Mobilizing Ca to enhance fruit quality:

Application of harpin αβ (ProAct®) in citrus orchards in Spain

Ángel Marín, Plant Health Care S.A. Spain.
amarin@planthealthcare.com
K. Staska, A. Dillon.

Agenda

- What is Harpin ab?
- What is the MOA of Harpin ab?
- Study Mobilizing Ca on citrus:
  - ✓ Technical approach
  - ✓ Economic approach
- Conclusions
- Acknowledgements

Main Discussion

- Can farmers potentially achieve a higher price by delaying citrus harvest until later in the season?
- If so, What inputs should they manage to achieve the best market window?
- What sort of pre and post harvest measurements could be established?

Harpin Technology: The Discovery

- Class of proteins, harpins, that function in nature as warning compounds
- Heat-stable, no cysteine, glycine-rich, linear, acidic protein
- Derived from “Hypersensitive Response & Pathogenicity N” gene (HrpN)
- Discovered by Zhongmin Wei et al. at Cornell University
- Featured on cover of Science Magazine
- In 2002, E.P.A. conferred the “Green Chemistry Challenge Award” granted by the President of the United States.

Wei, Z., Laby, R., Zumoff, C., Bauer, D., Ho, S.Y., Collmer, A. and Beer, S. (1992), “Harpin, elicitor of the hypersensitive response produced by the plant pathogen Erwinia amylovora”,Science, Vol. 2 No. 57, pp. 85-7.

Harpin aβ

Second Generation (2G)

Harpin aβ: 4 α, β domain protein from:

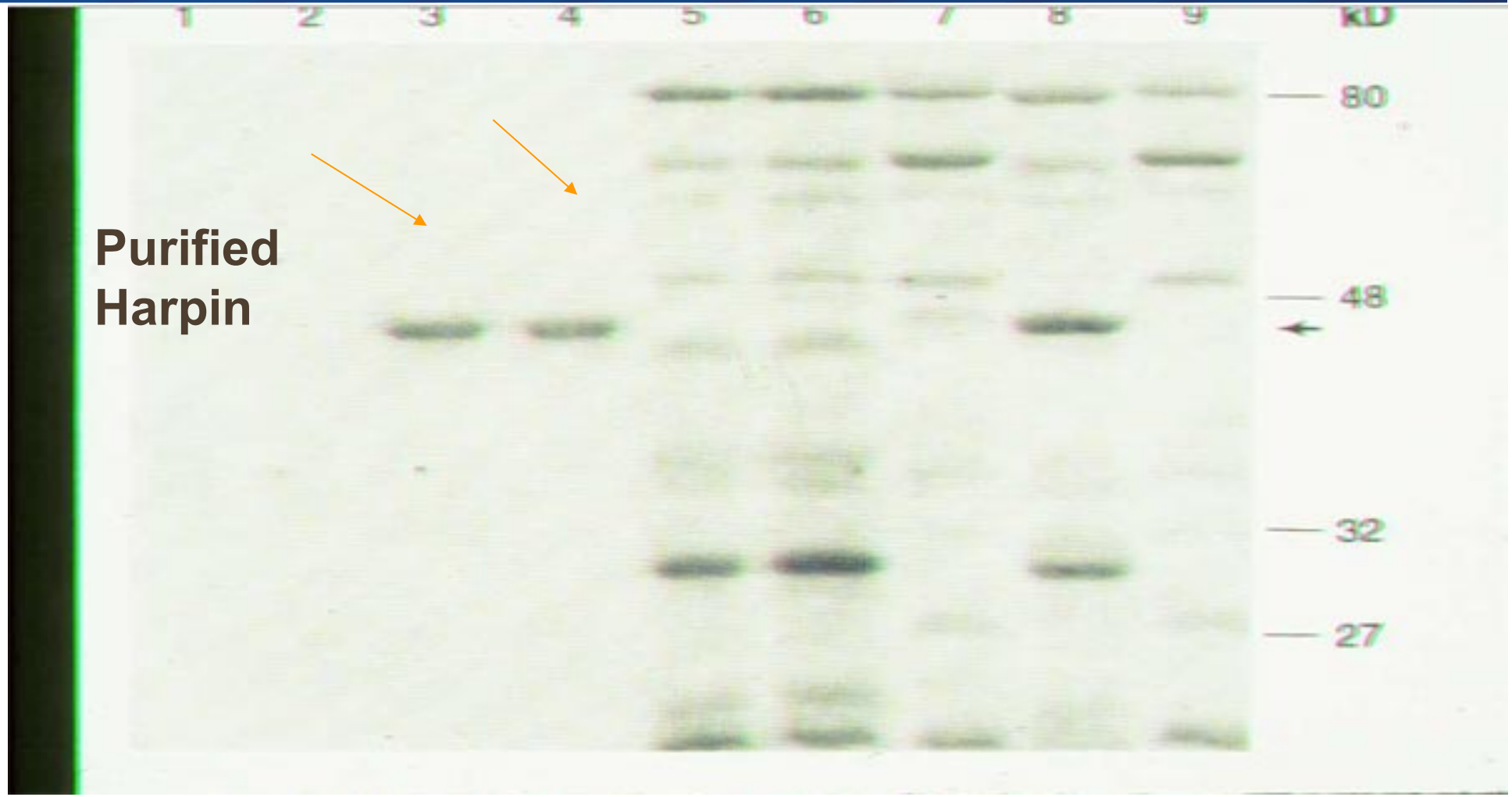
Súper-harpin 1 (Harpin aβ) HR index 5 nm,Growth index 2 ppm

