Joint Institute for Food Safety and Applied Nutrition

Influence of Pre-harvest Antibiotic Pesticide Treatment on the Native Microflora of Apple and Pear Blossoms, Leaves, Fruit, and Cider and its Implications for Food Safety

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Collaborators

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Background

Limited knowledge on relationship between unpasteurized apple cider safety and:

Good agricultural practices

- Known
  - -Avoid drops (2 log safety improvement)
  - -Use U.S. cider grade apples or better (<1 log improvement)

Good manufacturing practices

- Known
  - -Contaminated lots

Unpasteurized Apple Cider as a Public Health Problem
Cider-Associated Outbreaks

- *1974 Salmonella* (300 cases)
- *1980 E. coli* O157:H7 (23 HUS cases)
- *1993 Cryptosporidium* (213 cases)
- *1996 E. coli* O157:H7 (3 outbreaks)
  Cryptosporidium (213 cases)
- *1999 E. coli* O157:H7
- *2003 Cryptosporidium* (>5 cases)
- *2004 E. coli, Cryptosporidium, Camplobacter*

Fire Blight *(Erwinia amylovora)*

- Gram negative bacterial pathogen that attacks members of the *Rosaceae*

- Major problem in apple and pear orchards
- Lethal to many pear varieties
- Lethal to popular apple rootstocks
- Limits apple exports to Japan

- Treatments: Copper, Streptomycin and Oxytetracycline
- Streptomycin-resistant *Erwinia* epidemic in MI, 2001

Data Gaps:
What is the effect of fireblight controls on microbial ecology in the orchard?

Unknown : Influences of antibiotic use

- Effect on overall orchard microflora
- Effect on presence of Gram negative human pathogens responsible for illness outbreaks
- Organic pesticides ?

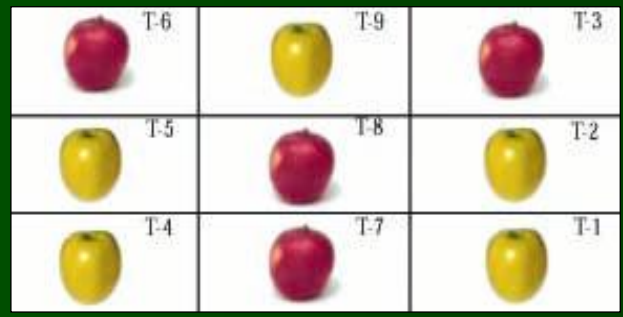
Does less pesticide reduce chemical risks but increase biological risks?

Plot 1: Unsprayed
"Transitional Organic"

Plot 3: Conventional
Spray Schedule

Experimental Design:

Plot 1: Unsprayed
"Transitionally organic"



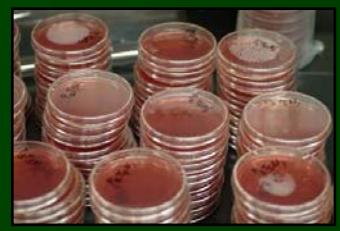
Plot 2: Early Sprays Only
Conventional sprays until June 15th,
Unsprayed July, Aug, Sept.

Plot 3: Conventional Spray

Experimental Procedure:

Duplicate sets of apples are collected from four locations on each tree in the three experimental plots.

“Wash” is prepared from surface microflora and 20 ml of water and 0.1% peptone buffer pH 6.8



Dilutions of the wash are plated onto general and selective media.

Populations are counted, recorded, and analyzed statistically using SAS.

Preliminary Experiment (2002)

Dependent Variables

- Yeast and Molds, BHIA, MacConkey, Total Coliforms

Independent Variables

- Pesticide Application
- Tissue (Leaf, Fruit and Shoot)
- Position within Tree

Samples taken in August, Sept. and Oct.

