8th Lecture Pathology

Principles of Postharvest Plant Disease Management

Take home message Multi-barrier concept for managing postharvest diseases



Ca = calcium, Heat = 38°C for 4 d, SB = sodium bicarbonate, Ant = antagonist, AntMX = antagonist mixture, Temp = low storage temperature, CA = controlled atmosphere storage

Prevention, suppression and eradication of postharvest decay

Fungicides

- Single synthetic active ingredient
- Well characterized chemically and toxicologically defined
- High efficiency

Classes of postharvest fungicides

- Compounds within each fungicide class have:
 - Similar chemical structures
 - A similar mode of action that targets either a single site or multiple sites in the biochemical pathways of the fungus

 Cross-resistance may occur among compounds within the same chemical class

Important older postharvest fungicides for citrus and pome fruits that are still being used today

Residual Fungicide	Class/Grouping	Crops	Decays
SOPP	Phenol	Citrus	Penicillium decay, sour rot
Thiabendazole	Benzimidazole	Citrus, pome fruit	Penicillium decay, gray molo
Imazalil	SBI-Imidazole	Citrus	Penicillium Decays

List of new and registered Postharvest fungicides

Common name	Chemical Group	Group name	Target Site	MoAction
Thiabendazole	Benzimidazoles	MBC fungicides (Methyl Benzimidazole Carbamates	β-tubulin assembly in mitosis	Cytoskeleton and motor proteins
Imazalil, Prochloraz	Imidazoles	DMI-fungicides (de-methylation inhibitors, SBI)	C14-demethylase in sterol biosynthesis (erg11/cyp51)	Sterol biosynthesis in membranes
Difenoconzole Propiconazole, Tebuconazole, Triadimefone	Triazoles	DMI-fungicides (de-methylation inhibitors, SBI)	C14-demethylase in sterol biosynthesis (erg11/cyp51)	Sterol biosynthesis in membrane
Pyrimethanil	Anilino-pyrimidines	AP fungicides (Anilino- Pyrimidines)	Methionine biosynthesis (proposed) (cgs gene)	Amino acids and protein synthesis
Azoxystrobin	Methoxi-acrylates	QoI-fungicides (Quinone outside inhibitors)	Complex III, cytochrome bc1 (Ubiquinol oxidase) at Qo site (cyt b gene)	Respiration

List of new and registered Postharvest fungicides

Common name	Chemical Group	Group name	Target Site	MoAction
Fludioxonil	Phenylpirroles	PP-fungicides (Phenyl Pyrroles)	MAP/histidine- kinase in osmotic signal transduction (os-2,HOG1)	Signal transduction
Netamycin (pimaricin)	Amphoteric macrolide antifungal antibiotics from <i>Streptomyces</i> <i>natalensis</i> or <i>S.</i> <i>chattanoogensis</i>	Polyene	Ergosterol binding	Lipid synthesis or transport/membrane integrity or function
Phosphorous acid and salts	Phosphonates	Phosphonates	Phosphate starvation signaling(?)	Host Plant defense induction
Guazatine; Iminoctadine Amlodipin Besilate	Bis-guanidine	Bis-guanidine	Multi-site contact activity	Membrane disruptors, detergents.

Chemical/class (Trade name)	Year introduced	Сгор	Decay/organisms	Methdos of Application	Residue Tolerance (mg/kg)	
	mtrouuceu			Application	(ing/kg)	
Aluminum potassium sulfatedodecahydrate (Alum, Potash Alum, Potassium Alum) Inorganci salt	1997	Bananas	Crown rot (fungal complex): Fusarium spp., Lasiodiplodia, Penicillium, Acremonium, Nigrospora, Colletotrichum, Ceraotcystis	Dips in de- latexing baths	Exempt	
Azoxystrobin (Azoxy) Quininine outside inhibitor	1996	Citrus	Penicillium spp.	Spray and drench	15	
(QoI;strobilurin)		Potatoes	Helmintosporium Fusarium Phytophthora	Spray and drench	8	
Azoxystrobin+ Fludioxonil QoI and phenyl pyrrole	2007	Citrus	Penicillium spp.	Spray and drench	15,10	
		Potatoes	Helminthosporium Fusarium Phytophthora	Spray and drench	8,6	
Azoxystrobin+Difenconazole+ Fludioxonil (Stadium) (Azoxy) Quininine outside inhibitor (QoI;strobilurin)	2013	Potatoes	Helmintosporium Fusarium Phytophthora	Spray and drench	8,4, 6	
Captan (N[(trichloromethyl)thio]-4- cyclohexane	1949	Apple, pears	Botrytis, Rhizopus and Colletotrichum	Dip or spray	25	1
Phtalimide		Sweet cherries	Storage pathogens	Dip or spray	100	