1 412 aa

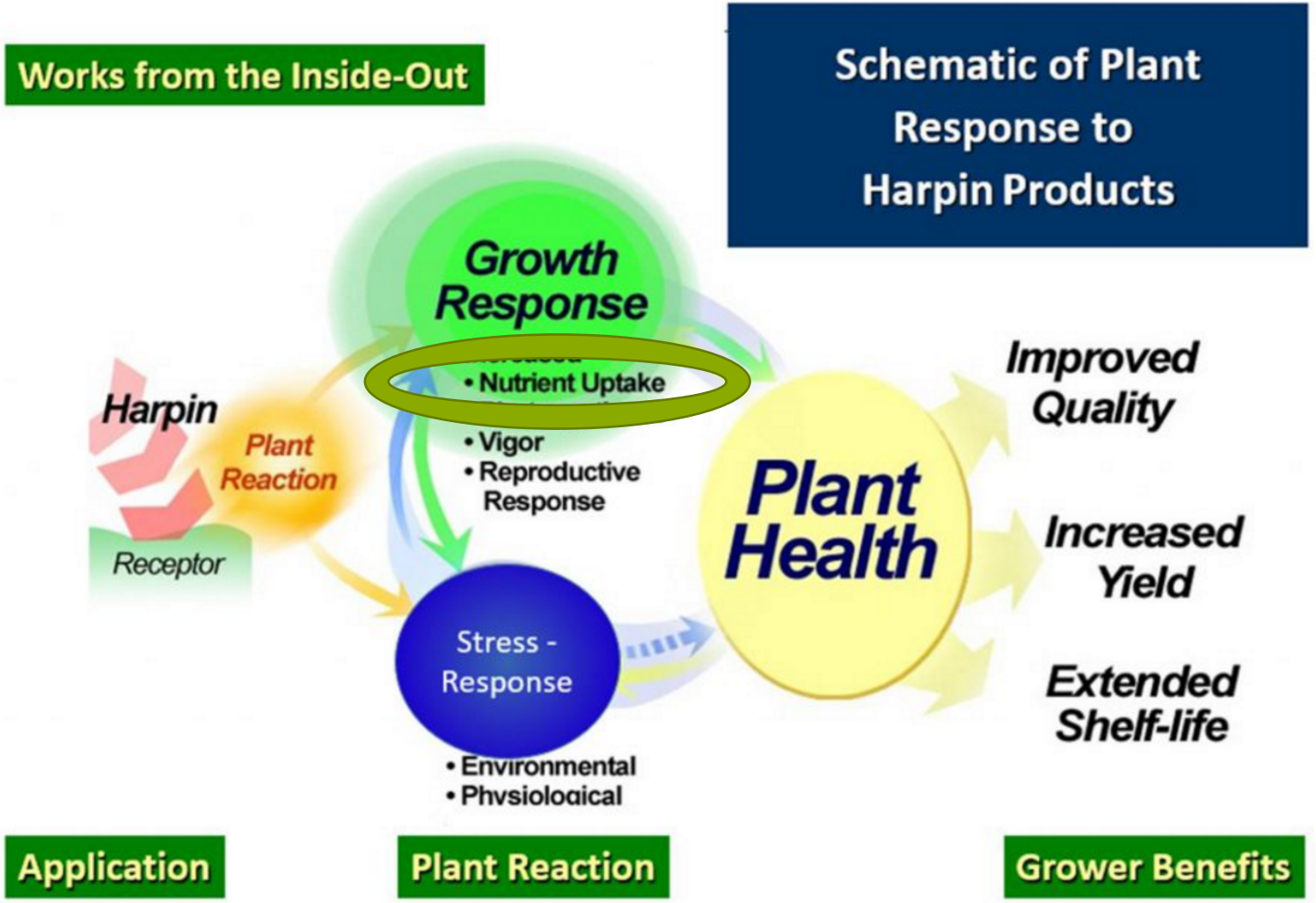
HrpW HrpZ PopA HrpN

HrpN -*Erwinia amylovora*.
HrpW -*Pseudomona syringae*.
HrpZ -*Pseudomona syringae*.
PopA -*Ralstonia solanacearum*.

Harpin Protein Shown in SDS PAGE



Harpin aβ Mode of Action



Plant Growth Gene Activation by Harpin

124 genes induced two fold or greater with statistical significance
Classes of genes with elevated expression

- General growth regulation genes

  -Camoldium and calmodulin-like proteins
  -Growth transcription factor
  -Sugar synthase, glucose, fructose and others
  -Embryogenesis enzymes
  -Ethylene response element-binding protein family
  -Hypocotyl elongation transcription factor

Plant Growth Gene Activation by Harpin

- Cell wall development genes
  - -Cell wall development kinases
  - -Gibberellin-induced cell wall proteins
  - -Auxin response genes
  - -Touch gene
  - -Leafy gene

- Stress tolerance
  - -Heat shock proteins
  - -Cold genes (COR)
  - -Salt tolerance zinc proteins

- Novel genes without known functions

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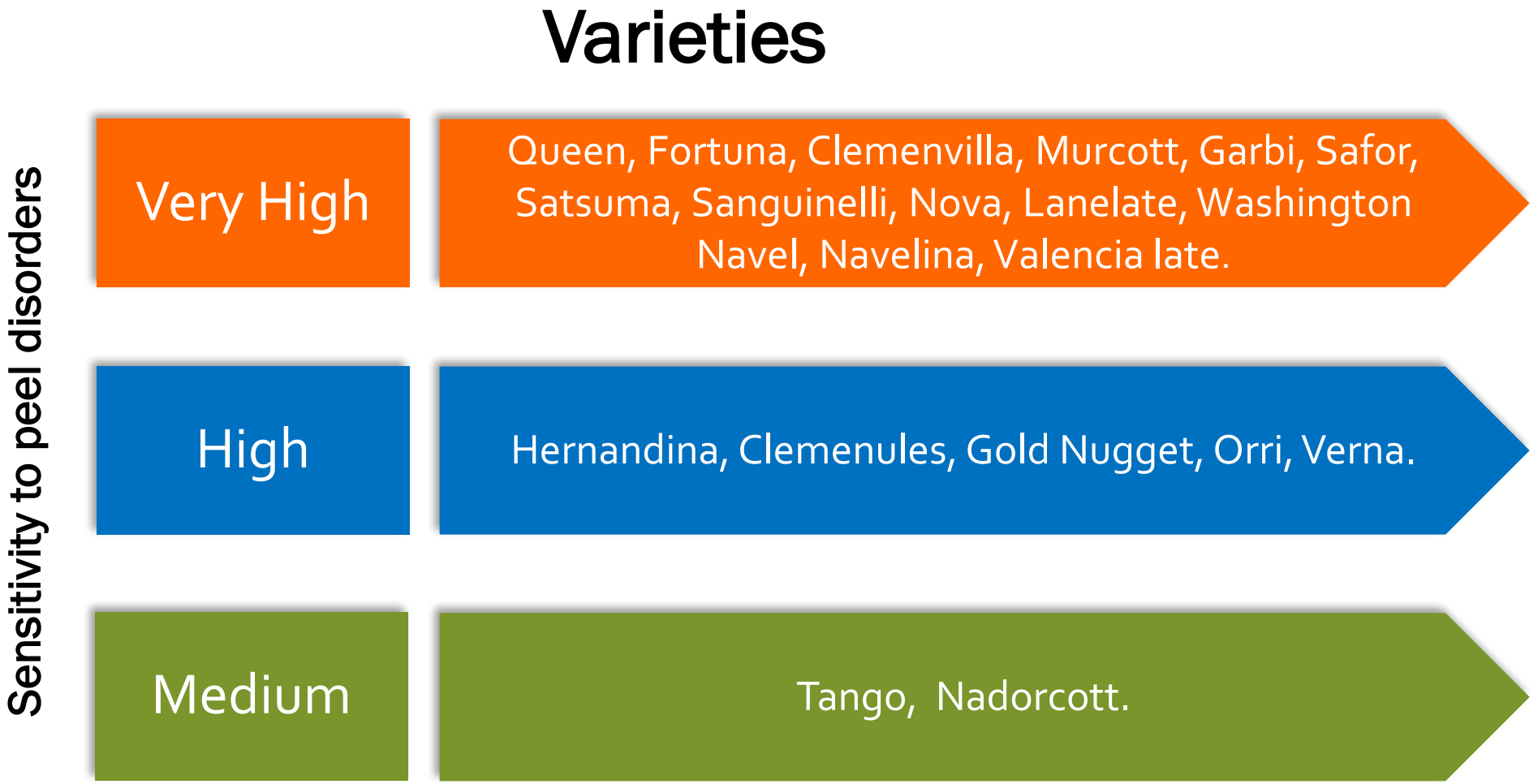
Citrus varieties sensitive to peel disorders



