Objectives

- Food safety outbreaks linked to fresh produce
- Human pathogens linked to fresh produce
- Sources of contamination
- Interaction of human pathogens with growing plants
- Current and future initiatives

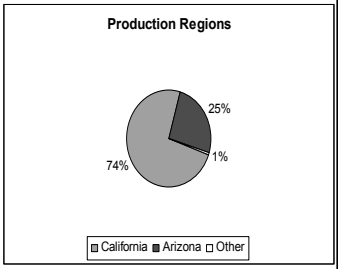
Fresh Produce Market

- Ready to eat Salads Market Growing at 10% per Year
- 6 Million Bags of fresh cut produce sold daily
- Current Market Value >US$70bn
- Greater Diversity of Produce Available (All Year Round)
- Centralized Production

Fresh Produce Industry

United States

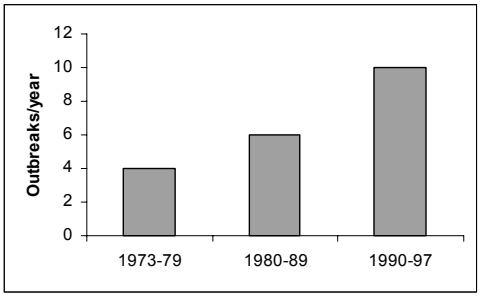
	Million $
Leafy Greens	2140
Tomatoes	1637
Potato	1247
Onions	905



Ontario

	Million $
Lettuce	9.91
Spinach	28.00
Sprouts	4.0
Tomatoes	245.45

Outbreaks Associated with Salad Vegetables



Source: Centre for Disease Control & Prevention, USA

Vehicle Categories 1996 - 2005

Category	Outbreaks	Illnesses
Processed	43	3,026
Produce	63	8,040
Sprouts	25	1,565
Seafood	120	2,567
Eggs	234	6,572

2006 *Annus horribilis* (North America)

- Sprouts (Ontario) Feb Suspected *Salmonella*
- Sprouts (Australia) Feb *Salmonella* 100 cases
- Lettuce June *E. coli* O121:H19 4 cases
- Sprouts Aug Suspected *Salmonella*
- Spinach Sept *E. coli* O157:H7 202 cases
- Carrot Juice Sept *Cl. botulinum* 6 cases
- Lettuce (Ontario) Oct *E. coli* O157:H7 30 cases
- Lettuce Oct 8, 500 carton recall due to suspected *E. coli*

- Tomatoes Oct- Nov *Salmonella* 400 cases
- Lettuce Nov- Dec *E. coli* O157:H7 132 cases
- Strawberries Nov Suspected *L. monocytogenes*
- Cantaloupes Dec Suspected *Salmonella*
- Spinach (Ontario) Dec Suspected *Salmonella*

1998-2006* Produce Outbreaks by Commodity

Tomatoes	11	Green onions	3
Cantaloupe	7	Mango	2
Melons	1	Almonds	2
Honeydew melon	2	Parsley	1
Raspberries	5	Basil	4
Romaine lettuce	4	Green grapes	1
Lettuce	10	Snow Peas	1
Mixed lettuce	1	Basil or Mesclun	2
Cabbage	1	Squash	1
Spinach	1	Unknown	3

*as of August 7, 2006

1998-2006 Produce Outbreaks

- **5 commodity groups make up >75 percent of produce related outbreaks**

Commodity	% produce outbreaks
Lettuce/leafy greens	30%
Tomatoes	17%
Cantaloupe	13%
Herbs (Basil, parsley)	11%
Green onions	5%
Total % of 5 top commodities	76%

Why the increase in Foodborne illness cases & Recalls

- Larger volume of product
- Increased awareness of food safety
- Better detection and investigation
- Lack of effective initiatives

FDA: 2 letters to California growers

Lack of urgency

Lack of understanding of human Pathogen:Produce interactions

Human Pathogens Linked to Produce

Escherichia coli O157:H7
Lettuce, Spinach, Sprouts

Salmonella
Tomatoes, Lettuce, Cantaloupe, Sprouts, Mangoes, Almonds

Shigella sonnei
Parsley, Lettuce, Green onions

Listeria monocytogenes
Cabbage

Cyclospora
Basil, Raspberries

Hepatitis A
Green onions, soft fruit

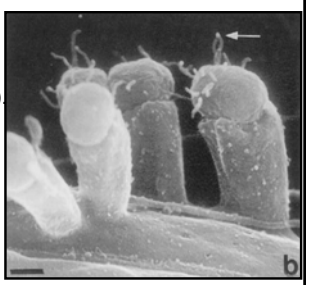
Why Pathogens Linked to Certain Produce?

- Unknown
- Pathogens within the environment?
- Pathogens adapted to produce?