Chemical/class (Trade name)	Year introduced	Сгор	Decay/organisms	Methods of Application	Residue Tolerance (mg/kg)
Cyprodinil+Fludioonil Anylinopyrimidien and phenylpyrrole	2013	Pomegranate	Botrytis cinerea	Dip or drenc	10
2,6-Dichloro-4-4nitroalniline (Botran) Dicarboximide	1959	Sweet potatoes	Rhizopus stolonifer	Spray or dip	10
Difenoconazole (only available in pre-mixtures) Demethylation inhibitor triazole	2013	Apples, pears	Peniclliun and Neofabrae	Dip, drench or spray	5
, , , , , , , , , , , , , , , , , , ,		Potatoes	Helminthosporium and Fusarium	Dip, drench or spray	4
		Citrus	Penicillium	Dip drench or spay	Not determined
Difenoconazole +Fludioxinil (Academy) Demethylation inhibitor-triazoe and phnylpyrrole	2014	Apples, Pears	Botrytis cinerea, Penicillium and Neofabrea	Dip or drech	5,5
Fludioxonil (Scholar) Pehnyl pyrrole	1990	Apples, pears	Botrytis, Penicillium and Rhizopus	Dip or spray	5
		Apricot, nectarines, peaches, plums,sweet cherris	Fruit rot pathogens as Monilia, Botrytis, Rhizopus, Mucor and Gibertella	Spray	5
		Citrus	Penicillium	Spray or drench	10
		Kiwifruit	Botrytis	Dip or spray	20

Chemical/class (Trade name)	Year introduced	Сгор	Decay/organisms	Methods of Application	Residue Tolerance (mg/kg)
Fludioxonil	1990	Pineapple and other tropical fruit Pomegranate Potatoes Sweet potatoes Tomatoes	Ceratocystis paradoxa and Penicillium Botrytis cinerea Helminthosporium and Fusarium Fusarium and Rhizopus Botrytis and Rhizopus	Dip or spray Dip or drench Dip or spray Dip or spray Dip or spray	20 5 5 3.5 5
Fludioxonil+Propiconazole Phenylpyrrole and demethylation inhibitors- triazoel	2012	Pineapple Stone fruits Tomato	Ceratocystis paradoxa and Penicillium Monilinia, Botrtytis, Rhizopus Botrytis, Rhizopus	Spray Spray and drench Spray and drench	20,4.5 5+4 5+3
Fludioxonil+TBZ (Scholar Max) Penylpyrrole and Methyl benzimidiazole carbamate (MBC)	2011	Apples, pears	Botrytis, Penicillium and Rhizopus	Dip or Spray	5+10
Imazalil (Fungaflor) Demethylation inhibitor- imidazole	1974	Citrus	Penicillium	Spray	10