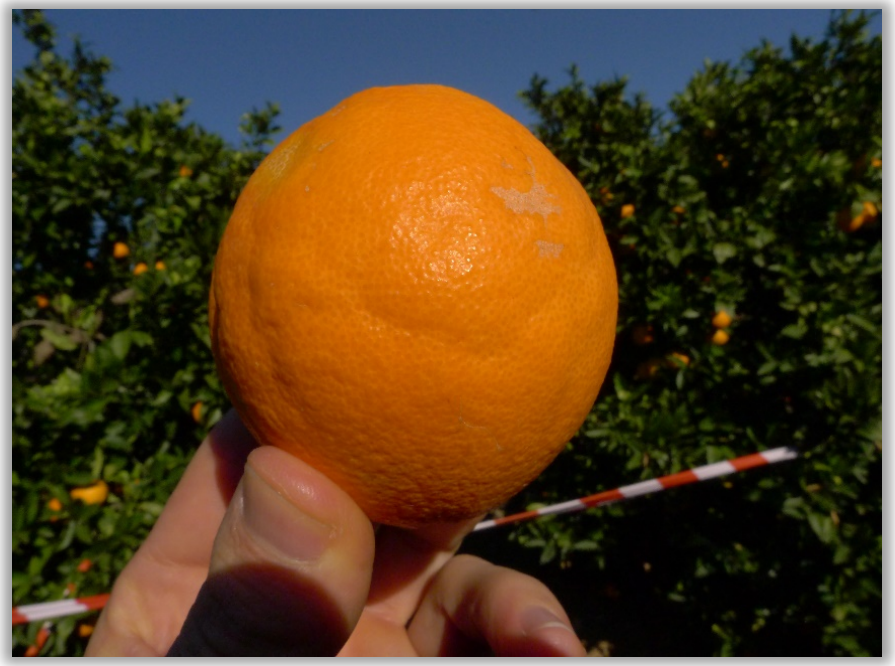
Source: PLANT HEALTH CARE, AGROVESA.

Calcium deficiencies in Citrus

- **Calcium content** has been associated to a number of pre and post harvest phisiological disorders.

QUEEN WITH WATER SPOT

NAVELINA WITH CREASING

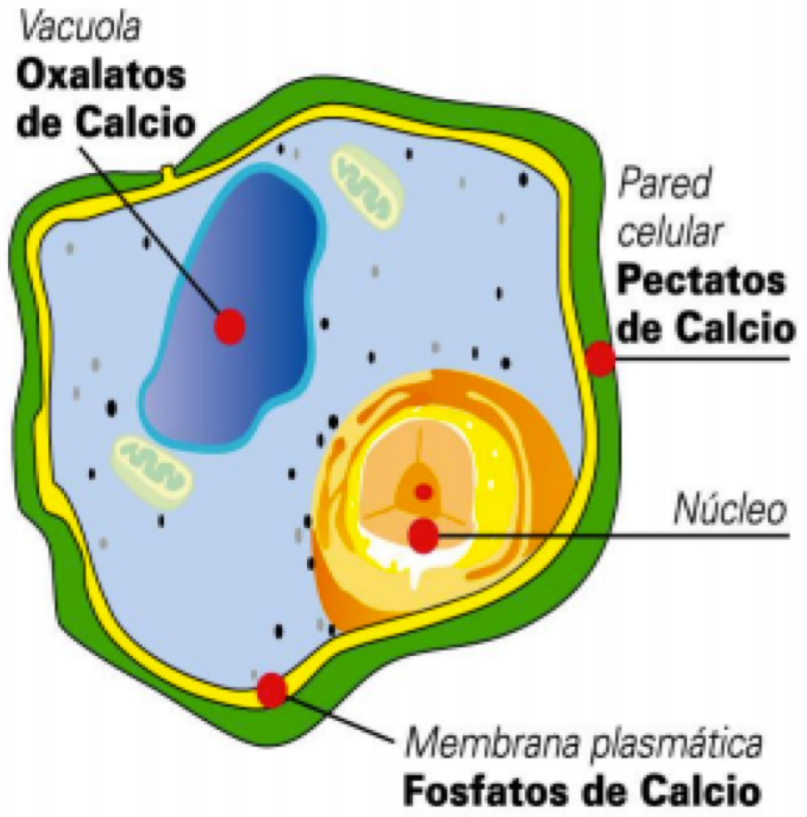


Source: IVIA, AGROVESA.

What is Bound Calcium?

- There are three forms of Calcium in plant cells

  - ✓ Pectines
  - ✓ Fosfates
  - ✓ Oxalates

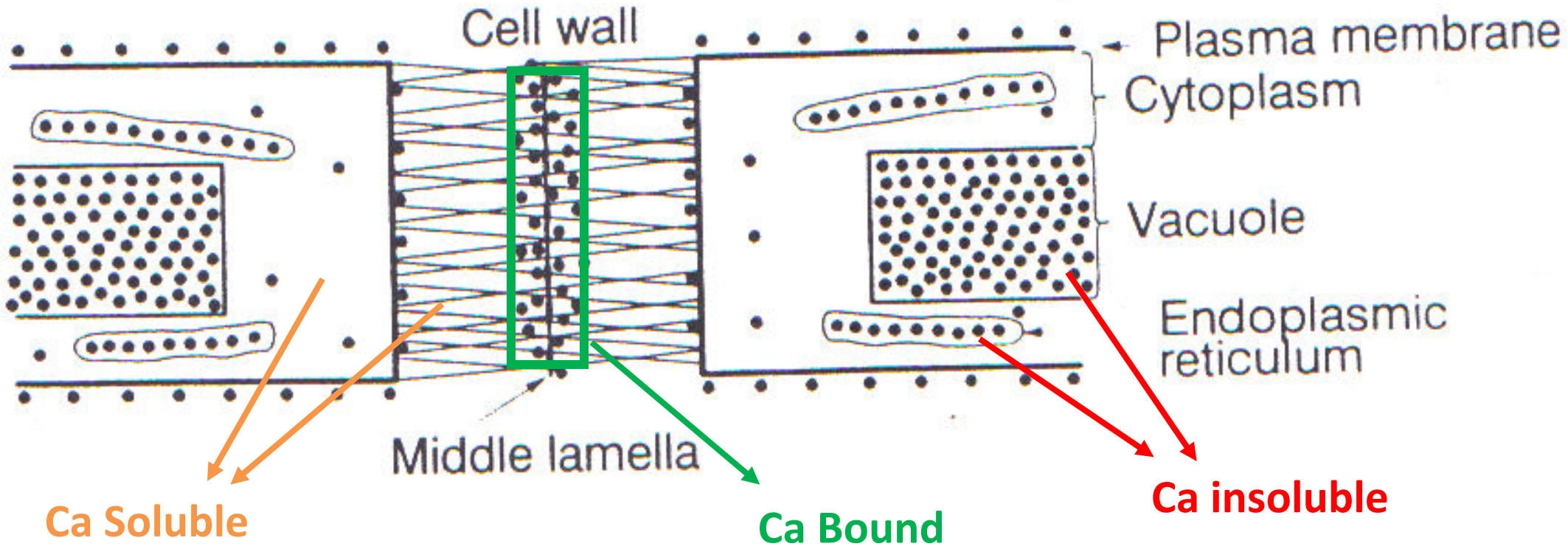


Source:Bonomelli et al 2008

Main factors determining the level of Bound Calcium

- Oxalic acid level
- Nitrate: Inhibits enzymes of oxidasa oxalic acid
- Light: Activity associated to nitrate reductasa enzyme
- Stress: Increases level of ethylene and free radicals
- Ability to transport calcium
- Xilems development
- Ca entry mechanisms into cell

