Human Pathogens on Plants:
Challenges and Approaches in Produce Safety

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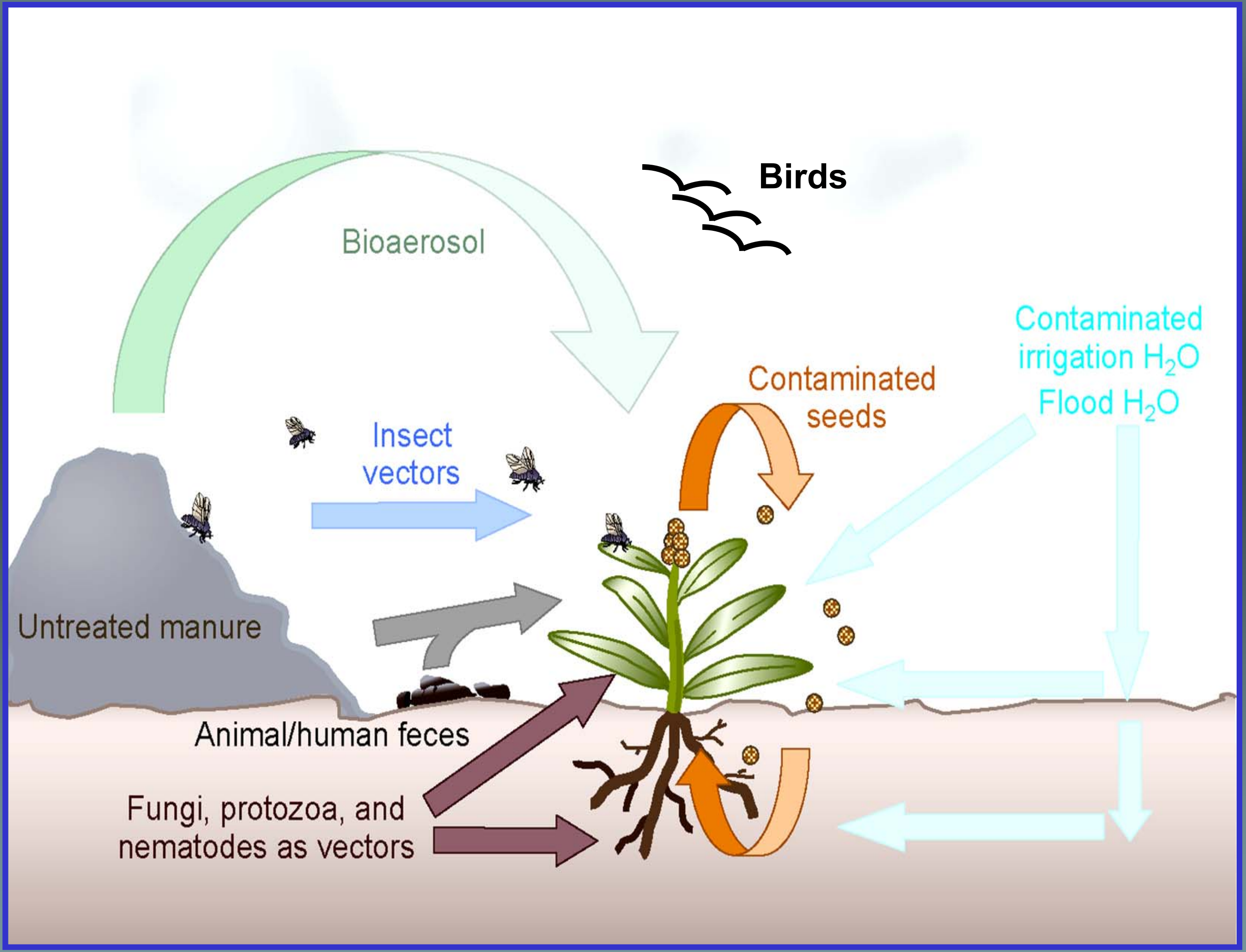
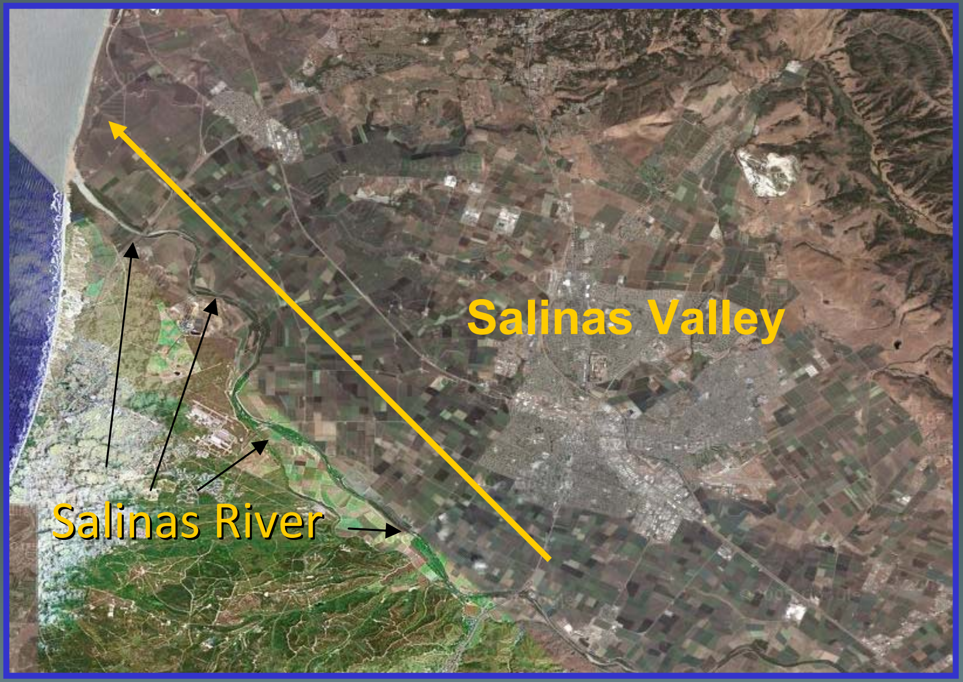
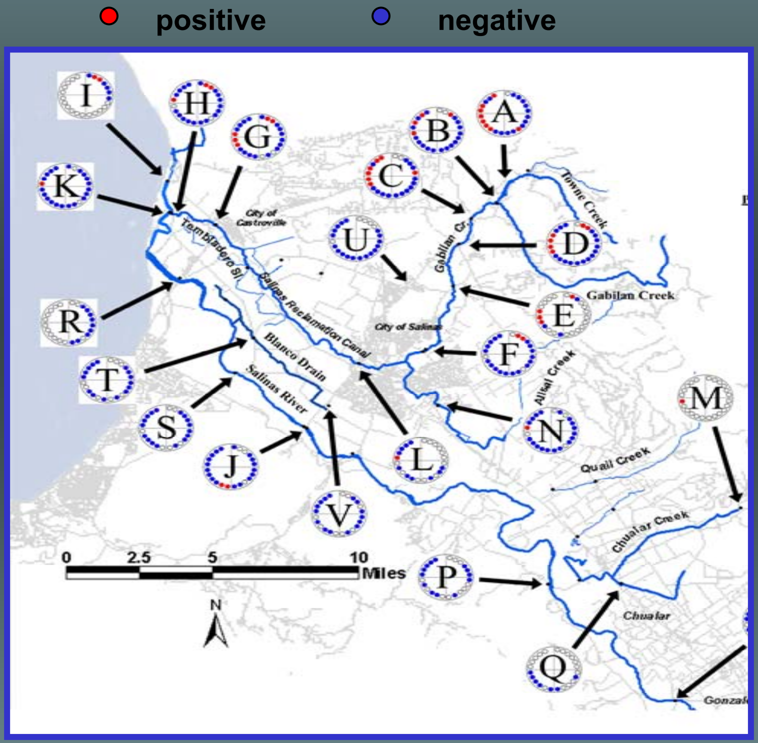
Potential sources of microbial contamination of crop plants



