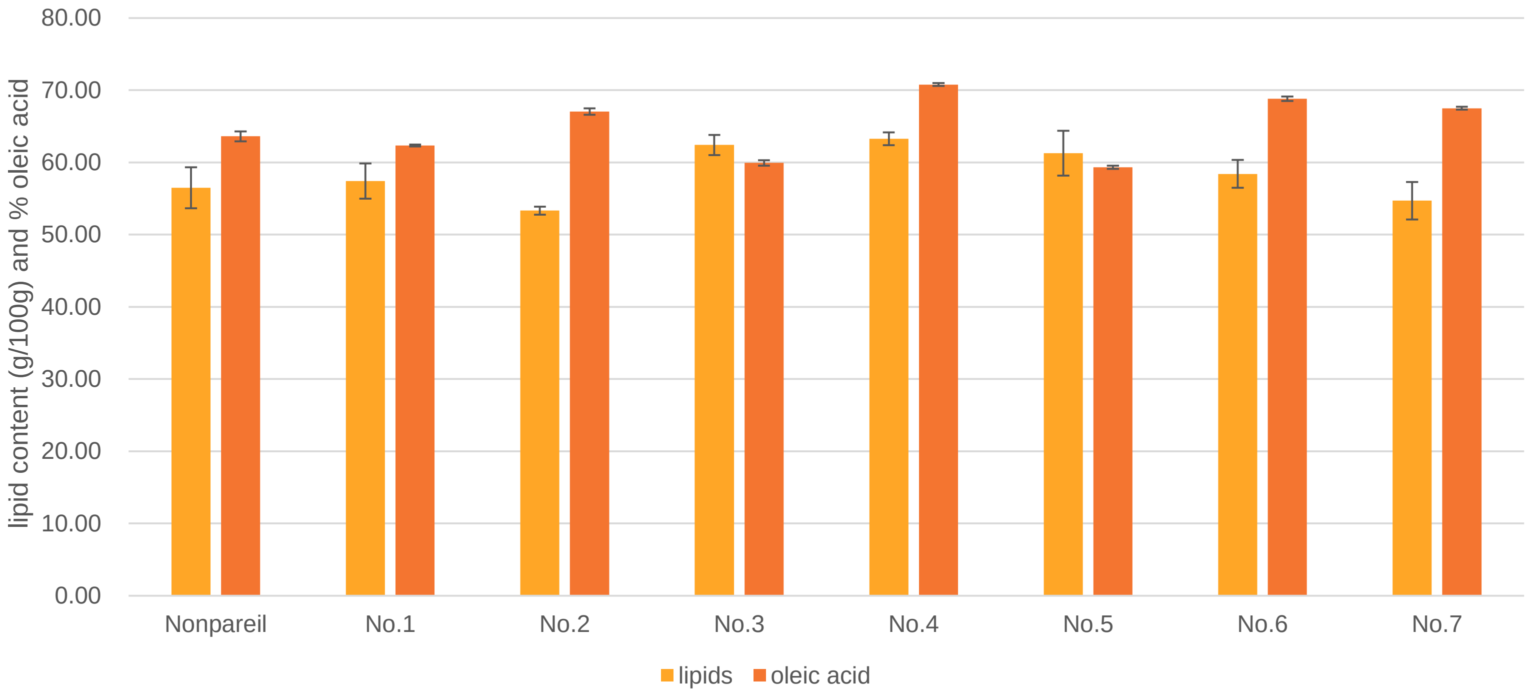
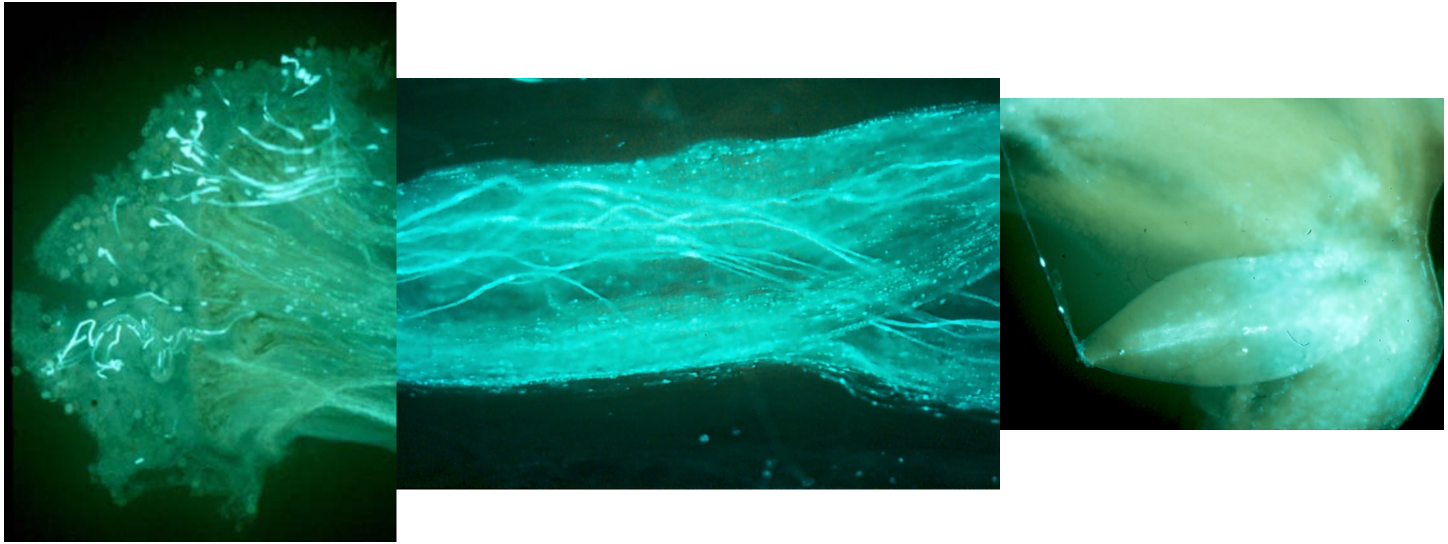

Cumulative Yields (kernel)
Yield (kg/ha)
24000
20000
16000
12000
8000
4000
0
2009
2010
2011
2012
2013
2014
2015
Nonpareil
No. 1
No. 2
No. 3
No. 5
No. 7

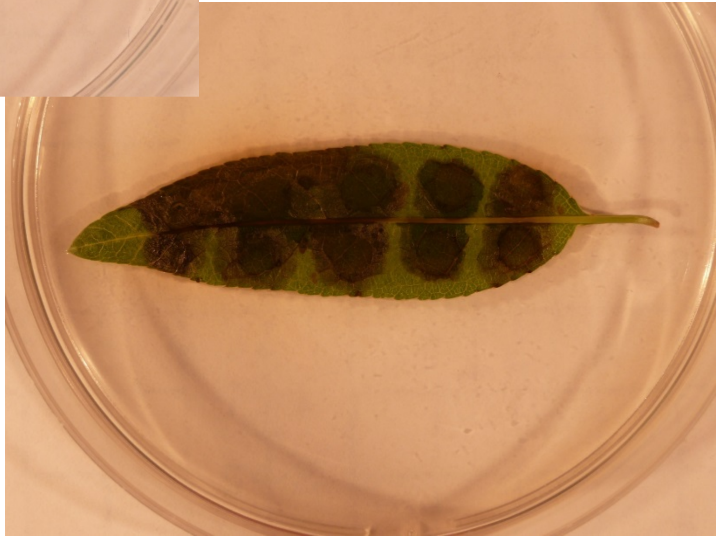
Lipid content and Oleic acid percentage of some selections