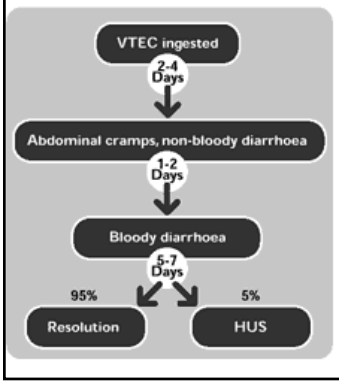
Human Pathogens Linked to Fresh Produce

Escherichia coli O157:H7

- Gram negative rod.
- Facultative anaerobic
- Temp 10 – 50°C (opt 37°C).
- Min a_W 0.93
- Remain viable at low pH especially at low temps.

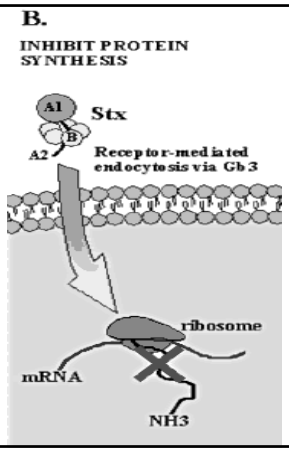


Symptoms



Low infective dose
10 – 100 cells

Heamolytic Uremic Syndrome



Sources of *Escherichia coli* O157:H7

- Manure from cattle and other ruminants
- Farm effluent
- Person-to-Person contact
- Wild animals

Shigella

Shigella dysenteriae
Shigella sonnei

Fecal-Oral route or via contaminated foods

Very low tolerance to environmental stress and typically only recovered in GI tract or fresh sewage

- Similar symptoms to EHEC
- Very low dose required (10 – 100 cells)
- Typically pathogen transferred directly from fecal material or food handlers

Salmonella

- Gram negative non sporulating rod
- Facultative anaerobic
- Temp for growth 5 46˚C (opt 35 37C).
- Min pH 4.5
- Min a_W 0.97

Taxonomy of *Salmonella*

- *Salmonella* group composed of very closely related serovars.
- Only two species *Salmonella enteritica* and *Salmonella bongori*
- Serotyping (antibody reaction) used to differentiate types.

Most Frequent Serotypes Associated with Food

- *S.* Typhimurium
- *S.* Enteritidis
- *S.* Dublin

Salmonella Typhimurium DT 104

- Why is DT 104 of concern ?
  - Multiple antibiotic resistance
    - Ampicillin
    - Chloramphenicol
    - Streptomycin
    - Sulfonamides
    - Tetracycline

Symptoms

- Typhoid or paratyphoid fever from *S. typhi* and *S. paratythi*

Salmonellosis

- Headache
- Fever
- Diarrhea
- Nausea
- Vomiting

- Infective dose 10^2 – 10^6
- Invasion of small intestine and colon by entering absorptive mucosal cells and mucosa associated macrophages.
- Grow inside fixed macrophages of liver and spleen.

Secondary Complications

- Arthritis
- Ms Reynard
- *Salmonella* from Chinese meal
- $2.5m Payout

Sources of *Salmonella*

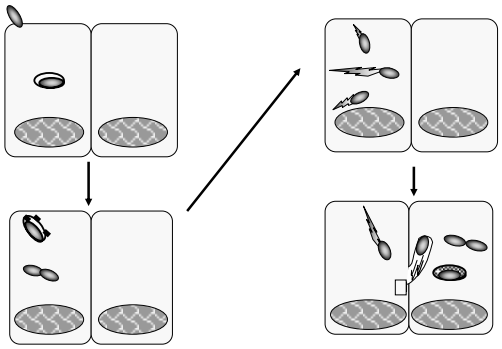
- Poultry and pig manure
- Sewage
- Wild animals
- Insects

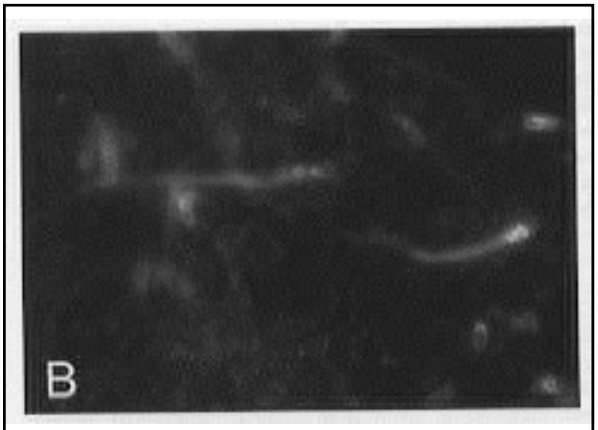
Listeria monocytogenes

- Gram positive non spore forming rod
- Facultative anaerobe
- Catalase positive
- Oxidase negative
- heamolytic

- Psychrotrophic
- Growth range 1 - 44˚C
- Opt temp 35-37˚C
- pH 5.0 – 9.6
  (opt 6 – 8) Survives at pH 4
- Min a_W 0.93
- Can survive in 25-30% NaCl solutions