Chemical/class (Trade name)	Year introduce d	Сгор	Decay/organisms	Methdos of Application	Residue Tolerance (mg/kg)
Imazalil+pyrimethanil (Philibuster) Imidazole and anylinopyrimidien	1974	Citrus	Pencillium spp	Spray	10+10
Phenylphenols-O-phenylphenol (OPP), Sodium o-pehnylphenate (tetrahydrate)(SOPP)	1936	Pears Citrus Cucumbers, and peppers	Bacteria and fungi <i>Geotrichum, Pencillium</i> spp., Stem end rots:Trichoderma and Phytophthora spp. Fungi	Dip, spray, or flood Wash, spray, dip or foam Spray	25 10 10
Phosphorous acid and Salts (Potassium and calcium phosphite)	2011	Citrus	<i>Phythophtora</i> and Penicillium	Drench, spray or dip	Exempt
Potassium sorbate (Sorbic acid, potassium salt) Organic salt		Prunes (dried) Citrus	Fungi Fungi	Dip Drench, spray or dip	GRAS GRAS
Propiconazole (Mentor)	2006	Apricots, nectarines, peaches, plums, sweet cherries Citrus Tomatoes Pineapple	Fruit rot pathogens: Monilia, Botrytis, Rhizopus spp.,, Geotrichum candidum Penicillium, Geotrichum citri Botrytis and Rhizopus Ceratocystis paradoxa	Spray or drench Spray or drench Spray or drench Dip or spray	4 8 3 4.5

Chemical/class (Trade name)	Year introduced	Сгор	Decay/organisms	Methdos of Application	Residue Tolerance (mg/kg)Pyrimethan il (Penbotec)
Pyrimethanil (Penbotec) Reduced Risk	2005	Citrus Apricots, nectarines, peaches, plums. Sweet cherries Apples and pears Pomegranates	Penicillium spp. Fruit rot pathogens: Monilia, Botrytis, Rhizopus spp. Botyttis, Pencillium and Neofabrea spp Botrytis cinerea	Spray or drench Spray and drench Spray and drench Dip and drechn	10 10 14 5
Sodium borate, sodium tetraborate (Borax), sodium carbonate (Soda Ash), and sodium bicarbonate (Baking soda) Inorganic salts reduce risk pesticide	1938	Grapefruit, oranges, lemons	Penicillium spp.	Dip, drech or spray, rinse with fresh tap water	exempt
Sodium or potassium bisulfite inorganic	-	Grapes fresh	Botrytis cinerea	Pads	10
Sulfur Inorganic	1800 BC	Bananas	Crown rot fungi	Paste	GRAS
Tebuconazole Demetylation inhibitor -triazole	1986	Sweet Cherry Plums	Monilinia, Botrytis, and Rhizopus Monilinia, Botrytis, and Rhizopus spp.	Spray and drench Spray and drech	5 1
Thiabendazole-TBZ-Mertect Methy Benzymidazole carbamate (MBC)	1968	Bananas Citrus Papayas Pome fruits (apples and pears)	Crown rot Penicillium spp. Stem- end rot Colletotrichum Penicillium, spp. Bull aye rot, Botrytis cinerea, Cluster rot and Nest rot.	Dip after dehanding and delatexing Drench or spray Dip or spray Dip, flood or spray	3.0 (0.4 in pulp) 10 (35 in the pulp) 5 10

Mode of actions

Non selective Respiration inhibitors

- Cu⁺⁺ and Cu⁺⁺ derivatives
- Sulfur
- Dithiocarbamates (Maneb, Thiram)
- Captan

All of them affect several enzymes of the respiratory systems and are considered as MULTISITE fungicides



Microtubules are polymers of tubulin that form part of the cytoskeleton and provide structure and shape to eukaryotic cells.



1) Mitchison T, Kirshner M. (1984) Dynamic instability of microtubule growth. Nature 312: 237-242.

2) Walker RA, O'Brien ET, Pryer NK, et al. (1988) Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. The Journal of Cell Biology 107:1437-1448. 3) Desai A, Mitchison T. (1997) Microtubule polymerization dynamics. Annual Review of Cell and Developmental Biology 13:83-117.

4) Dogterom M and Yurke B. (1997) Measurement of the force-velocity relation for growing microtubules. Science 278: 856-860.

5) Gittes F, Mickey B, Nettleton J, and Howard J. (1993) Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. The Journal of Cell Biology 120: 923-934.

6) Mirigian M, Mukherjee K, Bane SL, Sackett DL. (2013) Chapter 14 - Measurement of in vitro microtubule polymerization by turbidity and fluorescence. Methods in Cell Biology 115, 215-229.

7) Löwe J, Li H, Downing KH, Nogales E. (2001) Refined structure of alpha beta-tubulin at 3.5 Å resolution. Journal of Molecular Biology 313: 1045-1057.

