9^{th} Lecture Pathology

Curative effect using fungicides
(Therapy/erradication)

- Methods of application of fungicides
  - Gas phase
  - Water phase

Fumigation

- The objective of fumigation is to apply enough material in the form of gas at the infection place.
- Gas fumigant are polar type of compounds that concentrate in polar places
- Fumigation is affected by:
  - Time of exposure
  - Concentration of the compound

Application methods for postharvest fungicide treatments

Fogging

Fumigation techniques fitted to packaging

- <u>Sublimation of solid organic fungicides</u>

  Biphenyl, high vapor pressure

Biphenyl wrapping of citrus

Fumigation

Efficiency of fumigation is dependent on:

- Temperature (more diffusion)
- Humidity

SO_2

- Sulfur burning (1% SO_2 V/V)
- Sublimation of solid organic fungicides
- Volatilization of salts of weak acid (Hydrolysis of Na metabisulfite)

Chemical/class (Trade name)	Year introduced	Crop	Decay/organisms	Methdos of Application	Residue Tolerance (mg/kg)Pyrimethanil (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

Sulfur burning in the storage room

Fumigation techniques fitted to packaging

- Sublimation of solid organic fungicides

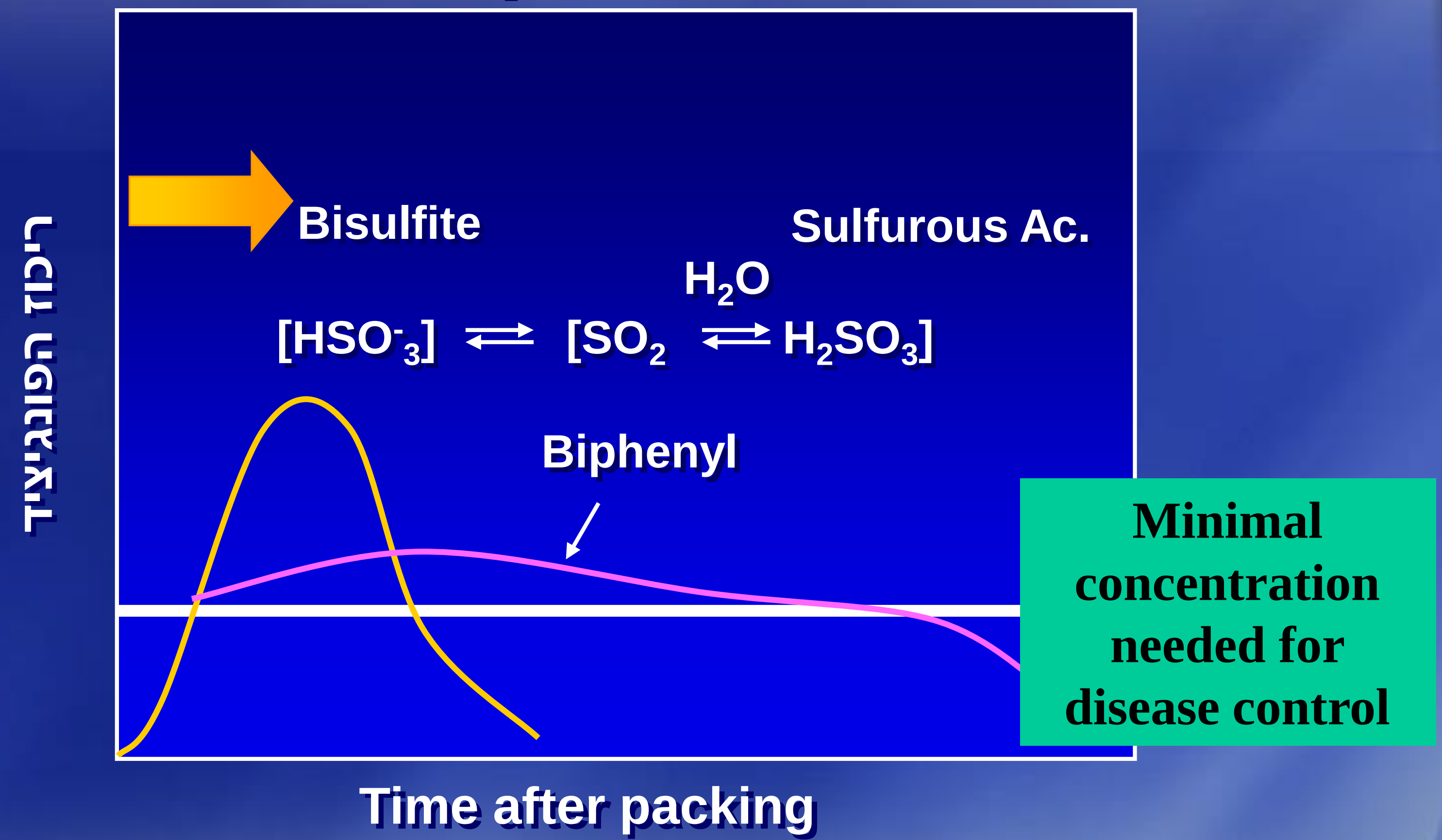
  Biphenyl, high vapor pressure

- Metabolism of volatile salts resulting from weak acids

  Production of SO_2 from Na-bisulfite and Na metabisulfite by hydrolysis

Bisulfite wrapping

Biphenyl vs. Bisulfite activity compounds



Bisulfite wrapping

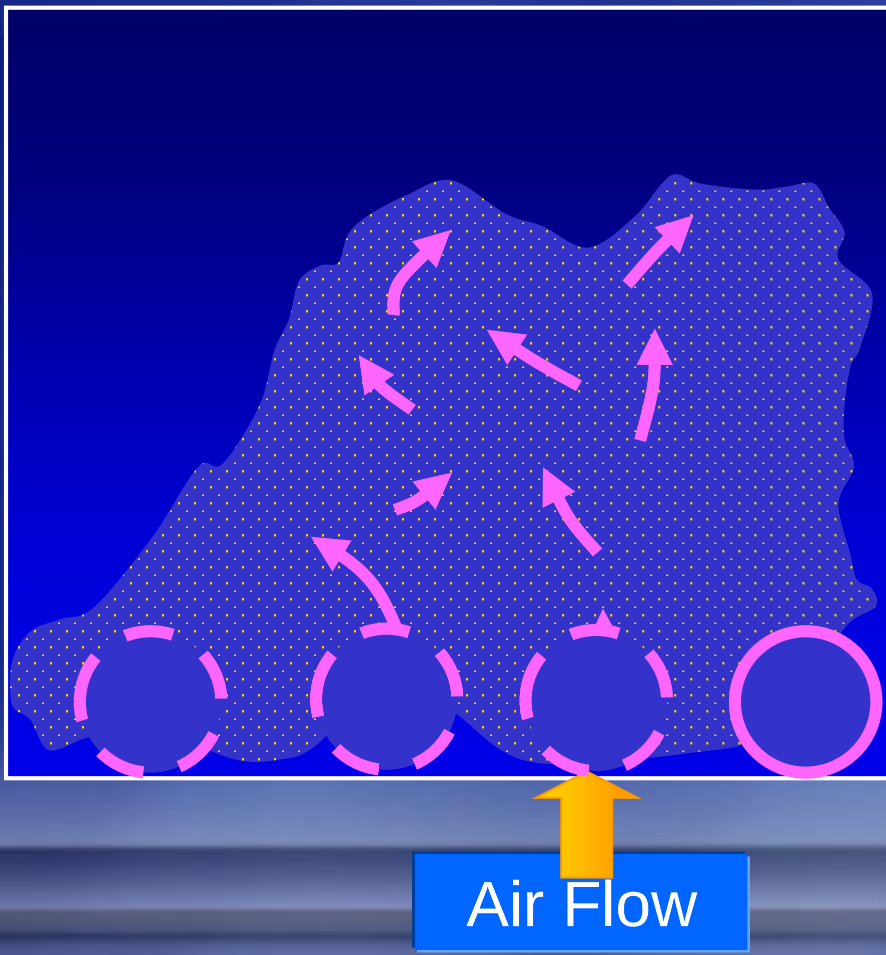
Method for application of fungicides

Fumigation / Smoke

- O_2Cl_4, H_2O_2 -Chlorine dioxide
  - Oxidative effect
- TBZ (Thiabendazole) in stored potatos
  - Inhibition of cell division

Chemical/class (Trade name)	Year introduced	Crop	Decay/organisms	Methdos of Application	Residue Tolerance (mg/kg)
Thiabendazole-TBZ-Mertect Methy Benzymidazole carbamate (MBC)	1968	Mushrooms Carrots Melons Potatoes	Dactylium. Mycogone, Trichoderman and Vertticillium spp Botrytis and Sclerotinia spp Fusarium spp. Fusarium spp.	Dip or spray Dip Dip Dip	40 10 15 10
Thiram Dithiocarbamates	1931	Bananas	Crown rot, stem end rot, surface molds, Fusarium Colletotrichum and Thielaviopsis spp	Spray, brush or paste	7
Triamdimefon (Bayleton) Demethylation inhibitor-triazole	1990s	Pineapple	Butt rot, Ceratocystis paradoxa	Dip or Spary	2

Application of fungicides by gas: Potato Storage Room



TBZ RESISTANT UP TO 320 C

- Curative application of fungicides
  - Gas phase
  - Water phase

Sanitize

- "Sanitize means to adequately treat cleaned surfaces by a process that is effective in
- destroying vegetative cells of pathogens, and
- substantially reducing numbers of other undesirable microorganisms,
- but without adversely affecting the product or its safety for the consumer"

Sanitize

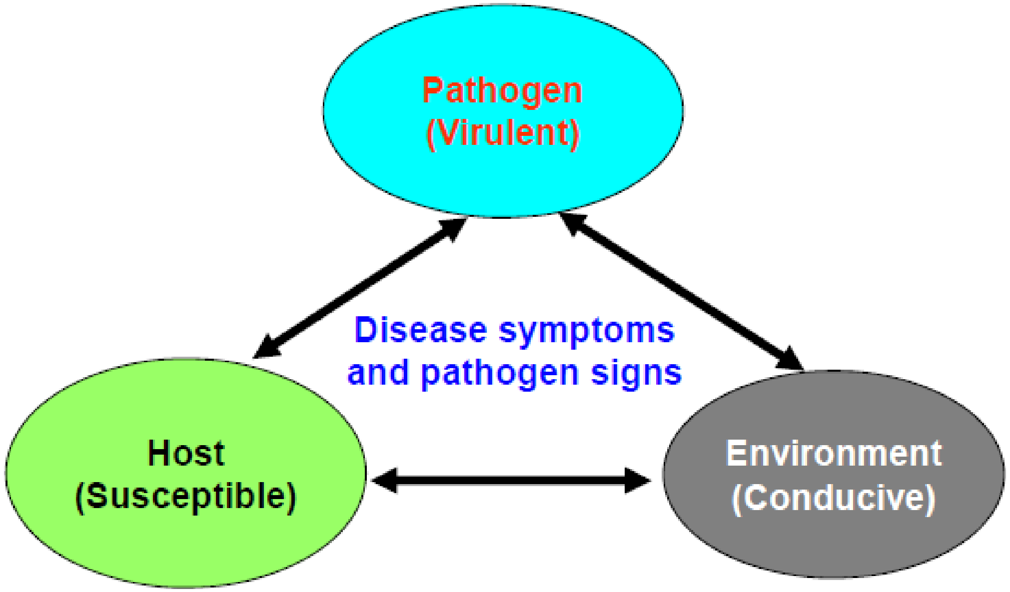
- Implicit in all definitions, sanitation does not mean a complete killing of all microorganisms. The terms “sanitizer” and “sanitizing agent” have been applied to many antimicrobial chemicals without considering their ability to meet a specific performance standard, such as a 5-log reduction of microorganisms on a treated product.

