Advances in Post-Harvest Treatments

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Citrus Australia – Technical Forum

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IMPORTANCE OF POST-HARVEST

Key Figures

10,5 billion by 2050 (1)

33% more human mouths to feed

Food supplies need to increase by **60%** (2)

Increasing agricultural productivity is critical but this may not be sufficient

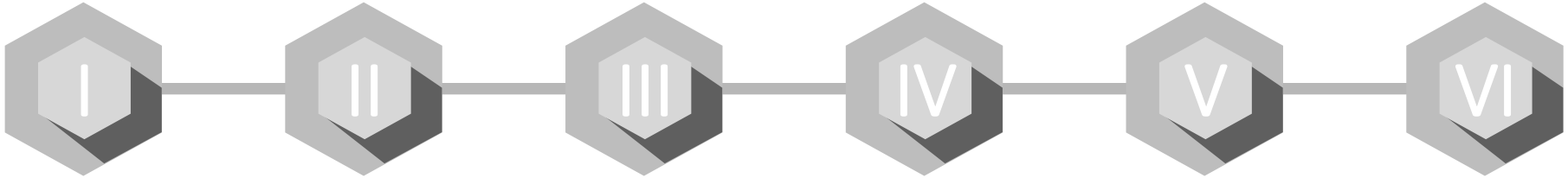
Limited land, water and increased weather variability due to climate change

95% of the R&D during the past 30 years focused on increasing productivity and only 5% directed towards reducing losses (2)

Waste reduction in postharvest process at farm, retail and consumer levels is needed

Sources:
(1) UN March 2013
(2) FAO 2012

POST-HARVEST FOOD LOSSES in supply chain flow



Harvesting

- Mechanization
- Production practices
- Decisions
- Moisture
- Season (temperature)

Food Storage

- Insects
- Molds
- Deterioration
- Shrinkage
- Moisture
- Temperature
- Improper handing
- Transport
- Time (duration)

Processing

- Removal of inedible portions
- Discarding of substandard products
- Visual based rejection
- Shrinkage
- Poor handling
- Package failures
- Transportation losses

Packaging

- Packaging failures
- Transportation losses
- Lack of packaging
- Improper packaging

Sales

- Improper portioning
- Supersizing
- Poor inventory
- Dented cans
- Seasonal items
- Temperature
- Infrastructure

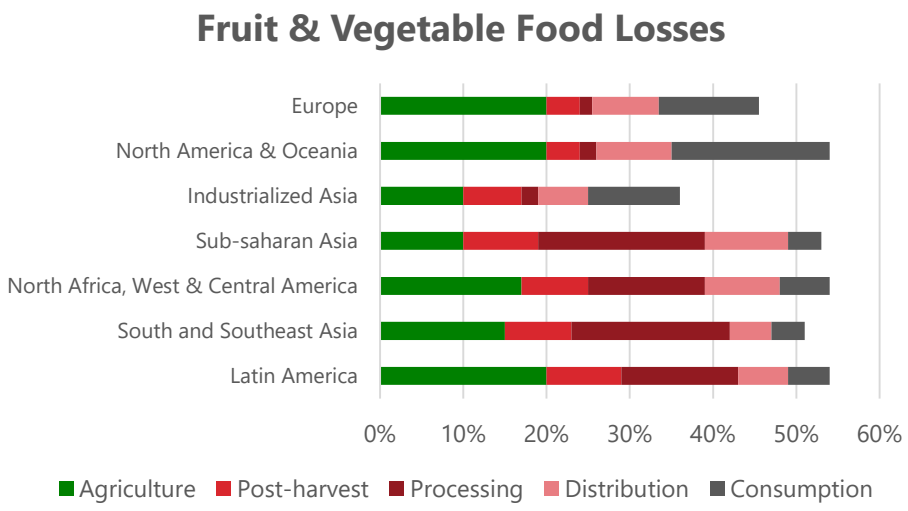
Consumption

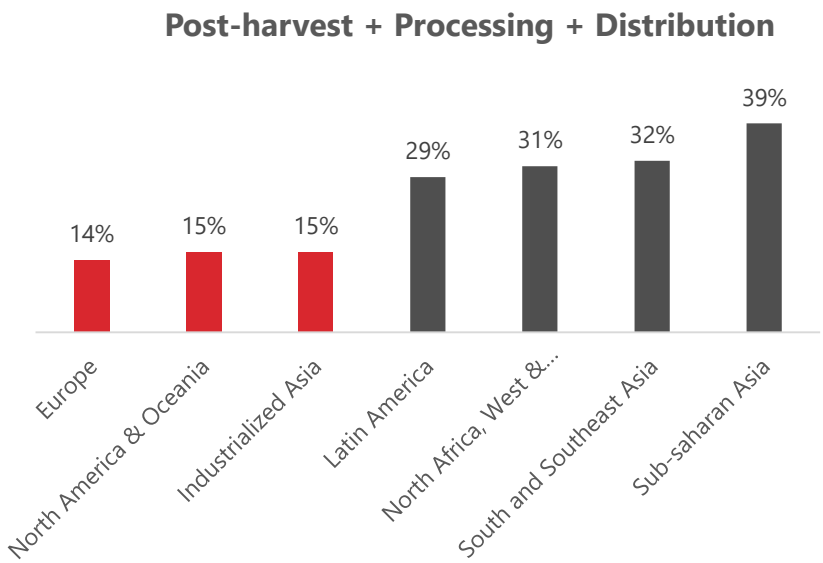
- Leftovers
- Expiration dates
- Use by dates
- Impulse buying
- Infrequent market visits
- Bulk purchase
- Poor portioning

Factors driving food losses

IMPORTANCE OF POST-HARVEST

45 % FRUIT & VEGETABLES FOOD LOSSES





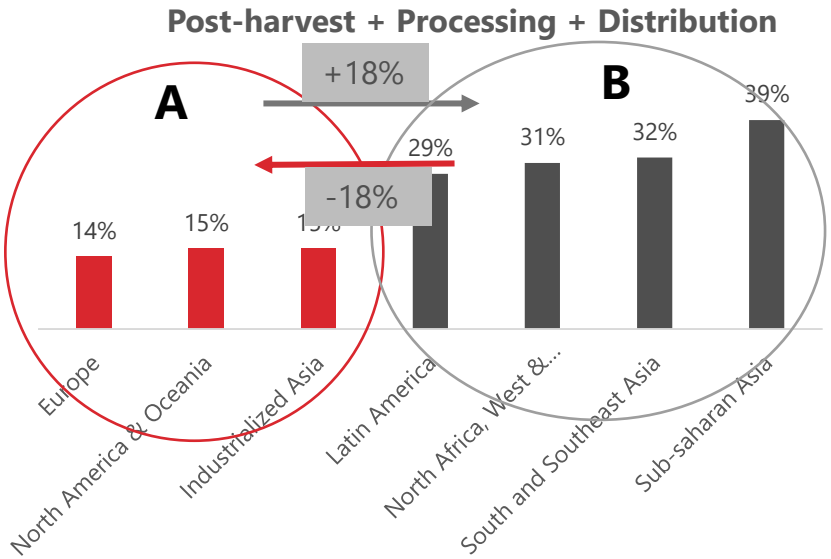
Source: FAO 2012

IMPACT OF POST-HARVEST

Citrus example

- If there is not an *advanced* PoH technology, the PoH losses are increased by 18% (difference of PoH losses between A-B)
- Considering 1$ as consumer market value for 1 kg of oranges in the market (1000$/ton)
- For each ton, 180$ would not achieve the market
- Assuming 9$ - estimated cost of PoH treatment per ton (average – Spanish market)
- If PoH treatment is applied, 171$ of the 180$ would achieve the market (taken PoH efficiency as 100%)
- For ~10 MTons of citrus exported in the group A, the benefit PoH technology would mean ~1710 M$
- In addition, fully developing PoH tech. in group B (4,1 Mtons)~712 M$