Self fertility

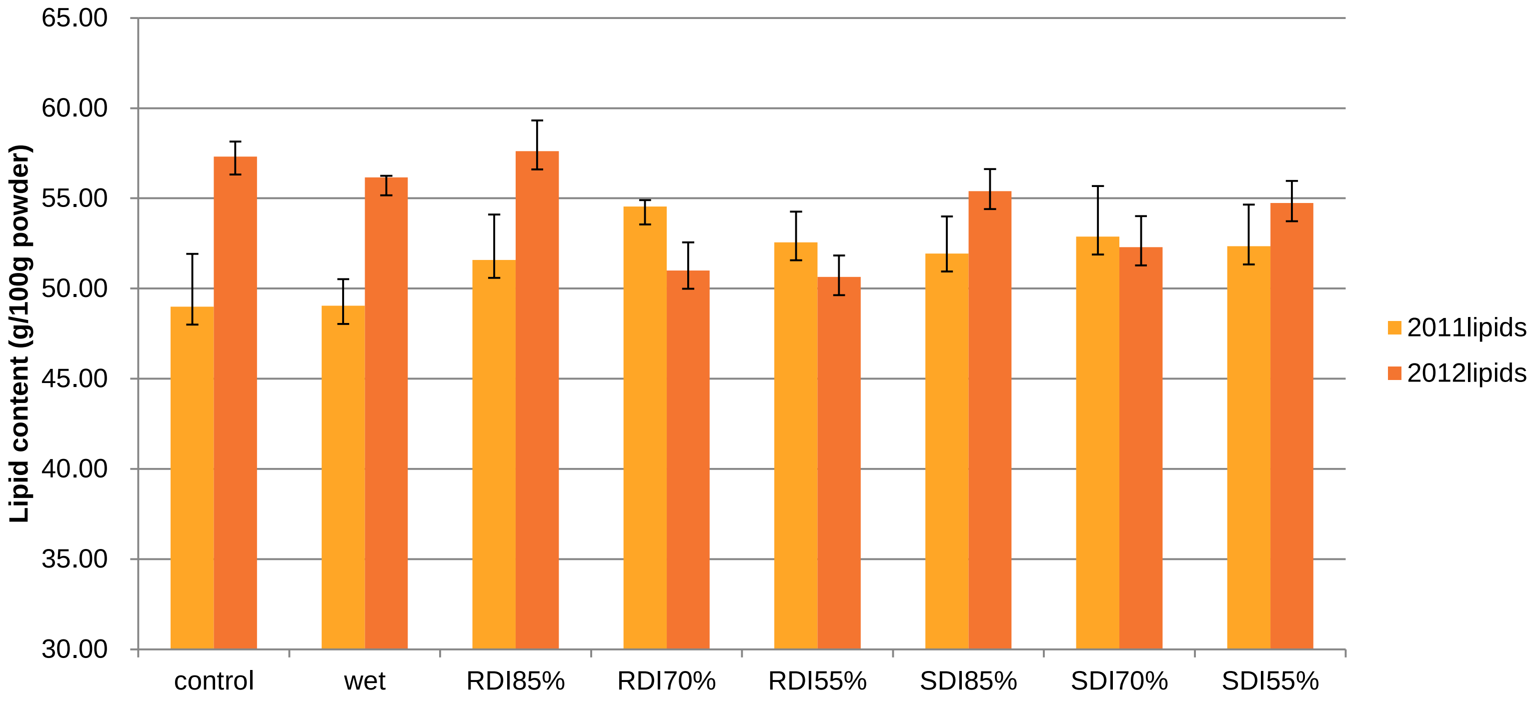


Bacterial spot assay




A5 114

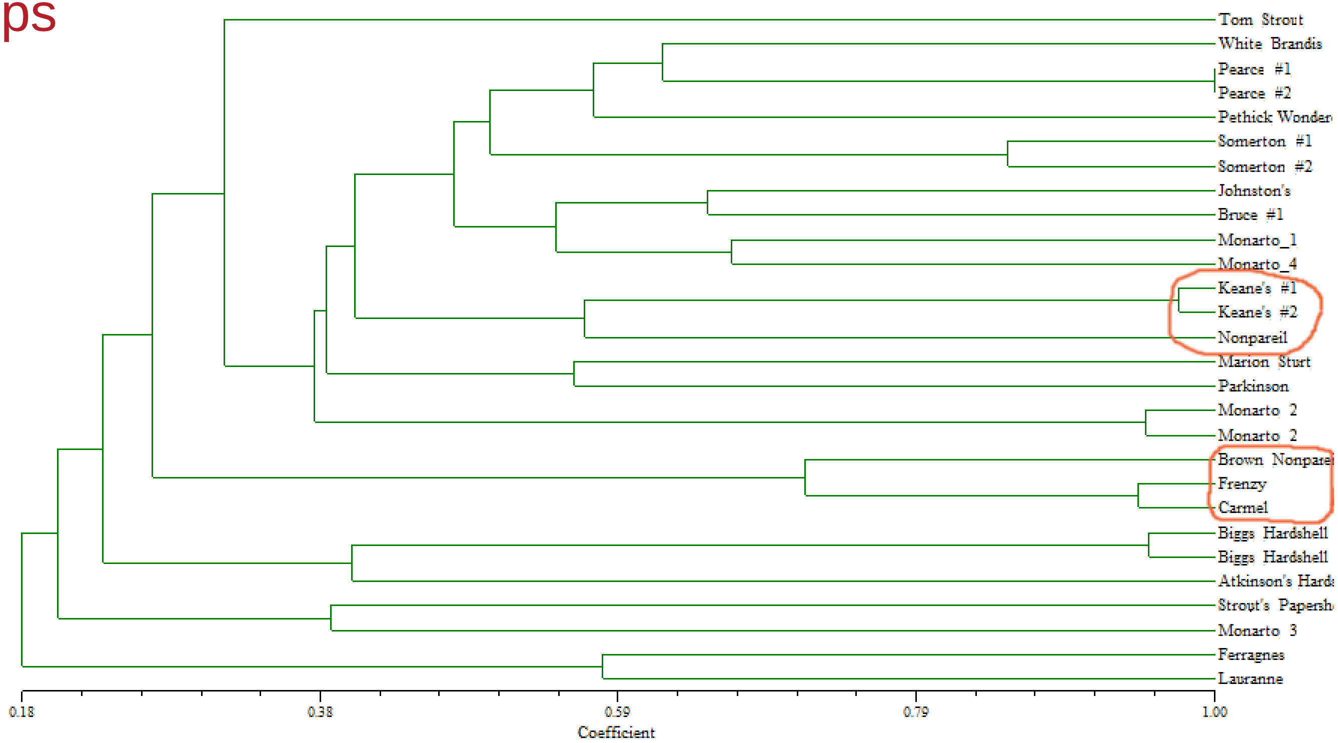
Effect of Deficit irrigation on lipid content



Fingerprinting methods for Germplasm Identification

- Initially screened 30 Single Sequence Repeat (microsatellite) markers
- Chose 19 of these, but some were not polymorphic, or they amplified more than one locus
- Finally used 13 SSR markers on 28 accessions
- Suitable for Plant Breeders Rights applications
- Genetic relationships

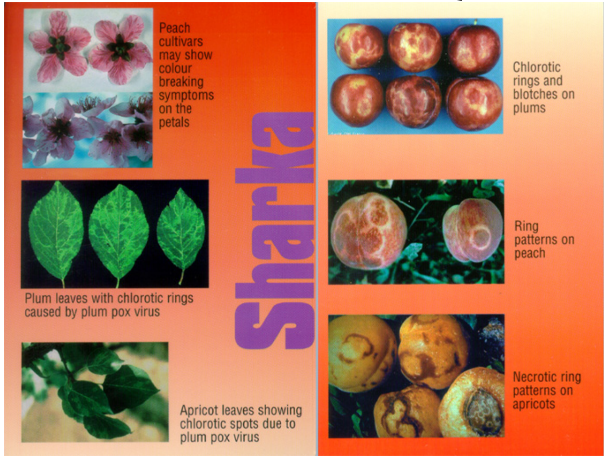
Dendrogram constructed from SSR marker data showing genetic relationships