Water Sanitation

Chlorine
Chlorine dioxide
Peroxides
Ozone
Others

Principle of Sanitation

- Reduce the pathogen inoculum to a sufficient low level that decay would not reach a level to cause appreciable losses



Chlorine as a treatment for sanitation

- Chlorine is a strong oxidizer
- Active against may organic molecules
- Sanitizer at concentrations higher than 50 ppm
- Strongly active at pH ranging from 6.5-8.5
- Sanitizer in Israel for carrots, tomato, persimmon, mango, partially processed fruits and leafy vegetables.

Mechanism of action

- **Oxidation reactions on the membrane surface may alter the permeability of the cell membrane such that the nutrient transport system is impaired**
- **Inactivation of the extracellular transport system will disrupt the electrochemical gradient of the cell, and ATP synthase is non-functional. Lack of ATP production will shut down multiple processes within the cell.**
- **Free chlorine may also oxidize proteins, denature proteins, oxidize amino acids, and/or inactivate enzymes**

hypochlorous acid

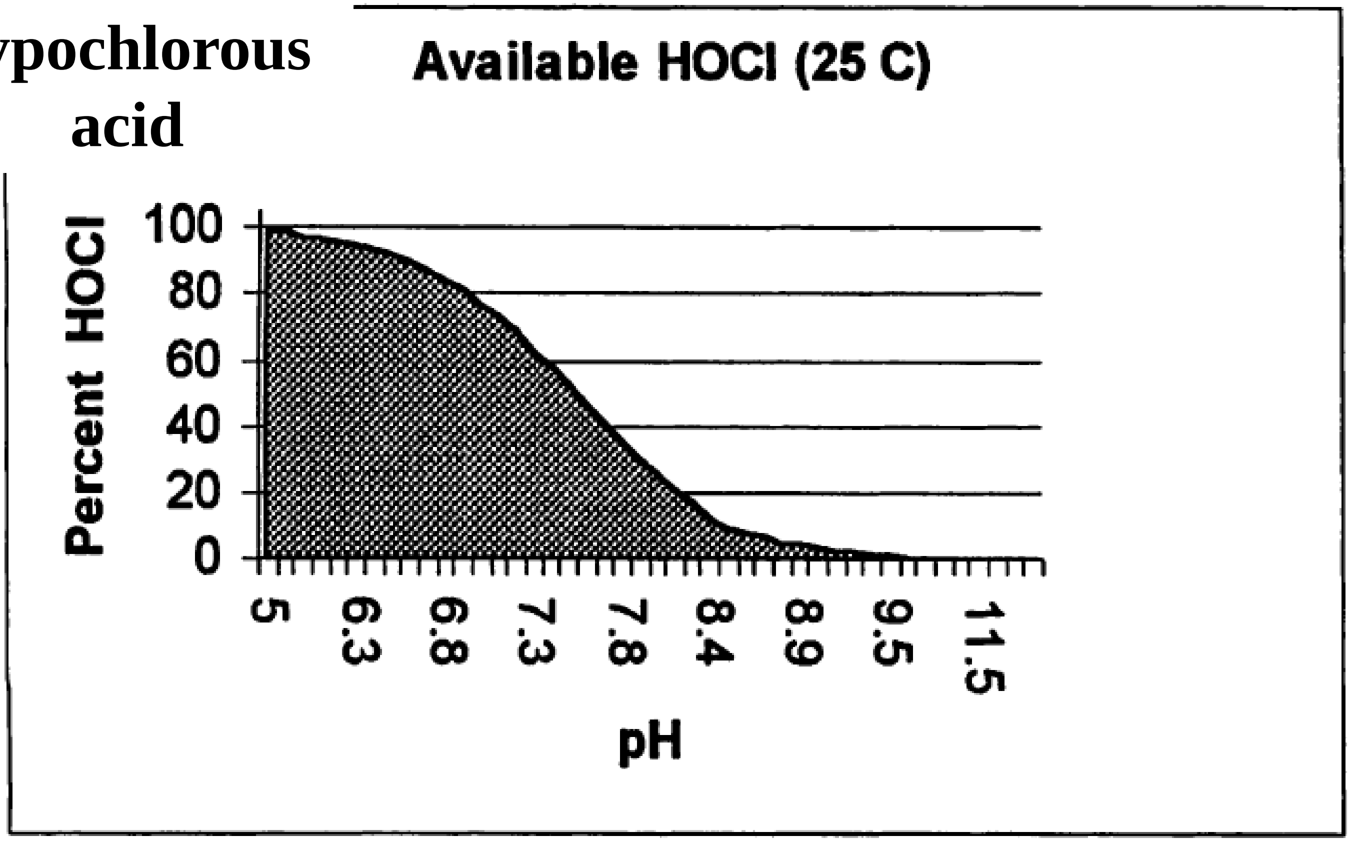


Figure 1. Influence of pH on dissociation of hypochlorous acid to hypochlorite ion at 25 (C. Data calculated from the following: percent HOCl = 100 X [1 + K_i/(H^+)], where K_i is the HOCl dissociation constant of HOCl at 25°C or 2.898 x 10^{-8} moles/liter (White, 1986).

Purpose of Chlorine sanitation

- Decay control
  - Destruct inoculum or reduce inoculum levels
- Fungicide resistance management
  - Reduce fungicide-tolerant populations to minimize the development of fungicide resistance

Chlorine treatment of lettuce

Chlorine treatment of tomato

Chlorine treatment on line

Chlorinated water for carrots before the packing line

Chlorinated water for carrots before the packing line

Bin Sanitation

Field bines contaminated by decay pathogens contribute to the build-up of inoculum in the drencher or in the packing line.

Condia surviving in storage bins are believed to be most important source of inoculum contributing to the selection for resistance to postharvest fungicides because storage bins cycle survived condia of *Penicillium expansum* from year to year.

Methods for Bin Sanitation

- Wash with chlorinated water
- Wash with other sanitizers
  - Chlorine dioxide, quaternary ammonium compounds, and others
- Steam
- Hot water treatment
- Fumigation

Bin sanitation - Hot water treatment

In-line Bin Sanitation: 77 C , immersion for 20 s

Sanitize with chlorine

- Destroying vegetative cells of pathogens,
- Reducing numbers of other undesirable microorganisms,
- Without adversely affecting the product or its safety for the consumer”

SOAPING WITH
Sodium orthophenyl phenyl tetra hydrate, SOPP

Applied in citrus at high pH

(pH 10-12)

Chemical/class (Trade name)	Year introduced	Crop	Decay/organisms	Methdos of Application	Residue Tolerance (mg/kg)Pyrimethanil (Penbotec)
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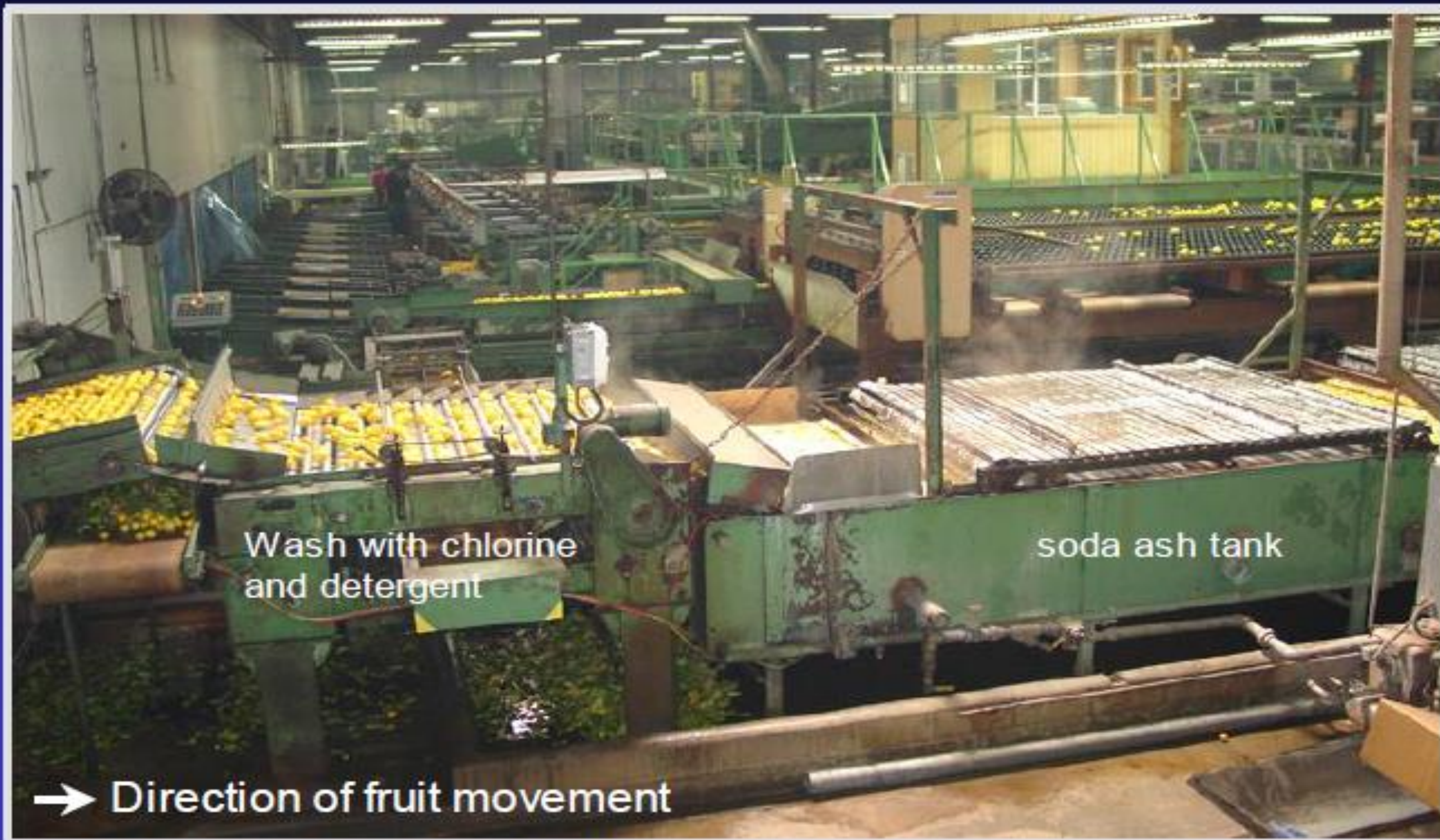
Usage of borax, sodium carbonate (soda ash), and sodium bicarbonate in postharvest treatments of lemons

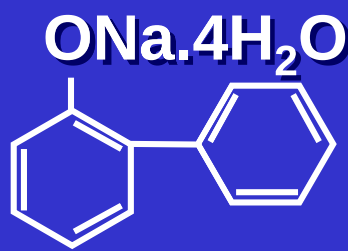
Treatment with heated soda ash

Water rinse after soda ash treatment

Usage of borax, sodium carbonate (soda ash), and sodium bicarbonate in postharvest treatments of lemons