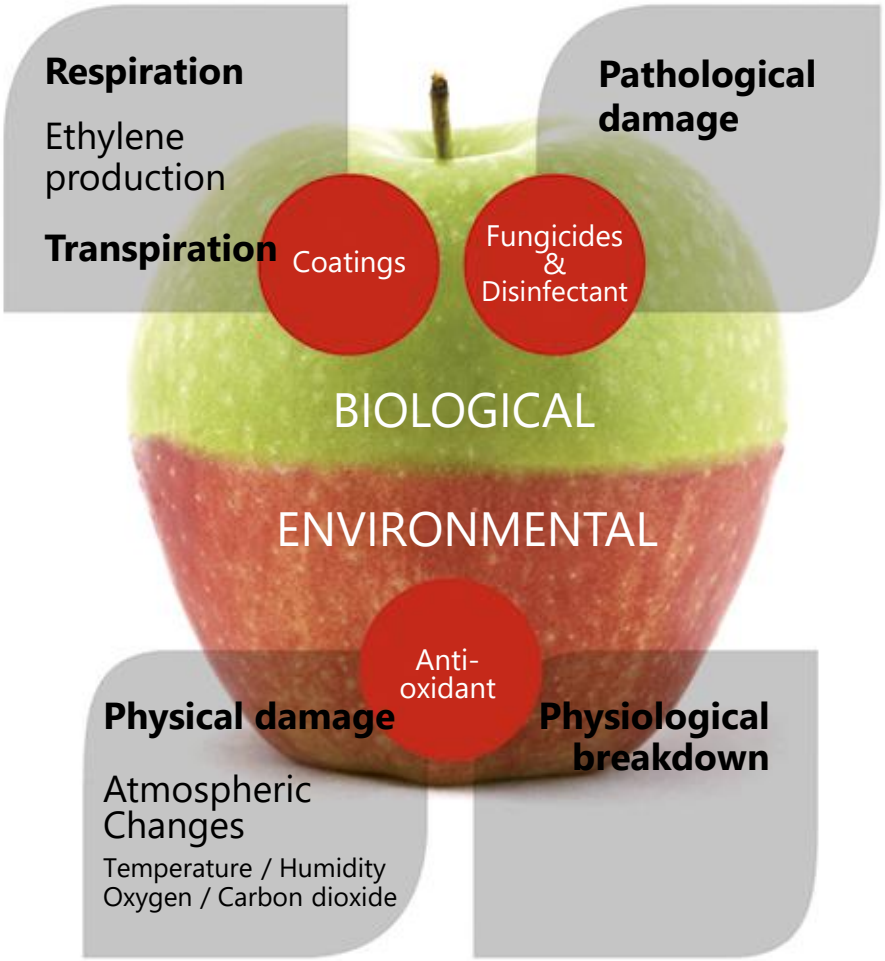
WW Citrus PoH impact amounts for ~2400 M$



WHY POST-HARVEST CARES?

Because harvested fruits:

- Are still living
- Continue to respire
- Loose water but cannot replace it
- Get diseased and infect Healthy Fruits
- Get injured and bruised by picking and handling
- Need rind protection to stay healthy



POST-HARVEST EVOLUTION

Stage 1- Production to consumption

Underdeveloped markets

- Domestic market
- Quick consumption
- Low value market
- Low profit

E.g. China, India, Brazil, Mexico

Stage 2- Production to export

Emerging markets

- Local market at the level of stage 1
- Higher value markets accessible by export
- It means longer distance and extend the fruit shelf life
- **Post-harvest technology is needed**

E.g. Argentina, Perú

Stage 3- Quality production

Mature markets

- Local market requires the same level of quality than the exported market
- Consequence: the PoH treatments do not distinguish between the fruit market destination

E.g. Europe, USA

Of course, intermediate steps among the described stages exist.

Sour Rot Management

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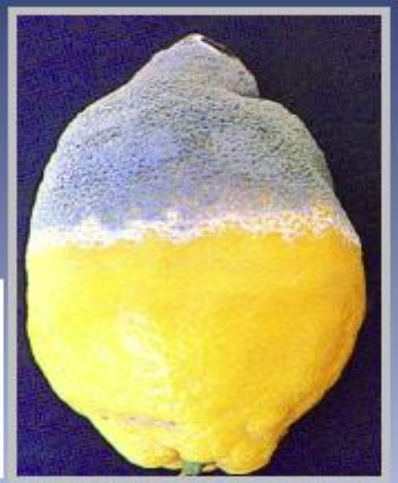
Sour Rot Management

Sour Rot Management

Sour Rot Management

Major postharvest decays of citrus

Green mold caused by *Penicillium digitatum* (most important on citrus)



Blue mold caused by *P. italicum* and green mold

Penicillium soilage

Penicillium spp. and *G. citri-aurantii* are wound pathogens

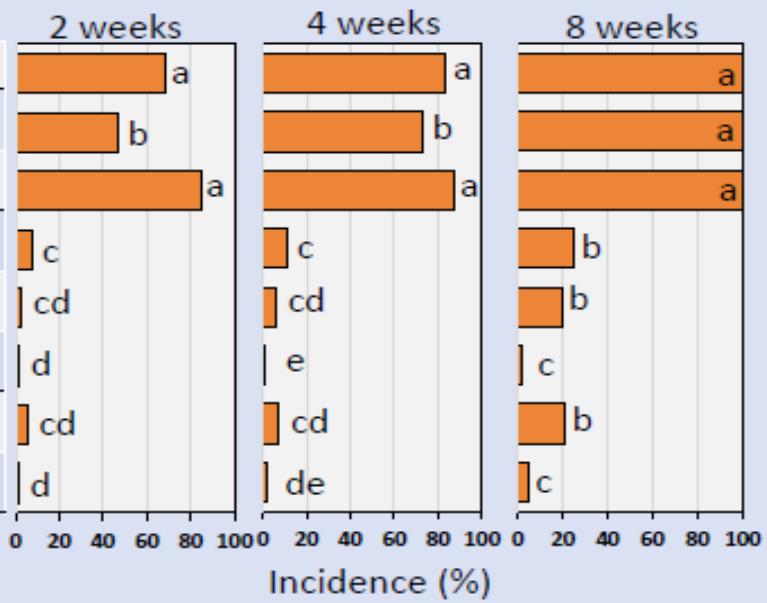
Sour rot caused by *Geotrichum citri-aurantii*

Sour Rot Management

Efficacy of commercial packingline treatments for managing sour rot

High Inoculum

Treatments	
Control	---
Soda ash 2% 115F	Line bath
250 ppm Imazalil	Hand drench
2000 ppm Mentor + 1000 ppm Graduate A+	Spray on line
3000 ppm Mentor + 1000 ppm Graduate A+	Spray on line
4000 ppm Mentor + 1000 ppm Graduate A+	Spray on line
500 ppm Mentor + 600 ppm Graduate A+	Hand drench
1000 ppm Mentor + 600 ppm Graduate A+	Hand drench



- -Fruit were inoculated on 8-24-16 with G. citri-aurantii at high (500,000 conidia/ml) or low (100,000 conidia/ml) concentrations. Conidia were suspended in lemon juice + 2 ppm cycloheximide. Fruit were incubated for 10 h at 12C, and then transported to Santa Paula on 8-25-16. Drench treatments were done 10-11:30 am, line treatments 12-3 pm.

** -Spray treatments were applied in storage wax (D202; diluted 4 gal in 55 gal total) + 300 ppm 2,4-D on the commercial packingline. Drenches were done by pouring a fungicide solution over fruit on a plastic rack. Control fruit and fruit treated by drenches were sprayed with diluted D202 upon arrival back in Riverside. Fruit were incubated at 12 C until evaluation. Incidence is: the number of fruit with decay per total no. of fruit evaluated.

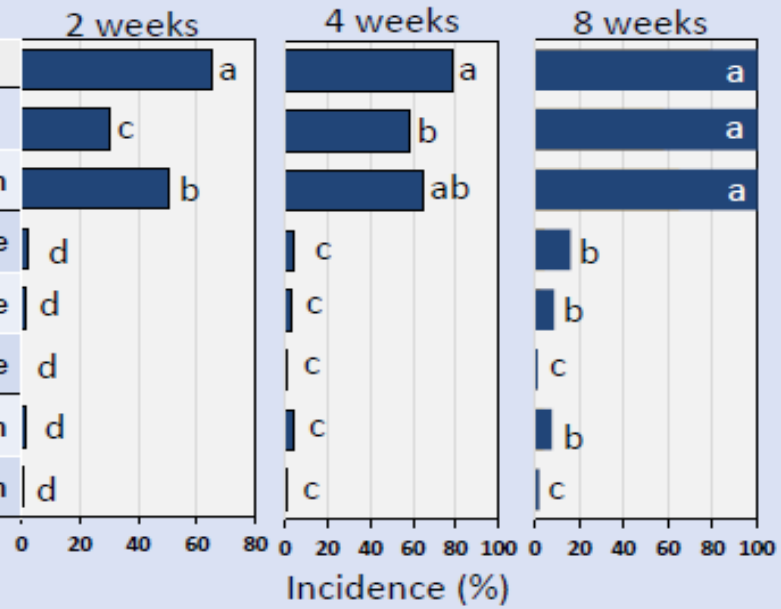
^ -Values followed by the same number are not significantly different based on analysis of variance and LSD mean separation procedures ($P > 0.05$).