Listeria Infection Process





Illness

Healthy individuals: Mild flu

High risk groups (young, pregnant, old, immuno-compromised:
Stillbirth or abortion
Meningitis
Septicemia
Pneumonia

- Infective dose for high risk groups 10^9
- 30% mortality rate
- Incubation period 1-4 weeks
- Illness can last 1-90 days

Sources of *L. monocytogenes*

- Decaying plant material
- Manure
- Drains
- Endemic within processing facilities

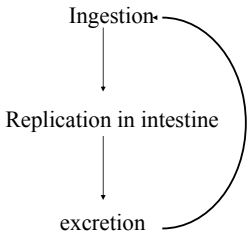
Human Parasites

- Protozoan
- Viruses

The majority require human host to replicate.

Infected handlers or human sewage

Route of infection-Faecal oral



Ingested orally by new host, from food or water

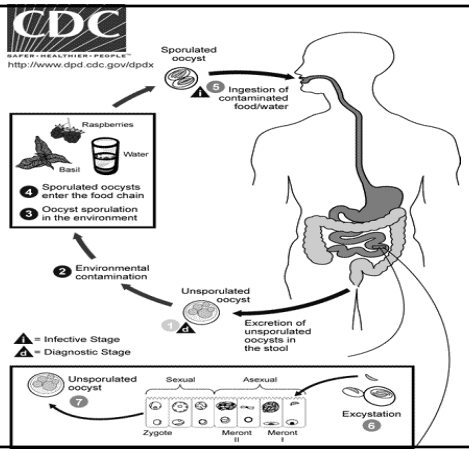
or

transferred to food via ill worker (carrier)

Infectious dose is normally low 10 - 100

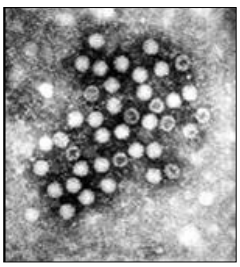
Cyclospora

- Increasing number of cases in Ontario
- Mexican basil
- Fecal contamination
- Person to Person less significant



Viral Hepatitis: associated virus
Hepatitis A and E

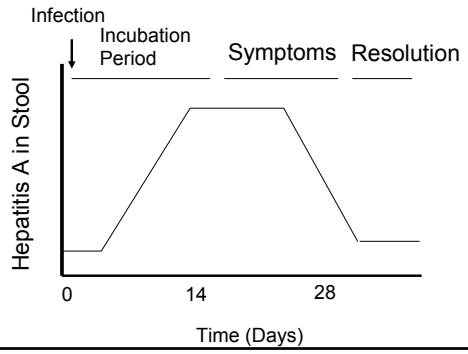
Most commonly associated with foods
•Jaundice
•Liver damage
•Abdominal discomfort
•Fever



Viral Hepatitis: associated virus
Hepatitis A

- Hepatitis A is usually a mild illness characterized by sudden onset of fever, malaise, nausea,anorexia, and abdominal discomfort, followed in several days by jaundice.
- Hepatitis A represents about 1/3 of all cases of viral hepatitis.

Infected persons can pass Hepatitis A without showing symptoms



- Transmitted via person-to person contact, water and through food (fruit a specific problem).
- 150, 000 cases a year in United States.
- In developing countries incidence in indigenous population is low, due to “childhood vaccination”, Vaccine is recommended for travellers.

Sources of Hepatitis A

- Infected food handlers
- Water contaminated with human sewage

Sources of Contamination

- Farm

Irrigation water
Run-off from effluent lagoons
Manure
Workers
Wild animals
Insects

Survival of Pathogens in Manure

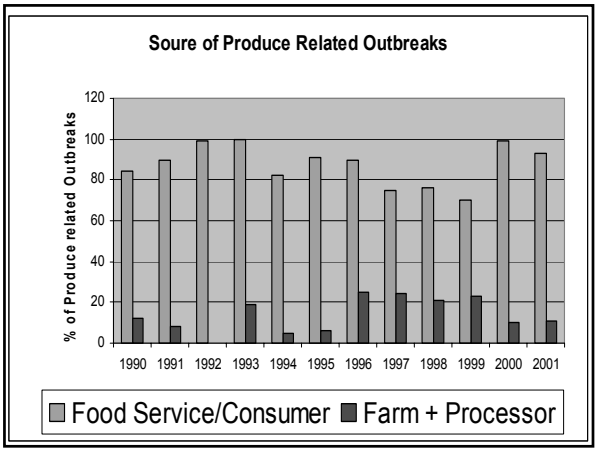
- *E. coli* O157:H7 >100 days
- *Salmonella* >200 days