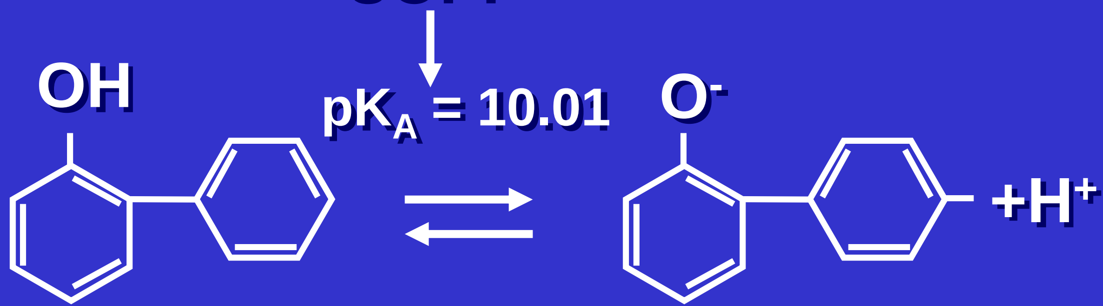


Sodium orthophenyl phenate tetra hydrate, SOPP



Very soluble in water and is hydrolyzed to

SOPP



HOPP

OPP

O-phenyl phenol

O- phenyl phenate

Phytotoxic<pH10

Non phytotoxic

Sodium orthophenyl phenate tetra hydrate, SOPP

1. Concentration of ortho phenyl phenol HOPP is dependent on the concentration of SOPP

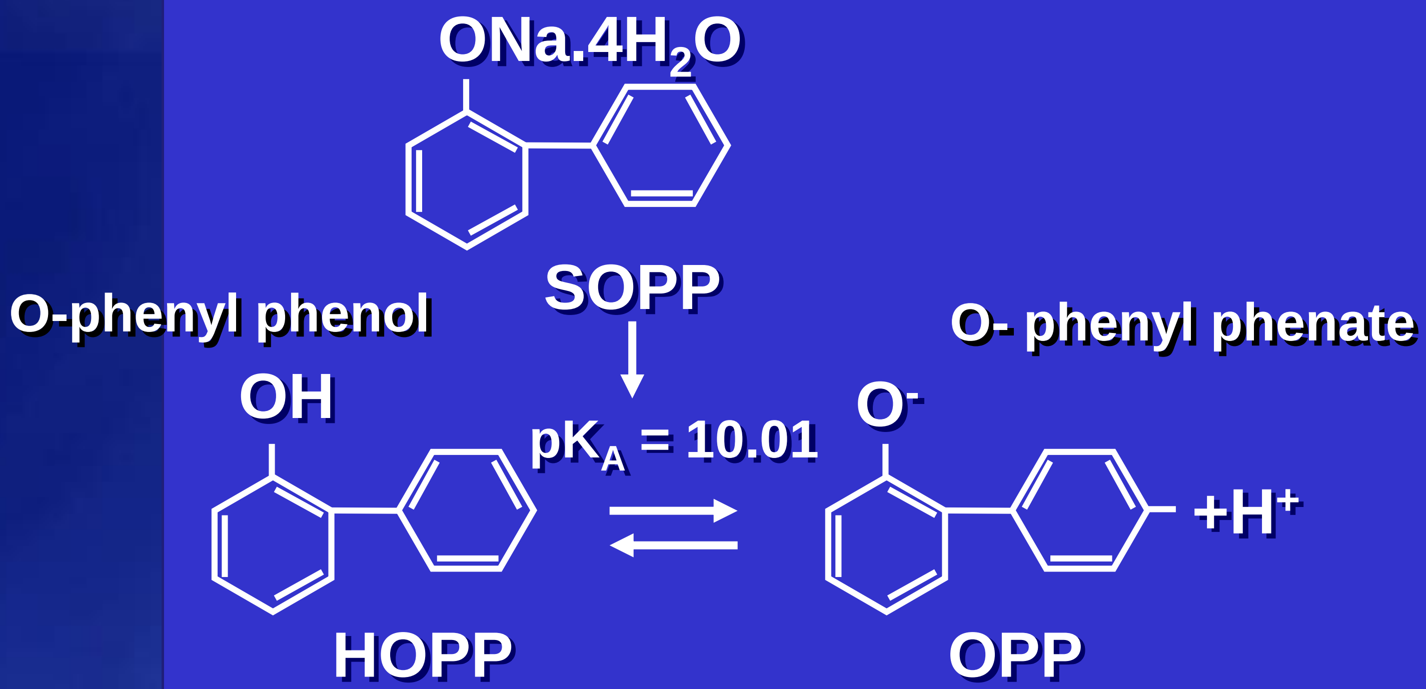
2. pH level

As the pH decrease → up to the pKa the concentration HOPP, ortho phenyl phenol, increases

OPP- ortho phenyl phenate is present at higher pH than the pKa affect microorganism but is not phytotoxic

HOPP- affect microorganism but it is also phytotoxic

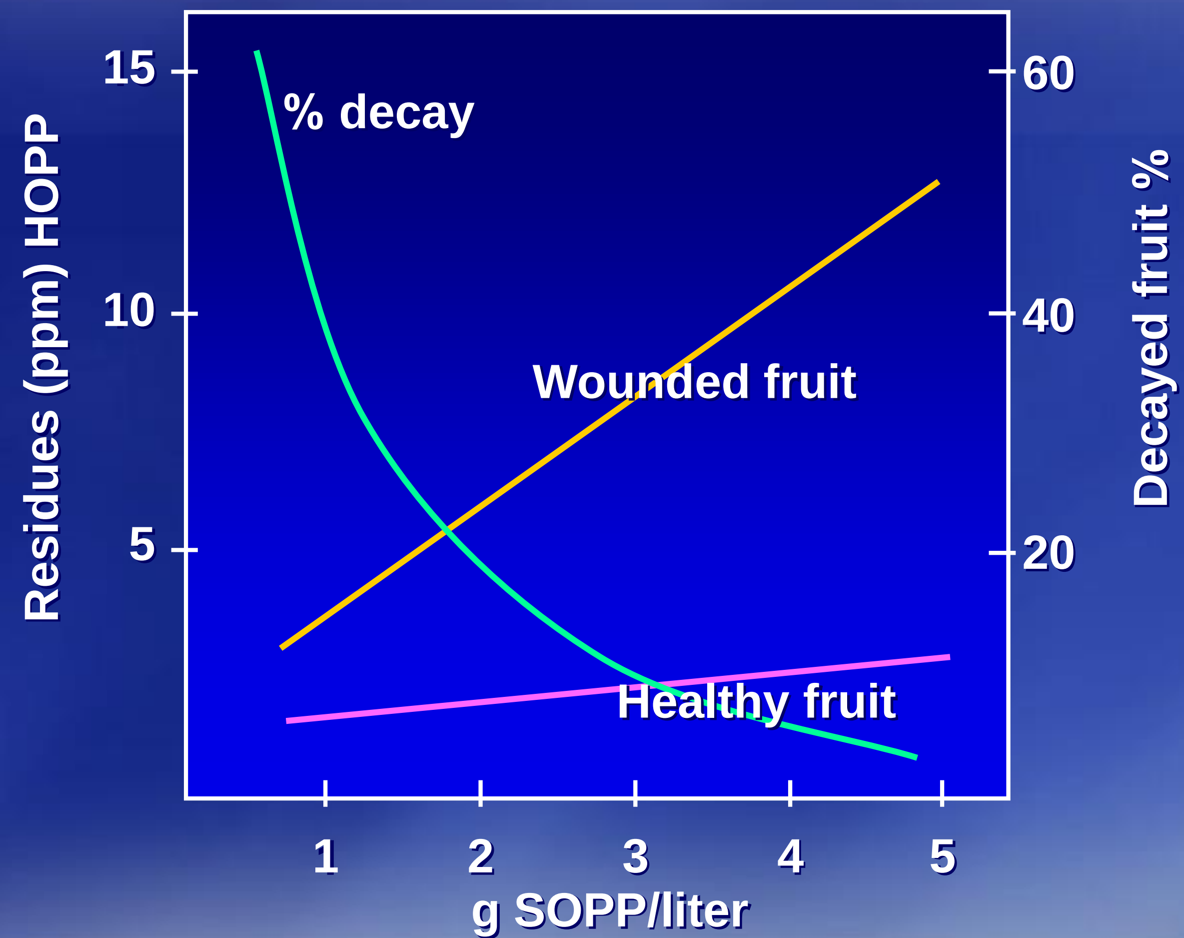
Sodium orthophenyl phenate tetra hydrate, SOPP



Mode of action:

1. Activity in solution on non wounded fruits
2. Activity on wounded fruits

SOPP activity and control



Altering the microenvironment

Sodium orthophenyl phenate tetra hydrate, SOPP

Advantages:

- Changes in pH by accumulation of alkali in infection sites on fruit surface
- Germination of spores is inhibited in the wound
- Heated solutions are more toxic

Disadvantages:

- Changes of pH may be reversed
- Fruit staining
- No residual activity

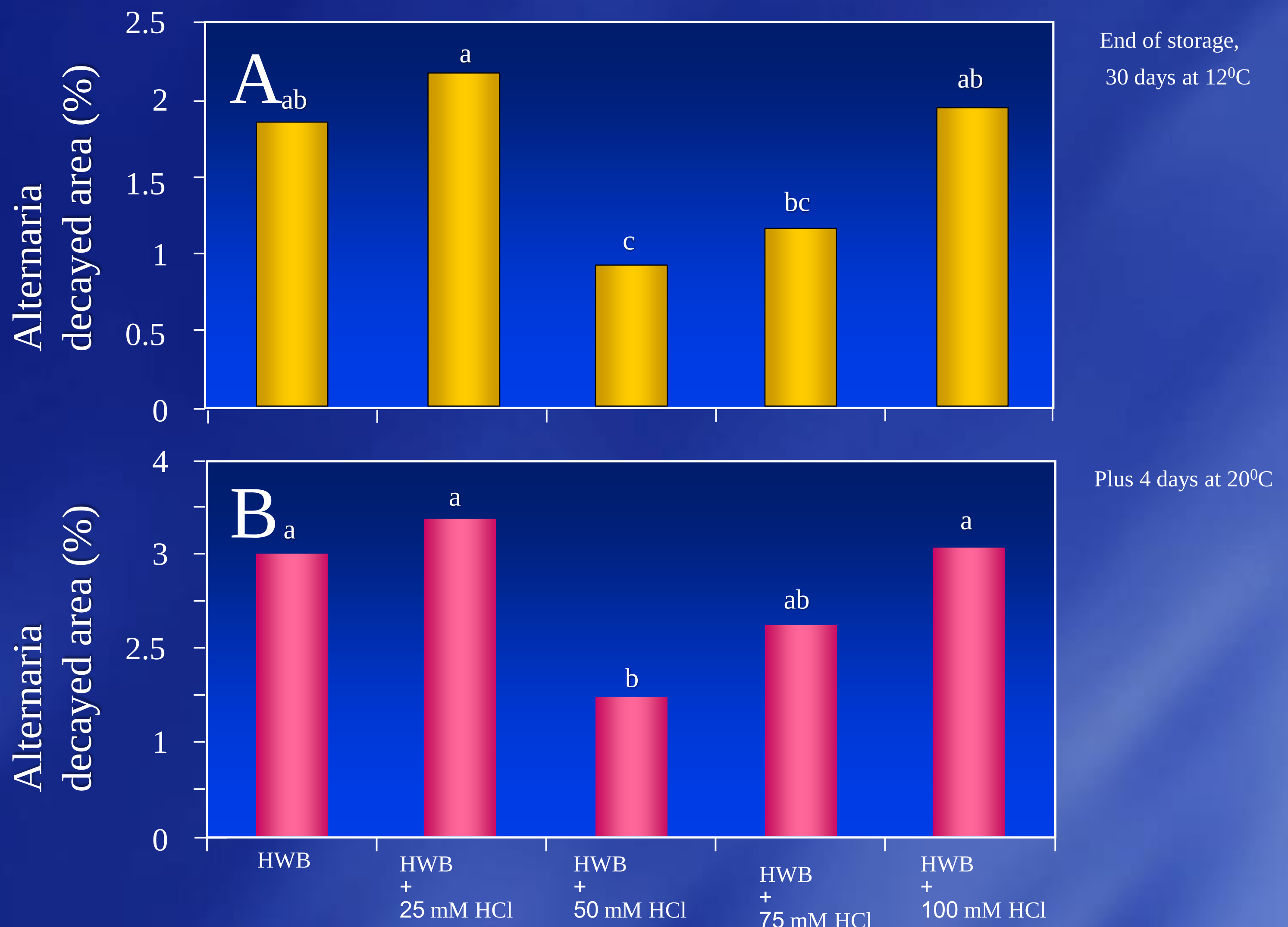
Altering the micro-environment

Treatments with indirect effect on the pathogen:

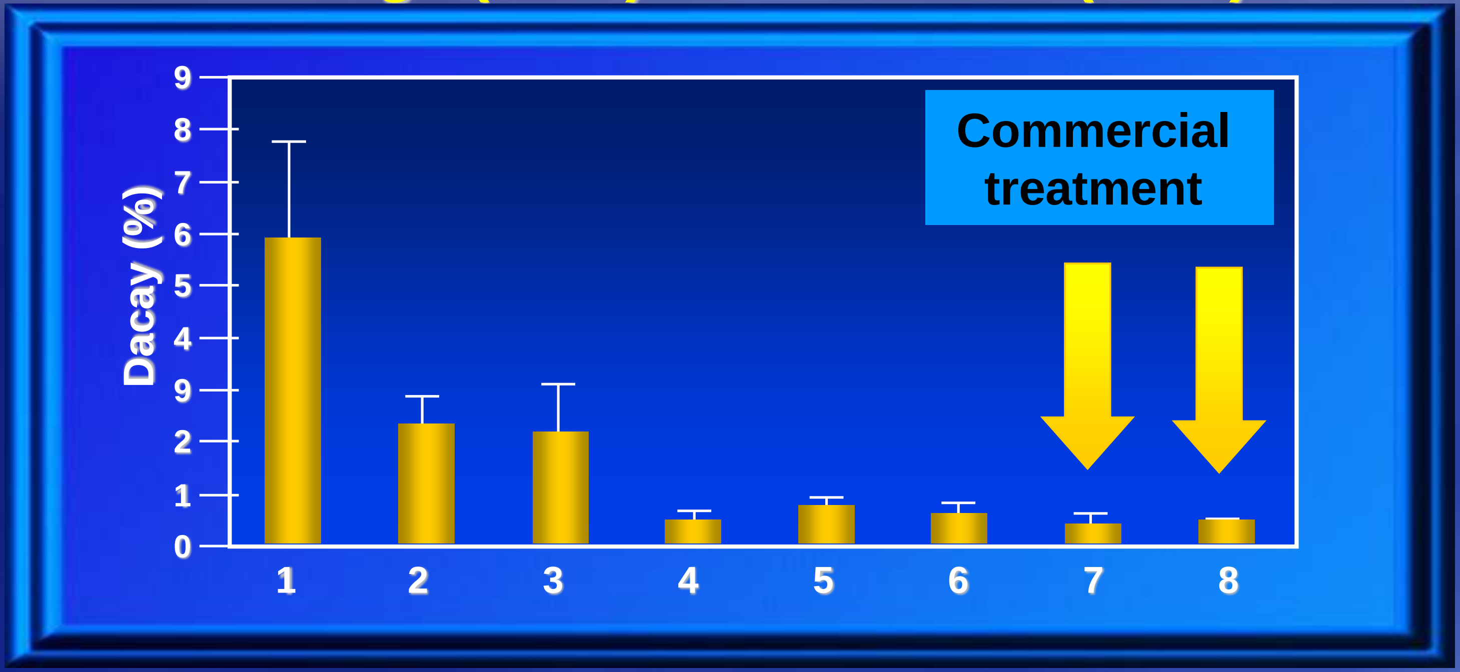
- **Alkaline solutions of borax, sodium carbonate (soda ash), and sodium bicarbonate**
- **Change of pH for prevention of NH_3 accumulation and effect on colonization**
- **Accumulation of acid in potential infection sites**

Postharvest treatments for the control of Alternaria rots (Alkalize pathogen)

Postharvest treatment for the control Alternaria side rots using HCl



The effect of neutralizers of host pH on the severity of Alternaria rot on 'Tommy Atkins' fruits after 4 weeks storage (14 C) and shelf life (20 C)



Untreated; 2. Untreated and waxed; 3. HWB and waxed; 4. HWB+ 0.2% .1 neutralizers and waxed; 5. HWB+ 0.3% neutralizers and waxed; 6. HWB+ 0.4% neutralizers and waxed and 7. HWB/ 225 µg/ml Prochloraz; 8. HWB/ 225 µg/ml Prochloraz and Chlorine spray.

Effect of acid conditions and fungal development

Methods for application of water soluble fungicides

Methods for application of water soluble fungicides

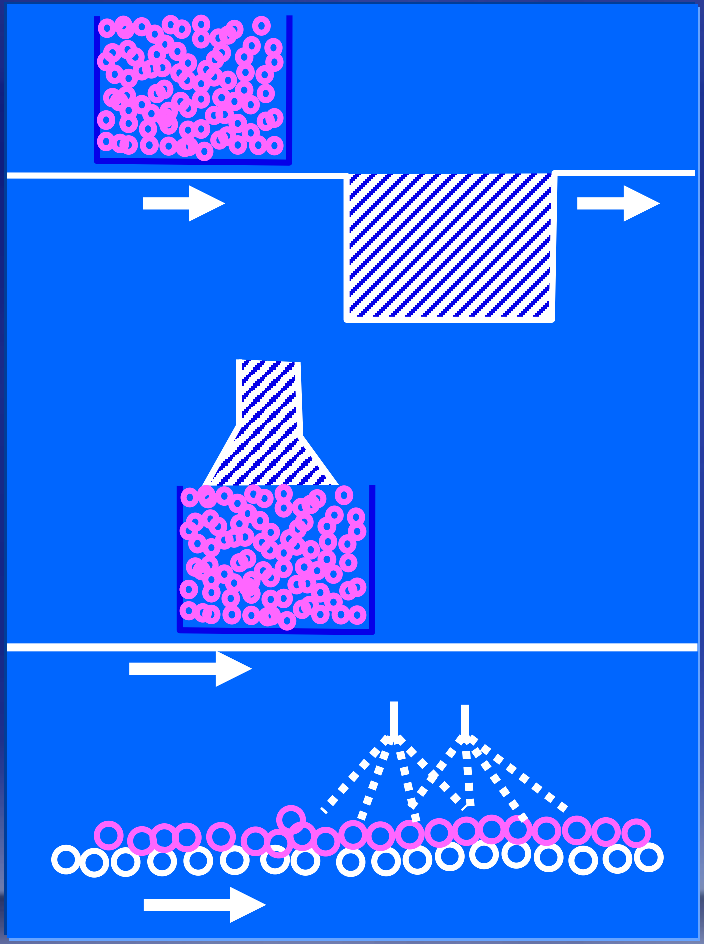
- DIP TREATMENT
- DRENCHING
- FUNGICIDE SPRAY
- FLOODER SOAPING AND FUNGICIDE APPLICATIONS
- WAXING AND FUNGICIDE APPLICATIONS

Common application treatments of fungicides

Dip Treatment

Drenching

Spray application



Application methods for postharvest fungicide treatments

Dip application

DIP TREATMENT

Applied in apples, pears, citrus, mango, persimmon, nectarines

Advantages:

Temperature regulation,

Full coverage

Dis- advantages:

Large amounts of fungicide suspensions

Because of quantities used fits to low price fungicides

Fungicides should be stable

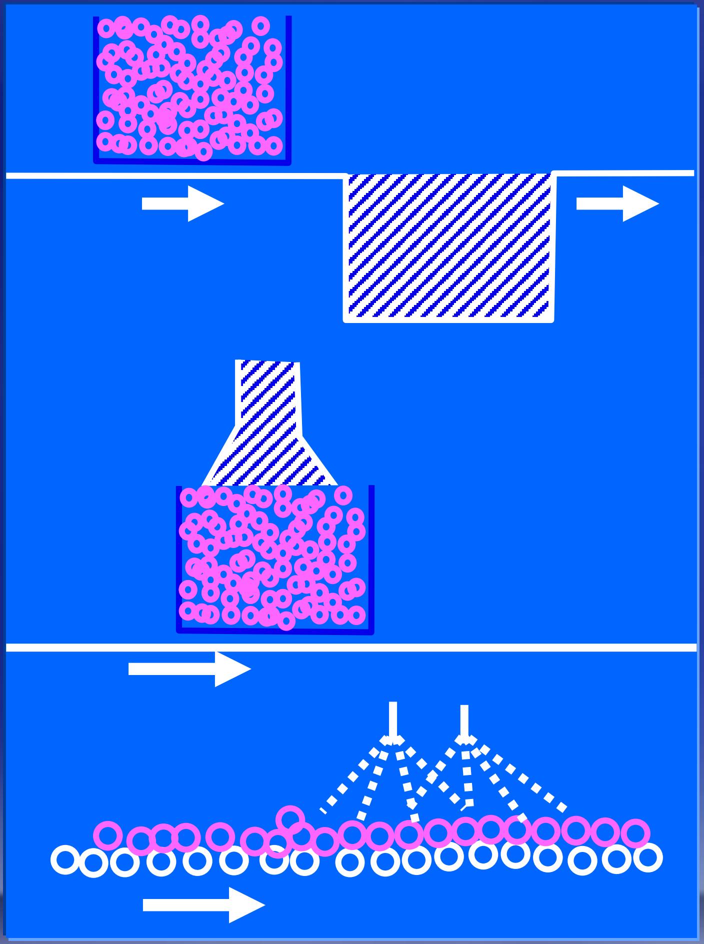
Simple method for quantity determination

Common application treatments of fungicides

Dip Treatment

Drenching

Spray application



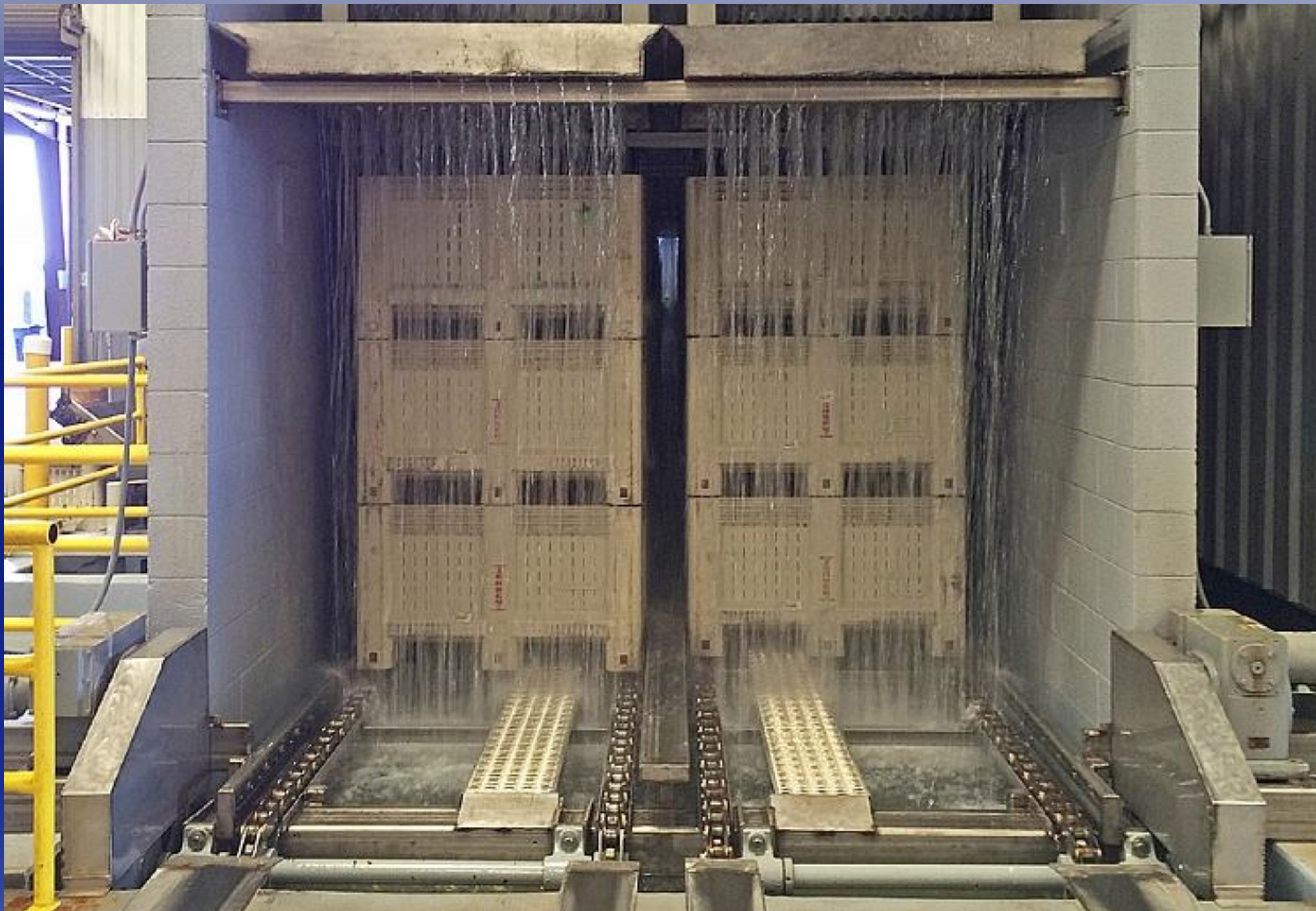
Drenching

Drenching

Drenching

1-DRENCHER

Immersion treatment for fruit and vegetables. The bins of product move forward on a roller conveyor through a curtain of chemical solution. This allows total coverage of the fruit with the treatment solution, which is then collected in a tank below for recycling via a high capacity pump.