Sour Rot Management

Efficacy of commercial packingline treatments for managing sour rot

Low Inoculum

Treatments		2 weeks	4 weeks	8 weeks
Control	---	a	a	a
Soda ash 2% 115F	Line bath	c	b	a
250 ppm Imazalil	Hand drench	b	ab	a
2000 ppm Mentor + 1000 ppm Graduate A+	Spray on line	d	c	b
3000 ppm Mentor + 1000 ppm Graduate A+	Spray on line	d	c	b
4000 ppm Mentor + 1000 ppm Graduate A+	Spray on line	d	c	c
500 ppm Mentor + 600 ppm Graduate A+	Hand drench	d	c	b
1000 ppm Mentor + 600 ppm Graduate A+	Hand drench	d	c	c



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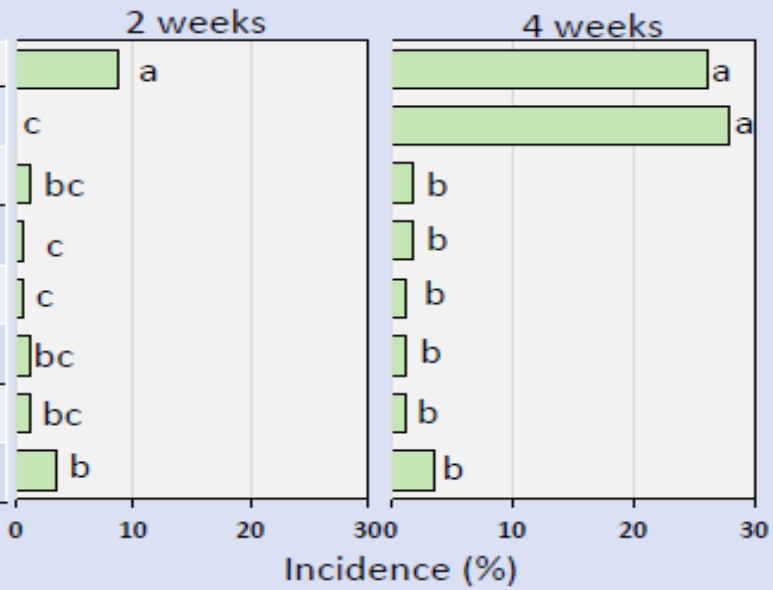
Sour Rot Management

Efficacy of commercial packingline treatments for managing natural incidence of Penicillium decays

Treatments		2 weeks	4 weeks
Control	---	a	a
Soda ash 2% 115F	Line bath	c	a
250 ppm Imazalil	Hand drench	bc	b
2000 ppm Mentor + 1000 ppm Graduate A+	Spray on line	c	b
3000 ppm Mentor + 1000 ppm Graduate A+	Spray on line	c	b
4000 ppm Mentor + 1000 ppm Graduate A+	Spray on line	bc	b
500 ppm Mentor + 600 ppm Graduate A+	Hand drench	bc	b
1000 ppm Mentor + 600 ppm Graduate A+	Hand drench	b	b



* -Spray treatments were applied in storage wax (D202; diluted 4 gal in 55 gal total) + 300 ppm 2,4-D on the commercial packingline. Drenches were done by pouring a fungicide solution over fruit on a plastic rack. Control fruit and fruit treated by drenches were sprayed with diluted D202 upon arrival back in Riverside. Fruit were incubated at 12 C until evaluation. Incidence is: the number of fruit with decay per total no. of fruit evaluated.

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Sour Rot Management

Fruit residues and solution concentrations from a commercial packingline trial for managing sour rot and Penicillium decays

Treatments		Residue of fruit (ppm)				Conc. of solutions (ppm)			
		Propi-conazole	Imaz-alil	Azoxy-strobin	Fludi-oxonil	Propi-conazole	Imaz-alil	Azoxy-strobin	Fludi-oxonil
Control	---	ND	ND	ND	ND	0	0	0	0
Soda ash 2% 115F	Line bath	ND	ND	ND	ND	0	0	0	0
250 ppm Imazalil	Hand drench	ND	1.1	ND	ND	0	260	0	0
2000 Mentor + 1000 Graduate A+	Spray on line	1.2	ND	0.9	0.8	1950	0	915	1050
3000 Mentor + 1000 Graduate A+	Spray on line	1.6	ND	0.8	0.8	2950	0	870	900
4000 Mentor + 1000 Graduate A+	Spray on line	3.7	ND	1.2	1.3	3660	0	808	760
500 Mentor + 600 Graduate A+	Hand drench	1.5	ND	0.8	0.8	326	0	188	160
1000 Mentor + 600 Graduate A+	Hand drench	2.8	ND	1	0.9	780	0	200	200

* -Spray treatments were applied in storage wax (D202; diluted 4 gal in 55 gal total) + 300 ppm 2,4-D on the commercial packingline. Drenches were done by pouring a fungicide solution over fruit on a plastic rack. Control fruit and fruit treated by drenches were sprayed with diluted D202 upon arrival back in Riverside. Fruit were incubated at 12 C until evaluation. Incidence is: the number of fruit with decay per total no. of fruit evaluated.

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Sour Rot Management

Top Export Markets	Propiconazole MRL, ppm	Additional MRL Information
USA	8	
Codex	Review in 2017	Citrus group CXL anticipated 3Q2018
1. Canada	8	
2. Japan	0.05	FA designation anticipated 2019[a]
3. Korea	6[b]	
4. Australia	7	
5. Hong Kong	CXL	Codex deferral anticipated
6. China	CXL?	No import tolerance process, possible conversion from Codex when established
7. New Zealand	Current 0.1 default	Defer to Codex in absence of domestic MRL
8. Chile	0.05	
9. Philippines	CXL	Codex deferral anticipated
10. Mexico	8	
Other Countries of Interest:		
EU	6	

Update on Resistance

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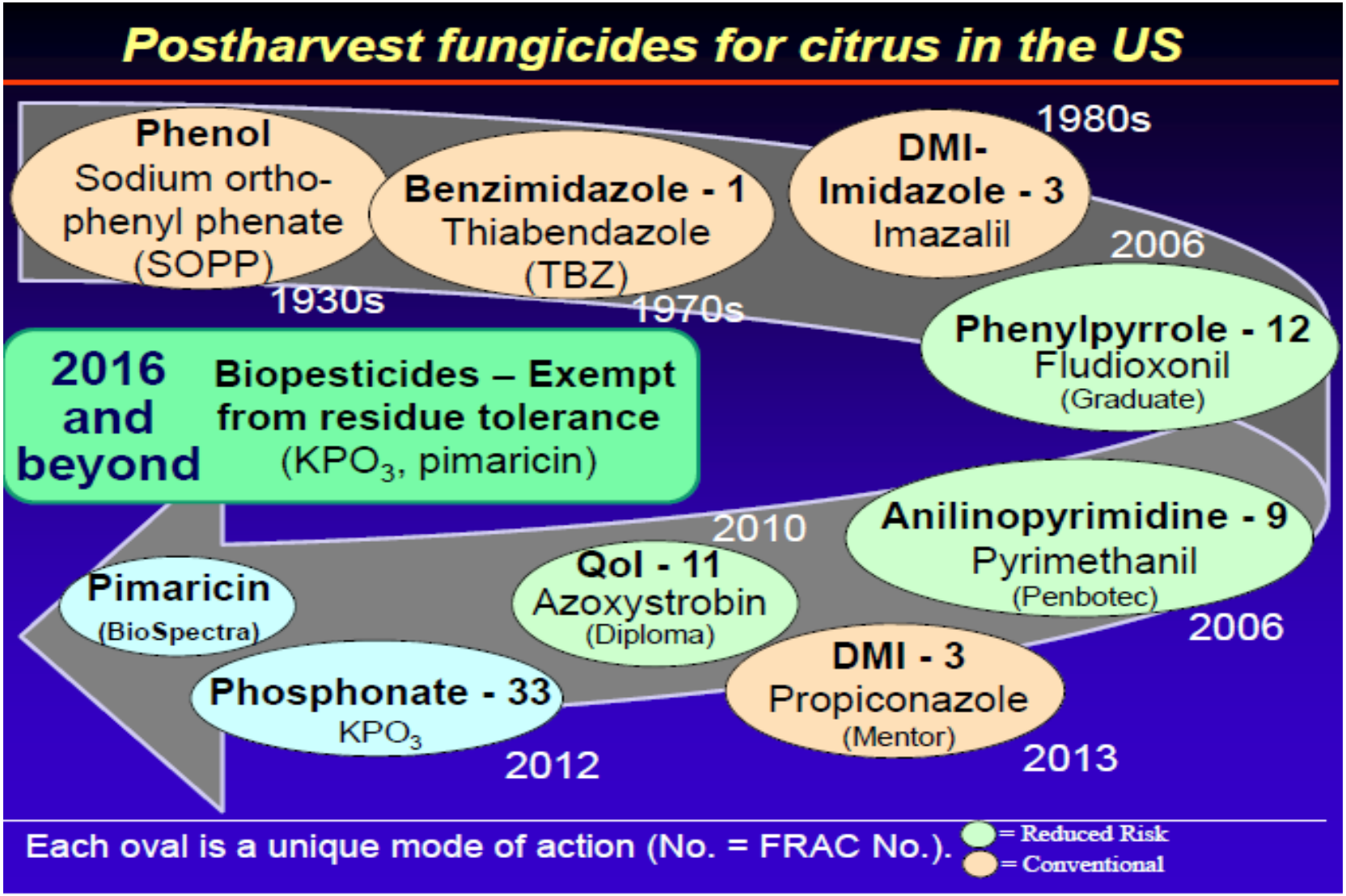
Update on Resistance