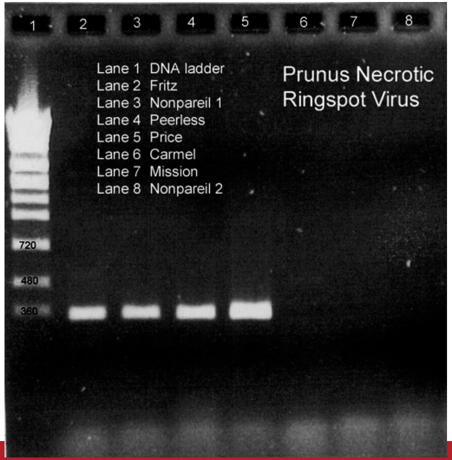
Molecular markers

Specific primers for detection of

Prunus necrotic ringspot virus
Prune dwarf virus
Plum Pox virus



Primers based on coat protein sequences



Specific primers designed from the introns for S-allele identification

Specific allele	Primers	Nucleotide sequence (5'→3')	Amplified specific band from PCR product
S1	S1F S1R	CTC TTT AGC ATT TTA GTT TTT AG CTG AGA CAT CCA AGC AAT ATA G	488
S5	S5F S5R	GGC TCT TTG TTT TTC TAG TTA C GCA ACA TCC AAG CAA TAA ATC	75
S7	S7F S7R	ACC ATA TAA CAT CGT GTT GC GAG GAT AAT ATG GTA CAT TC	425
S23	S23F S23R	ATT GTC ATC TGA AGA CCA TAT AC TGA GAC ATC CAA GCA ATA TAT AC	437
Sf	SfF SfR	GTG CCC TAT CTA ATT TGT TGA C GAC ATT TTT TTA GAA AGA GTG	459

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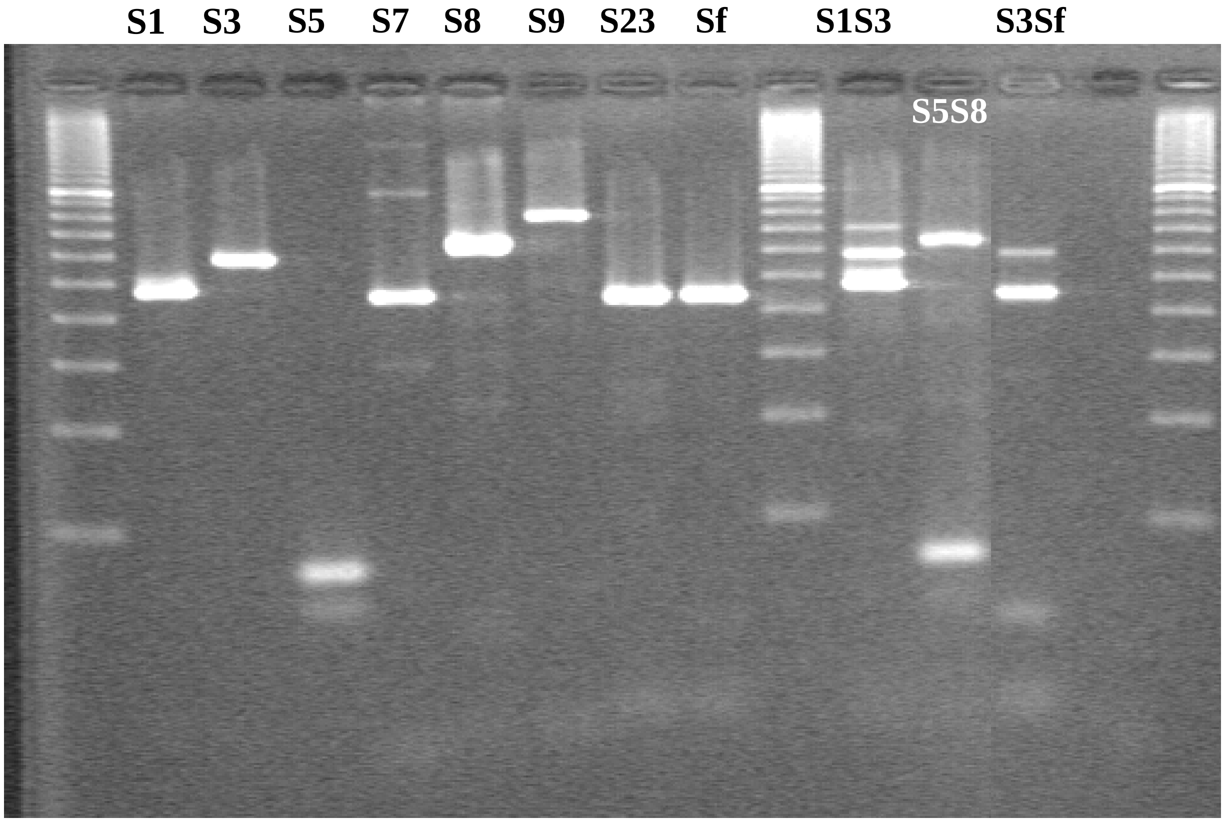
S-allele identification



S-allele identification in almond cultivars using specific primers

Cultivar	Reported alleles	S1	S3	S5	S7	S8	Sf	S23
Ramillete	S6 S23							√
Lauranne	S3 Sf		√				√	
Chellaston	unknown				√			√
Pethick Wonder	unknown							√
Johnston’s	S23 S?							√
McKinlay’s	unknown				√	√		
Parkinson	unknown							√
Pearce	S23 S?					√		√
Somerton	unknown	√						√

S-allele identification methods

- First intron primers:

PaCons1-F 5’-(A/C)CTTGTTCTTG(C/G)TTT(C/T)GCTTTCTTC-3’

Ref: Sonneveld et al, 2003

EM-PC1consRD 5’-GCCA(C/T)TGTTG(A/C)ACAAA(C/T)TGAA-3’

Ref: Ortega et al, 2005

- Second intron primers:

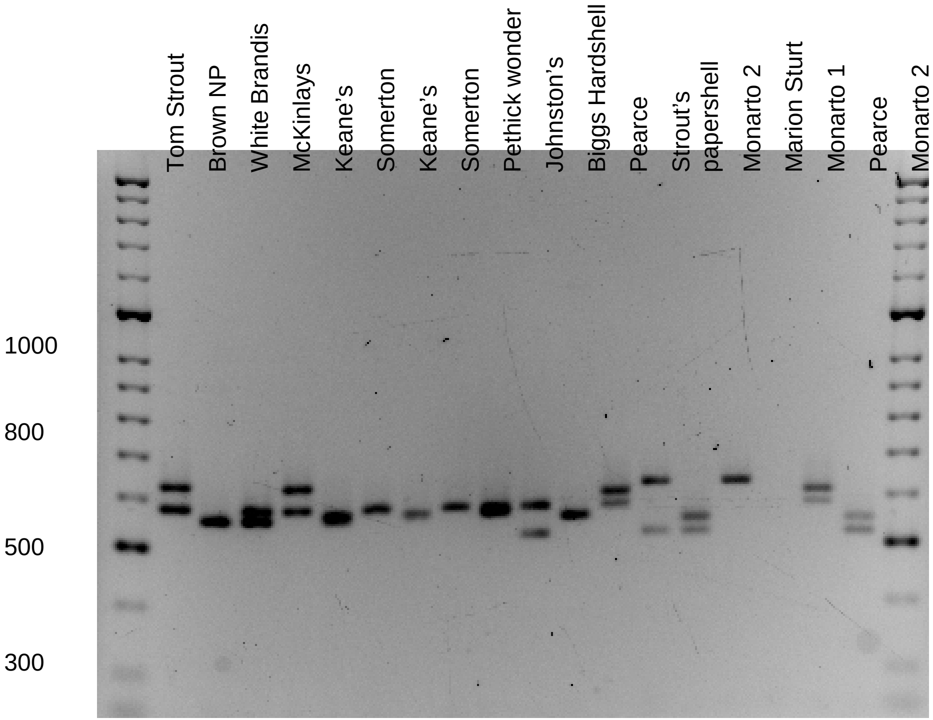
EM-PC2consF 5’-(TCAC(A/C)AT(C/T)CATGGCCTATGG-3’