DRENCHING

Applied in apples, citrus

Advantages
- Simple to run
- Many bins can be treated
- Easy to keep fungicide concentration

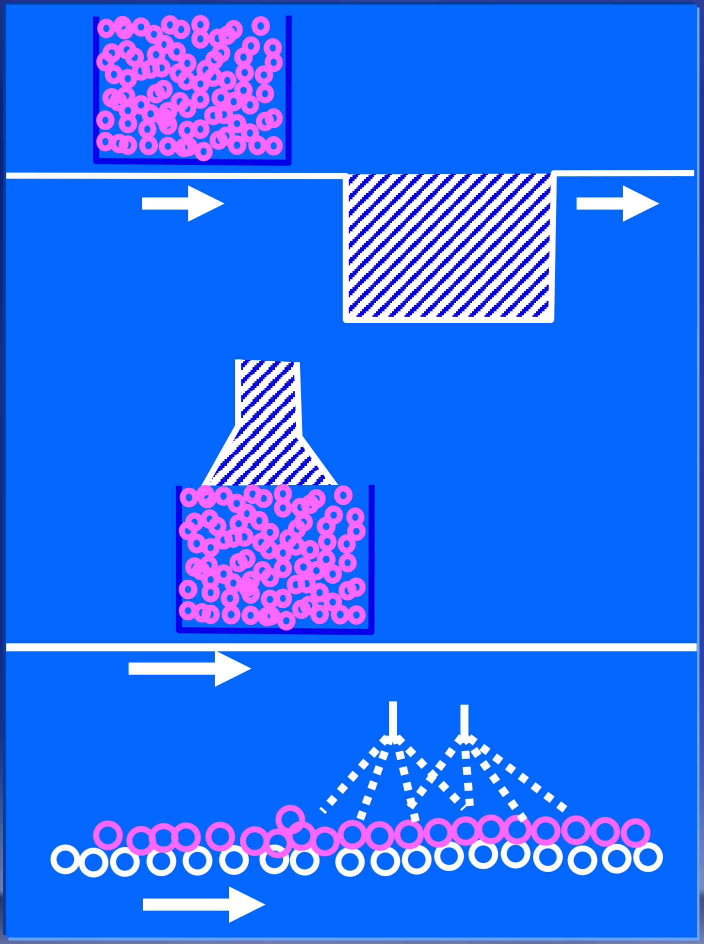
Dis-advantages
- No temperature regulation
- Not fully efficient because of short contact

Common application treatments of fungicides

Dip Treatment

Drenching

Spray application



FUNGICIDE SPRAY

Applied in citrus, mango,

Advantages - Used for new and expensive fungicides
- Reduced problems of fungicide stability (pH stability)

Applications - Roller conveyors
- Brush conveyors

Application of postharvest fungicides treatments

- **High volume application :250-500 liter/ton fruit (Drenching)**

- **Low volume applications: 15-70 liter/ton fruit (Spray on line)**

Low volume application systems have become more popular because of very little run off and no disposal problems

Application methods for postharvest fungicide treatments

High-volume spray application ('T-Jet')

Application methods for postharvest fungicide treatments

Low-volume spray application
(Controlled droplet application - CDA)

Methods for application of water soluble fungicides

- DIP TREATMENT (טבילה)
- DRENCHING (קילוח)
- FUNGICIDE SPRAY (ריסוס)
- FLOODER SOAPING AND FUNGICIDE APPLICATIONS
- WAXING AND FUNGICIDE APPLICATIONS

Flooder

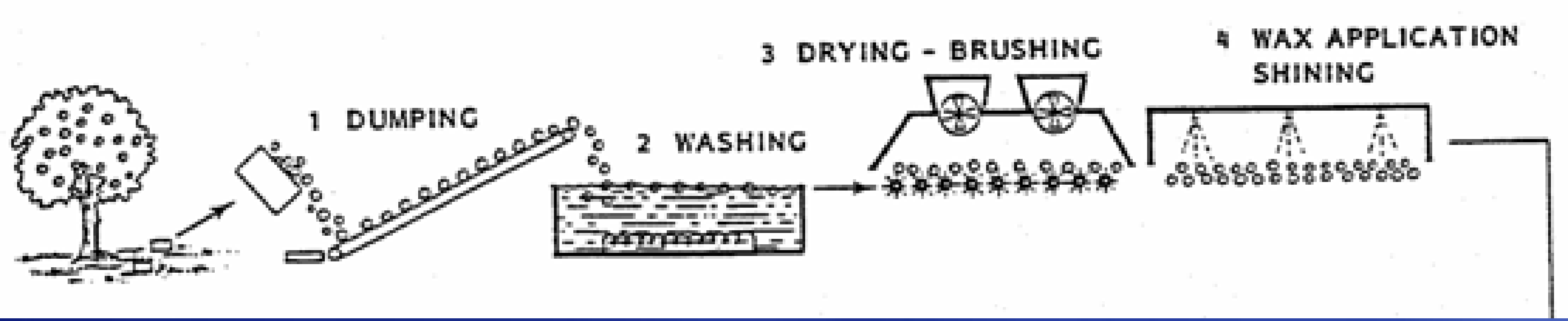
On-line Flooder, on-line application of fungicides

On-line Flooder, on-line application of fungicides

On-line drench is a system for on-line application of fungicides. This fungicide application system easily

1. Is used in water tanks and on-line applicators
2. Very efficient in terms of effectiveness and efficiency
3. Decrease in consumption of fungicide since it may be recycled
4. Reducing treatment variability by always keeping fungicide concentrations constant.

Waxing



1 DUMPING
2 WASHING
3 DRYING - BRUSHING
4 WAX APPLICATION SHINING

WAXING AND FUNGICIDE APPLICATIONS

Applied in citrus, mango and melons

Advantages: Easy to apply

Dis advantages: Fungicide sensitivity to alkaline conditions

Reduce activity

Application of postharvest fungicides

- **Aqueous applications**

- **Application in wax-oil emulsions**

1. **Not all the coating are considered food grade**
2. **Increase shine of fruits**
3. **Importance for prevention of water loss while permitting gas exchange**

Common fruit coatings used in postharvest treatments

Type of wax	Characteristics			Use on specific crops			
	Prevention of water loss	Gas exchange	Shine of fruit*	Citrus	Nectar./ Peach/ cherry	Plum	Pome
Mineral oil non-emulsified	+++	+	+++		+	+	
Mineral oil emulsified	++	++	+++		+	+	
Polyethylene	+++	+++	+++	+			
Vegetable oils	++	++	++		+	+	
Carnauba	+++	+++	++	+	+	+	+
Shellac	+	+/-	+++	+			+
Wood rosin blends	+	+/-	+++	+			

- Shine of fruit is not important for peaches and plums.
- Carnauba coatings are made from leaves of the Brazilian life tree. Shellac coatings are made from insect exudates. Wood rosins (ester derivatives) are extracted from pine trees.
- Mixtures of polyethylene, carnauba, shellac, and wood rosins are also used on citrus.
- Mixtures of carnauba and shellac are also used on pome fruits.

Summary of postharvest treatments

Common:
- **Drenchers**
- **High volume sprayers**
- **Low Volume sprayers**

Less common:
- **Dips**
- **Flooders (citrus)**
- **Foamers (SOPP)**
- **Fumigators**
- **Paper wraps (Biphenyl)**
- **Box liners (Grapes)**

Methods for application of water soluble fungicides

- DIP TREATMENT
- DRENCHING
- FUNGICIDE SPRAY
- FLOODER SOAPING AND FUNGICIDE APPLICATIONS
- WAXING AND FUNGICIDE APPLICATIONS

Some examples of postharvest treatments

Chemical/class (Trade name)	Year introduced	Crop	Decay/organisms	Methods of Application	Residue Tolerance (mg/kg)
Fludioxonil Phenyl piyrole Reduce Risk Fungicide	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 Prochloraz Demethylation inhibitor	1974	Citrus Mango	Penicillium Alternaria	Spray Spray	10

Effect of fungicides on stem end rot in mango fruits in Israel

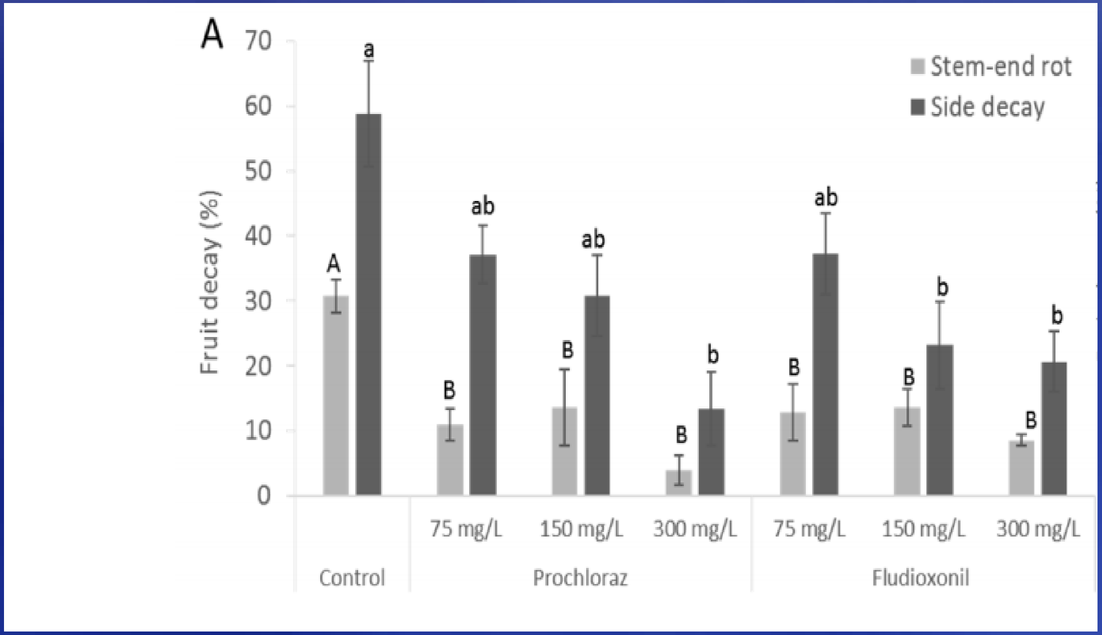
Control

Sportak 150 ppm

Schoolar 150 ppm

Fungicide residues

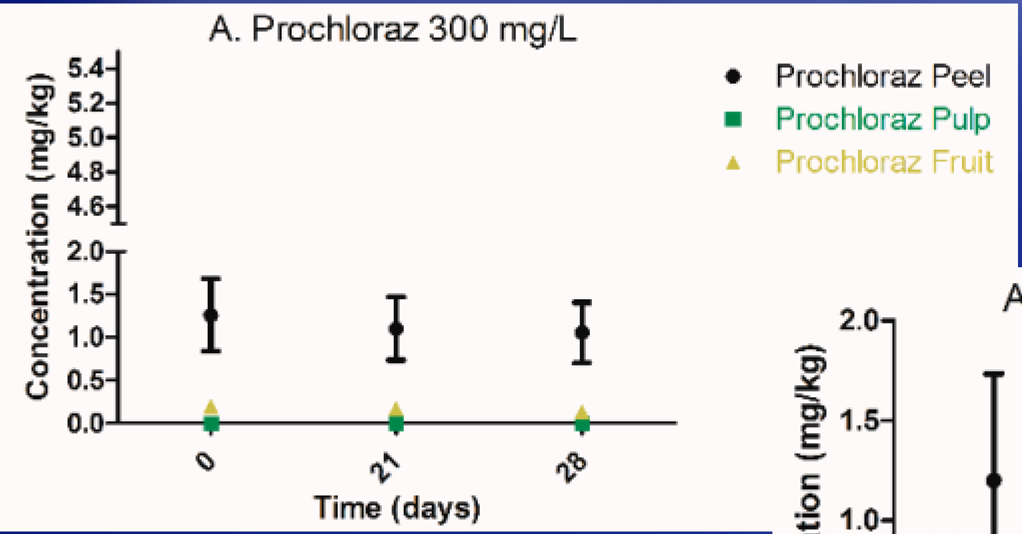
Postharvest fungicide treatments of avocado in Israel