Brandl. Ann. Rev. Phytopath. 2006

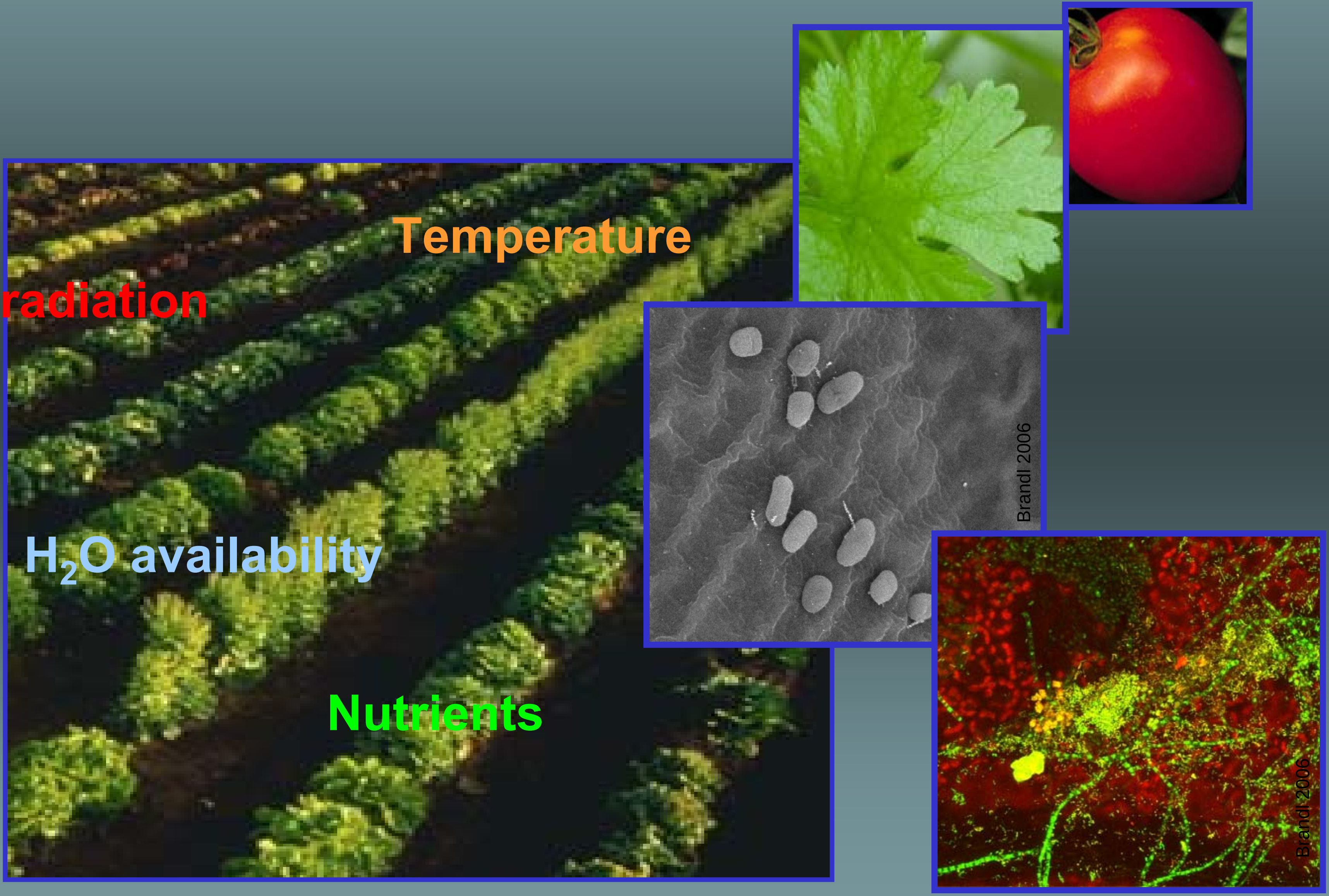
Salinas, California: source of several *Ec*O157:H7 epidemics linked to lettuce