Determining bound calcium to pectines



Source: AGQ

Effects of Harpin in Citrus

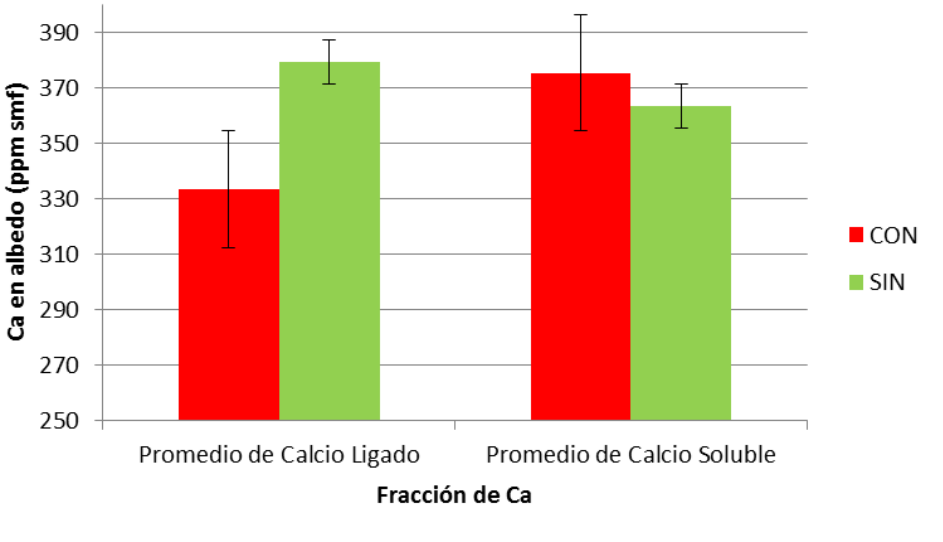
- Previous studies related Harpin with Peel alteration: PIXAT (water spot).

  Legaz, F., Quiñones, A., Martínez, B., Gardey, P. 2005. Levante agricola.

- Determination of Bound Ca to pectate in fruit peel.
  http://www.agq.com.es/

WITH CREASING

WITHOUT CREASING



Scientific papers for Harpin technology on citrus

- Legaz, F., Quiñones, A., Martínez, B., Gardey, P. 2005. ***Efecto de las proteínas harpin sobre las alteraciones no patológicas de la corteza del fruto "pixat" de clementina de Nules.*** Levante Agrícola: Revista internacional de cítricos, ISSN 0457-6039, Nº. 382, 2006 , págs. 277-282.
- Iglesias, D., Navaza, J.M., Ibañez, V., Talón, M. 2005. ***Efecto de las proteínas harpin sobre el desarrollo vegetativo de cítricos de las variedades clementina de nules y nova***. Levante Agrícola: Revista internacional de cítricos, ISSN 0457-6039, Nº. 378, 2005 , págs. 384-393.
- PHIRI, Z.P. 2010. ***Creasing studies in citrus.*** Dissertation presented for the degree of Master of Science in Agriculture at Stellenbosch University.
- David Reboutier and François Bouteau 2008. ***Harpins and ion channels modulations.*** LEM; EA 3514; Université Paris Diderot; Case 7069; Paris, France.
- Janaynna Magalhaˆes Barbosa-Mendes a, Francisco de Assis Alves Mouraˆo Filho a,Armando Bergamin Filho a, Ricardo Harakava b, Steven V. Beer c, Beatriz Madalena Januzzi Mendes d,2009. ***Genetic transformation of Citrus sinensis cv. Hamlin with hrpN gene from Erwinia amylovora and evaluation of the transgenic lines for resistance to citrus canker.*** Scientia Horticulturae 122 (2009) 109–115.
- Lucon C., Guzzo, S., de Jesús C., Pascholati S., de Goes A. 2010. ***Postharvest harpin or Bacillus thuringiensis treatments suppress citrus black spot in 'Valencia' oranges.***
- Agostini J.P., Bushong P.M, Timmer L.W. 2003***. Greenhouse Evaluation of Products that Induce Host Resistance for Control of Scab, Melanose, and Alternaria Brown Spot of Citrus.*** The American Phytopathological Society. D-2002-1112-01R.

Trial Sites – Alicante and Seville

Queen Mandarin - Characteristics

- Satsuma-Clementine hybrid unknown origin
- Medium vigorous tree
- Fruit weight: 100-130 g
- Diameter: 65-75 mm
- Peel: 2.3 - 3.0 mm
- Intense orange colour
- Juice %: 50-55
- Brix: 11-14
- High production
- Harvest: January 1st to March 10th

Source: IVIA

Queen – Alicante (2015)

Objetives: Reduce water spot by increasing cell wall calcium with ProAct applications
Crop: Mandarin *Citrus unshiu*
Variety: Queen
Rootstock: Citrange carrizo
Trees per ha: 500 trees/ha
Age: Began production in 2012.
Location: Dehesa de Pino Hermoso, Orihuela, Alicante
Soil type: Clay loam
Rates: ProAct foliar application 150 g/ha
Thesis:

T0=Grower standard
T2= Grower standard + 3 ProAct apps in April, July and September

Sampling date: November 17th, 2015
Analysis dates – Start and Finish: 19 – 26 of November 2015
Materials and Methods: Lab Analysis powered by AGQ Labs. Espect ICP-OES, gravimetry.

Queen – Seville (2017)

Objetives: Reduce water spot by increasing cell wall calcium with ProAct applications
Crop: Mandarin *Citrus unshiu*
Variety: Queen
Rootstock: Citrange carrizo
Trees per ha: 416 trees/ha
Age: Began production in 2017.
Location: La Romana farm. Utrera, Seville
Soil type: Sandy loam
Rates: ProAct foliar application 150 g/ha
Thesis:

T0=Grower standard
T2= Grower standard + 3 ProAct apps in August and November

Sampling date: November 30th, 2017
Analysis dates – Start and Finish: 2 – 12 of December 2017
Materials and Methods: Lab Analysis powered by AGQ Labs. Espect ICP-OES, gravimetry.