Update on Resistance

Fungicide Resistance

FRAC defines resistance as an acquired, heritable reduction in sensitivity of a fungus to a anti-fungal agent. Resistance can be evaluated at the *cellular*, *organismal*, or *population level*.

Studies in field or practical resistance (at the population level) involve reduced efficacy of a fungicide that was previously demonstrated efficacious and confirmation at the organismal level showing a heritable reduction in sensitivity.

- *Reduced sensitivity - small reductions in sensitivity that have little or no impact on fungicide performance.*
- *Resistance - large reductions in sensitivity that affect the efficacy of the fungicide (i.e., loss of control)*

Update on Resistance

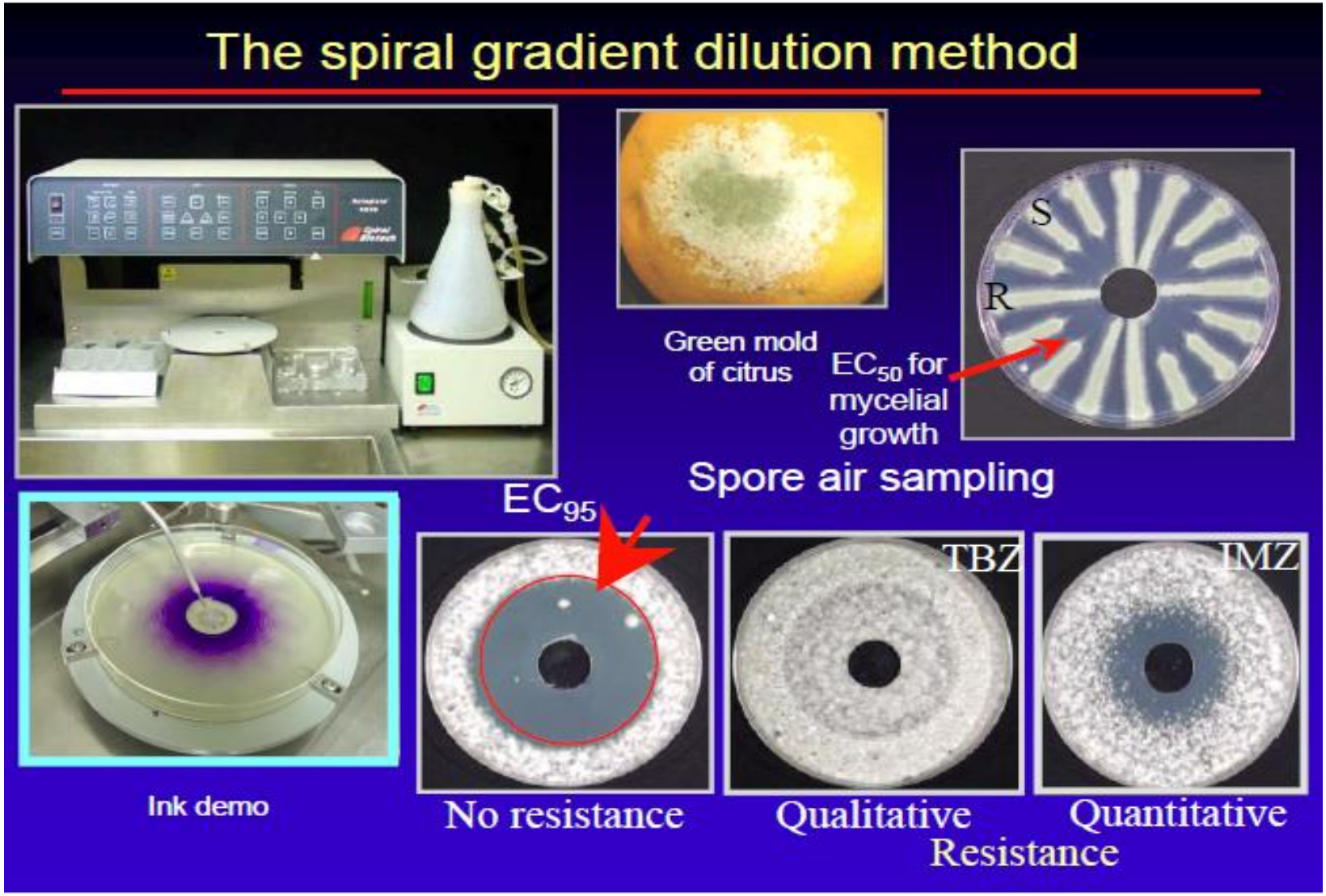
Development and Types of Fungicide Resistance

Development of fungicide resistance is a result of evolutionary processes where a change in the environment (i.e., use of a fungicide) provides a selection pressure for mutations or genetic variants that have an advantage in survival and reproduction.

Baseline describes the sensitivity of a population before it is exposed to the fungicide under field conditions.

- *Qualitative (discrete) resistance – isolates with significantly higher EC_{50} values than the baseline average.*
- *Quantitative (continuous) resistance - isolates with a gradual change toward higher EC_{50} values.*

Update on Resistance

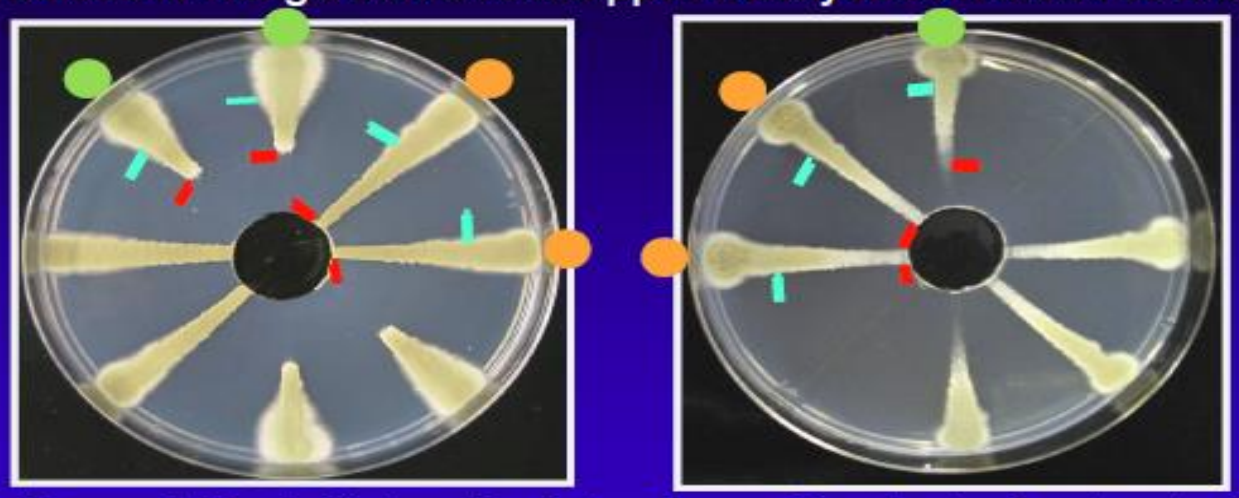


Update on Resistance

Sensitivity testing of monitoring samples

Spiral gradient dilution assays visualize growth over a wide fungicide concentration range

Fludioxonil Range 0.002 to 0.2 ppm Azoxystrobin 0.006 to 0.8 ppm range



- Sensitive reference isolate
- Suspect resistant isolate
- EC_{50}
- EC_{95}

- Recent *P. digitatum* isolates from Graduate A+-treated fruit commonly show slightly increased EC_{50} values and increased EC_{95} values for azoxystrobin and fludioxonil.
- Presence of reduced sensitivity to azoxystrobin and fludioxonil indicates a non-specific detoxification mechanism.