Cooley *et al*. PLoS ONE, 2007

Bacteria in the plant environment



Temperature

UV radiation

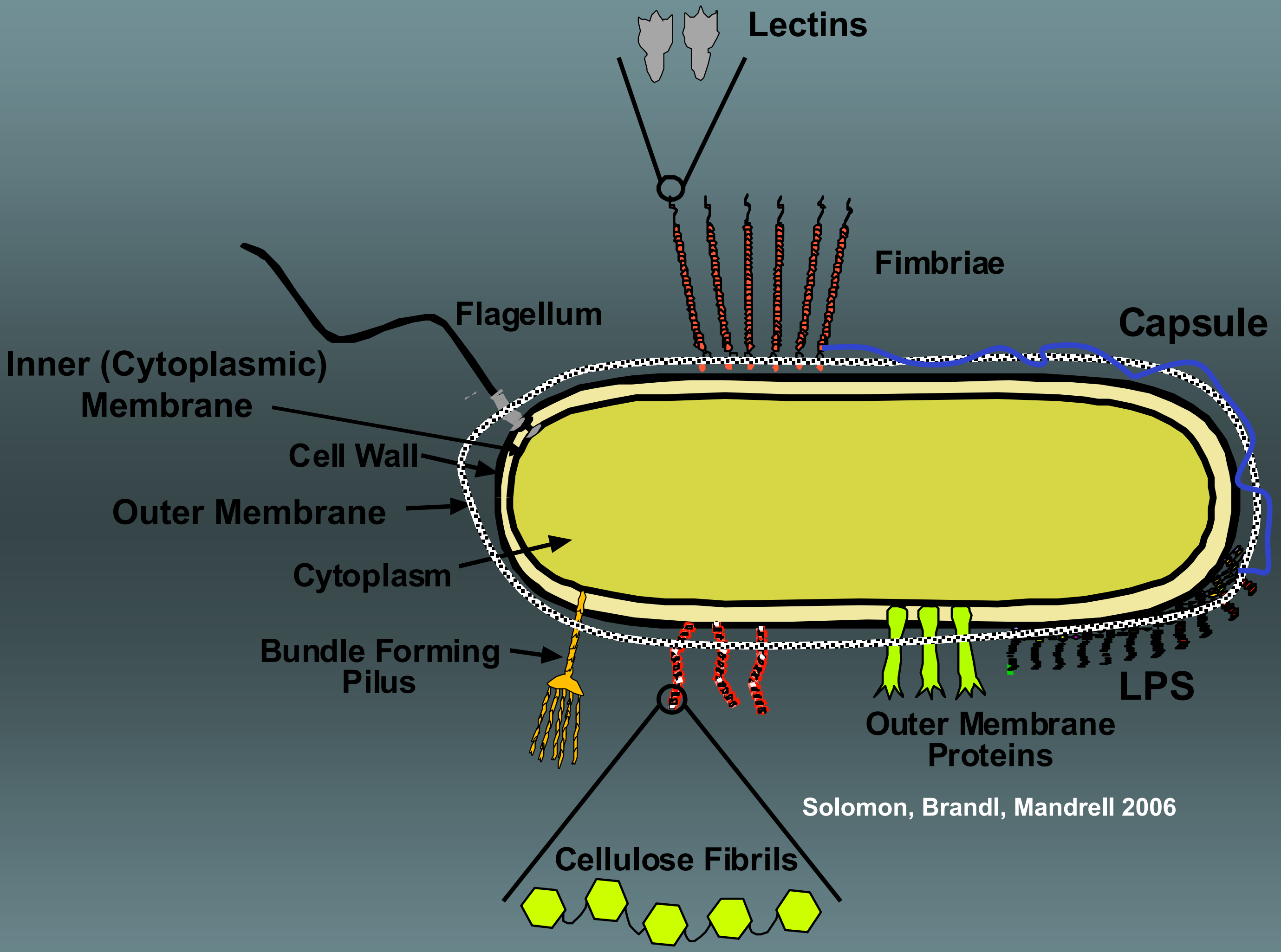
H_2O availability

Nutrients

Brandl 2006

Brandl 2006

Bacterial attachment to plants

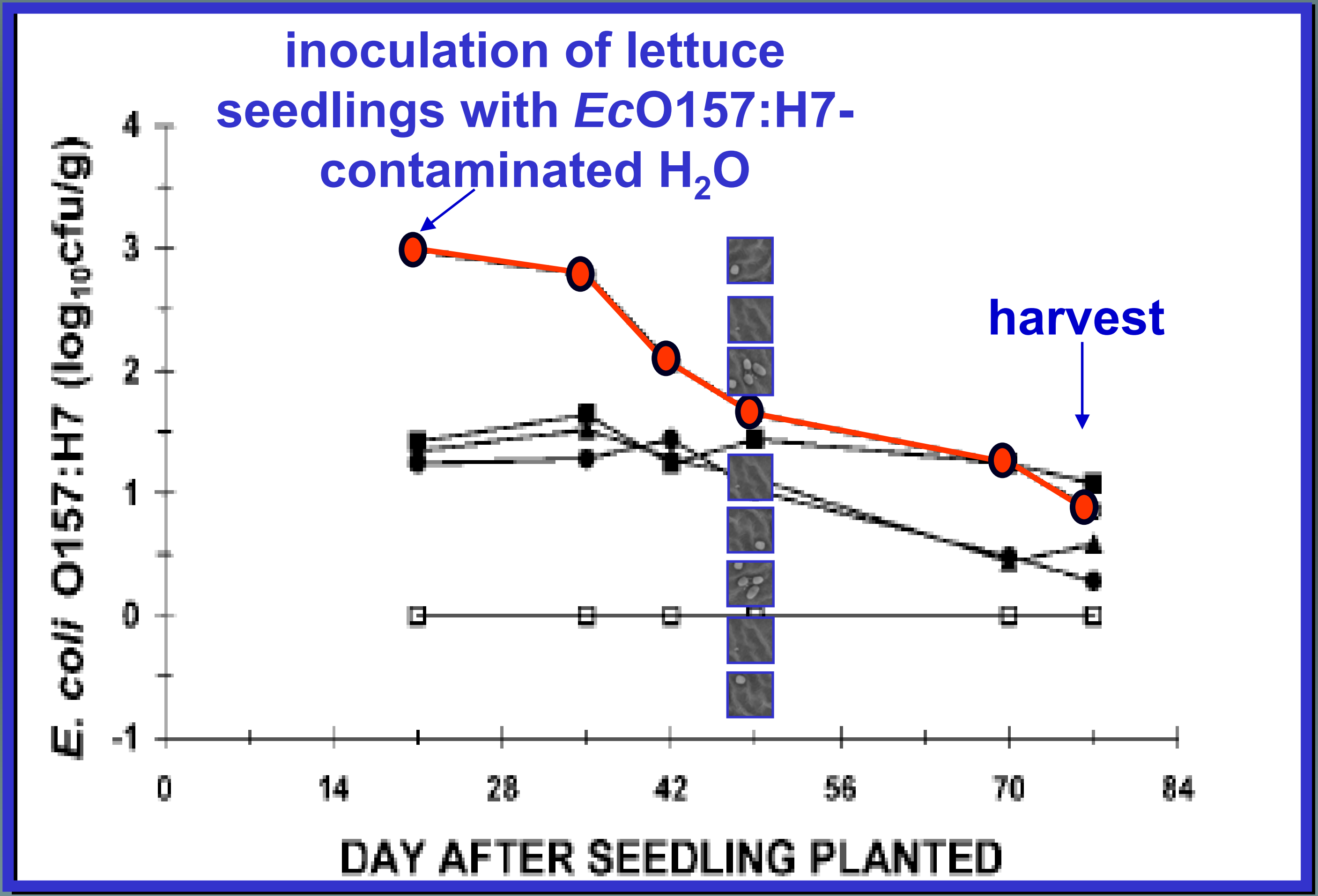


Solomon, Brandl, Mandrell 2006

Passive mechanisms:
Hydrophobicity
Surface charge

- ☐ Multifactorial
- ☐ Variations among pathogens, strains, and within a given strain
- ☐ Variations among plant species, cultivars, and plant parts

Human pathogens survive on plants in the field



inoculation of lettuce seedlings with *Ec*O157:H7-contaminated H_2O

harvest

Islam *et al*. 2004

Do enteric pathogens grow on plants?

water

- Irrigation
- Dew
- Rain
- Guttation fluids

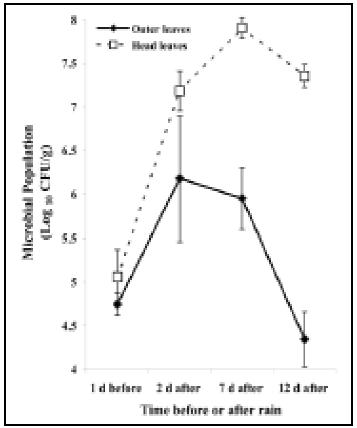


Figure 2—Microbial population in heads and outer leaves of iceberg lettuce after a rainfall event. Bars indicate standard deviation.

Fonseca 2006

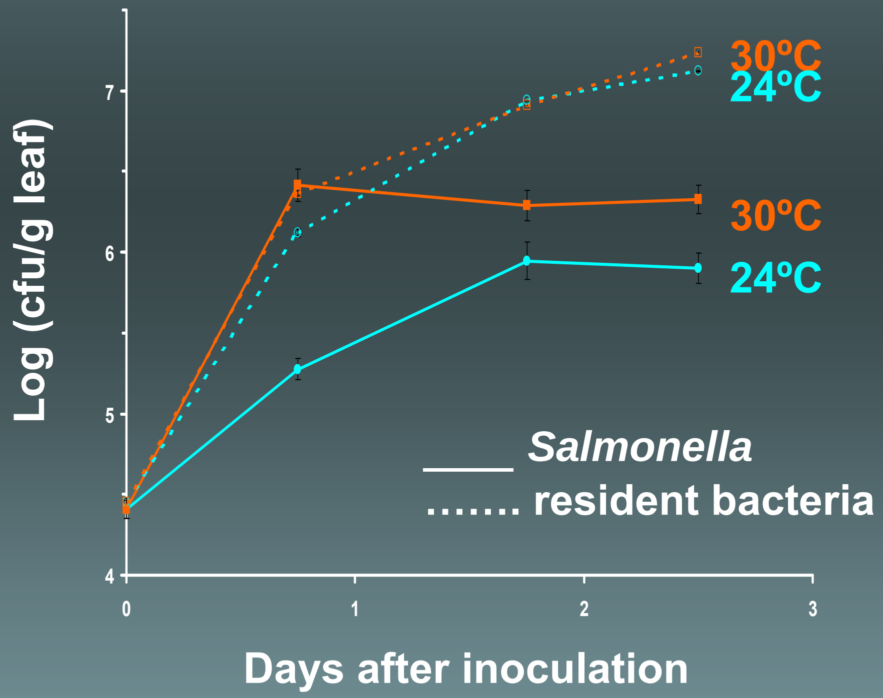
nutrients

- Plant surfaces are nutrient-poor, but oases of nutrients exist
- Availability affected by plant factors and microflora