Ref: Sutherland et al, 2004

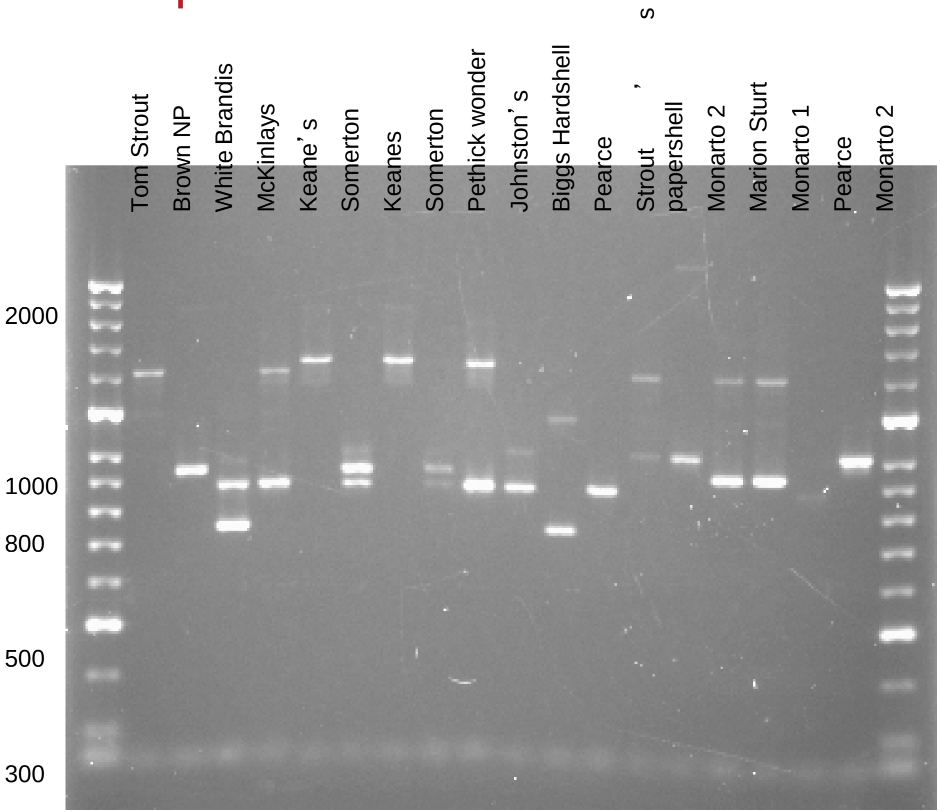
EM-PC3consRD 5’-A(A/T)(C/G)T(A/G)CC(A/G)TG(CT)TTGTTCCATTC-3’

Ref: Sutherland et al, 2004

Results – First intron product



Results – Second intron product



S genotypes deduced from amplification products of some Australian cultivars

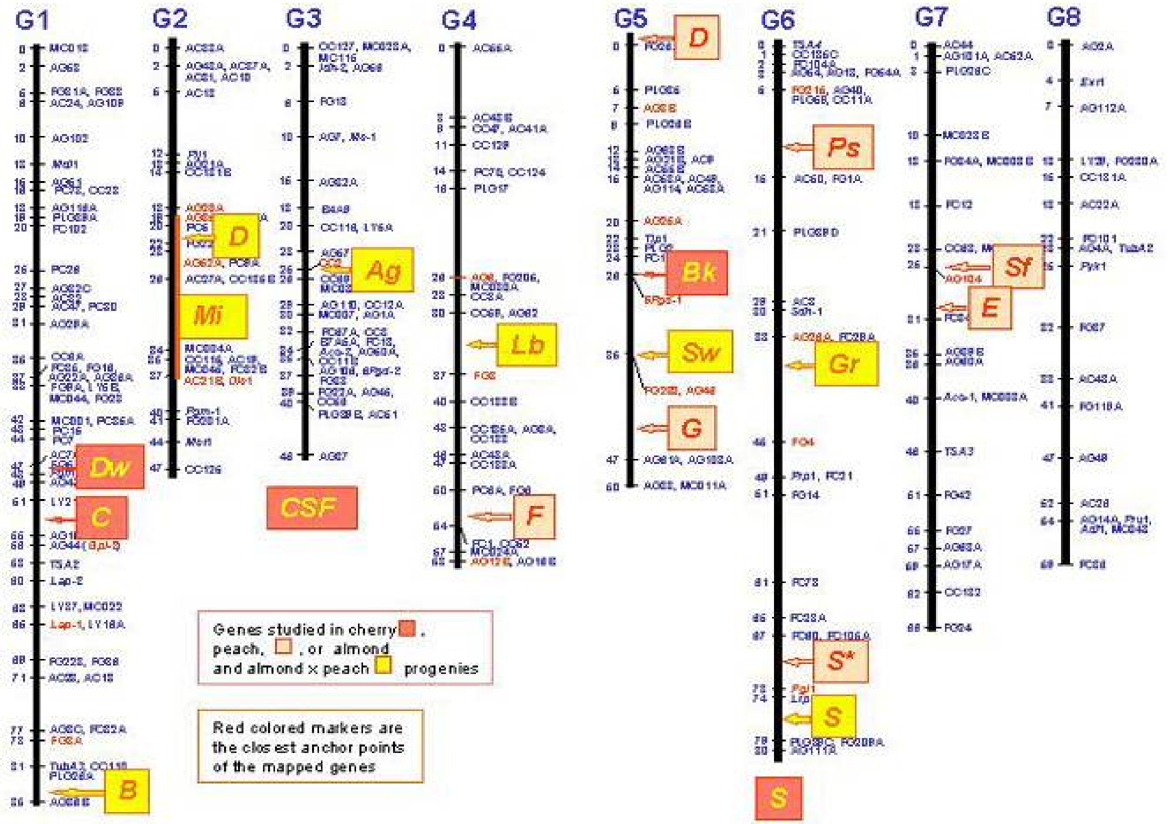
Cultivar	First intron bands		Second intron bands		*S*-alleles	Possible parents/ancestors
Tom Strout	371	419	1000	1200	$S_{13/19}$ S_{22}	Kapareil/Jordan
Somerton	375	-	685	742	S_{1} S_{23}	Ne Plus U/Jordan
Brown NP	878	346	745	1750	S_{1} S_{7}	NePlusUltra/NP
Strout's Papershell	433	325	1217	833	S_{22} S_{25}	Jordan/?
Johnston's	379	321	710	813	S_{23} S_{25}	Jordan/?
Pethick Wonder	371	381	700	1317	S_{23} S_{27}	Jordan/?
Bigg's	554	1015	358	1143	S_{6} S_{14}	Peerless/Jordanolo
White Brandis	346	367	558	692	S_{6} S_{23}	Peerless/Jordan
Keane's	358	381	1711	1333	S_{7} S_{27}	Nonpareil/?
Pearce	360	384	2133	685	S_{8} S_{23}	Nonpareil/Jordan
Frenzy	-	405	283	2233	S_{5} S_{8}	Carmel/NP

Genetic mapping

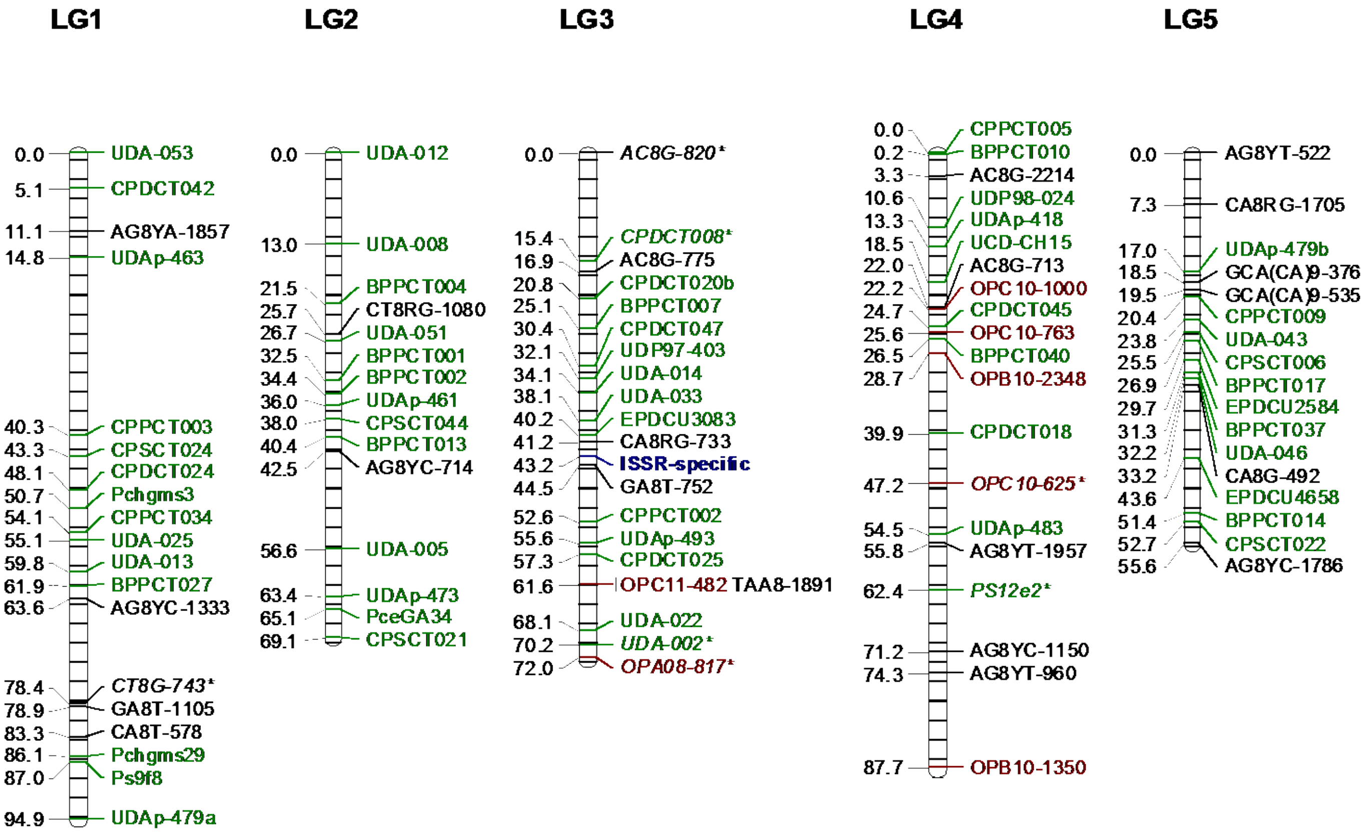
Almond population Nonpareil x Lauranne
182 individual progeny
Measurements for 6 years on nut traits