Biostimulant application schedule

Table 1. Biostimulant treatment and application schedules across both sites

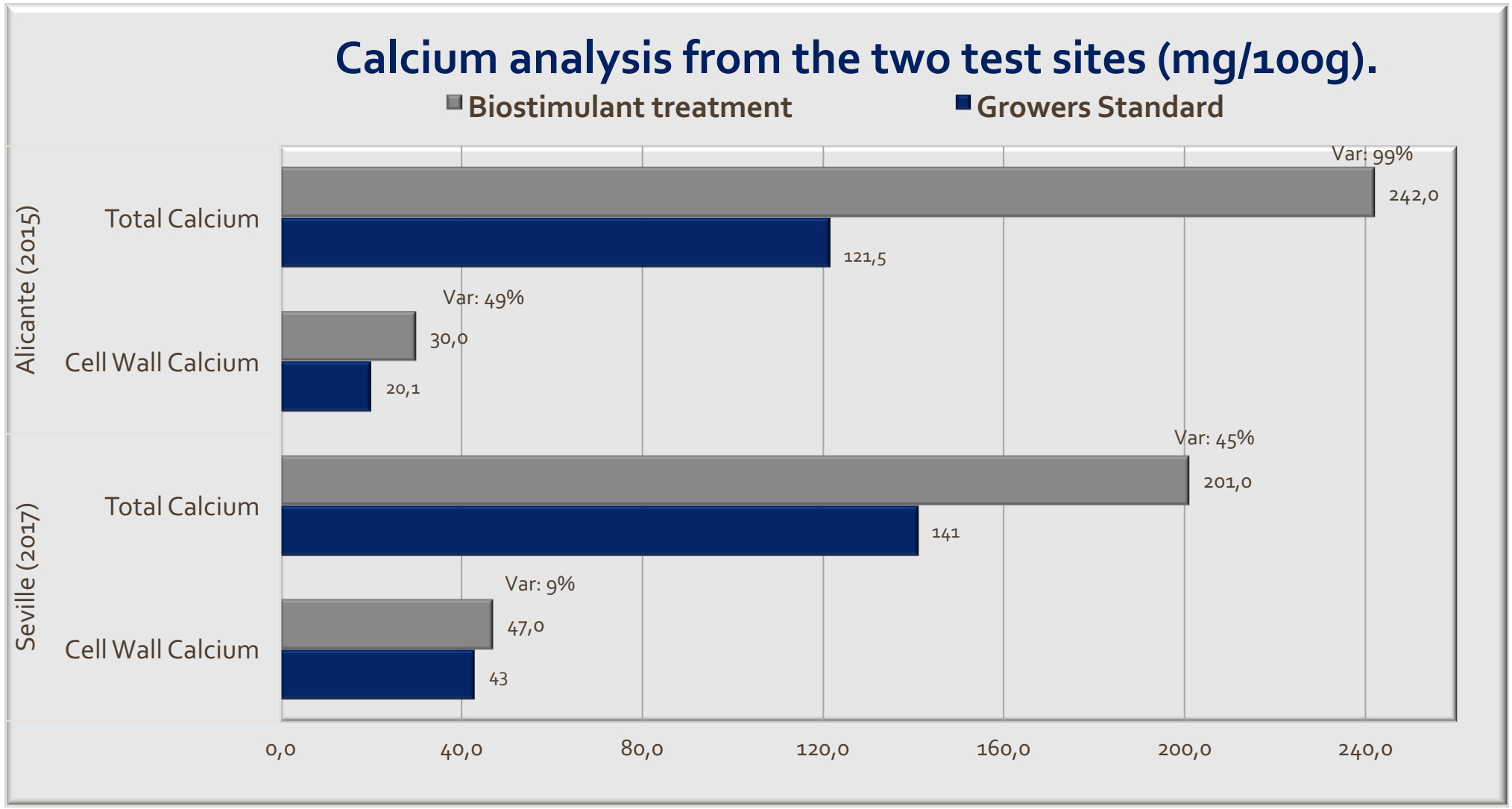
Site	Treatment	Dose	Application dates
Alicante, Spain 2015	ProAct®	3 x 150g/Ha	1. Flowering (GS65) - 30 April 2015 2. Flowering + 12 wks. - 28 July 2015 3. Flowering + 20 wks. – 21 Sept. 2015
Seville, Spain 2017	ProAct®	3 x 150g/Ha	1. Fruit development (GS75) - 15 Aug. 2017 2. Fruit development + 2 wks. – 25 Aug. 2017 3. Fruit development + 13 wks. – 15 Nov. 2017

Results

Table 2: Calcium analysis from the two test sites.

Site	Crop	Parameter (mg/100g)	Growers Standard	Biostimulant treatment	% Increase
Alicante, Spain 2015	Mandarin variety Queen	Total Calcium	121.5	242.0	99
		Cell Wall Calcium	20.1	30.0	49
Seville, Spain 2017	Mandarin variety Queen	Total Calcium	141.0	201.0	45
		Cell Wall Calcium	43.0	47.0	9

Results


Calcium analysis from the two test sites (mg/100g).
Biostimulant treatment
Growers Standard
Alicante (2015)
Total Calcium
Var: 99%
242,0
121,5
Cell Wall Calcium
Var: 49%
30,0
20,1
Seville (2017)
Total Calcium
Var: 45%
201,0
141
Cell Wall Calcium
Var: 9%
47,0
43
0,0
40,0
80,0
120,0
160,0
200,0
240,0

Harvest - Alicante

- Control January 18, 2016

- Treated March 15, 2016

Photos: Filgueiras (2016).

Seville – Fruit size measurements

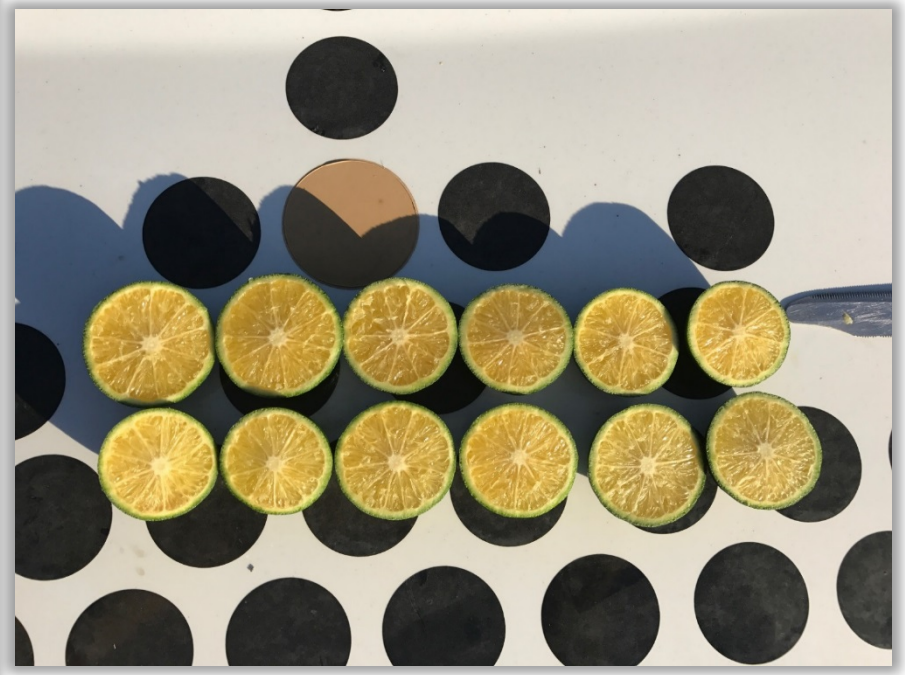


Photo: Parias (2017).