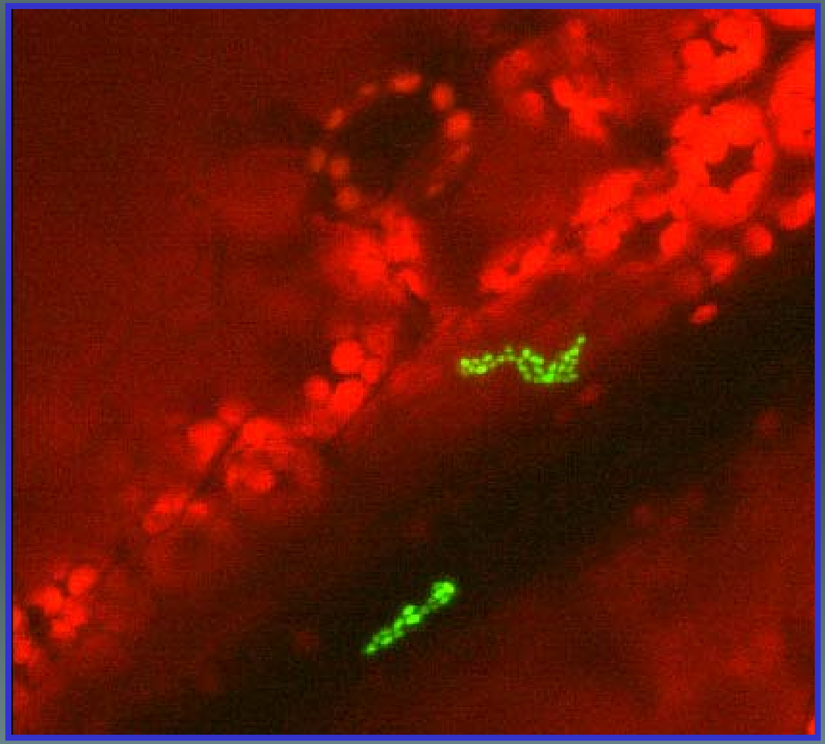
warm temperature

Role in seasonality of outbreaks?

Growth under warm and wet conditions

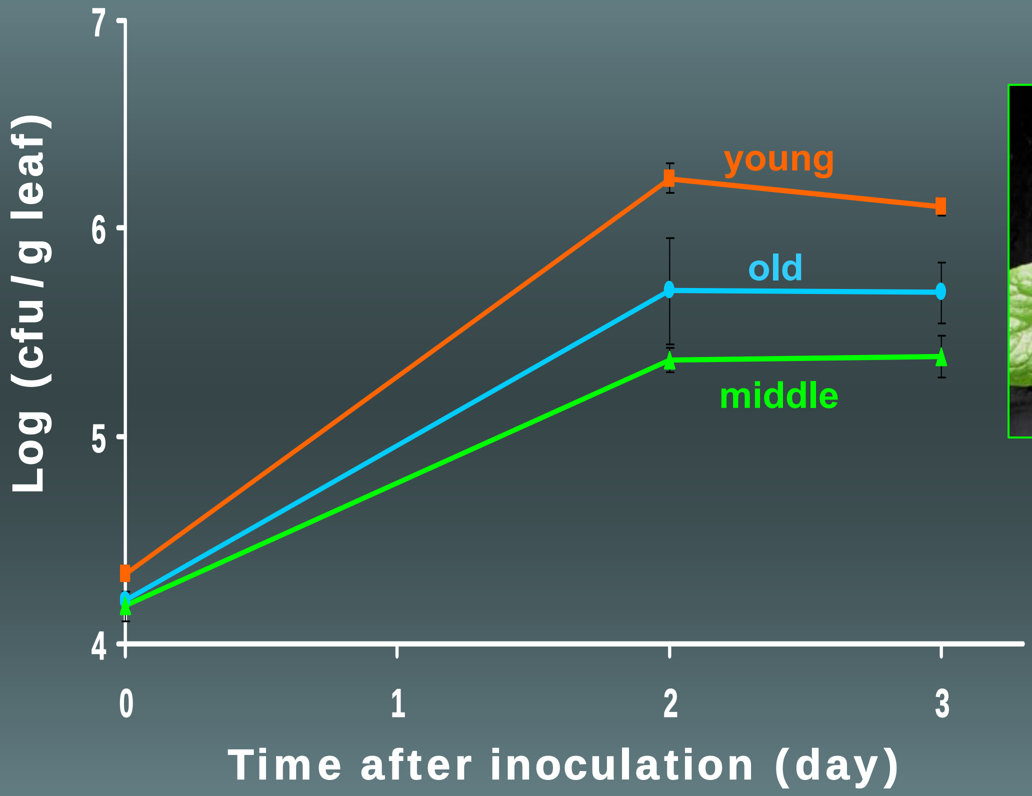


Salmonella on cilantro leaves



Brandl and Mandrell 2002

Effect of leaf age on colonization of Romaine lettuce by *E*cO157:H7

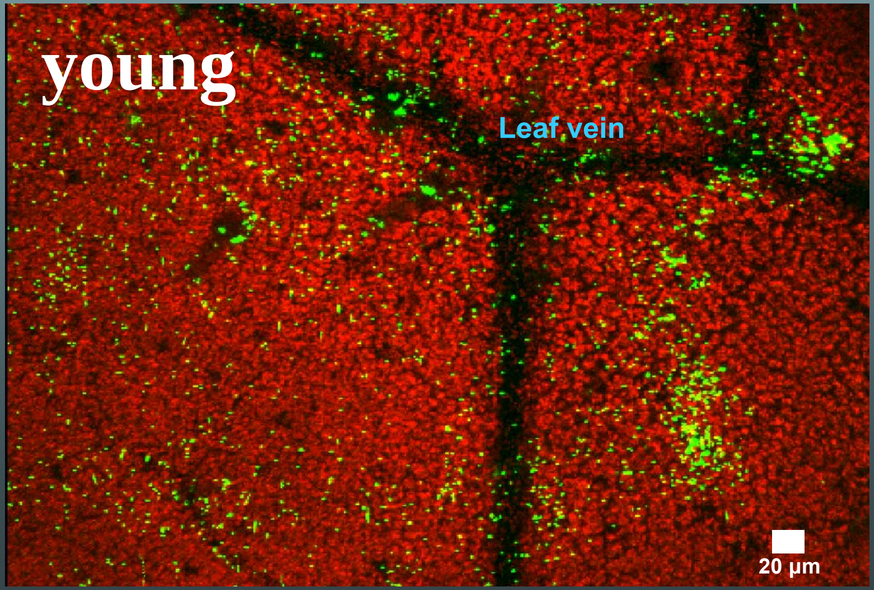


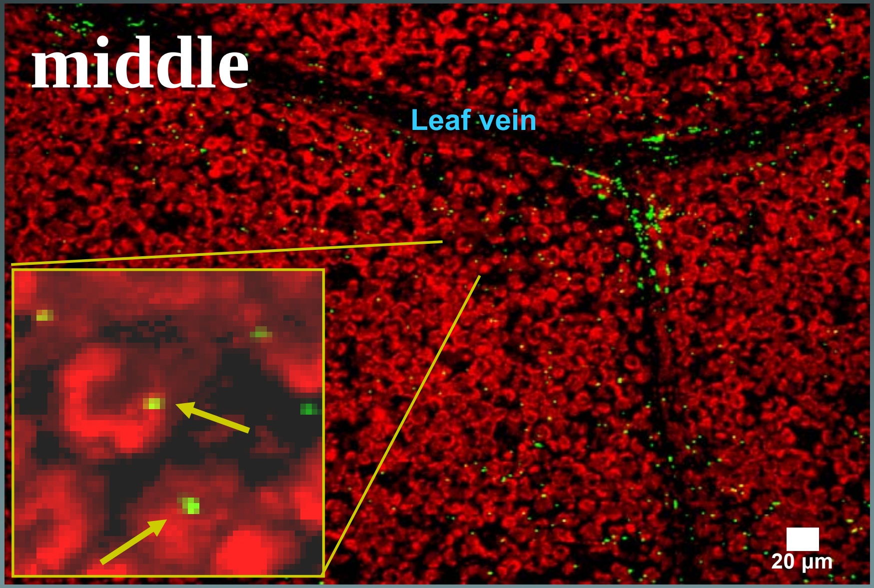
Pre- and post-harvest contamination:

Young (heart) leaves promote greatest pathogen populations sizes*

*Same trends observed with *Salmonella*

Brandl and Amundson 2008





Nitrogen availability is higher on the surface of young leaves

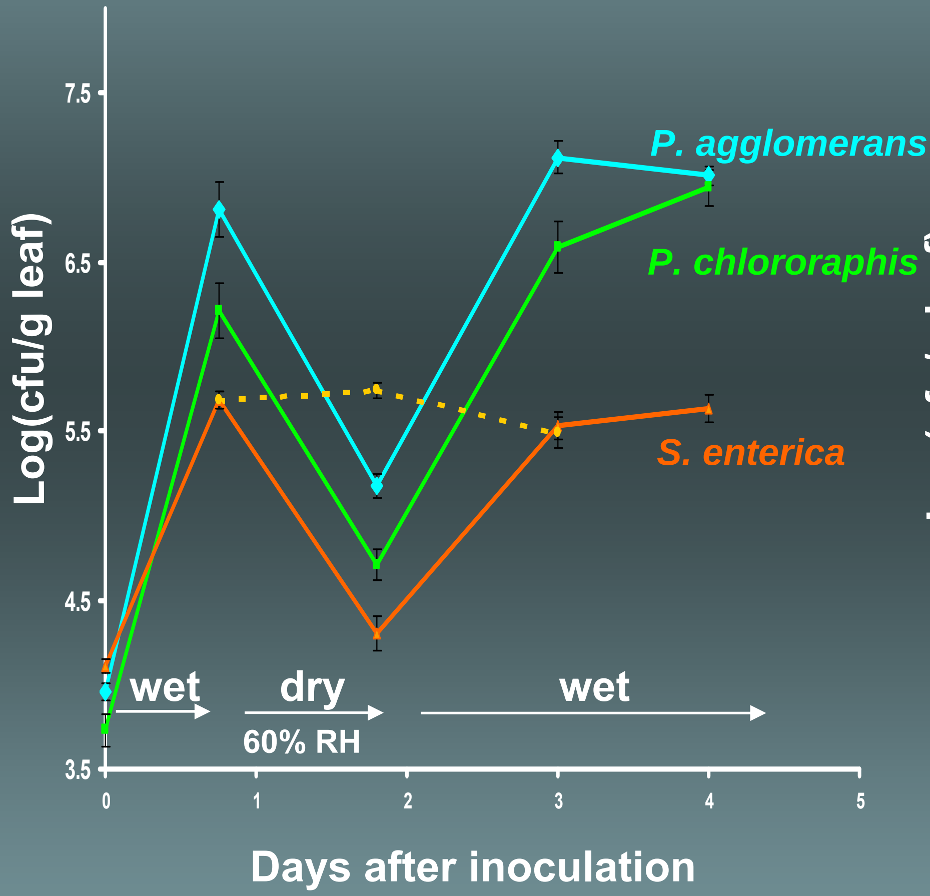
- ☐ Elemental analysis of leaf exudates
- ☐ *Ec*O157 growth is increased on middle leaves by addition of nitrogen to the inoculum, but not by addition of a carbohydrate

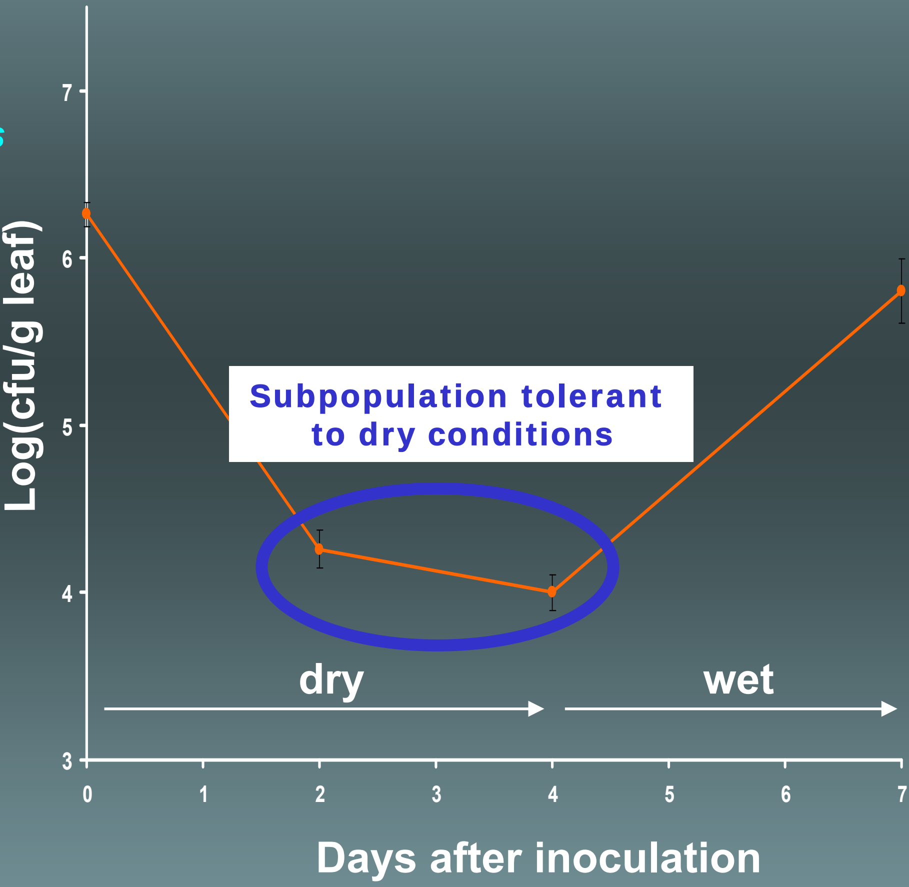
Elemental analysis of leaf surface washings

	N (µg/g leaf)	C (mg/g leaf)
Young leaf	71	2.8
Middle leaf	29	2.3

Brandl and Amundson 2008

Desiccation tolerance of *Salmonella* on leaves





Brandl and Mandrell 2002

Role of UV radiation

Total surface and internalized *Ec*O157 in lettuce leaves in the field

Log (CFU/g)

	Spray Treatment	Day 0	Day 14
Lower surface }	Abaxial, sunny	4.52	2.07
Upper surface }	Adaxial, sunny	6.04	0.10

Derived from Erickson *et al* 2010

Suslow spinach study with inoculation at twilight showed greater *Ec*O157 survival in the field than in previous studies:

→ effect of less UV radiation and desiccation?