Update on Resistance

Sensitivity testing of monitoring samples

- Shifts in EC_{50} and EC_{95} values for mycelial growth:

Fungicide	Increase in EC_{50} values*	Increase in EC_{95} values
Azoxystrobin	3 - 20x	3 – 10x
Fludioxonil	2 - 4x	5 - 35x

* - In comparison with baseline isolate

** - Shifts in EC_{50} values of 50-100 are considered resistant.

- Incidence of less sensitive isolates:

Year	No. isolates tested	% isolates with increased EC_{50} and EC_{95} values
2015	34	85.3
2016	16	94.1

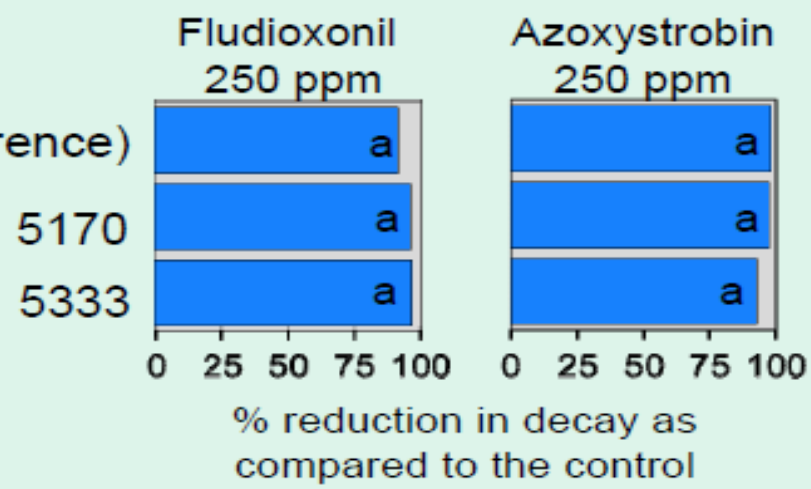
The significance of less sensitive isolates for decay control was evaluated in fruit inoculation studies.

Update on Resistance

Fruit inoculation studies with less sensitive isolates of *P. digitatum*

Isolate	EC_{95} azoxystrobin	EC_{95} fludioxonil
Pd (reference)	0.4 ppm	0.03 ppm
5170	1.7 ppm (= 4.2 x)	1 ppm (=33 x)
5333	1.6 ppm (= 4 x)	0.75 ppm (=25 x)

Fruit were inoculated, treated after 13-14 h, and stored for 7 weeks at 12C



Isolates were pathogenic and virulent (sporulating on fruit).

Data demonstrate high performance and no reduction in decay control!

Update on Resistance

Competitiveness of resistant isolates of P. digitatum

Co-inoculation studies with resistant and sensitive wildtype isolates

Isolate	Recovery of resistant phenoptype
Highly resistant to pyrimethanil	14.8 - 84.1%
Highly resistant to fludioxonil	0
Moderately resistant to fludioxonil	0 - 2.1 %

- Pyrimethanil-resistant isolates sometimes had a reduced competitiveness but still were recovered at a high frequency.
- Fludioxonil-resistant isolates lacked of competitiveness in the presence of wildtype isolates.
- Similar results were obtained for *P. expansum*.
- Fludioxonil most likely has several target sites
- Resistance mutations have pleiotropic effects – reduced fitness (Forster et al. 2012. Phytopathology 102(S6): S6.9.)

Update on Resistance

Summary

- Reports on fludioxonil resistance in *P. digitatum* in California (Kim et al. Plant Dis. 99:1447, 2015) could not be substantiated by Syngenta or Adaskaveg labs.
- Currently, resistance (reduced sensitivity) to fludioxonil in citrus packinghouses is only found in air populations of *P. digitatum*.
  - Reduced virulence and pathogenicity (Kanetis et al. 2010, Phytopathology 100:738-746)
  - Reduced competitiveness is a fitness penalty (Forster et al. 2012. Phytopathology 102(S6): S6.9)
- Isolates from Graduate A+ - treated fruit commonly show increased EC_{50} and EC_{95} values for fludioxonil and azoxystrobin.
  - Decay caused by these isolates still can be effectively controlled using registered rates. (No practical resistance)

Update on Resistance - Management

- The most effective way to combat fungicide resistance is to mix or alternate fungicides with different modes of action (classes of fungicides).
- Each class should be limited to 1 applications/fruit lot.
- Although the use of fungicide pre-mixtures can be a step in the right direction, unless both mixture components have activity against a particular pathogen, they will act like single-fungicides in the selection process. *Thus, if resistance exists against one component, selection is high against the second component.*

Update on Resistance - Management

- Sanitation washes – minimize pathogen populations from fruit and equipment
- SBC – modify micro-environment, additive decay control together with fungicides
- Fruit sorting, decay removal – reduce development of decay and increase of pathogen populations
- Timely application of treatments (within 24 h of harvest)
- Isolate set-back and hold-back fruit from packinghouse
- Use any class of fungicide only once on a fruit lot
- Use effective application methods based on the characteristics of the fungicide (e.g., contact vs. systemic, activity against decay and sporulation, compatibility with fruit coatings, …)

Storage Room Sanitations

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Room Sanitizers

1. **Control Room**
2. **ClO_2**
3. **Decon 7**
4. **H_2O_2 + quats**

Room Sanitizers

Sept 31 Saticoy disinfection test Oct 4, 2015

Germination of *Penicillium digitatum* spores after decontaminating treatments

ClO_2 Room A Location	Germinated	Total examined	Percentage germinated	Comment
West intake	0	500	0.0	
East intake	0	500	0.0	
1	0	500	0.0	
2	0	500	0.0	
3	0	500	0.0	
4	0	500	0.0	
5	0	500	0.0	
6	0	500	0.0	
7	0	500	0.0	
8	0	500	0.0	
9	0	500	0.0	
10	0	500	0.0	
11	8	600	1.3	
12	16	400	4.0	
13	0	500	0.0	
14	1	500	0.2	
		Mean =	0.3	
		StDev =	1.0	

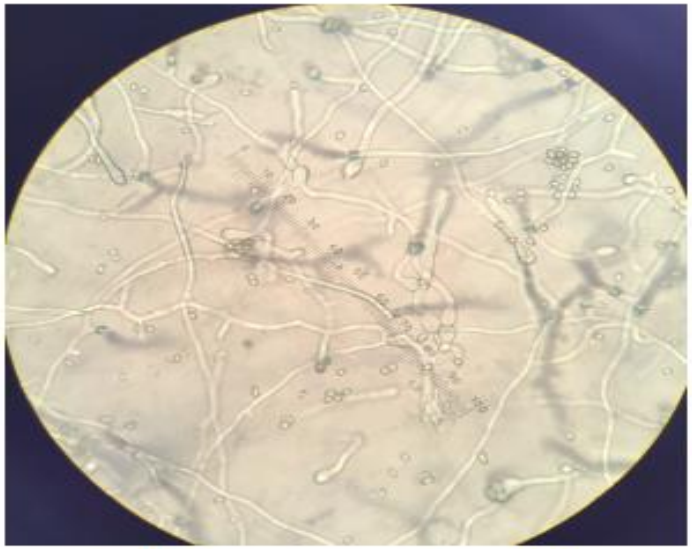
CONTROL Room C Location	Germinated	Total examined	Percentage germinated	Comment
West intake	45	66	68.2	
East intake	34	52	65.4	
1	49	87	56.3	
2	38	79	48.1	
3	53	113	46.9	
4	49	92	53.3	
5	51	93	54.8	
6	63	93	67.7	
7	59	138	42.8	
8	37	72	51.4	
9	36	71	50.7	
10	35	70	50.0	
11	55	99	55.6	
12	59	98	60.2	
13	44	86	51.2	
14	41	69	59.4	
		Mean =	55.1	
		StDev =	7.4	