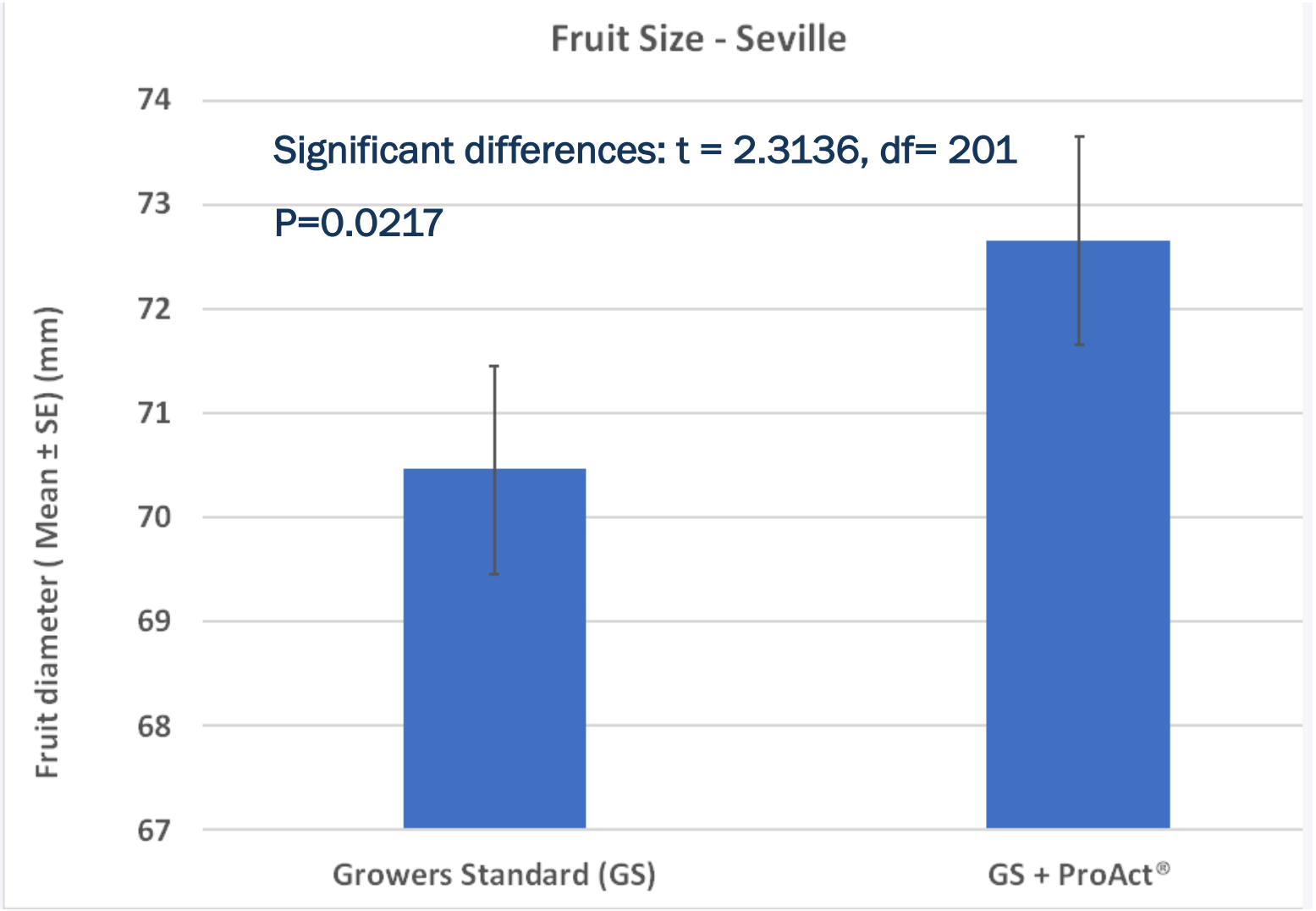
Average fruit size - Seville

GS= 70.45+-0.69 mm (n=104)

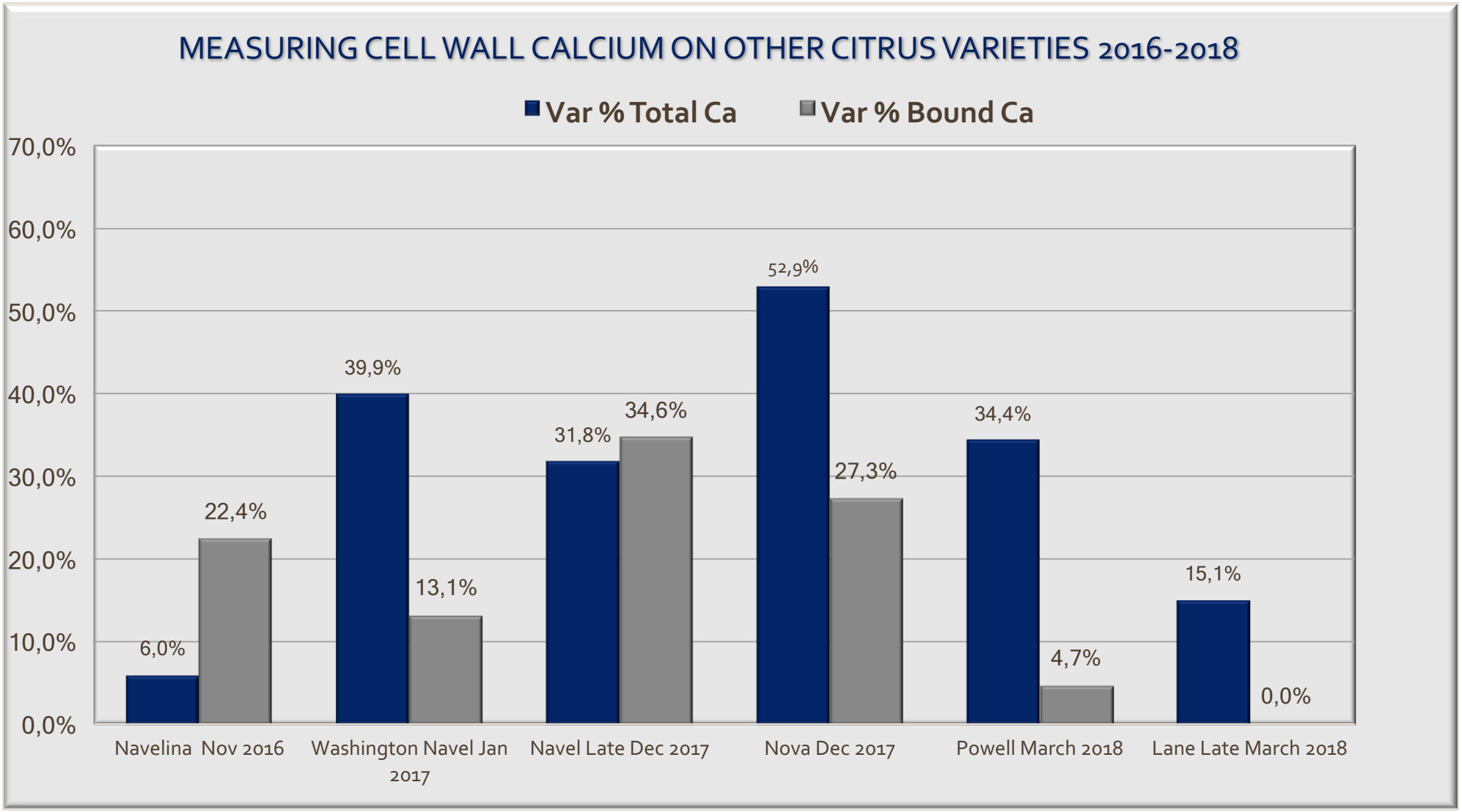
GS+ProAct=72.66+-0.66 mm (n=99)

Size/Calibres

1XXX (78+ mm)
1XX (67/78 mm)
1-1X (63/74 mm)
2 (58/69 mm)
3 (54/64 mm)
4 (50/60 mm)
5 (46/56 mm)
6 (43/52 mm)



% Of cell wall calcium on Harpin αβ treated compared to non-treated on other varieties 2016-2018