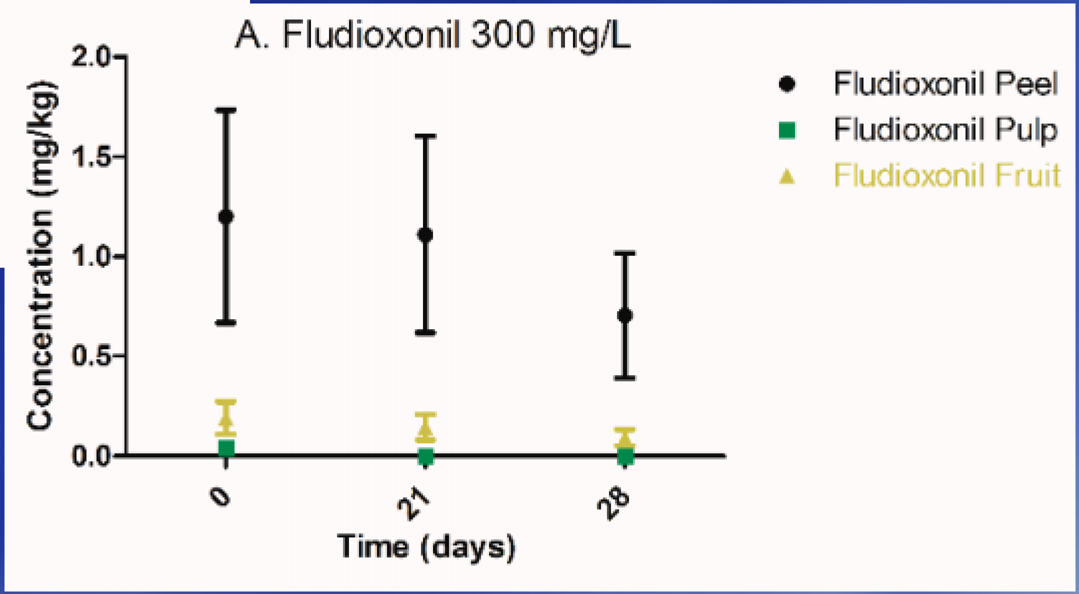


Postharvest fungicide application on avocado fruit and its effect on natural stem-end rot. The fruit was treated with prochloraz or fludioxonil at different concentrations (300, 150, 75 mg/L); fruit was stored at 5 °C for 21 days and for additional 7 day period (SL) at 20 °C

Shimshoni et al., 2020, Foods

Fungicide accumulation in the peel and fesh of avocado fruits



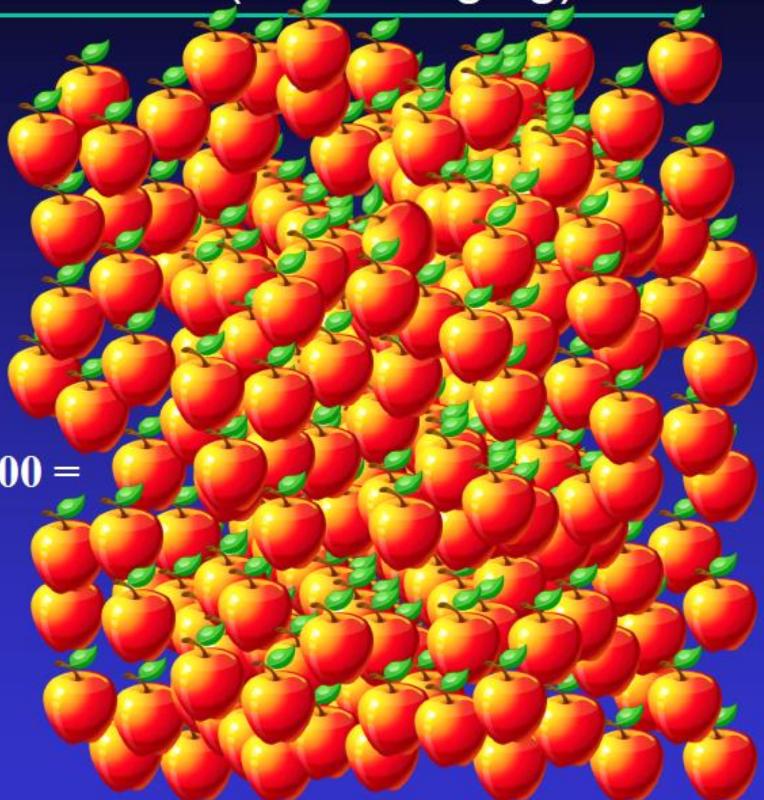


Shimshoni et al., 2020

How many apples does someone need to eat to reach the LD_{50} of fludioxonil (>5000 mg/kg)?

1 ppm = 1 mg/kg
or 1 mg/10 apples

X 5000 =



50,000 apples/Kg x Body weight (70 kg for an adult)=3.5 million apples!

Altering the micro-enviorment

- Treatments with a direct effect on the pathogen:

  <u>Fungicides</u>: Direct toxicity

  <u>Biocontrol</u>: Competition, antibiosis, parasitism

Microbiome interaction

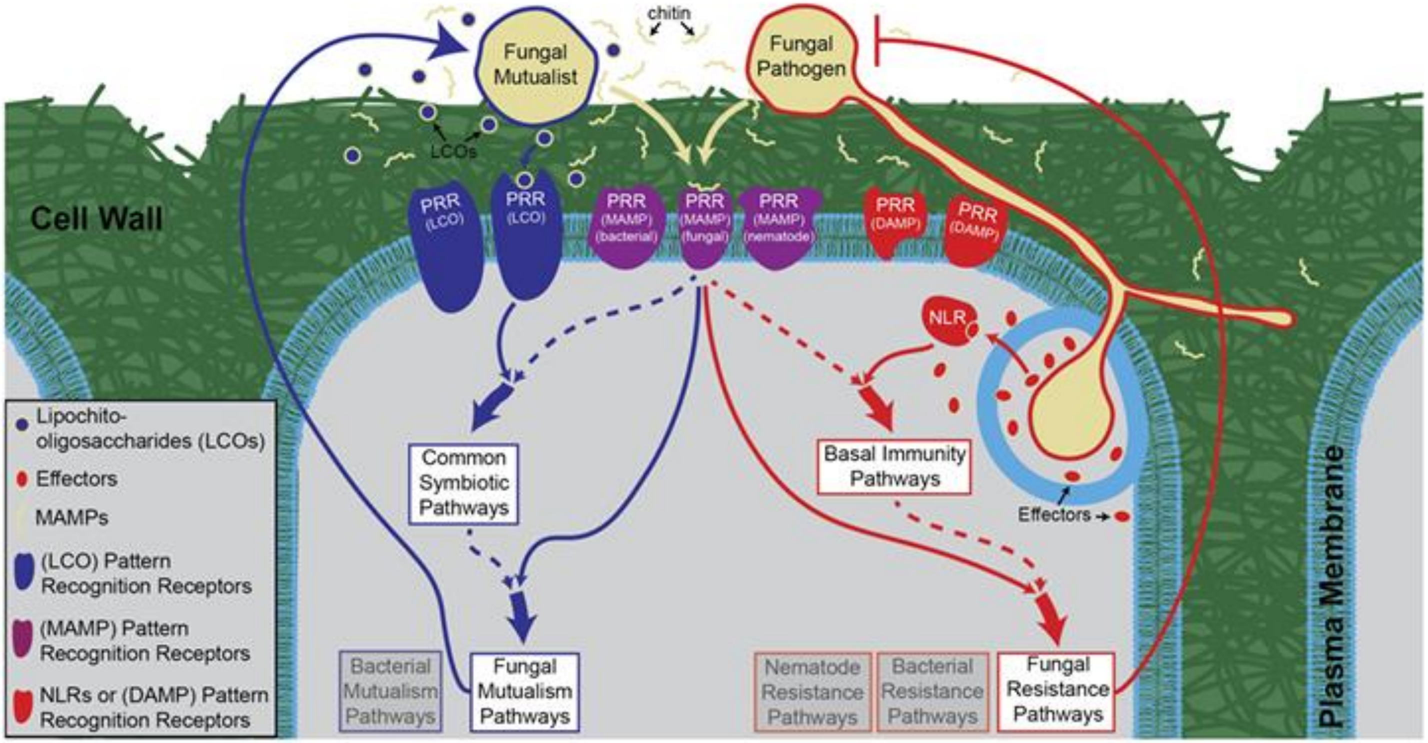


Fig. 2. Multiple receptor inputs contribute to specific and robust immunity and symbiosis responses. To decide whether to engage in immunity or symbiosis, a plant must identify the type of microbe it is interacting with and determine whether that microbe is mutualistic or pathogenic. Robust activation of a specific pathway depends on the combined input of microbe-associated molecular patterns (MAMPs) and one or more lifestyle associated factor (e.g., damage-associated molecular patterns (DAMPs), effectors, lipochitooligosaccharides [LCOs]). In this example, chitin informs the plant that the symbiont is a fungus, and LCOs or effectors inform the plant whether it is a mutualist (blue components and arrows) or pathogenic (red components and arrows). The central boxes represent basal immunity pathways and common symbiotic pathways, which include the respective signaling cascades, posttranslational modifications, and gene induction required for initiation of an immune or mutualistic response. At the bottom are the microbe-specific responses for resistance against or mutualism with a specific type of microbe. Activated pathways are indicated by white boxes and inactive pathways are shown in gray. Dotted lines indicate weak signaling and activation, while solid lines reflect stronger interactions. Wide arrows represent either a synergistic or additive response. PRR = pattern recognition receptor, NLR = Nod-like receptor.

Biocontrol: Competion, antibiosis, parasitism

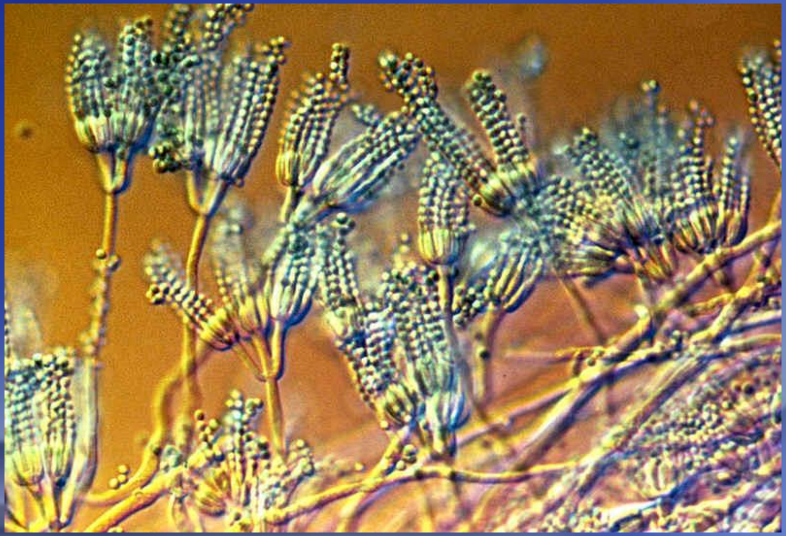
- Development is driven by safety concerns
- Difficult to transfer laboratory efficiency to commercial scale
- Efficiency is generally in-consistent
- Two product registered:

1. **Aspire** (Candida oleophila) no longer manufactured)
2. **Bio-Save** (Pseudomonas syringe), still in use

Biological Control:competition

Utilization of antagonistic microorganisms of the pathogen

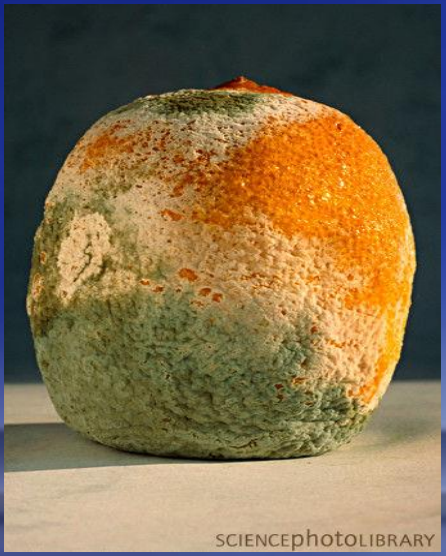
- The **antagonistic microorganism can compete** with the pathogen for nutrients at the wound site.
- The yeast *Pichia guilliermondii* **development is much faster** in grapefruit peel than the *Penicillium digitatum* spores and inhibit their germination.