Preliminary Results (2002)

Significant Effects

- Pesticide Usage

  (Half log reduction with Pesticide Usage)

- Position within Tree

Non-significant Effects

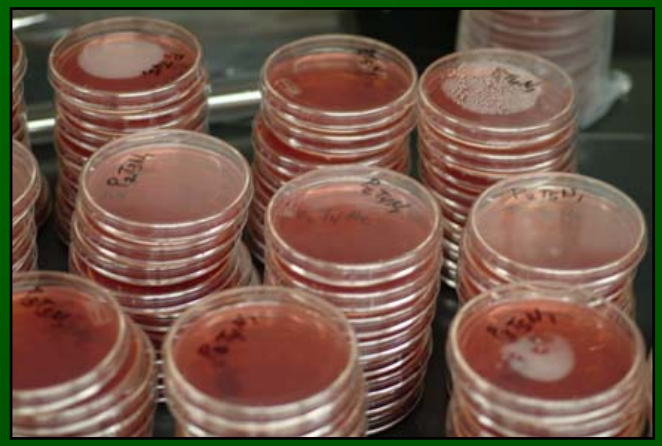
- Leaf and Fruit Counts Similar
- Shoot Counts Extremely Variable

Less-selective Media

Brain Heart Infusion Agar (BHIA)
Total aerobic count

3M Petri Film
Yeasts and Molds

Selective media



MacConkey
(Gram negative bacteria)

Petri Film Total Coliforms
(30 - 37C)

Total Aerobic Count

BHIA (2003) Log CFU / Apple (SE)

Treatment	Early-Season	Late-Season
Full Spray	4.38 (.06)	4.35 (.03)
Half Spray	4.49 (.06)	4.39 (.02)
No Spray	4.55 (.06)	4.59 (.03)

Selective Media for Gram Negative Bacteria

MacConkey(2003) Log CFU / Apple (SE)

Treatment	Early-Season	Late-Season
Full Spray	3.30 (.03)	4.23 (.05)
Half Spray	3.31 (.04)	4.57 (.05)
No Spray	3.53 (.05)	4.81 (.05)

Selective Media 3M Petri Film

Total Coliforms (2003) Log CFU / Apple (SE)

Treatment	Early-Season	Late-Season
Full Spray	1.93 (.03)	2.75 (.05)
Half Spray	1.74 (.03)	3.06 (.05)
No Spray	1.79 (.03)	3.27 (.05)

Laboratory ‘Cider’ (2003)

Plate Counts Log CFU / cm^2

Treatment	BHIA	MacConkey	Total Coli
Full Spray	2.4	1.6	1.3
Half Spray	3.1	2.5	2.3
No Spray	3.0	2.4	2.5

Discussion:

- Is the log reduction a significant GAP?
- How does it affect human pathogens?

Option 1:

Reduce risk by direct kill of human pathogens ?

Option 2:

Increase risk for human pathogen survival if native microflora are no longer occupying competing niches ?

Organic Production and GAPs.

To be profitable, organic orchards require cider production.

What are the implications on food safety caused by the change from conventional to organic production systems?

Wye Experimental Organic vs. IPM (Conventional) Orchard

AREAS OF RESEARCH INVESTIGATION

JIFSAN FUNDING

Food Safety Issues
Fruit Quality

AGROECOLOGY FUNDING

Specialty Crop Potential
Economics

Insecticide, Herbicide,
Fungicide and Fertilization
Requirements and Recommendations

ORGANIC VS. CONVENTIONAL PLOTS

Design:

- Randomized orchard blocks: 2.5 acres
- 50 feet between conventional and organic conditions – (MDA)
- Organic Certification anticipated in fall, 2005

ENT.	OLY.	GAL.
GLD.	NIT.	FUJ.
LIB.	ATA.	COR.

O-5 ORGANIC

ENT.	OLY.	GAL.
GLD.	NIT.	FUJ.
LIB.	ATA.	COR.

C-5 CONVENTIONAL

FUJ.	NIT.	GLD.
COR.	OLY.	LIB.
GAL.	ATA.	ENT.

C-4 CONVENTIONAL

FUJ.	NIT.	GLD.
COR.	OLY.	LIB.
GAL.	ATA.	ENT.

C-4 ORGANIC

LIB.	ATA.	COR.
ENT.	NIT.	FUJ.
GLD.	OLY.	GAL.

O-3 ORGANIC

LIB.	ATA.	COR.
ENT.	NIT.	FUJ.
GLD.	OLY.	GAL.

C-3 CONVENTIONAL

FUJ.	OLY.	ENT.
GAL.	ATA.	LIB.
COR.	NIT.	GLD.

C-2 CONVENTIONAL

FUJ.	OLY.	ENT.
GAL.	ATA.	LIB.
COR.	NIT.	GLD.