- Processing Facility

Water
Food handlers
Processing environment

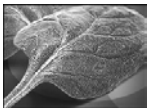
- User interface

Cross-contamination (cutting boards)
Handling
Temperature abuse



Interaction of Human Pathogens with Produce

Spinach *E. coli* O157



- Salinas Valley
- 26 States and within Canada
- 199 confirmed cases
- 3 Deaths

>$100m in loss sales and production

- Previous outbreak in California 2003

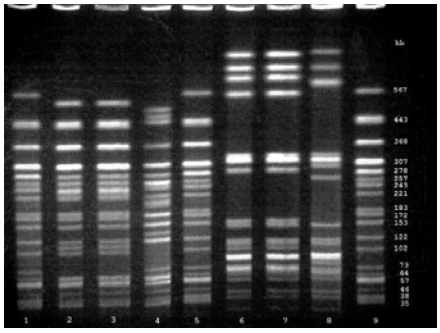
16 cases (2 deaths)

Rapid Response

- Traceability
- Molecular typing

Rapid connection between cases and trace back

PFGE Banding Pattern

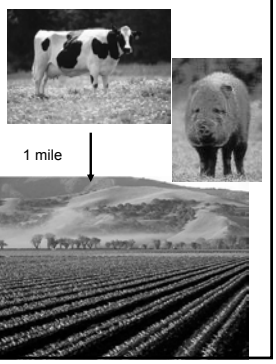


Salinas Valley CA

What they think happened

E. coli O157:H7 genotype

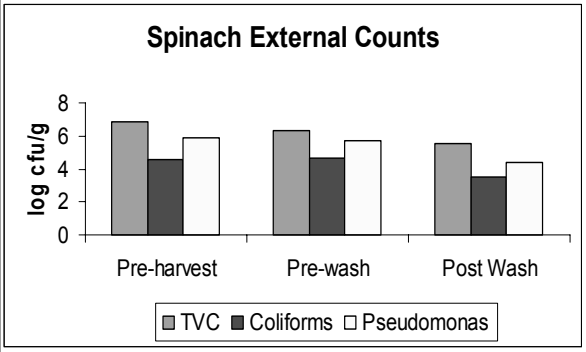
- Infected persons
- Product
- Processing facility
- Cattle ranch near spinach field.



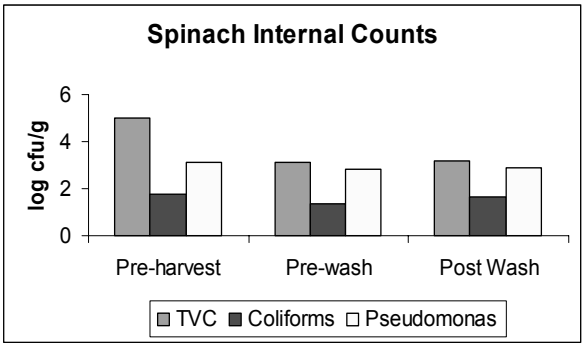
Pre-Washed Spinach

How Effective is Washing?
Does Triple Wash Mean Anything?

Commercial Post-Harvest Washing

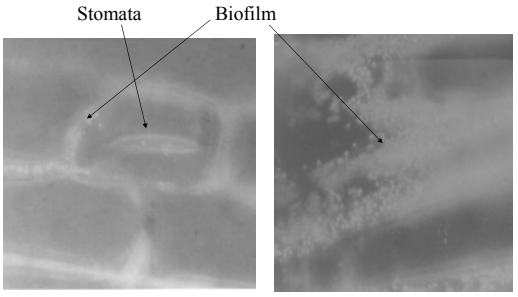


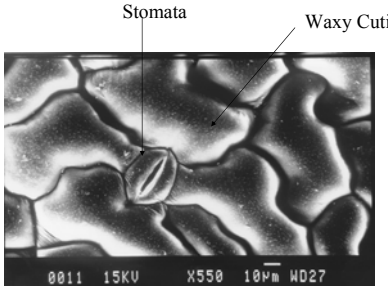
Commercial Post-Harvest Washing



Post-Harvest Washing

- Cross-contamination as opposed to decontamination.
- Hypochlorite rapidly sequestered
- Internalized populations protected
- Biofilms

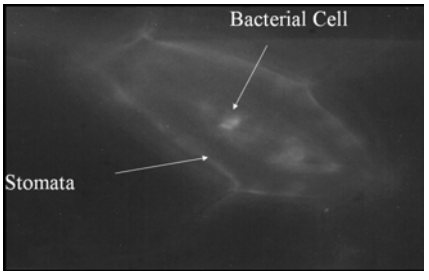




Upper Side of washed Spinach Leaf

Location of Bacteria Surviving Biocidal Washing

•BacLight Nucleic Acid Staining



Human Pathogens as Endophytes

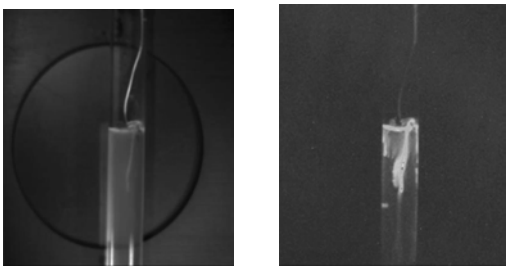
- Could human pathogens become integrated into plant endophytic microflora?
- Protected against UV and desiccation in the field
- Protected against post-harvest biocidal washing