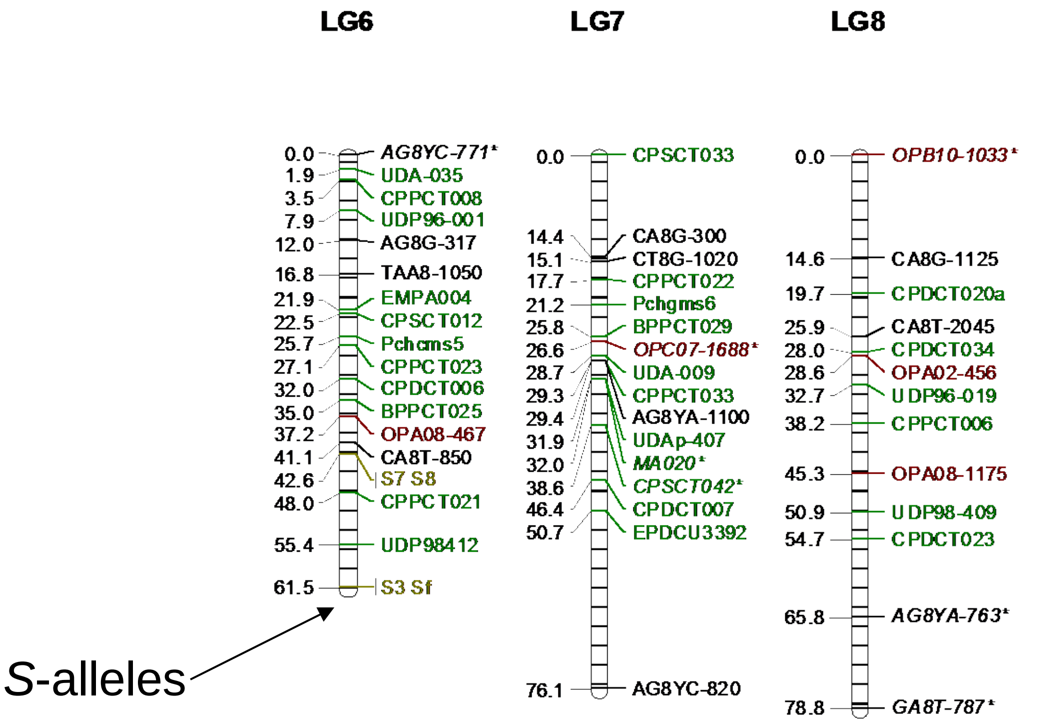
- in-shell weight
- kernel weight
- % doubles
- kernel thickness
- kernel shape
- shell thickness
- testa colour
- testa pubescence
- kernel taste
- vitamin E
- fatty acids

Prunus reference map



Almond integrated map

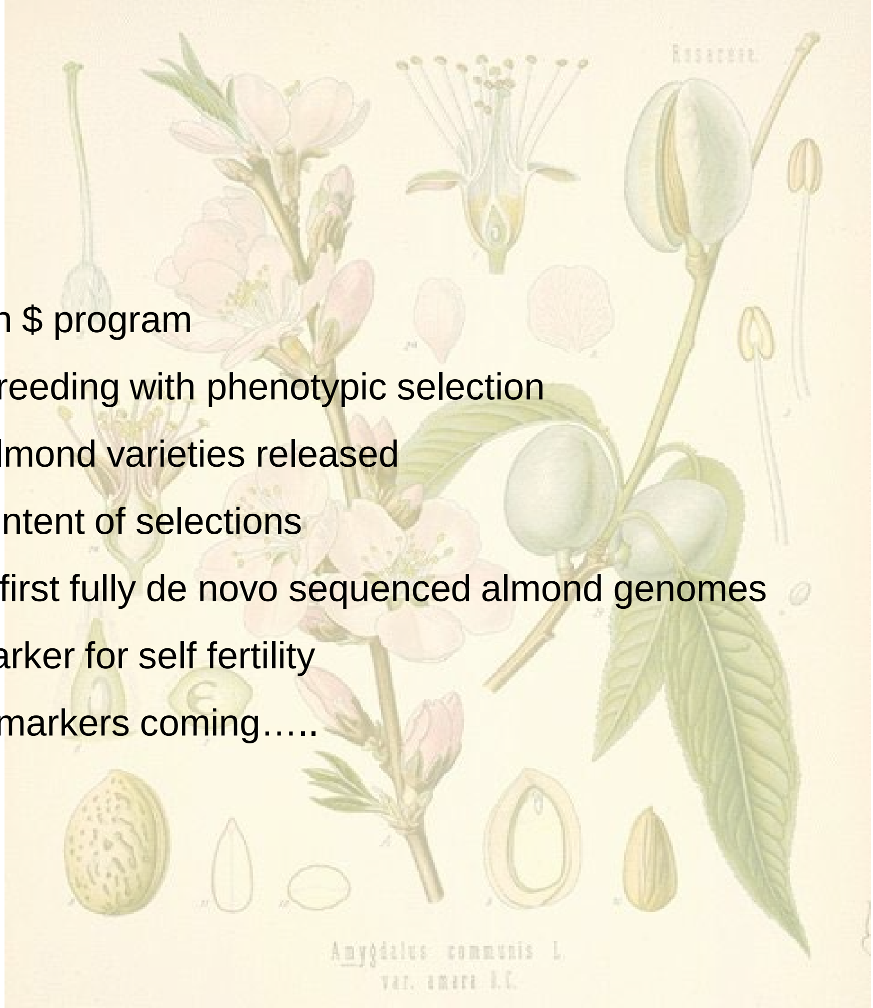




Integrated genetic map of almond cross ‘Nonpareil’ × ‘Lauranne’. Individual marker colour coded: Black ISSR, magenta RAPD, blue SCAR, olive S-alleles and green for microsatellites

Summary

- Multi-million $ program
- Classical breeding with phenotypic selection
- Five new almond varieties released
- Nutritive content of selections
- One of the first fully de novo sequenced almond genomes
- ‘Perfect’ marker for self fertility
- With more markers coming…..