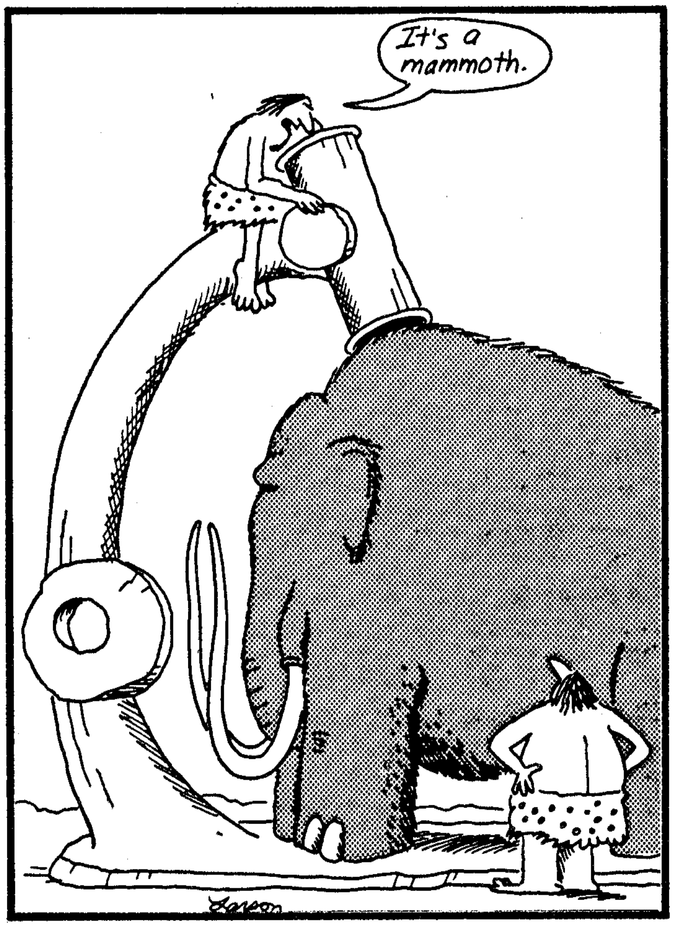
Gutierrez-Rodriguez *et al* 2011

The needle in the hay stack

- Rare successful immigration events
- BUT even few surviving cells count because

1) plant sites suitable for pathogen multiplication
2) low infectious dose of some enteric pathogens eg *Ec*O157

Are we looking at the right scale?



Early microscope

G. Larson

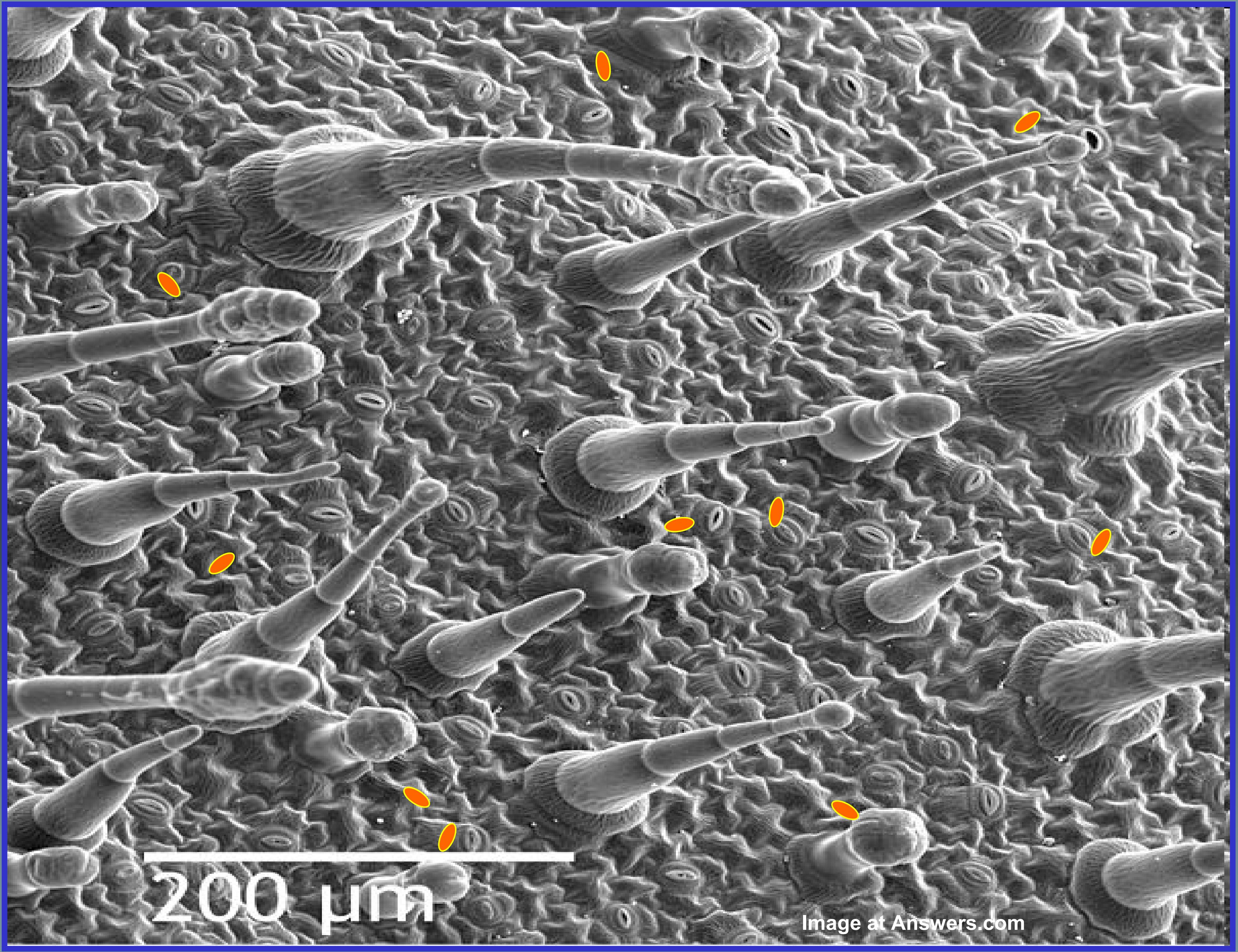
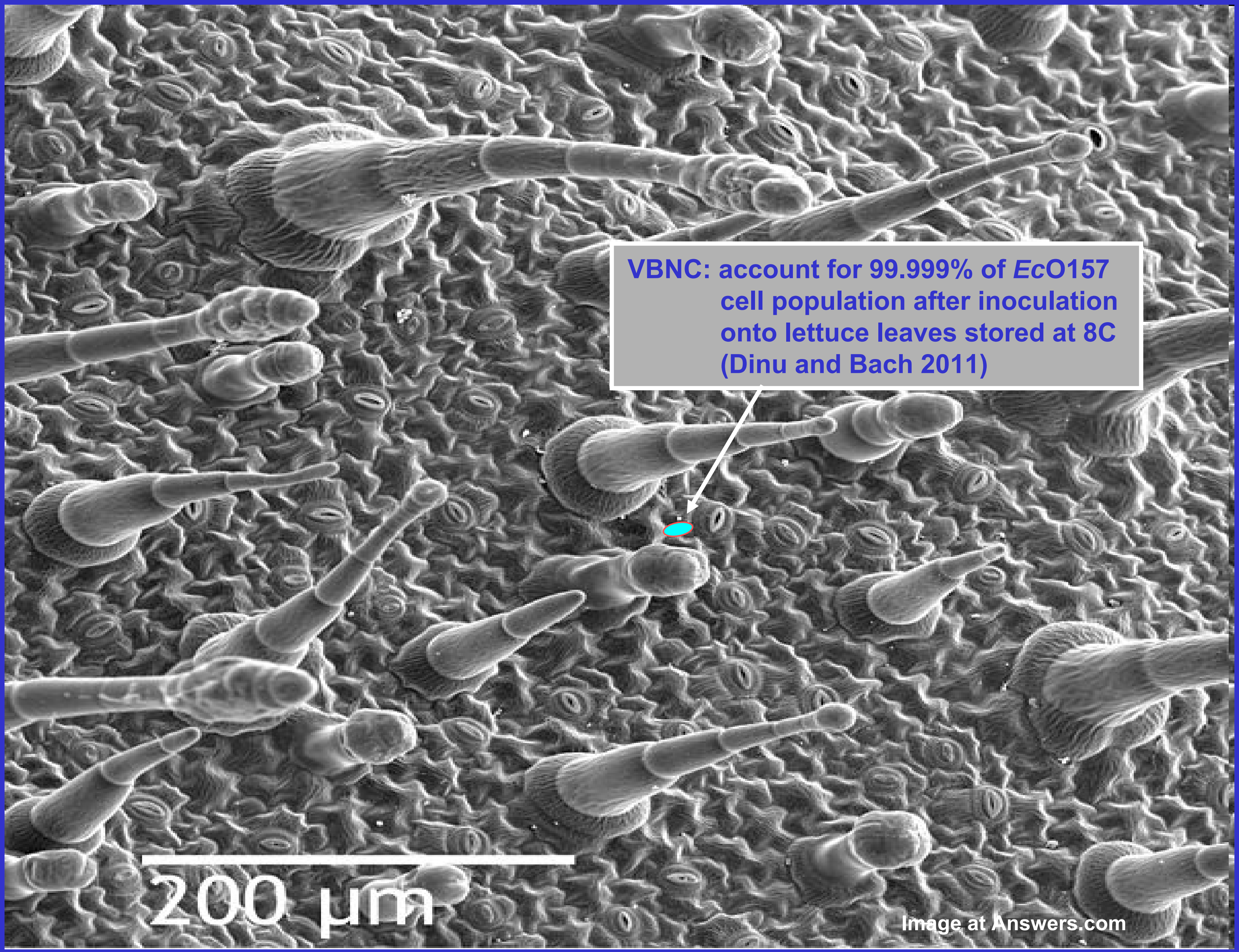