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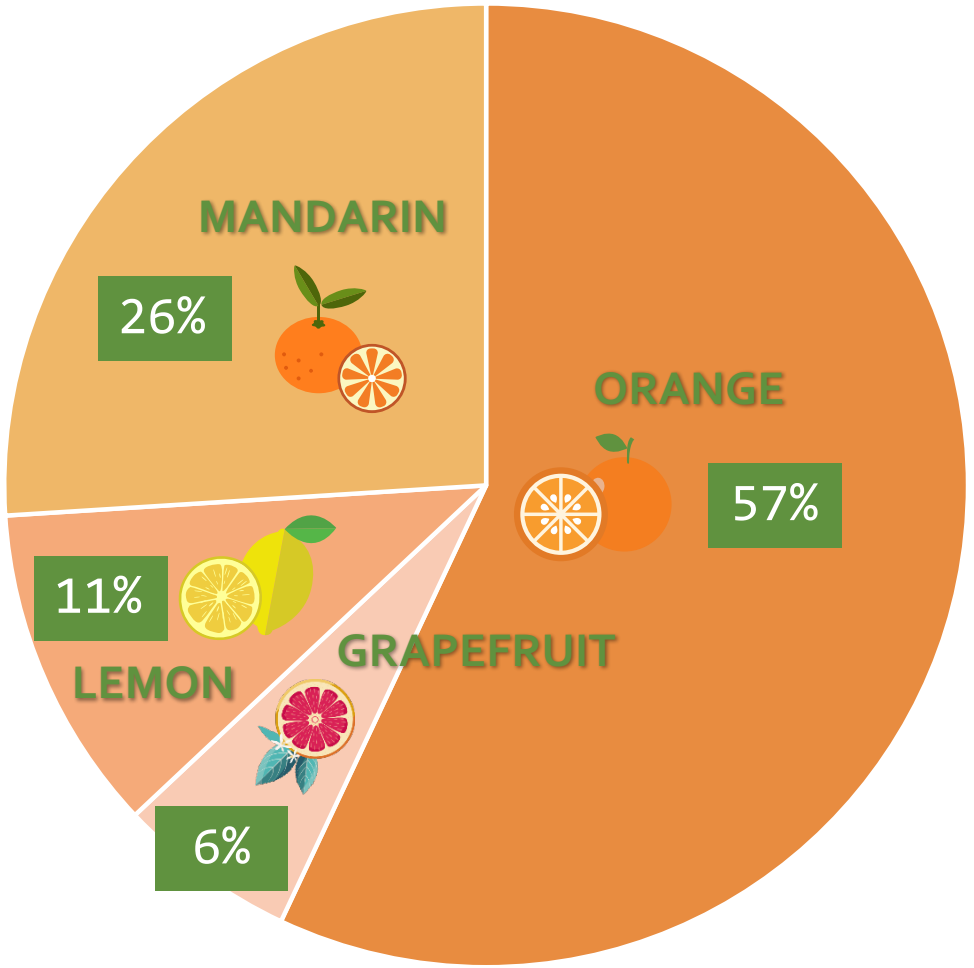
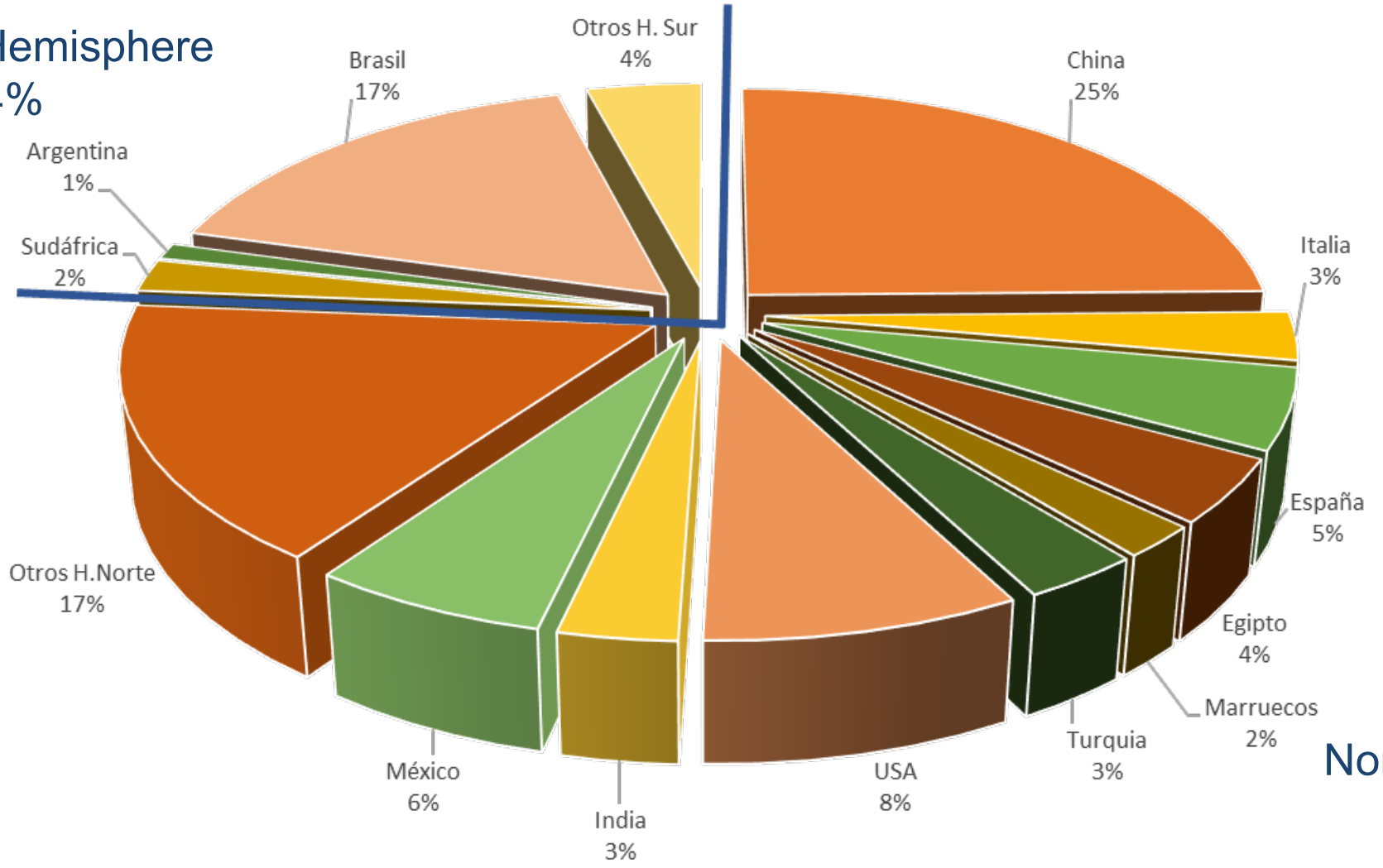
World citrus production



Production 2013/2014. Source: FAO 2015

Citrus world geographic distribution by production

Southern Hemisphere
24%



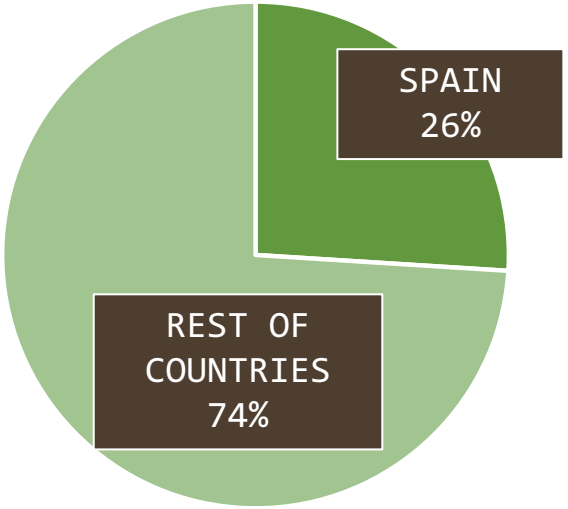
Northern Hemisphere
76%

Source: FAO 2015

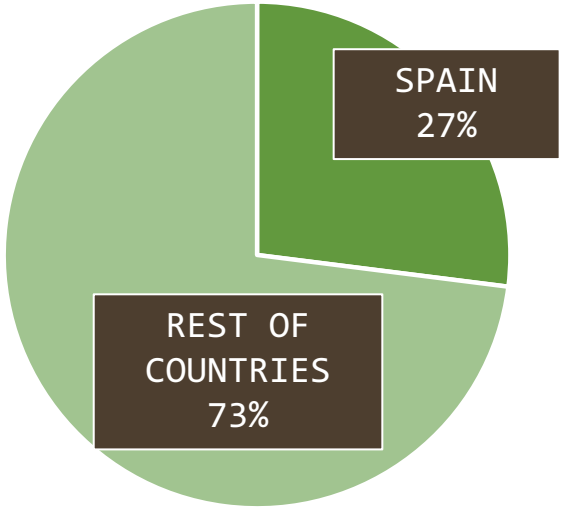
Citrus world players

Spain: 1st fresh citrus world exporter (orange, mandarin, lemon)