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Michael Coates,
University of South Australia

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Green Harvesting

Michael Coates

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Horticulture Innovation Australia

Agricultural Machinery Research and Design Centre (AMRDC)

Professor John Fielke

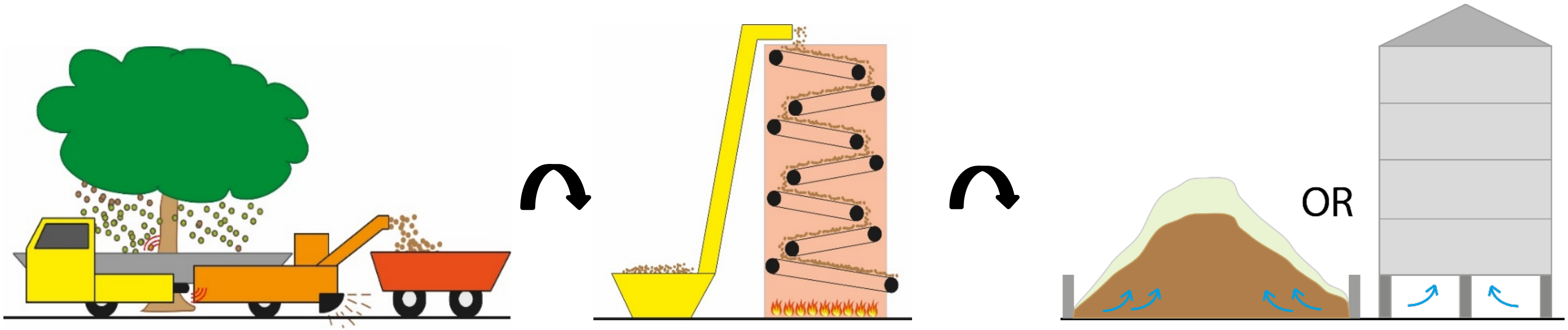
Dr Maryam Shirmohammadi

Michael Coates

University of South Australia

What is green harvesting?

1. Shake and catch (fruit not hitting the ground)
2. On-farm hulling (keeping hulls on farm)
3. Mechanical dehydration
4. Aerated on-farm storage



Shake and Catch | Dehydration | Storage

Benefits

- Harvesting independent of the weather
  - Avoids fruit damage from rain events
- Reduces mould / bacteria exposure
- Reduces pest infestation
- Easier to remove hull whilst green
  - Retain hulls of farm (nutrient value)
  - Reduced storage and transport costs
- Eliminate sweep and pickup passes
- Dust reduction

http://www.abc.net.au/news/2015-04-29/rain-allows-for-second-almond-harvest/6427720
http://honeywithoutflowers.blogspot.com.au/2012/10/harvest-fog.html

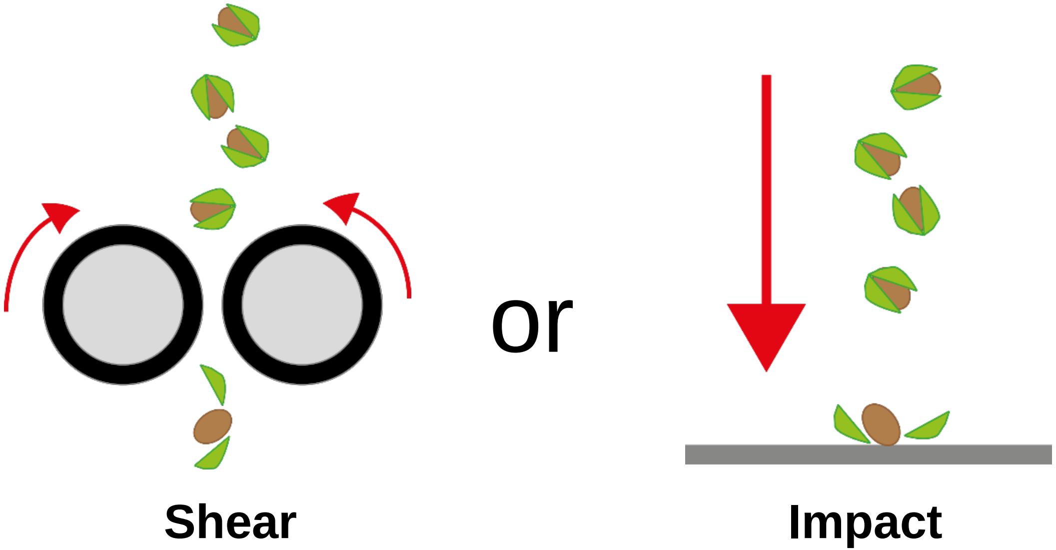
Shake and catch

Modify existing equipment (side by side)

http://www.theolivecentre.com/Olive-Equipment/Mechanical-Harvesters/COE-Olive-Harvesters.html

In-field hulling

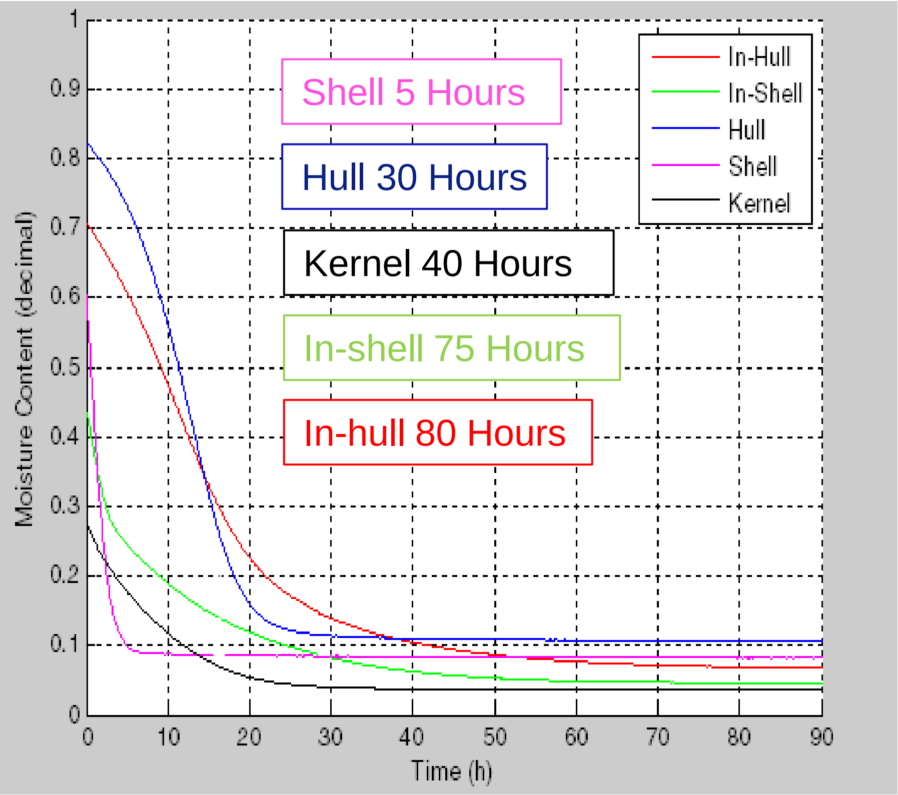
- Moisture dependant
- Hull separation
- Hull milling



Mechanical Dehydration

- Drying rates (diffusion)
  - Constant rate
  - Falling rate
- Continuous / Batch flow dryers
  - IR
  - Forced air
- Managing the moisture gradient
  - Intermittent drying
- Remove the hull before dehydrating