Interaction of *E. coli* with Growing Spinach Plants

Potential routes:

- Seed
- Growth Matrix (soil/hydroponic solution)

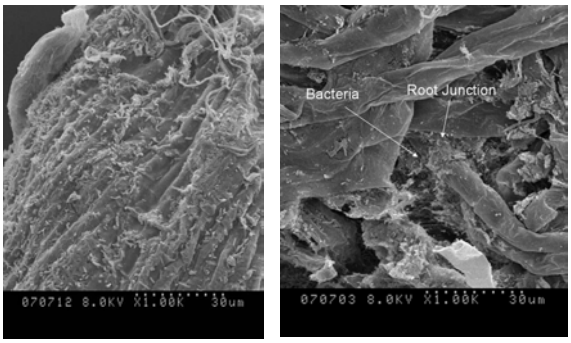
Bioluminescent *E. coli*

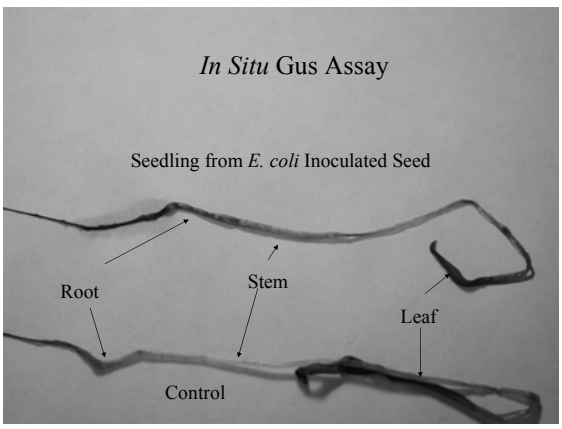


Light Dark

Day 15

E. coli on Spinach Roots



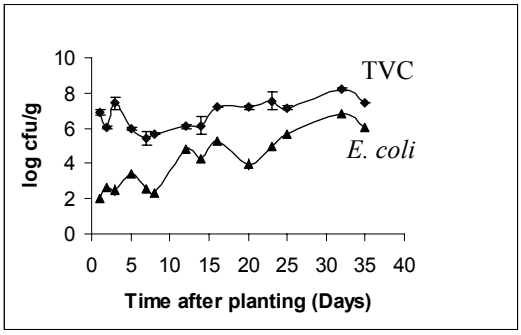


Spinach Plants	TAC Log cfu/g		*E. coli* O157 Log cfu/g	
	Surface	Internal	Surface	Internal
Day 9	5.97	2.31	5.91	2.46
Day 49	5.49	2.36	5.51	ND

Conclusions from Inoculated Seeds

- Internalization of *E. coli* during early stages of germination
- *E. coli* established on/within roots and surface of leaves in mature plants

Inoculated Soil



Counts on Spinach

Log cfu/g

Days after Planting	TVC		*E. coli*	
	Wash	Extract	Wash	Extract
12	6.3	6.0	3.4	2.2
14	5.4	5.7	2.2	ND
16	6.6	3.9	6.0	ND
20	5.9	4.7	3.2	ND
23	6.4	5.2	4.7	ND
25	6.8	5.1	4.7	ND
32	6.4	4.3	5.2	2.1*
35	7.5	3.9	6.3	2.9*

1 plant positive from a batch of three tested

ND: < 1 log cfu/g

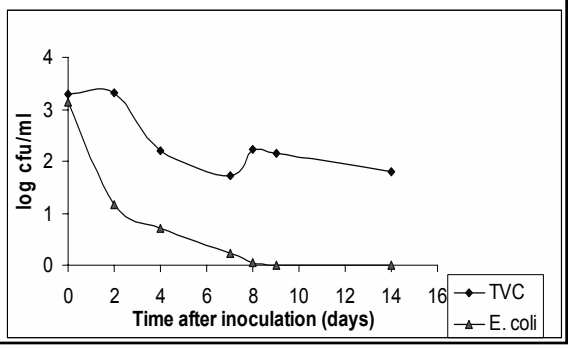
Conclusions from Contaminated Soil

- *E. coli* proliferates in soil over the duration of plant cultivation.
- *E. coli* becomes established internally/ externally on roots. Surface of leaves.
- Low level of internalization in mature plants

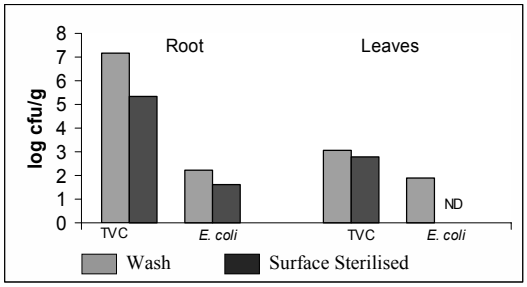
Spinach Cultivation

- Nutrient Film Technique (NFT) Hydroponic System
- Soil- free cultivation
- Safer?