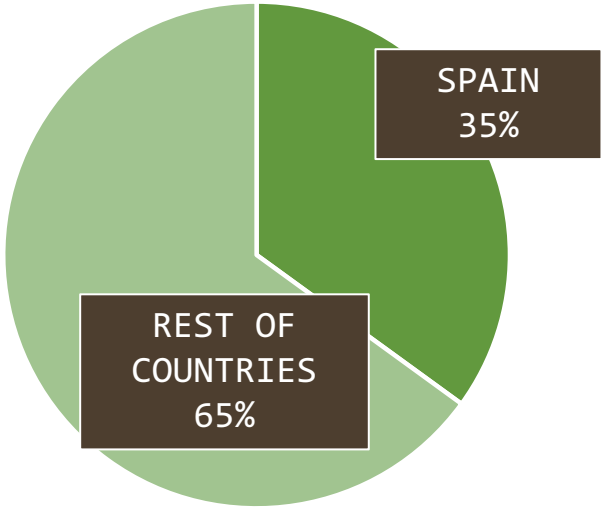
CITRUS WORLD EXPORTS
2012/13: 15.181.100 Tm



ORANGE WORLD EXPORTS
2012/13: 6.873.200 Tm



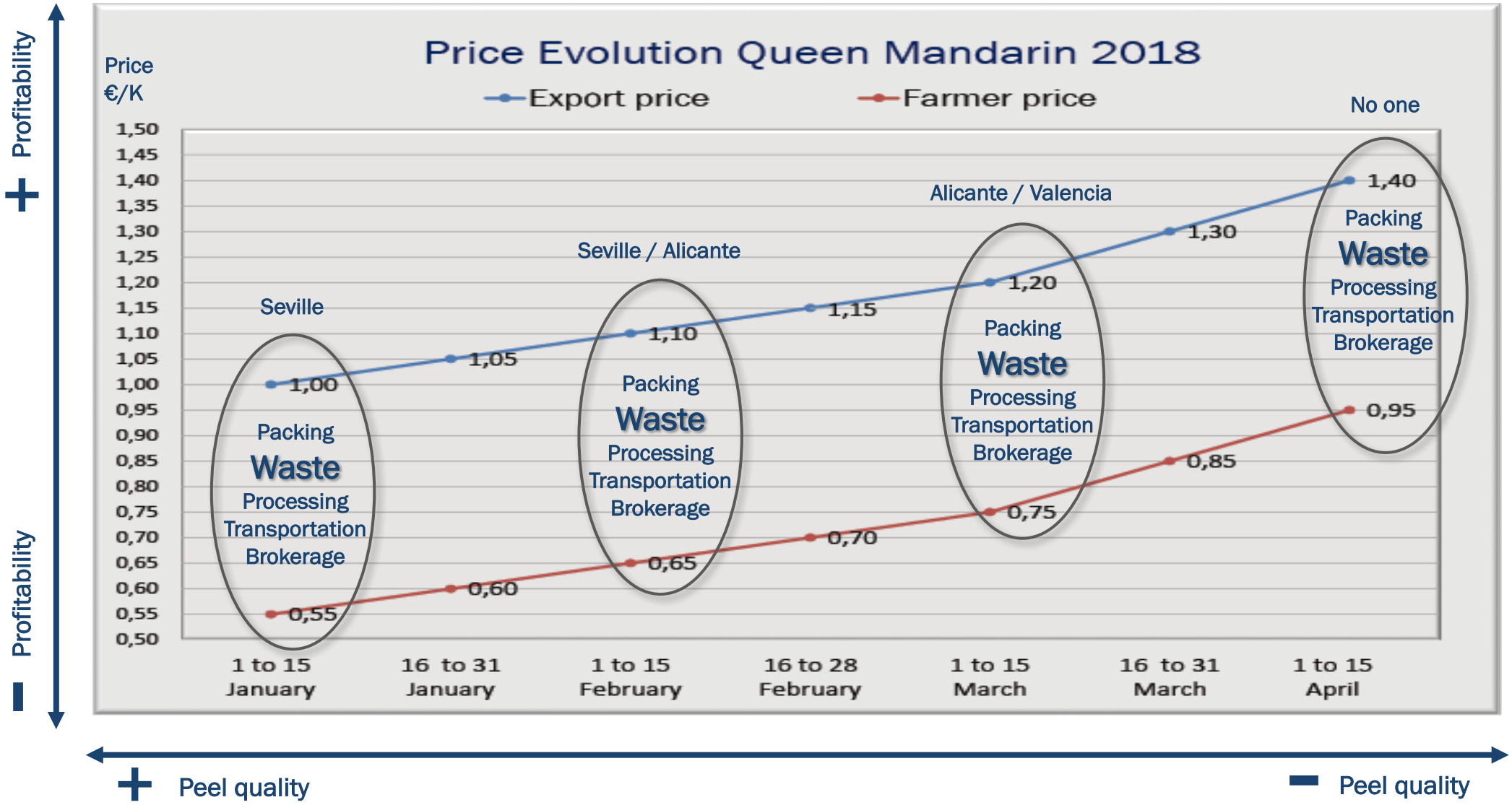
MANDARIN WORLD EXPORTS
2012/13: 4.617.000 Tm



45% of the world citrus exports market is controlled by ES, ZA & TR

Source: FAO 2015

Approx. Price trends queen mandarin 2018



Farm operation cost/ha (´000 Euro)

ALICANTE OPERATIONS 7,8

OTHERS 0,8

FERTILIZER 0,9

PESTICIDES 1

WATER 2,9

SERVICE OPERATIONS 1,6

HERBICIDES 0,6

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CONCLUSIONS

- Transcriptome studies have shown that harpins induce the expression of genes involved in cell wall development, cellular communication, signal transduction and defence responses (Livaja et al., 2008).

- Under laboratory conditions, it has been shown that Harpins have the ability to induce a Ca flux into plant cells (Vidhyasekaran, 2016).

- At fruitset the transpiration rate of fruit is at its highest, and it is during the early stages of fruit development that most Ca^{2+} is delivered to fruit (Montanaro et al., 2012). It is therefore not surprising that the greatest percentage increases in both total Ca^{2+} and cell wall Ca^{2+} were observed on the site where the first ProAct® application occurred just before fruit set (Alicante). At the Seville site the first application of ProAct® did not occur until BBCH GS 75, by which time average fruit size was 47 mm.

- The importance of increasing available Calcium early in the season to improve fruit quality has previously been demonstrated by Treeby and Storey (2002), who observed that early and mid season sprays of Calcium were better than late season applications to prevent the citrus rind disorder albedo breakdown.

Acknowledgements

The authors thank the farms (Explotaciones Agrícolas La Perdiz S.L. and El Jardín de Almayate) for trial sites and their technical staff who kindly cooperated on the trials.

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Many thanks!

Questions?

Ángel Marín
amarin@planthealthcare.com

Plant Health Care España S.A.

Managing Director: Angel Marín (Appointed January 2012)

Angel Marín took over Spanish branch in January 2012. Mr. Marín joined the Plant Health Care group in 2002. He spent 10 years in the Mexican subsidiary where he ended up being Sales & Marketing Manager. Mr. Marín was responsible for re-introducing first generation of harpins Messenger in Mexico and Colombia respectively. He also led Myconate introduction in Mexico. Mr. Marín holds a bachelor degree on International Business (Universidad Iberoamericana) and a MBA from Monterrey Tech (ITESM).