Decon 7 Room B Location	Germinated	Total examined	Percentage germinated	Comment
West intake	74	112	66.1	
East intake	54	85	63.5	
1	0	500	0.0	
2	1	500	0.2	
3	1	500	0.2	
4	0	500	0.0	
5	19	600	3.2	
6	39	268	14.6	
7	1	500	0.2	
8	0	500	0.0	
9	0	500	0.0	
10	5	500	1.0	
11	30	340	8.8	
12	0	500	0.0	Few spores
13	0	500	0.0	Few spores
14	22	110	20.0	
		Mean =	11.1	
		StDev =	21.8	

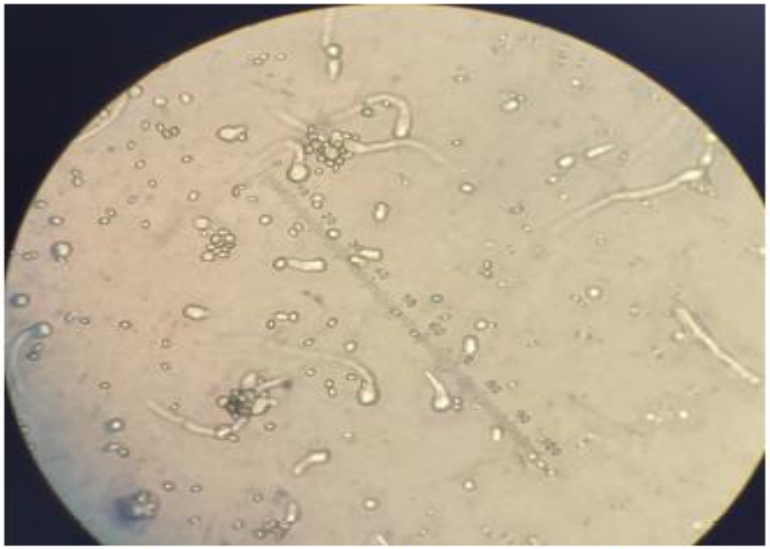
H_2O_{2+quat} Room D Location	Germinated	Total examined	Percentage germinated	Comment
West intake	42	60	70.0	
East intake	54	90	60.0	
1	24	225	10.7	Some recovering
2	31	75	41.3	
3	39	131	29.8	
4	34	111	30.6	
5	90	149	60.4	
6	43	95	45.3	
7	51	215	23.7	
8	37	83	44.6	
9	54	152	35.5	
10	39	125	31.2	
11	15	174	8.6	Some recovering
12	34	186	18.3	
13	43	145	29.7	
14	27	150	18.0	
		Mean =	34.9	
		StDev =	17.9	

Room Sanitizers

Appearance of spores after 24 hours on potato dextrose agar



CONTROL – note long germ tubes



TREATED – note thick, short germ tubes

Figure 1. Note appearance of treated conidia after incubation on potato dextrose agar. Often among those that survived treatment germination was abnormal.

Room Sanitation

Maintenance

- Slow release chemistry
- Low doses of CLO2
- 90 Day treatment typical
- Room is safe for employees

WIPEOUT

- Fast acting chemistry
- High levels of CLO2
- 3-6 hour treatment time
- Room must remain sealed

Room Sanitation

Main Treatments	Wipeout	Maintenance
Step 1	Clean organic soil load off walls and floors.	Place drum on output side of coils, hydrocell
Step 2	Ensure seal on room is adequate	Turn on Fan
Step 3	Mix parts A/B in vibratory mixers and turn on	Replace after 90 days or more
Step 4	Seal room and wait the allotted time	
Step 5	Vent room and clean up any spilled product	

Development in Bio-Fungicides

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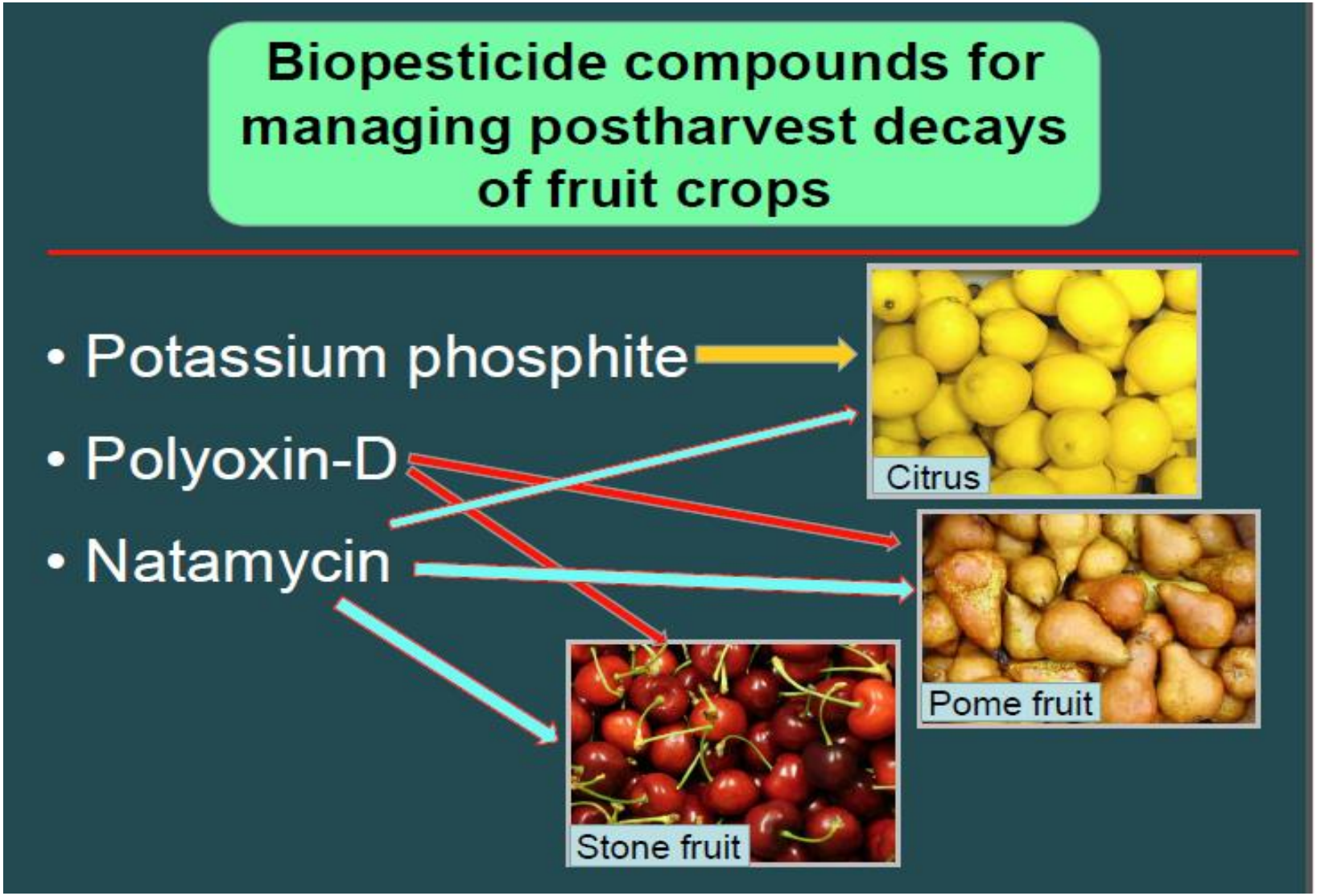
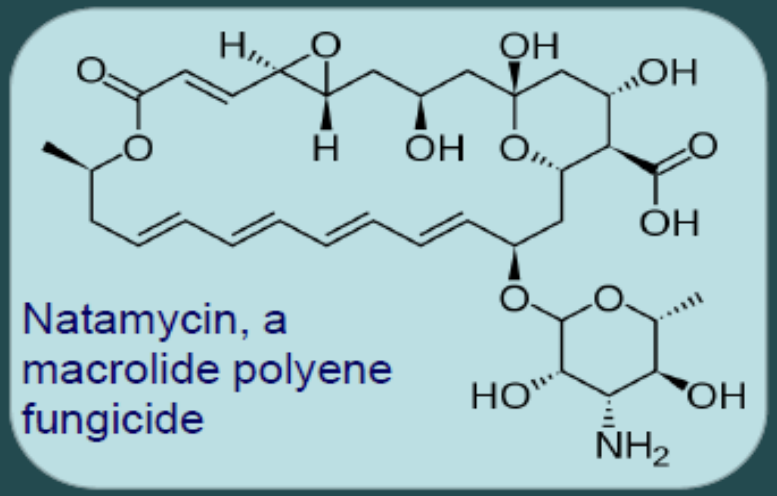
Bio pesticides



Natamycin, a new fungicide for postharvest management of fruit decays

Natamycin (Pimaricin) is used as a food preservative and is a naturally occurring antifungal compound produced during fermentation by the soil bacterium *Streptomyces natalensis* and other *Streptomyces* spp.