E. coli (10^3 cfu/ml) Inoculated into Nutrient Solution

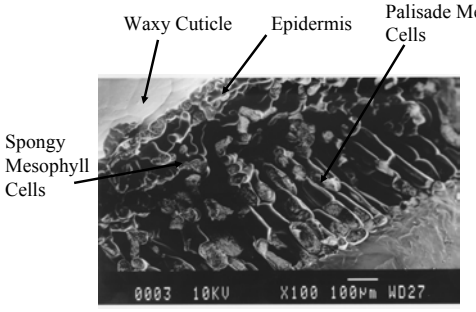


Bacterial Counts From Spinach Plants



ND: <1 log cfu/g

Vacuum Cooling



Cross Section of Unwashed Leaf

Can pathogens be internalized into Spinach?

- Yes, in seedlings but not in mature plants.
- Pathogens can find entry via natural openings and cut edges
- Internalization only part of the problem
- Is vacuum cooling safe?

Foodborne Illness Outbreaks Linked to Tomatoes

***Salmonella* serotype**	**Year**	**Total cases**	**Deaths**
Javiana	1990	174	0
Montevideo	1993	84	0
Baildon	1998	85	3
Javiana	2002	141	0
Newport	2002	297	0
Beranderup Javiana	2004	561	0
Typhimurium	2006	181	0

Inoculate Flowers of Growing Plants

0.1 ml 7 log cfu/ml

Screen for *Salmonella* External and Internal

Variety: Abigail VFFT

Serovar	Total Batches Tested	Surface (% Positive)	Internal (% Positive)
Javiana 5913	15	8 (53%)	4 (26%)
Javiana 6027	15	14 (93%)	6 (40%)
Montevideo	10	4 (40%)	9 (90%)
Newport	9	7 (78%)	4 (44%)
Enteritidis	9	3 (33%)	1 (11%)
Senftenberg	10	5 (50%)	0
Typhimurium	8	3 (38%)	0
Hadar	9	7 (78%)	5 (56%)
Infantis	11	4 (36%)	1 (1%)
Dublin	9	7 (78%)	2 (22%)

Survey of Tomato Growing Regions in Florida and Mexico

- *Salmonella* Montevideo
- *Salmonella* Javiana

Soil
Water
Packing plant
Feces of wild animals

Human pathogens adapted to environments outside the host?

Sprouted Seeds

- Ontario 2005
  > 600 cases of salmonellosis linked to mung bean sprouts

34 outbreaks linked to alfalfa and other sprouted seeds since 1990

Contaminated seed implicated in majority of cases

Sakai City, Japan, in 1996

Radish sprouts contaminated with *E. coli* O157:H7.

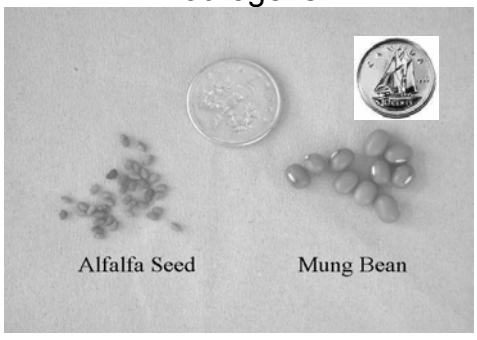
>6000 cases 13 deaths

Further 4000 cases reported in other cities

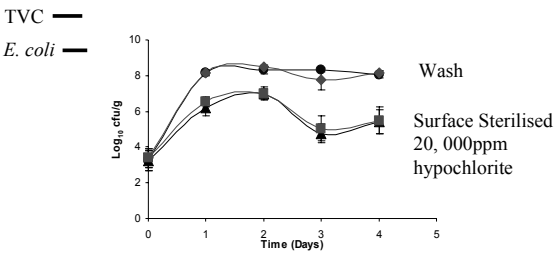
Sprout Outbreaks 1996-2004

Year	Alfalfa	Clover	Mung Bean	Cases
1996	1	1		650
1997	3	1		277
1998	3	1		48
1999	5	2		389
2000	——	——	1	75
2001	1		2	88
2002	1		1	21
2003	5			52
2004	2			33
Total: 27 Outbreaks			1633 Cases	