Image at Answers.com

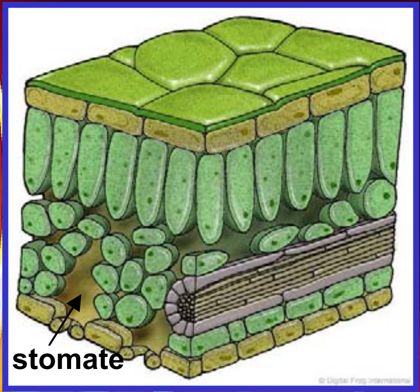


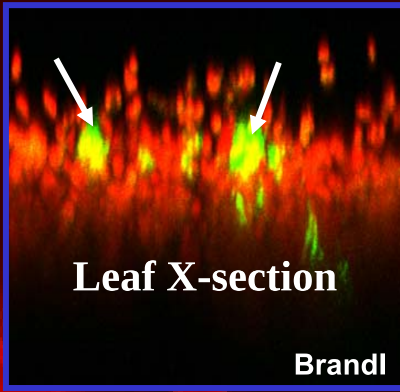
VBNC: account for 99.999% of *Ec*O157 cell population after inoculation onto lettuce leaves stored at 8C (Dinu and Bach 2011)

Image at Answers.com

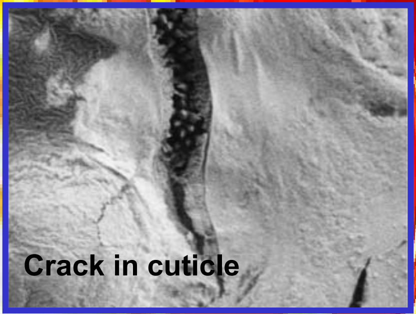
Internalization into plants

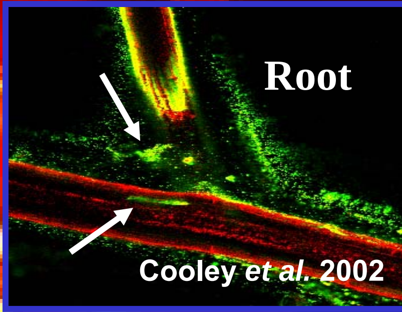
- Protection from stresses, sanitizers, and decontamination treatments
- Vectoring if internalization into seeds
- Increased availability of nutrients in some tissues
- More difficult to detect during testing





Brandl





Cooley *et al.* 2002

Natural openings

Stomata
Cracks in the cuticle
Junction of lateral roots
Flowers (tomato)
Stem scars of fruit (tomato, apple, melon, orange, mango, nuts)

Gu *et al.* 2011

Promoted during hydrocooling, washing and processing

Lesions: the perfect niche for opportunists

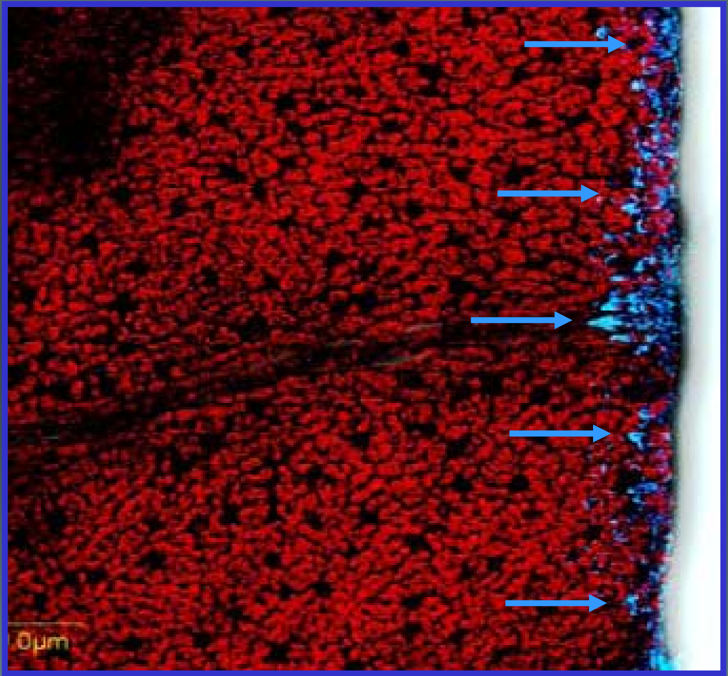
Attachment of *Ec*O157 to lettuce leaves:

- preferential attachment to cut edges

After chlorine treatment:

- highest survival deep in damaged tissue
- intermediate survival at cut edges and in stomata
- lowest survival on leaf surface

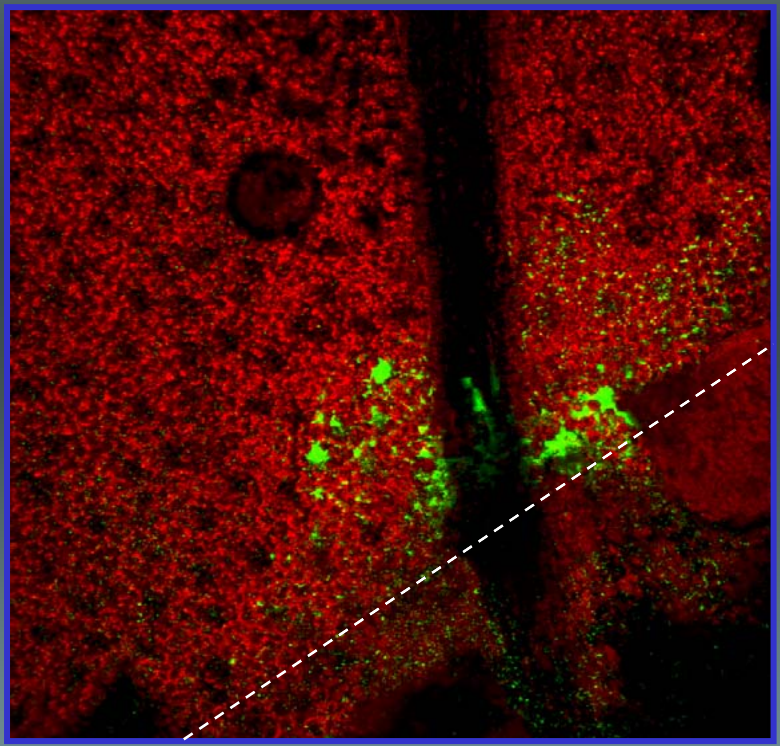
Seo and Frank 1999 JFP
Takeuchi and Frank 2001 JFP