Natamycin, a macrolide polyene fungicide

- Discovered in soil samples from Pietermaritzburg, S. Africa 1954 and described by Struyk in 1957
- Very low solubility in water
- Effective at MIC (minimum inhibitory concentration) of <10 ppm for most fungi.
- Used in dairy (e.g., cheese) and meat products as a preservative (E235).

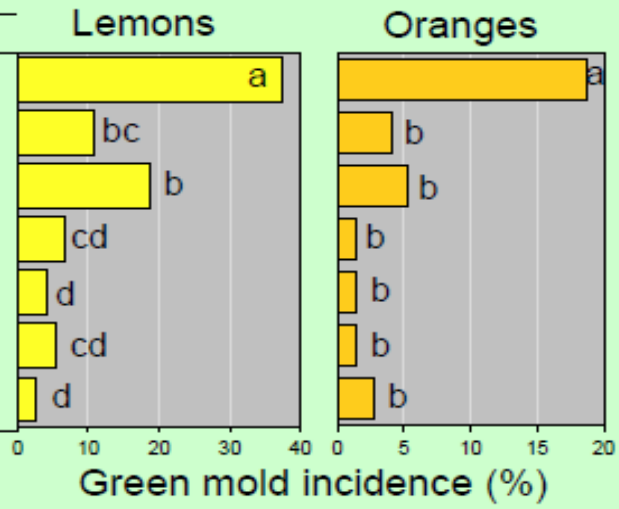
- Commonly used to treat keratitis, but no other medicinal uses.
- Safety – no acute toxicity with an LD_{50} (rat) ≥ 2300 mg/kg (metabolites also non-toxic)

Bio pesticides

Evaluation of EXP-13 for management of green mold of citrus

Laboratory fruit inoculation studies with pimaricin and fludioxonil

Treatments	
Drench	Fruit Coating
Control (Water)	None
Pimaricin 50WP	None
Pimaricin 50WP	Carnauba
Pimaricin 5SC	None
Fludioxonil SC 125 ppm	None
Pimaricin WP + Fludioxonil SC	None
Pimaricin WP + Fludioxonil SC	Carnauba



- Fruit were treated 12 h after wound inoculation
- Pimaricin was used at 1000 ppm

Control

Fludioxonil (Graduate) 125 ppm

Pimaricin 1000 ppm

Activity not as high as registered fungicides, no sporulation control, and the activity was affected by the fruit coating, but…..

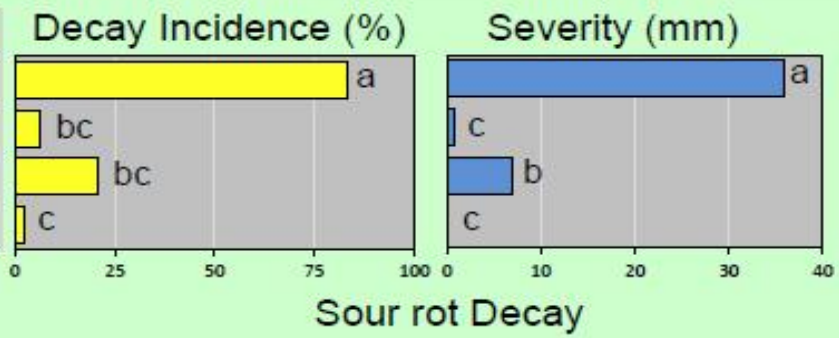
- Both formulations were efficacious protective treatments
- Pimaricin is an excellent mix partner

Bio pesticides

Effectiveness of pimaricin on sour rot of lemons

Fruit inoculation studies with pimaricin and propiconazole

Treatment	Conc. (ppm)
Control	---
Pimaricin 50WP	1000
Propiconazole 45WP	256
Pimaricin + Propi.	1000+256



Control

Pimaricin

Propiconazole:

- Mentor is currently the only highly effective fungicide for control of sour rot.

Pimaricin:

- Effective against sour rot
- Highly effective in combination with propiconazole

New Application Methods

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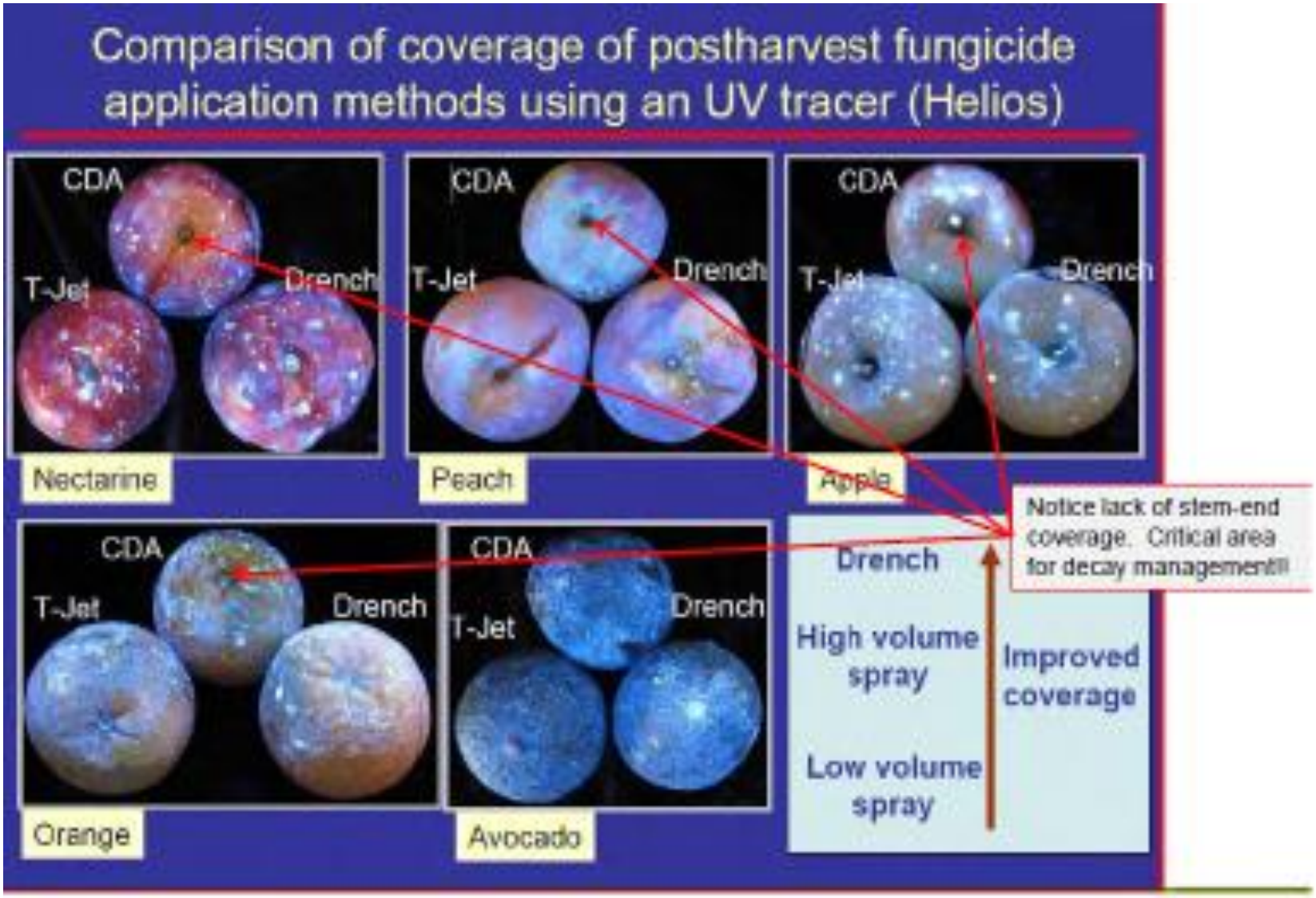
Application Methods