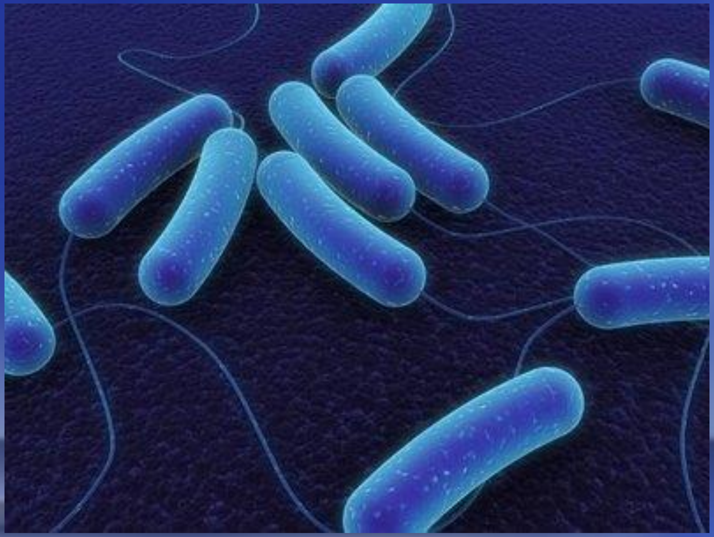


Biological Control: antibiosis

Utilization of **antagonistic microorganisms** of the pathogen

- **Reduction of the rate of decay** development in storage.
- The application of the antagonistic microorganisms may apply by **spray in the orchard or by dipping after harvest**.
- **Secretion of antibiotic compounds** by the antagonistic microorganisms: e.g. the bacteria *Bacillus subtilis* can inhibit the development of major fungi in citrus.

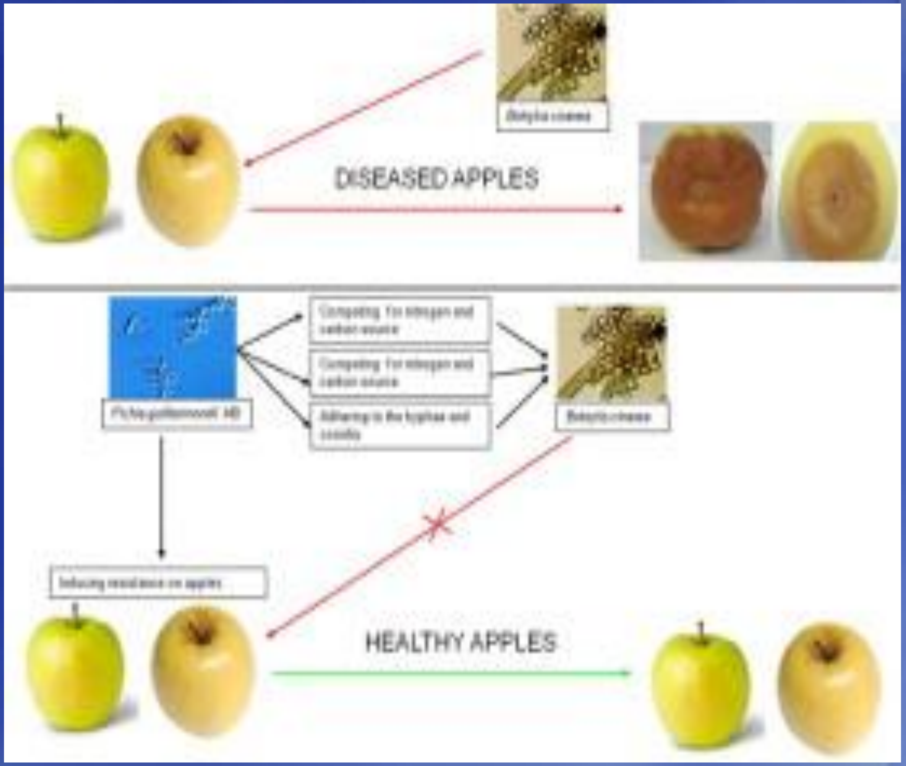




Biological Control: induce defenses

Utilization of antagonistic microorganisms of the pathogen

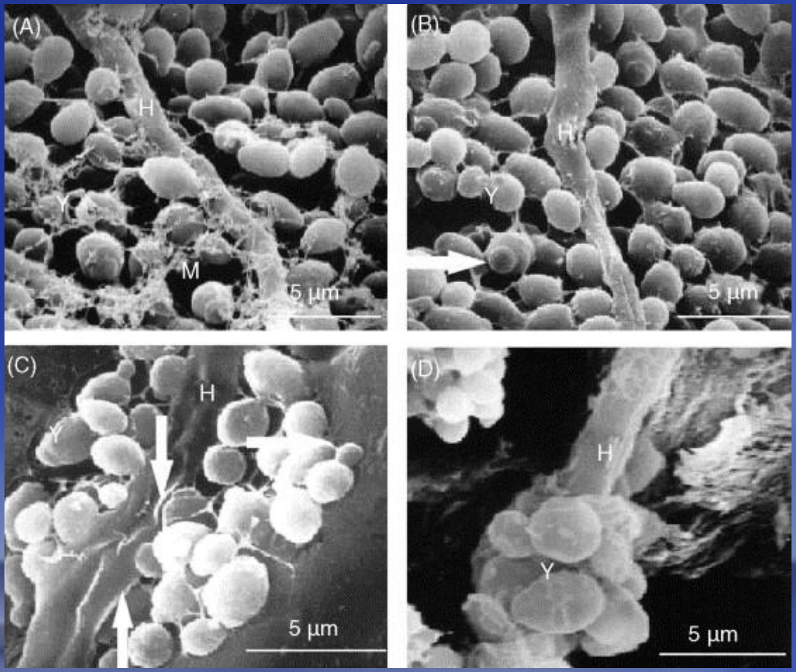
- Antagonistic microorganisms can induce the defense mechanisms of the fruit.
- The yeast Pichia induced ethylene production in citrus peel and increased PAL enzyme activity.

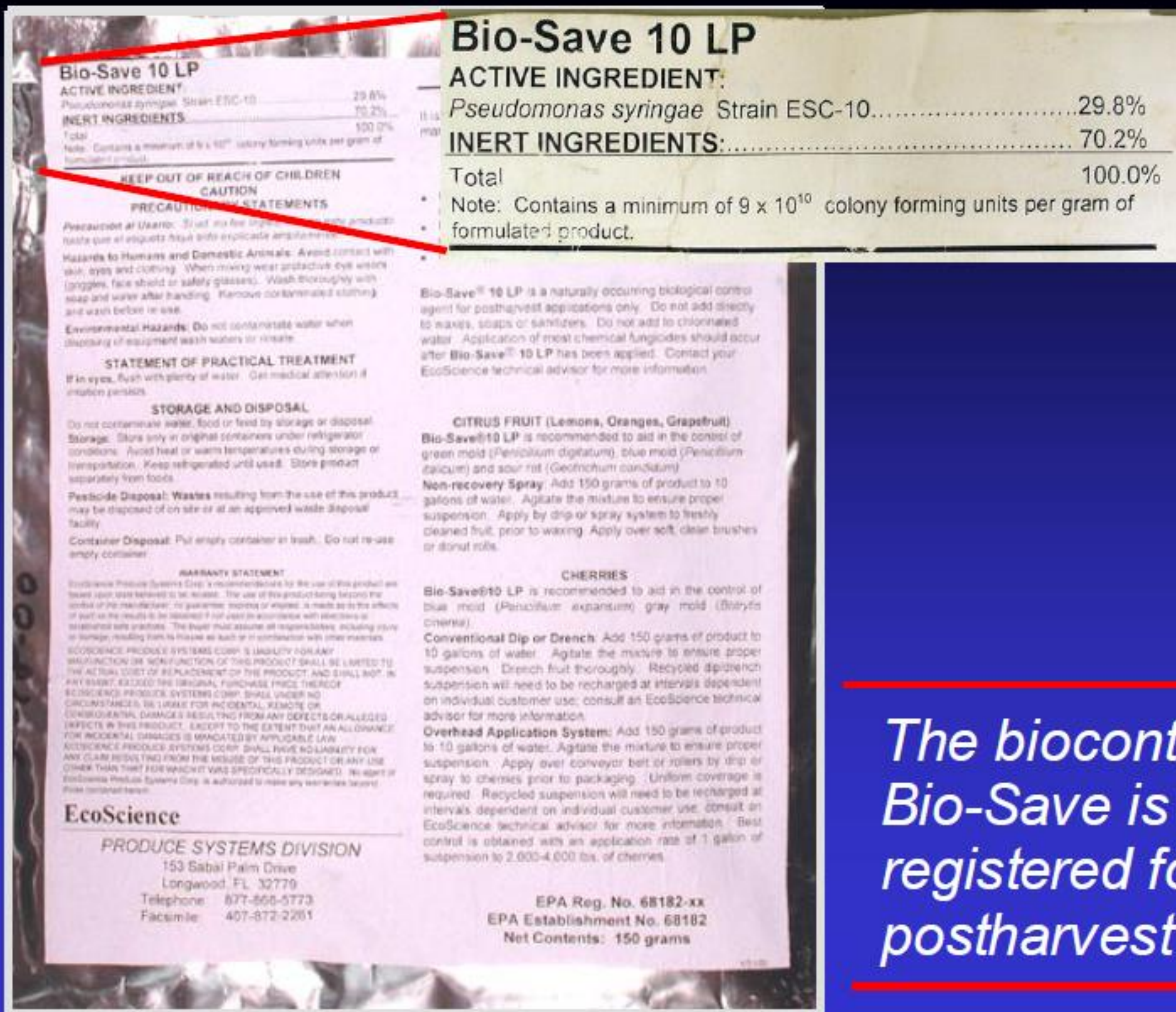


Biological Control: direct effect

Utilization of antagonistic microorganisms of the pathogen

- The antagonistic microorganism can directly affect the pathogen.
- It was demonstrated that the **Pichia is able to stick to fungi Mycelium.**





The biocontrol Bio-Save is registered for postharvest use

Spectrum of Activity of Biocontrols for Postharvest Decay Control

Biocontrol	Organism	Crops	Decays
Bacteria	*Pseudomonas syringae*	Apples, pears, citrus	Penicillium Decays
		Sweet cherry	Gray mold, Penicillium decays
Yeast	*Candida oleophila*	Pome fruit	Penicillium Decays
		Citrus	Penicillium Decays

Biocontrol products registered in other countries

- YieldPlus (*Cryptococcus albidus*) – developed in South Africa for pome fruit
- Avogreen (*Bacillus subtilis*) – South Africa for avocado
- Shemer (*Metschnikowia fructicola*) – Israel for apricot, peach, citrus, grapes, pepper, strawberry, sweet potato

- Several other products such as **Candifruit** (*Candida sake*), **NEXY** (*Candida oleophila*), and **Boni-Protect** (*Aureobasidium pullulans*) are in development.