Carmel 1% hull split @ 50°C

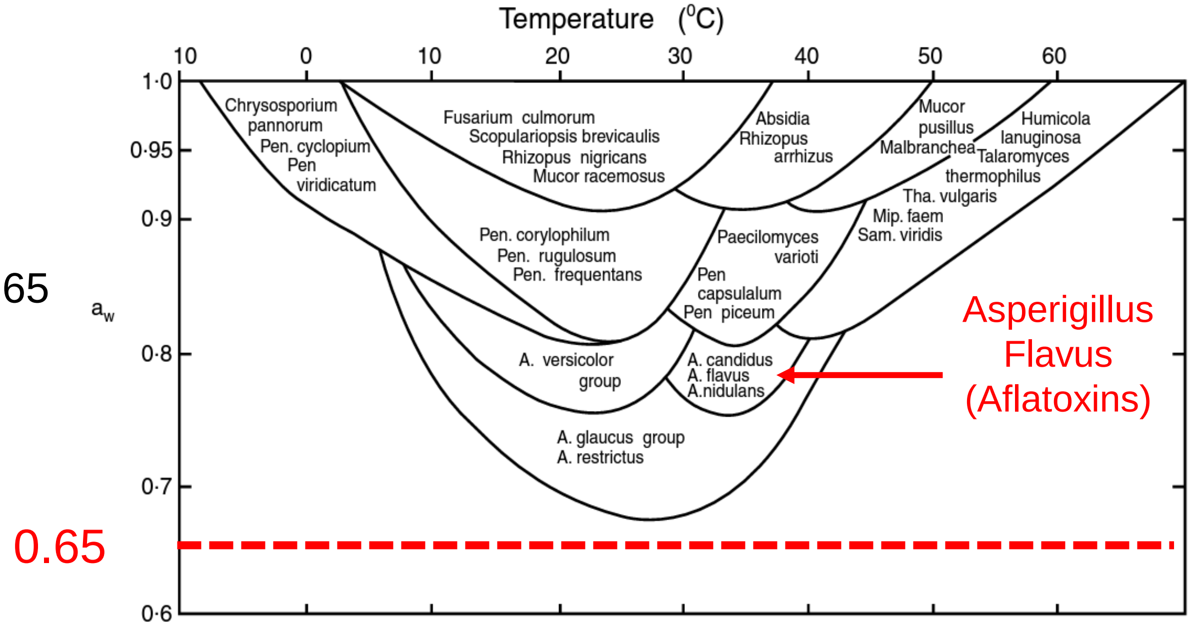


On farm storage

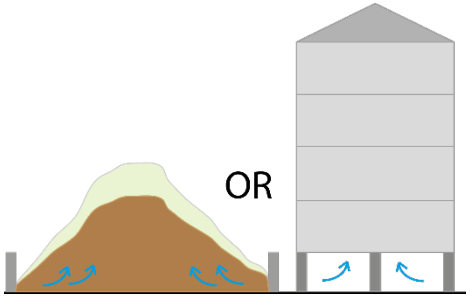
Safe storage of in-shell

- Silo
- Covered stockpile

Maintain water activity below 0.65



(Lacey, J S.T., ad Edwards M.A., 1980)



Feasibility testing (July 2015)

- Shake study
- Skin quality
- Cavites
- Brown centres

Strain Ranch, Arbuckle CA

July 2015 – Professor Patrick Brown, UCDavis and Professor Zhongli Pan, UCDavis

3 rows of 4 trees

Shake 1 row per week followed by a re-shake in the following week.

1 bucket (6 kg sample) for each tree was examined for:

- Moisture content
- Insect damage
- Fruit hull split distribution
- Tree damage
- Skin (pellicle) damage

The re-shake was done to determine fruit left on the tree (future mummies).

Strain Ranch, Arbuckle CA

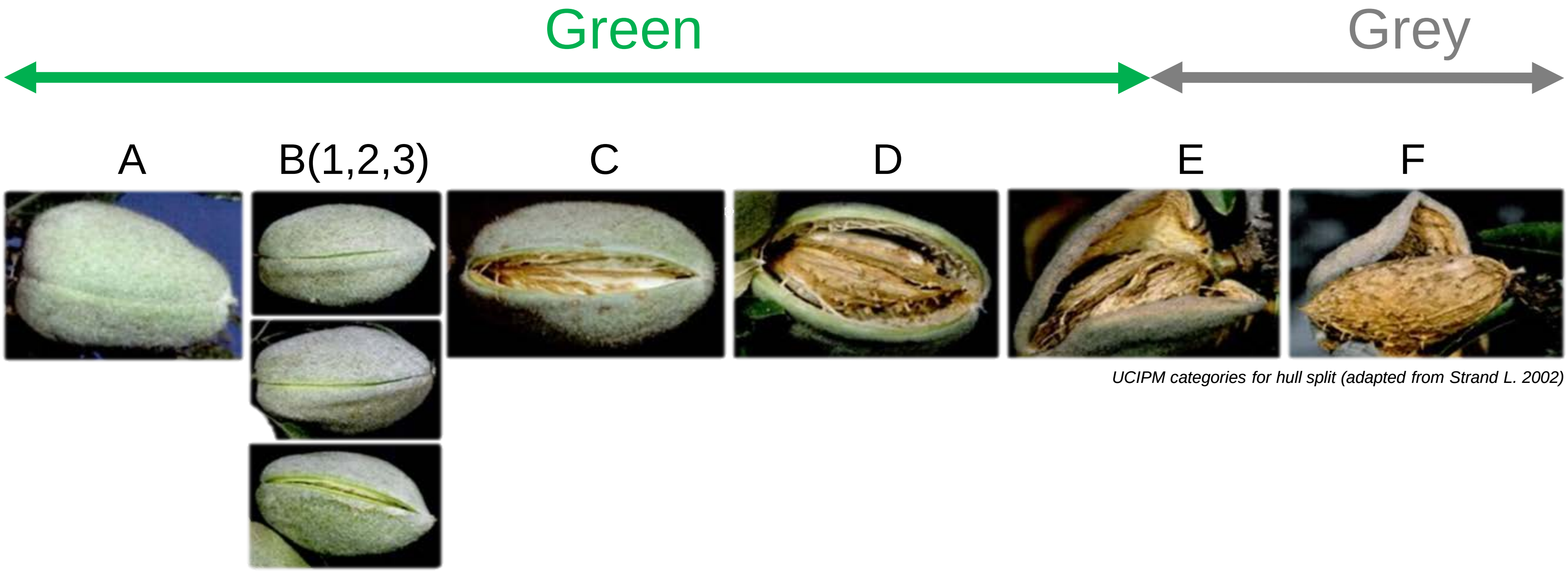
For early shaking:

- Stopped irrigation 5 days before shake to prevent barking.
- Normal shaking cycle on 10 year old, drip irrigated trees.
- No tree damage was observed.
- Simular or less fruit remained on tree after first shake.

	5 weeks out	4 weeks out	3 weeks out
Re-shake (remaining fruit)	42 ± 9	4 ± 1	30 ± 16

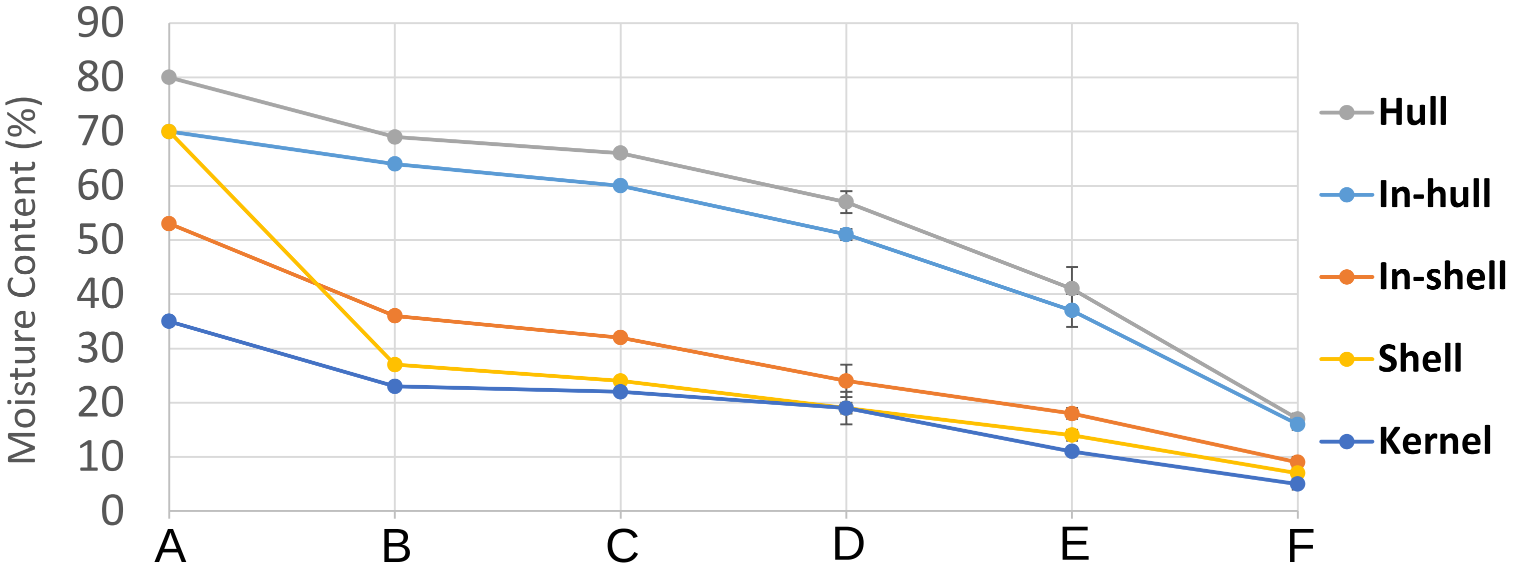
Stages of hull split

Transitioned to the UC IPM (Integrated Pest Management) (Strand L, 2002)