Inhibition of microtubules assembly:

ASSEMBLY OF MICROTUBULES

a-TUBULIN O $\bullet \beta$ -TUBULIN

DIMERS

- Disappearance of microtubules:
- Displacement of mitochondria from hyphal apices
- Disappearances of Spitzen-korper
- Reduction of linear growth
- Metaphase arrest of all mitosis

MoA Benzimidazoles



Figure 1 MoA of MBC-fungicides. (A) Schematic drawing of the effect of MBC-fungicides on microtubule dynamics. (B) Fluorescent microtubules in U. maydis cells, treated with the solvent dimethyl-sulfoxide (DMSO), 1 µg/ml benomyl and 1 µg/ml carbendazim for 30-45 minutes. Open arrowheads indicate short microtubule fragments. Scale bar is given in micrometres.

Demethylation inhibitor-imidazoles affecting sterols production



Mechanism of action of Imidazoles and Triazoles



The function of sterols in the membrane



- Cell reproduction
- Cell permeability and water loss
- Activity of membrane bound enzymes
- Inhibition of cell growth
- Cell death

Theoretical mechanism of fludioxonil action



We postulate that instead of acting directly upon a prototypical hybrid histidine kinase (HHK), fludioxonilinstead creates a stress state in affected cells. Stress intermediates derived from this state are then detected via their modification of sentinel cysteines present in the HHK, inducing a structural shift that favors the phosphatase activity of the HHK over the kinase activity. Dephosphorylation of Ypd1 directly activates the HOG1 cascade, interfering with cell cycle and glycerol production pathways and swiftly rendering the cell non-viable.

Strobilurines

Mode of action:

- Inhibitors of Ubiquinone-cytochrome C reductase- the cytochchrome bc₁ complex (complex III)
- The fungicide bind to the center of the ubihydroquinone oxidation center of the cytrochrome b

The results:

No transfer of electrons from the ubiquinol to the cytochrome-c

Respiration inhibitors





Summary of last lecture



Development of resistance to post-harvest treatments The main problem: identifying inefficiencies to the fungicide as a problem of resistance

- Use of the wrong fungicide
- Use of the wrong concentration
- Reduction of the quality of the fungicide because of long storage
- Application not at the right time (the day after harvest)
- Resistance to the fungicide

Reasons for developing resistance to post-harvest treatments

- Specificity of the mechanism of action
- Use for several years of the same fungicide
- Use of fungicides with similar mechanism of action
- Duration of contact with the pesticide
- Reducing the concentration of the fungicide during long periods of storage

Qualitative Resistance development

QUALITATIVE RESISTANCE BUILD-UP



Qualitative resistance: Pathogen population changing from a sensitive pathogen strain to an insensitive pathogen strain. (Modified from Hewitt, 1998)

Repeated application..... result in predominantly resistant population

Quantitative Resistance development

QUANTITATIVE RESISTANCE BUILD-UP



Quantitative resistance: Pathogen population with a range of sensitivity shifting to insensitive over time. (Modified from Hewitt, 1998)

Repeated application..... result in increasingly insensitive population!!!!

Examples of developing resistance against fungicides

- Benzimidazoles
- Development of a single mutation in a single amino acids
- Inhibitors of Sterol biosynthesis
- Development of genes activation proteins encoding for an internal pump that secretes the fungicide out of the cell

Point mutations in resistant strains

Partial amino acid sequence of B-tubulin as predicted from the DNA base sequence (codon 239-243) from a benomyl-resistant (R) and a sensitive (S) strain of Saccharomyces cerevisiae; Schizosaccharomyces pombe, Neurospora crassa and chicken.