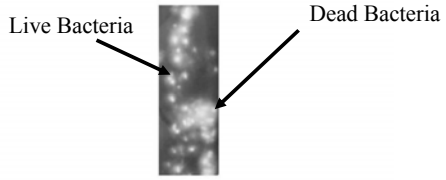
Seeds are Main Source of Pathogens

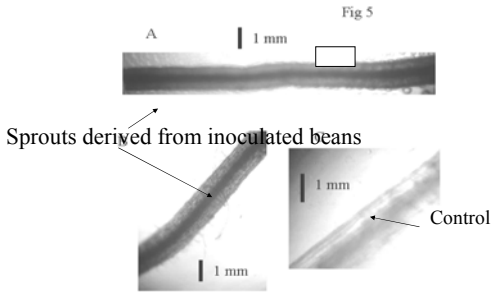


Growth of *E. coli* during mung bean sprouting



Apoplastic Fluid from Surface Sterilised Bean Sprouts





GUS Assay

Seed Decontamination

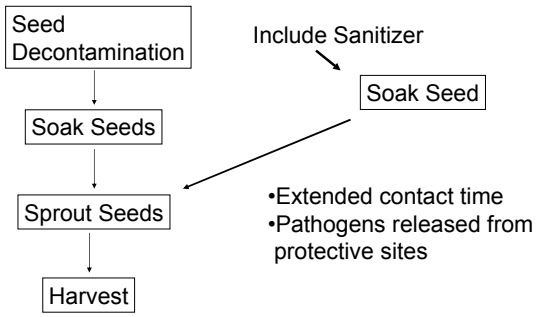
- Calcium hypochlorite
- Acidified sodium chlorite
- Peroxyacetic acid + hydrogen peroxide
- Hot water pasteurization
- Heat treatment
- Irradiation
- Calcinated Calcium
- Organic acids

All have failed to successfully decontaminate seeds without adversely affecting seed germination.

Why are seeds so difficult to decontaminate?

- Protective sites on the seed coat
- Low number of survivors (<1 MPN/g) can proliferate during the first 24h of sprouting.
- Need to preserve seed viability

Different Approach



Calcium Hypochlorite (20, 000ppm) Vs Germin-8-or (200ppm)

Treatment of mung beans	*E. coli* O157:H7		*Salmonella*	
	Count Log cfu/g	Enrichment	Count Log cfu/g	Enrichment
Calcium hypochlorite (20, 000ppm, 20mins)	8.59	NT	7.96	NT
Germin-8-or (200ppm)	ND	ND	ND	ND

Initial loading: 3-4 log cfu/g ND <1 cfu/25g

Botulism Carrot Juice

- 6 cases (4 USA; 2 Toronto)
- 28 cases per year
- Home canning
- Vegetables in oil

Clostridium botulinum

- Gram positive anaerobic rod.
- Forms resistant endospores
- Temperature range:
- Proteolytic 10-48˚C (opt 37˚C)
- Non-proteolytic 3.3-45˚C (opt 30˚C)
- aw min: 0.93
- pH min: 4.6

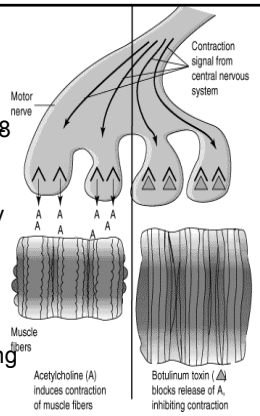
- Loss of muscle function
- Incubation period: 18 36 hours
- Symptoms:

Loss of muscle activity

Weakness in eye muscle

Slurred speech

Difficult swallowing, breathing and moving limbs.



Possible Sequence of Events

Botulism spores in soil

Pasteurization:

- Inactivated natural anti- microbial constituents
- Reduction in competitive microflora
- Activation of spores
- Outgrowth of *Cl. botulinum* with product held at elevated temperatures.

Future Directions

Lettuce Safety Initiative (Aug 2006)

- Review current procedures
- Rapid response to outbreaks
- Documentation (on-farm HACCP)
- Introduce regulations

Post-harvest Intervention

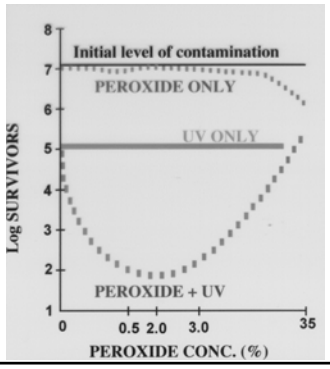
- Can contamination in the field be prevented? No
- Is testing the answer? Yes, but cannot provide total assurance.
- Washing is ineffective

Decontamination of Fresh Produce

- Pre Wash: Potable water to remove visible soil

Biocidal wash
- 200ppm sodium hypochlorite
- Organic acids
- Peroxyacetic acid
- Acidified Sodium Chlorite
- Ozonated water