UCIPM categories for hull split (adapted from Strand L. 2002)

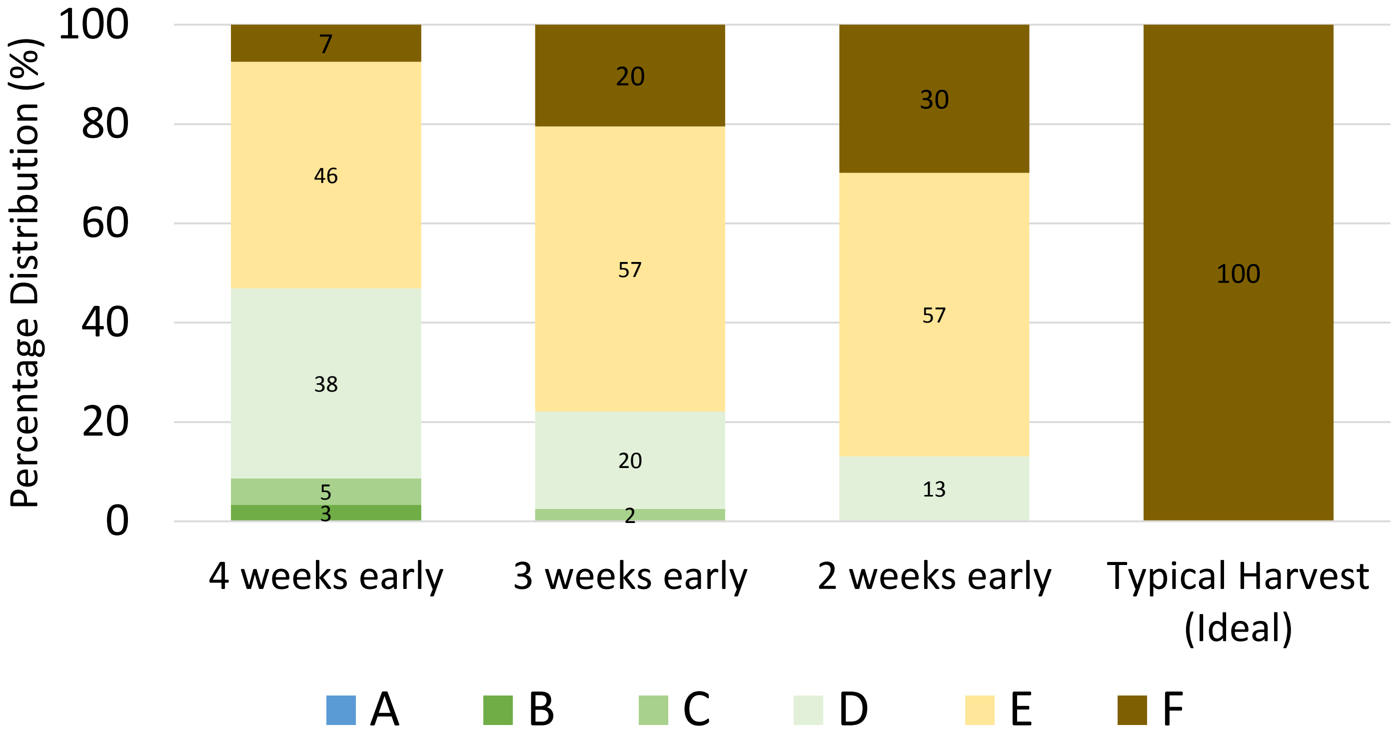
Stages of hull split



UCIPM categories for hull split (adapted from Strand L. 2002)

Category

Fruit category distribution



Observable pest damage

Carob moth / Navel orange worm

Eggs were visible in D and E,
Kernel damage was only visible in the F category

Week 1 showed 1 in 10 kernels infested in F
No observed eggs in A-D

Week 2 and 3 showed 1 in 20 kernels infested in F,
Eggs observed in D, small worms in E

http://www.ozanimals.com/Insect/Carob-Moth/Apomyelois/ceratoniae.html
https://www.studyblue.com/notes/note/n/grapes--orchards/deck/2417207

Skin quality after drying

Green harvested kernel (A-B) dried outside of the shell showed:

- Uneven colour
- Inconsistent texture
- Detached pellicle

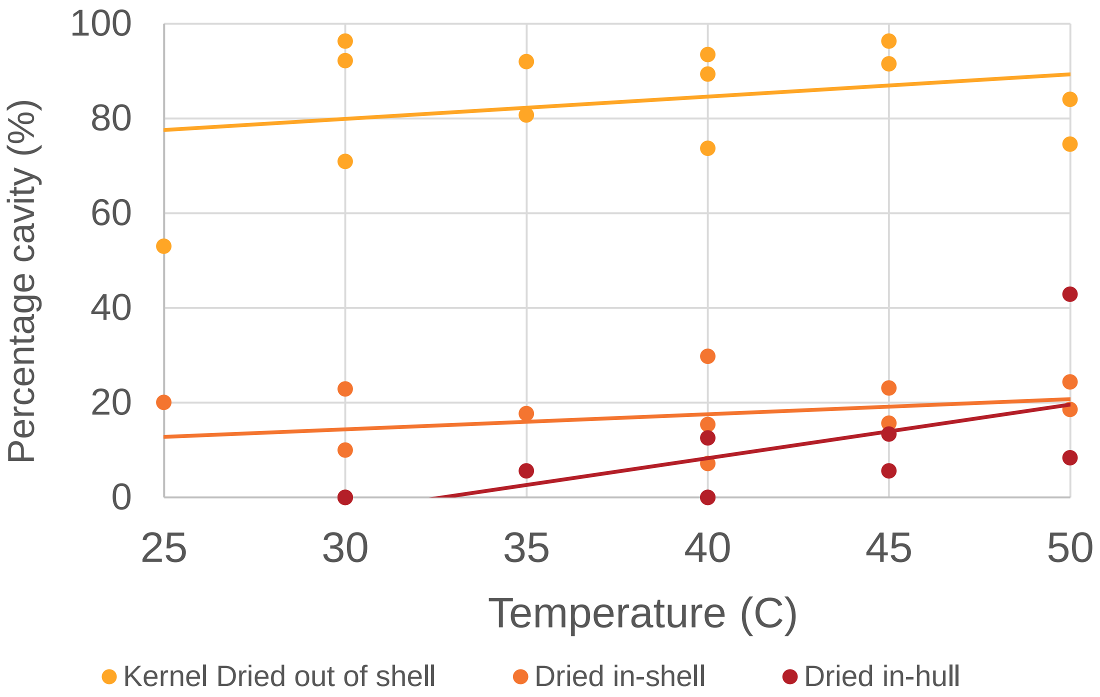
High temperature drying (≥ 60°C) of in-shell showed:

- Flaky pellicle

Low temperature drying (≤ 40°C) of in-shell showed:

- Consistent golden colour with no pellicle damage

Drying – Avoiding cavities



- Cavities exist in current drying methods
- Avoid rapid removal of moisture

Drying - Avoiding brown centres

Category **D** in-hull
(~19% Kernel MC)

100%

Category **E** in-hull
(~11% Kernel MC)

20%

Category **F** in-hull
(~5% Kernel MC)

0%

held at 40°C for 48 h.

Cannot take green fruit and hold at high temperature while drying.

Need to replicate best field drying conditions (intermittent drying)

Green harvest feasibility

Can be done if:

- Harvest in the E category (11% kernel moisture)
- Intermittent drying (>15% kernel moisture)
    - Manage rate of moisture loss to prevent cavities
    - Maintain low kernel temperature to prevent brown centres

What is next for the Australian 2016 season.

Separation of hull from in-shell.

In-shell dehydration modelling of the late (D,E and F) categories.

Transportable version of the huller, dryer and a small aerated silo.

Repeat of the pilot trial on a larger scale.

Thank you

Professor John Fielke – The University of South Australia

Almond Board of Australia

Professor Patrick Brown - UC Davis Plant Sciences

Prof. Zhongli Pan - UC Davis Agricultural Engineering

Costas, Jubilee, Strain Ranch

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Questions?

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