		<u>239</u>	<u>240</u>	<u>241</u>	<u>242</u>	<u>243</u>
S. cerevisiae S	~ -	ser -	leu -	arg -	lyr -	pro - ~
S. cerevisiae R	~ -	ser -	leu -	his -	lyr -	pro - ~
S. pombe	~ -	ser -	phe -	arg -	phe -	pro - ~
N. crassa						pro - ~
Chicken	~ -	cys -	leu, -	arg -	phe -	pro - ~

Point mutations in resistant strains

Partial amino acid sequence of 8-tubulin as predicted from the DNA base sequence (codon 165-169) from a benomyl-resistant (R) and a sensitive (S) strain of Neurospora crassa, Saccharomyces cerevisiae, Schizosaccharomyces pombe, and chicken

	<u>165</u>	166	<u>167</u>	<u>168</u>	<u>169</u>
N. crassa R S. cerevisiae S. pombe	 ala - ala - ala -	thr - thr - thr -	<u>tyr</u> - phe - phe -	ser - ser - ser -	val - ~ val - ~ val - ~ val - ~ val - ~

Behavior of benzimidazoleresistant molds in the world!!!

	Location	Pathogenicity	Competition	Reference
P. italicum	Israel	Similar	Stable	Gutter et al.
P. digitatum	Israel	Similar	Stable	Gutter et al.
P. digitatum	USA, Ca	Similar	Decreased	Eckert et al
P. expansum	Israel	Reduced	Decreased	Prusky et al.
P. expansum	NY state	Not tested	Decreased	Rosenberg et al.

Strategies for disease control and prevention of developing populations resistant to pesticides

Sanitation

Prevention of Selective Pressure

- **1.** Prevention of the use of very selective fungicides
- 2. Prevention of mixtures of fungicides with the same mode of action
- **3.** Use of fungicides in a combined treatment with agricultural ones
How to manage resistance

• Rotate, Rotate, Rotate

Alternate MOA groups to remove isolates that develop an insensitivity to a specific MOA. This rotational process ensures the longevity of fungicide efficacy.

• <u>Restrict use</u>

Maintain application limits during each season/year. Practice guidelines of MoA for consecutive applications will help reduce resistance development.

• <u>Apply the correct rates</u>

Under-application can lead to fungal pathogens that are not fully controlled, creating a greater chance of survival and adaption of the disease.

<u>Use preventative applications</u>

Preventative application maintains a much lower pathogen population. This reduced number of pathogens limits the opportunity for natural mutations that lead to resistance development.

Incorporate multi-site MOA

Consider tank mixing low-risk, multi-site fungicides to expand target sites of the fungicide mix.

<u>Practice integrated disease management</u>

Employ sound cultural practices and other treatments,, dew removal, mowing, ensuring adequate airflow and shade reduction.

Support new technology

Many new fungicides released are from existing MOA but use new chemicals with new MOA or combination products, but offer little to aid resistance management.

Rules for fungicide treatments

Proper use to ensure food and environmental safety, as well as high-quality nutritious fruits and vegetables.

Prevention of resistance in pathogen populations to fungicides

- Rotate between fungicide classes
- Use labeled rates
- Limit the total number of applications
- Education of spectrum of activity
- Sanitation is essential in an integrated management program

When should we apply the fungicide

Different approaches for disease control according to the etiology of the disease:

- 1. Diseases that infect the host before harvest, remain quiescent and develop after harvest
- 2. Diseases that infect by wounding at harvest and develop after harvest





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Symptoms



Core Rot infection during flowering



Open calycine tube in starking



Figure 1: Core not symptoms found in Starking apples a) movidy core, b) dry core rot and c) wet core rot.

Red Delicious





Open calycine tube in Red Delicious **Close calycine tube in Golden**

i i abie

The effects of bromuconazole and difenoconazole, alone and with captan on control of moldy-core in apple fruit in 2004

Treatment and concentration (% v/v)	% of fruits infected with moldy-core		
Control	8.0 a		
Bromuconazole 0.08 (3 sprays) (Vectra)	4.0 b		
Bromuconazole 0.08 + captan 0.25 (3 sprays)	2.0 c		
Difenoconazole 0.02 (3 sprays) ^(Dividend)	5.0 ab		
Difenoconazole 0.02 + captan 0.25 (3 sprays) ^a	1.0 c		
Bromuconazole 0.08 (7 sprays)	6.0 ab		
Difenoconazole 0.02 (7 sprays)	5.0 ab		