Comparison

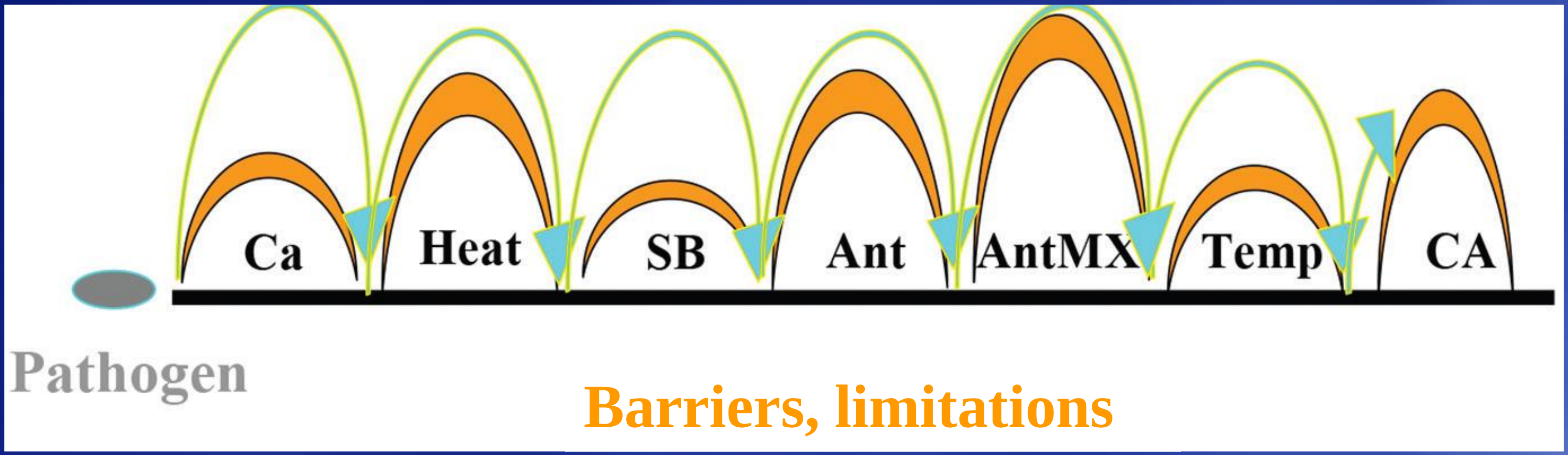
Prevention, suppression, and eradiction of postharvest decays

Fungicides vs. biological controls

Fungicides	Biological controls
Single synthetic active ingredient	Mixtures of active and inactive ingredients. Active ingredient often unknown.
Well characterized chemically and toxicologically	Chemically and toxicologically often poorly characterized, but considered natural.
Efficacy generally high	Efficacy variable

Take home message

Multi-barrier concept for managing postharvest diseases

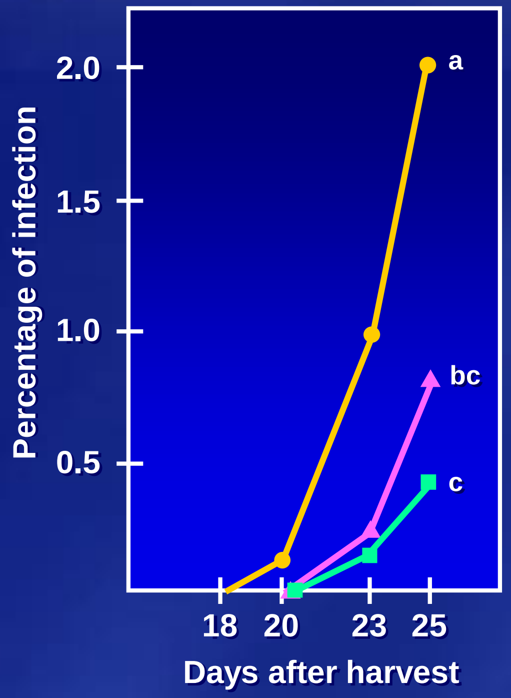


Ca = calcium, Heat = 38°C for 4 d, SB = sodium bicarbonate, Ant = antagonist, AntMX = antagonist mixture, Temp = low storage temperature, CA = controlled atmosphere storage

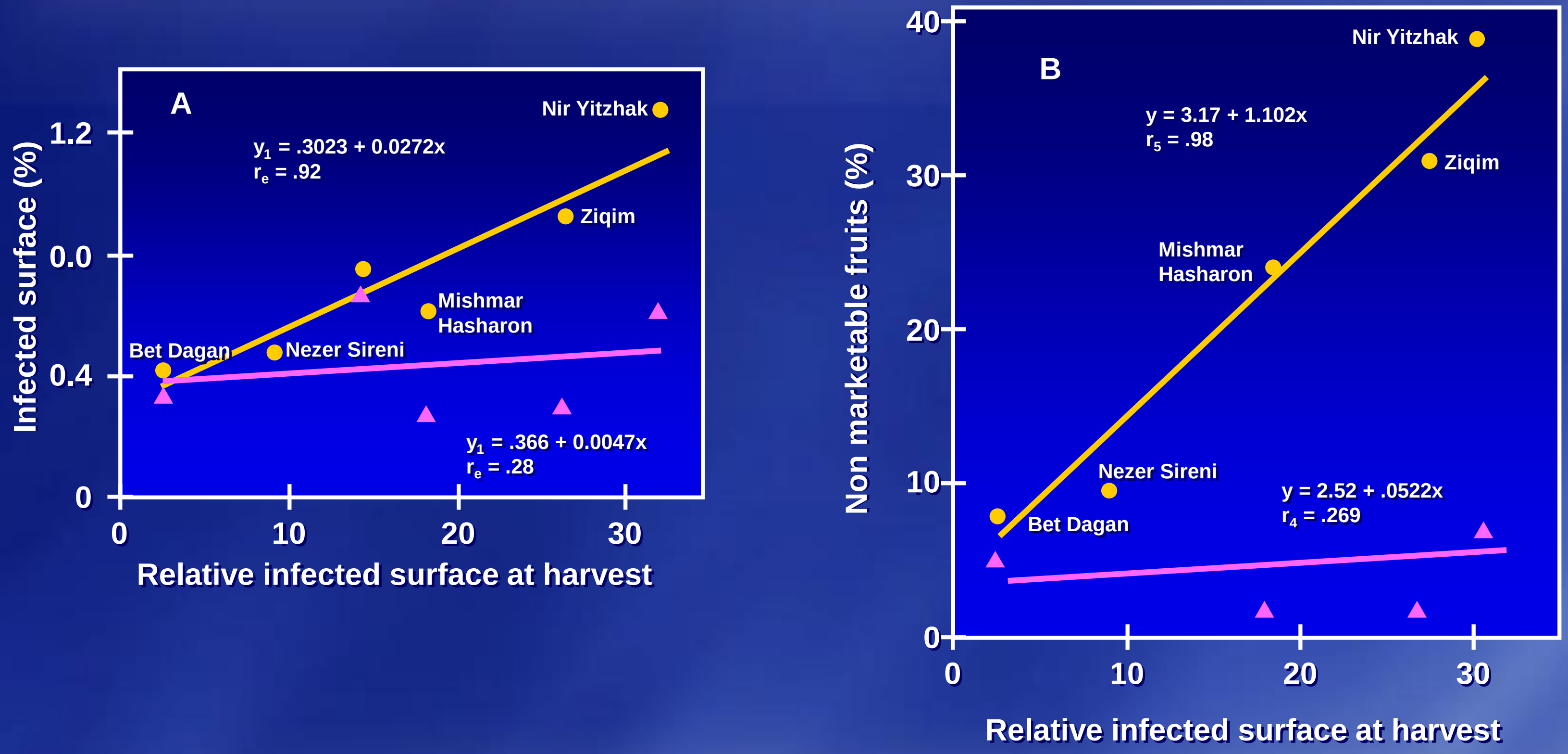
Thank you for attending the course!!!!!

Preharvest vs. Postharvest treatments

A comparison between the pre harvest and postharvest treatment efficiency



A comparison between the pre harvest and postharvest treatment efficiency



Preharvest vs. Postharvest treatments

Postharvest treatment is better more efficient and cheaper

Integration of postharvest treatments

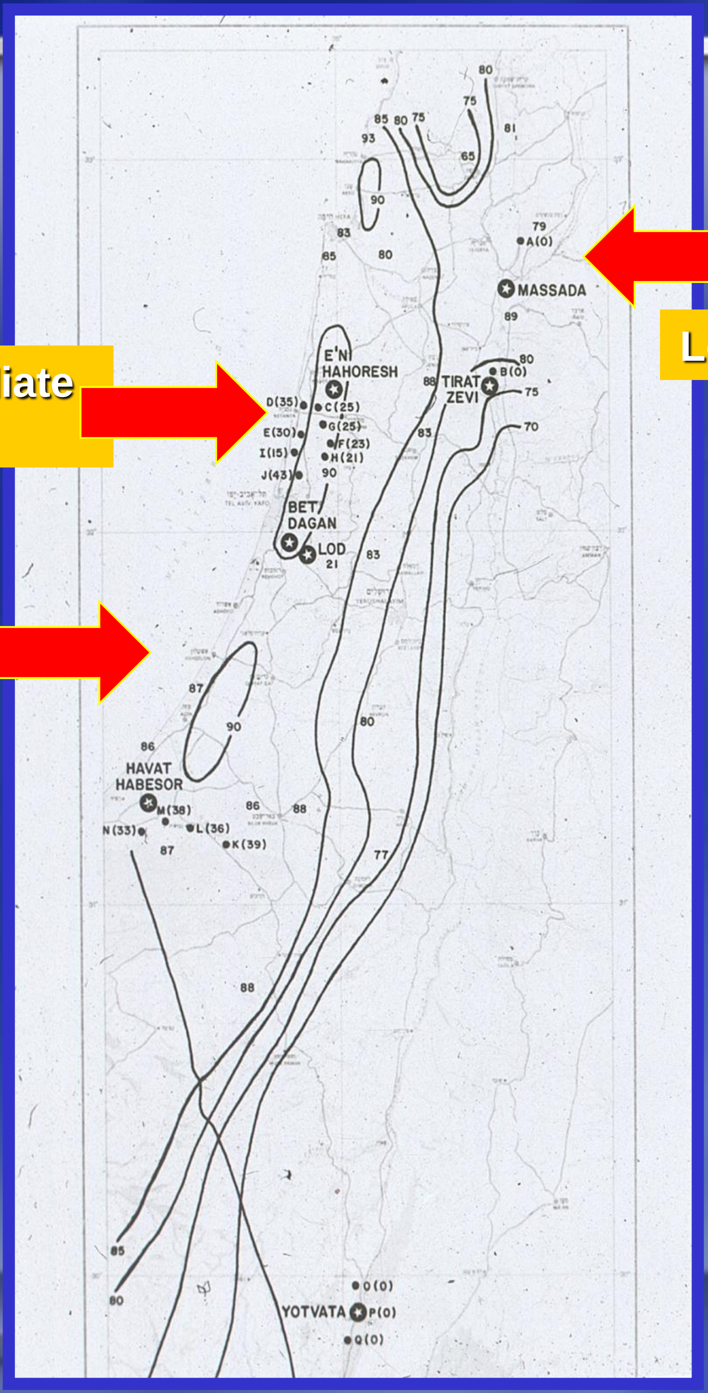
Hot water treatment
Fruit brushing
Fungicide spray
and
Waxing

Postharvest treatment include application of hot water brushing (HWB) techniques at different temperatures in combination with fungicide

- HWB (Physical)
- HWB with 225 µg/ml Prochloraz (Physical+chemical)
- 900 µg/ml Prochloraz spray (Chemical)
- Waxing

A different postharvest treatments for each agricultural region

The relation between the RH in the orchard and the relative quiescent infected area at the end of the growing season



Intermediate RH

High RH

Low RH

The effect of HWB, prochloraz and waxing on the severity of Alternaria rot on Tommy Atkins.