Application methods for postharvest fungicide treatments

Flooder

CDA

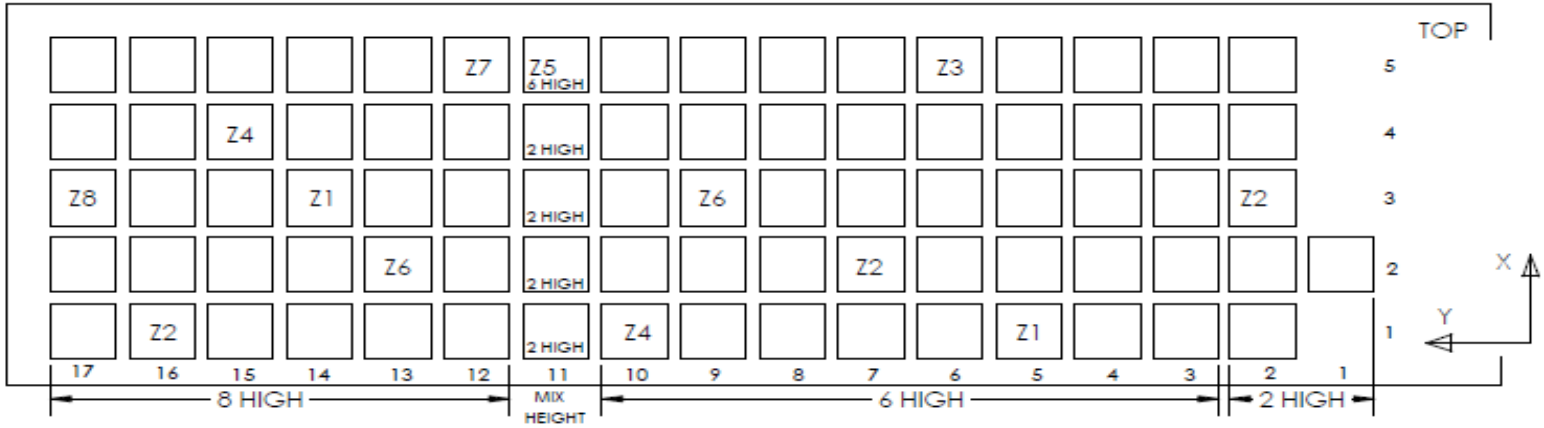
Application Methods

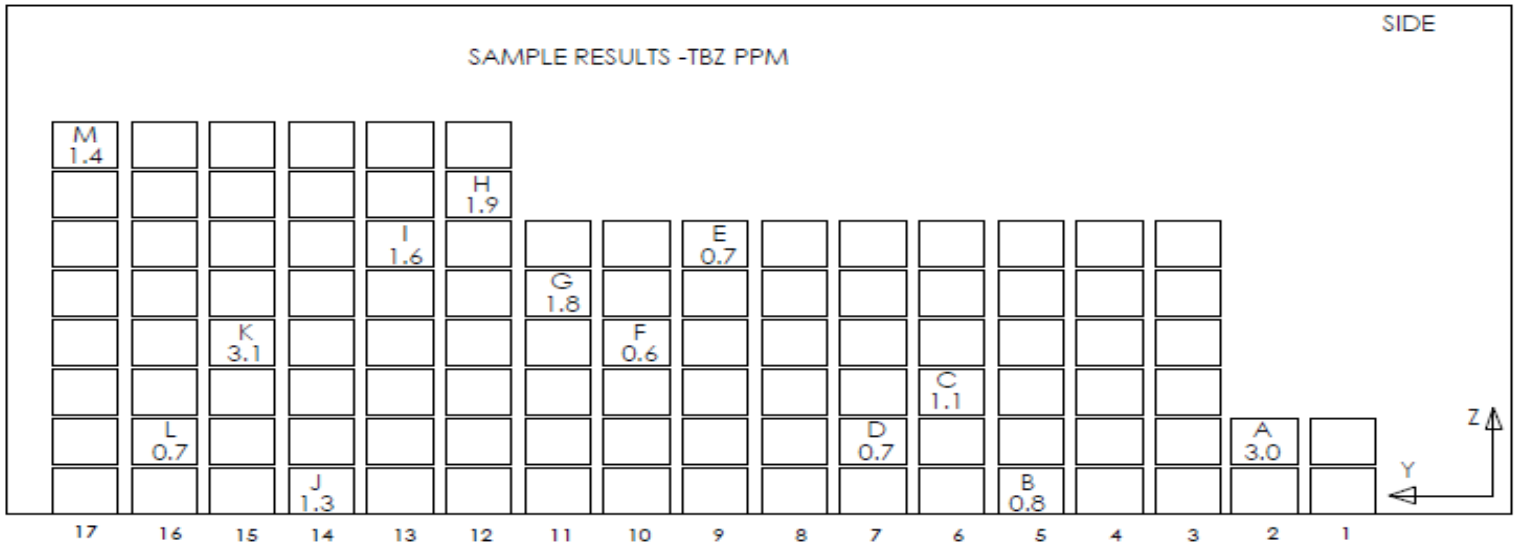


Application Methods

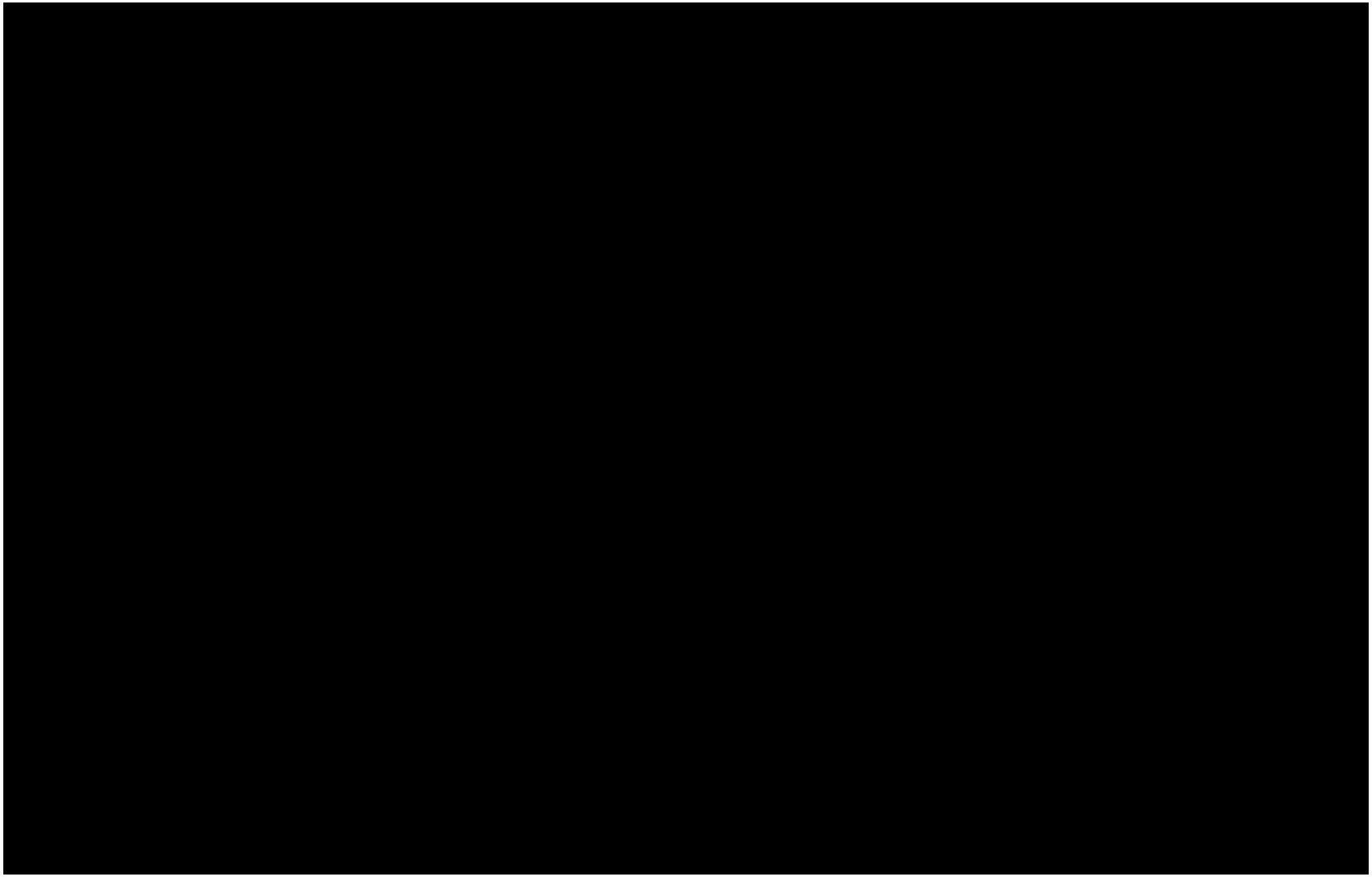
Application Methods

New Application Methods - TBZ Residue Distribution





New Application Methods



Acknowledgment

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Thank You Citrus Australia

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