Three foliar sprays of each fungicide or of a tank mixture were applied in 2004 on: 31st March, at the pink-cluster stage (15–20% bloom), 3rd April (60% bloom) and 5th April (80% bloom)

Time study of colonization of A. alternata ni elppa detaertnu dna detaert-edicignuf fo stiurf seert

Treatment and concentration (% v/v) ^a	<u>% of fruits colonized by A. alternata at various days after full bloom</u> ^b			
	35	78	97	132 (harvest)
Control	7.0 a	26.0 a	36.0 a	58.0 a
Bromuconazole 0.08ª	0.0 a	0.0 b	20.0 b	37.0 b
Bromuconazole 0.08 + captan 0.25 ^a	0.0 a	0.0 b	16.0 b	22.0 c

 2nd example of prevention of postharvest diseases by preharvest treatments

Stem End rot attack during flowering and during fruit growth Botryodiplodia, Colletotrichum



Improved disease management system for mango anthracnose and stem-end rot (Philippines)

- The protectant fungicide, mancozeb, applied at least four times during the early fruit development, 35–75 days after flower induction (DAFI), provided 53% control of fruit rot incidence and 83% disease severity.
- One or two applications of systemic fungicide (azoxystrobin) at early fruit development (35–45 DAFI) provided adequate fruit protection. Additional application of azoxystrobin gave high level of fruit protection or complete freedom from fruit rot infection.
- Azoxystrobin applied three times during fruit development achieved 95–99.6% control of fruit rot while five sprays of mancozeb attained 50–75% control.

• 3rd example of prevention of postharvest diseases by preharvest treatments

Postharvest decays of pomegranates and kiwifruit

Gray mold caused by *Botrytis cinerea*





Infection through flower parts



Infection through cut stem ends at harvest



Botrytis in Strawberries



Infection of Botrytis in strawberries



Spread of Botrytis in strawberries field



The basic circadian pattern of airborne conidium dispersal in *Botrytis cinerea* in a raspberry plantation. Dispersal occurs when the relative humidity is rising or falling rapidly. h B.S.T. = Hours British Summer Time. From Jarvis (1962a)









Mechanism of infection in the field



strawberry plantation. Conidia are mostly dispersed on days when picking occurs. From Jarvis (1962e)

Different approaches for disease control of Botrytis in strawberries

Timing of fungicide applicationAmount of fungicide application

A possible approach for disease control in strawberries field



Number and date of sprayings



4th example of prevention of postharvest diseases by preharvest treatments

Alternaria in mango attack during all the period of fruit growth



Evaluation of latent infections in mango fruits



Fig. 1. Development of latent infection in discs sampled from different zones in control and maneb-treated mango fruits. No. 1 indicates the zone proximal to the stem end, and No. 6 the zone distal from stem. \Box - control; \Box - maneb treated.

Treatments with the protectant maneb treatment for the control of Alternaria infection during fruit growth



Fig. 3. Relative infected-surface of mango fruits, cv. Haden, by Alternaria alternata in fruits untreated (O) or treated with the protectant fungicide maneb, three (\triangle) or four (\Box) times, during the growing season. Arrows indicate the day of treatment. Statistical analysis (Newman-Keuls multiple range test) was done for each sampling date. Points on graph ascribed by no letters in common are significantly different at 5% level.

Effect of pre-harvest fungicide treatment on of area of quiescent infections corrected with quiescent infections without corrections



Fig. 4. Effect of maneb sprays during fruit growth on the post-harvest percentage infection (black spot development) in mango fruits. Untreated fruits (O); treated with maneb, three (\triangle) or four (\Box) times. Points on graph ascribed by no letters in common are significantly different at 5% level.

The relation between the area covered with quiescent infections and the number of hours with relative humidity over 80% during all the period of fruit growth



The relation between the local RH and the level of infection at each region





The relation between the percentage of unmarketable fruits and the level of quiescent infections found in the orchard

5th example of prevention of postharvest
diseases by preharvest treatments

Alternaria alternata attacks during all the period of fruit growth specially in the last months of fruit growth





Summary: The prevention of postharvest diseases by preharvest treatments is possible but is dependent on:

• Timing of Fungicide application

Concentration of fungicide treatments