C-2 ORGANIC

GLD.	ATA.	GAL.
ENT.	OLY.	COR.
LIB.	NIT.	FUJ.

O-1 ORGANIC

GLD.	ATA.	GAL.
ENT.	OLY.	COR.
LIB.	NIT.	FUJ.

C-1 CONVENTIONAL

NINE CULTIVARS

3 Disease resistant apple cv

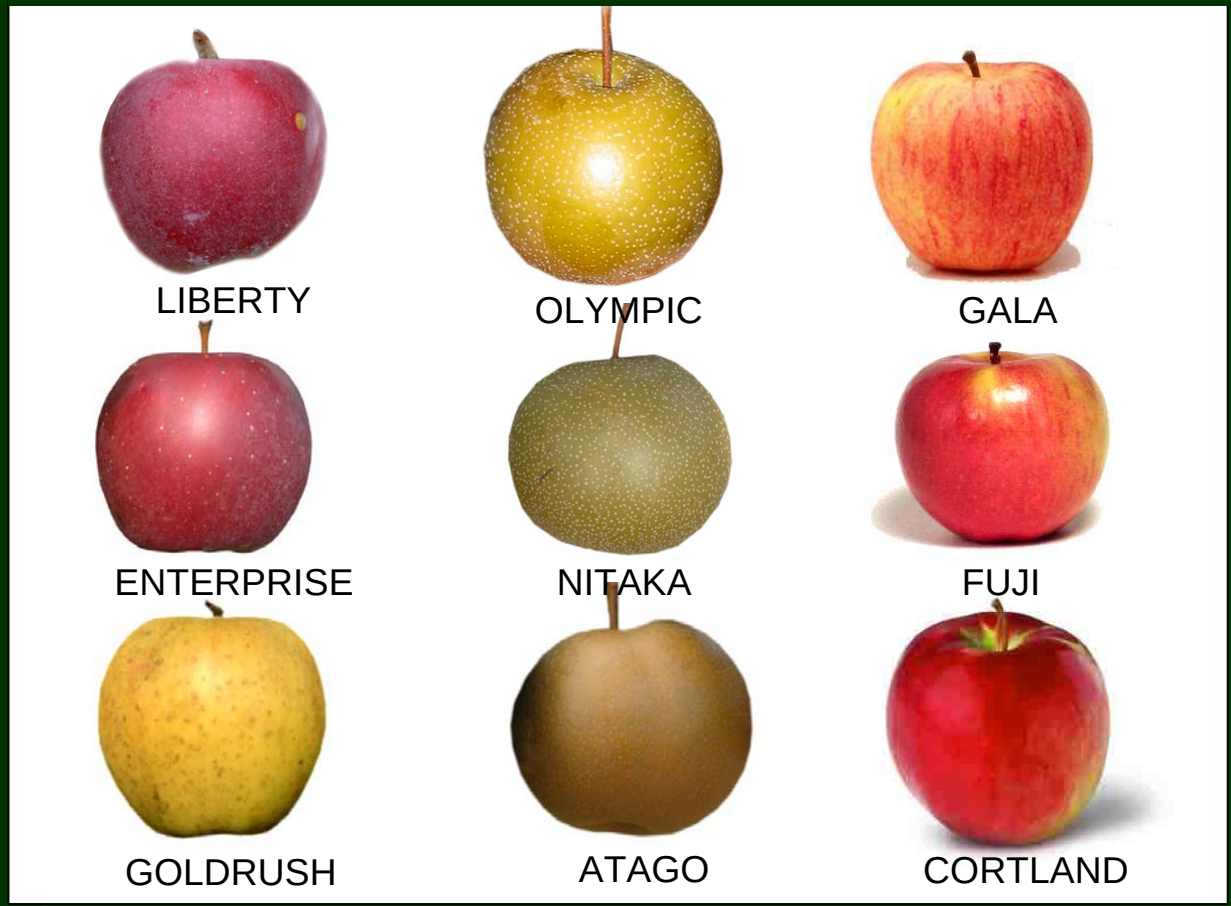
LIBERTY, GOLDRUSH, ENTERPRISE

3 Asian pear cv

OLYMPIC, ATAGO, NITAKA

3 Commercial apple cv

GALA, FUJI, CORTLAND



Pressure treated wood for tree support
in the conventional plots

“Strips” prepared with herbicide,
Gramoxone

Fertilized w/Calcium nitrate

Aluminum poles for tree support
In the organic plots

Dedicated “organic” product sprayers
used for all applications.