Synergistic Action of UV and Hydrogen Peroxide

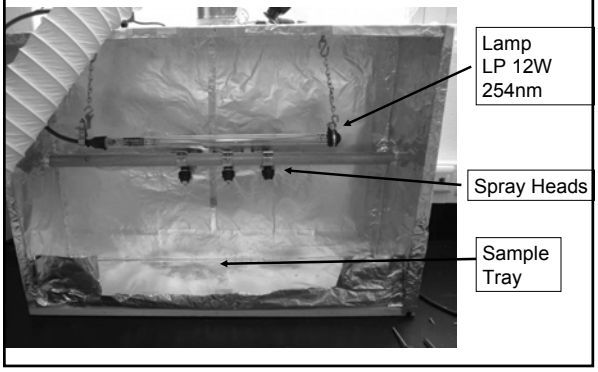


Inactivation of Different Bacteria on Agar Plates

Bacterium	Log Count Reduction			
	UV_{254}	UV_{254} 1% H_2O_2	UV_{254} 1.5% H_2O_2	UV_{254} 2% H_2O_2
Pectobacterium carotovora	2.25	ND	ND	ND
Escherichia coli O157	2.83	4.20	ND	ND
Pseudomonas fluorescens	2.50	4.60	ND	ND
Salmonella Montevideo	0.52	4.97	4.75	4.84
Aeromonas hydrophila	2.32	ND	ND	ND
Listeria monocytogenes	ND	ND	ND	ND

Initial loading 6 log cfu
ND Not Detected

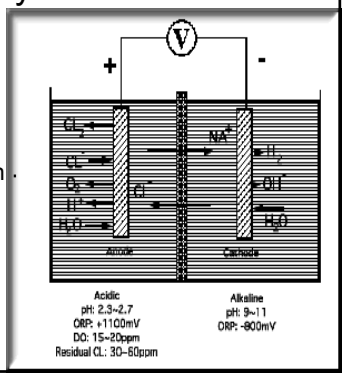
Prototype System



Spinach	Log Count Reductions	
	External	**Internal**
***Escherichia coli* O157:H7**		
UV:H_2O_2	4.75 ± 0.85	0.63 ± 0.15
Hypochlorite	0.46 ± 0.07	- 0.05 ± 0.01
Salmonella		
UV:H_2O_2	3.65 ± 0.12	0.89 ± 0.18
Hypochlorite	0.48 ± 0.08	- 0.34 ± 0.34

Electrolyzed Water

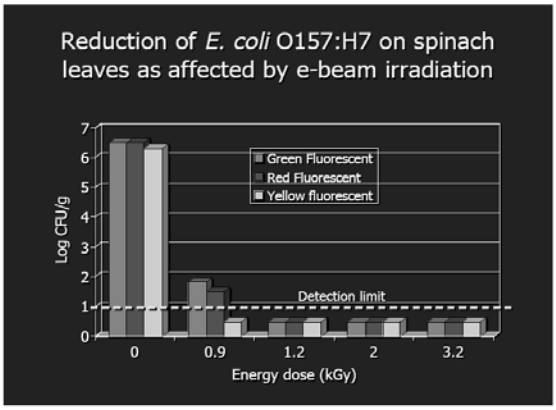
- Generates chlorous acid
- 2 log cfu reduction in lettuce inoculated with *Salmonella*



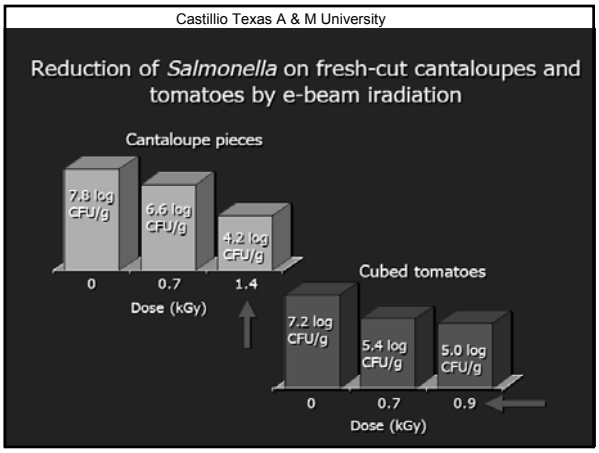
Irradiation

- Cobalt 60
- E beam
- Consumer acceptance?

Castillio Texas A & M University



Castillio Texas A & M University



Final Thoughts

- Fresh produce represents a continuing food safety issue.
- One outbreak is one too many
- Greater understanding on routes by which human pathogens enter and disseminate through produce chain
- Focus on interventions
- Regulation (Industry or Government led)?

The Next Few Teleclasses

February 21	***Infection Control in the Endoscopy Clinic*** … with Dr. Richard Everts, Nelson Marlborough Health Service
February 22	***Best Practice for Hospital Construction Management*** … with Andrew Streifel, University of Minnesota
March 6	***Tuberculosis in the Modern Age*** … faculty to be announced
March 8	***Voices of CHICA*** … with CHICA-Canada Board Members & Guests
March 22	***A Year of Cleaner, Safer Care – A Worldwide Experience*** … with Dr. Didier Pittet, World Health Organization, Geneva

For the full teleclass schedule – www.webbertraining.com
For registration information www.webbertraining.com/howtoc8.php