Mowers cleaned before use in organic
plots.

PLANTING YEAR: PEARS PLANTED 4/17/03 APPLES PLANTED 4/20/03

ORGANIC MATERIALS 2003

4/30/03	Fertilize Nitro Mix
6/6/03	Herbicide: All Down
6/10/03	Herbicide: 20% acetic acid
7/4/03	Insecticide: Pyganic
7/7/03	Herbicide: All Down
7/10/03	Fertilizer: Nitro Mix
7/13/03	Pyganic & Sulfur
7/25/03	Pyganic, Sulfur & Surround
9/16/03	Pyganic, Sulfur & Surround

IPM ORCHARD MATERIALS 2003

3/24/03	Herbicide: Roundup
4/30/03	Fertilize $Ca(NO_3)_2$
5/29/03	Herbicide: Gramoxone
7/2/03	Herbicide: Roundup
7/4/03	Imidan, Captan, Provado
7/10/03	Calcium Nitrate
7/17/03	Captec & Provado
7/30/03	Captec, Sulfur, Imidan, Nova
8/19/03	Captec, Sulfur, Imidan, Nova
9/6/03	Captec, Provado, Topsin
9/16/03	Roundup

ORGANIC MATERIALS 2004

4/10/04	Pyganic, Sulfur, Surround
4/20/04	Pyganic, Sulfur
4/30/04	Sulfur
5/5/04	Sulfur, Pyganic & Surround
5/13/04	Sulfur, Pyganic & Surround
5/24/04	Sulfur, Pyganic & Surround
6/7/04	Pyganic, Sulfur, Surround, Entrust
6/17/04	Pyganic, Sulfur, Surround, Entrust
6/24/04	Fertilize McGeary 5-3-4
7/15/04	Pyganic, Sulfur, Surround, Entrust
7/22/04	Pyganic, Sulfur, Surround, Entrust

cont.

CONVENTIONAL MATERIALS 2004

4/10/04	Warrior, Dithane, Rubign
4/20/04	Solican & Geamoxone
4/30/04	Dithane & Topsin
5/5/04	Mancozeb & Imidan
5/13/04	Mancozeb, Imidan & Topsin
6/7/04	Imidan, Thiodon, Captec, Topsin
6/28/04	Imidan, Nova, Provado, Captec
7/15/04	Imidan, Nova, Provado, Captec
7/22/04	Imidan, Captec, Topsin, Vydate
8/4/04	Roundup
8/5/04	Imidan, Captec, Topsin, Thiodon
8/20/04	Captec, Topsin & Sevin

ORGANIC

CONVENTIONAL

AUGUST 25TH HARVEST OF ATAGO PEARS-16 months after planting orchard.

Preliminary Greenhouse Study, 2004

Purpose- To develop a protocol for testing applications of coliform bacteria under controlled conditions.

March. Plant 50 apple trees in container nursery in ‘soilless’ mix.

June. FSIS clearance sought to use GFP and K12 strains under controlled conditions.

Preliminary Greenhouse Study, 2004

July and August. Greenhouse background/safety tests. Swab for coliforms 4 separate occasions. Low background level found in greenhouse. Log 2-log 3 levels in floor drains.

August. Move container grown trees into greenhouse and begin shoot bioassays.

FACTORIAL STUDY OF K12 SURVIVAL EFFECT OF ALUMINUM FOIL

Preliminary Greenhouse Study, 2004

September. Controlled inoculations using K12 suspended in solution with non-ionic surfactant allow uniform wetting, and controlled treatments.

A factorial testing incubation temperature (22 vs 37) and humidity demonstrated that aluminum foil covers over beakers held at 22 C allowed recovery of inoculated K12 for 7 to 10 days.

Research to be completed in early-2005

- Compare counts in ‘cider’ made from Unsprayed and Sprayed trees at Keedysville orchard
- Compare bacterial counts in ‘cider’ made from organic and conventional apples and pears from Wye
- Test the effects of organic pesticides survival of K12 and/or GFP on greenhouse-grown shoots in the laboratory


CAUTION