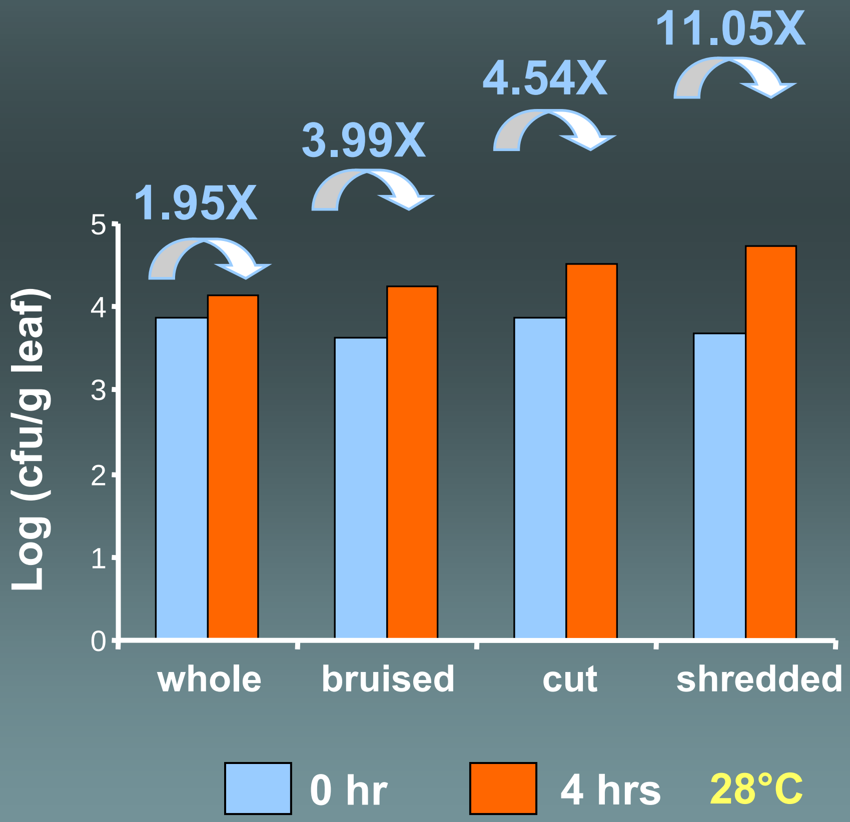
CFP-*Salmonella* on cut lettuce leaf
2h attachment

Kroupitski *et al* JAM 2009

Internalization and growth of *Ec*O157 in damaged Romaine lettuce leaves

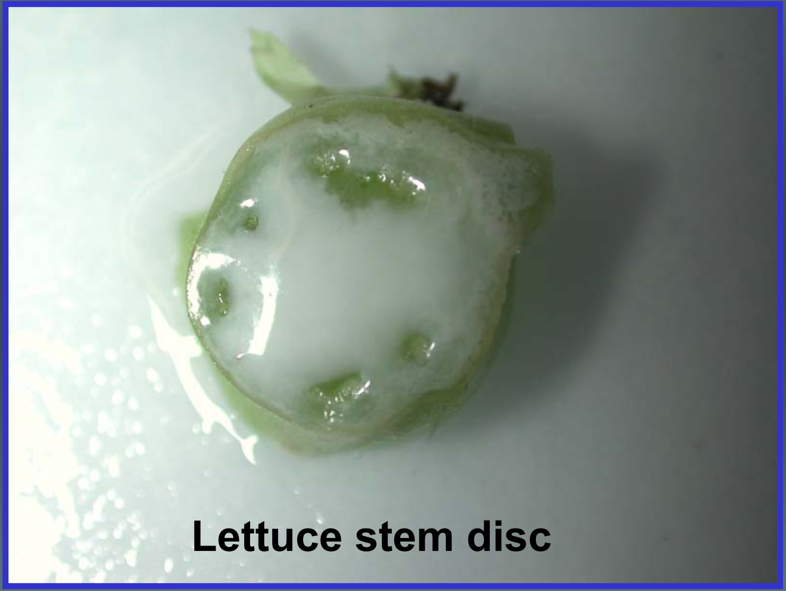


24 hr incubation, 28°C



Brandl 2008

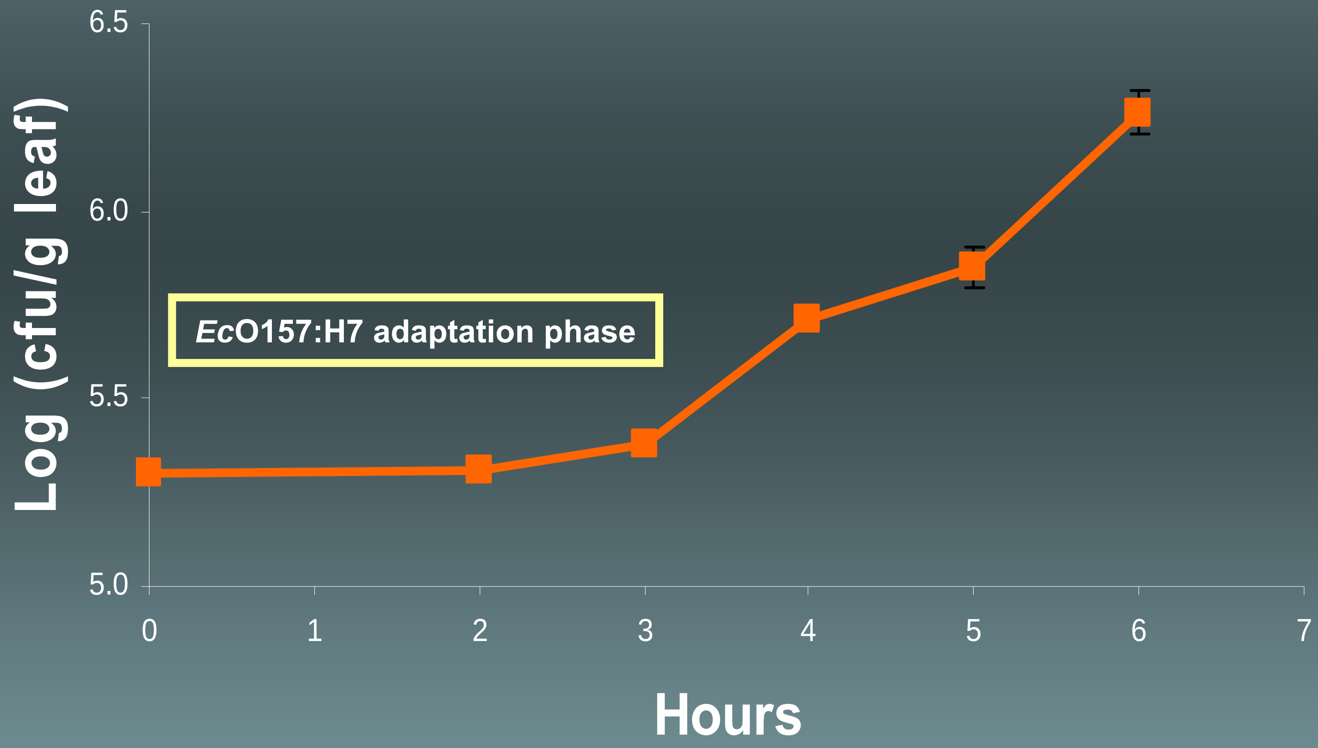
Growth in lettuce cut stem and latex


Lettuce stem disc

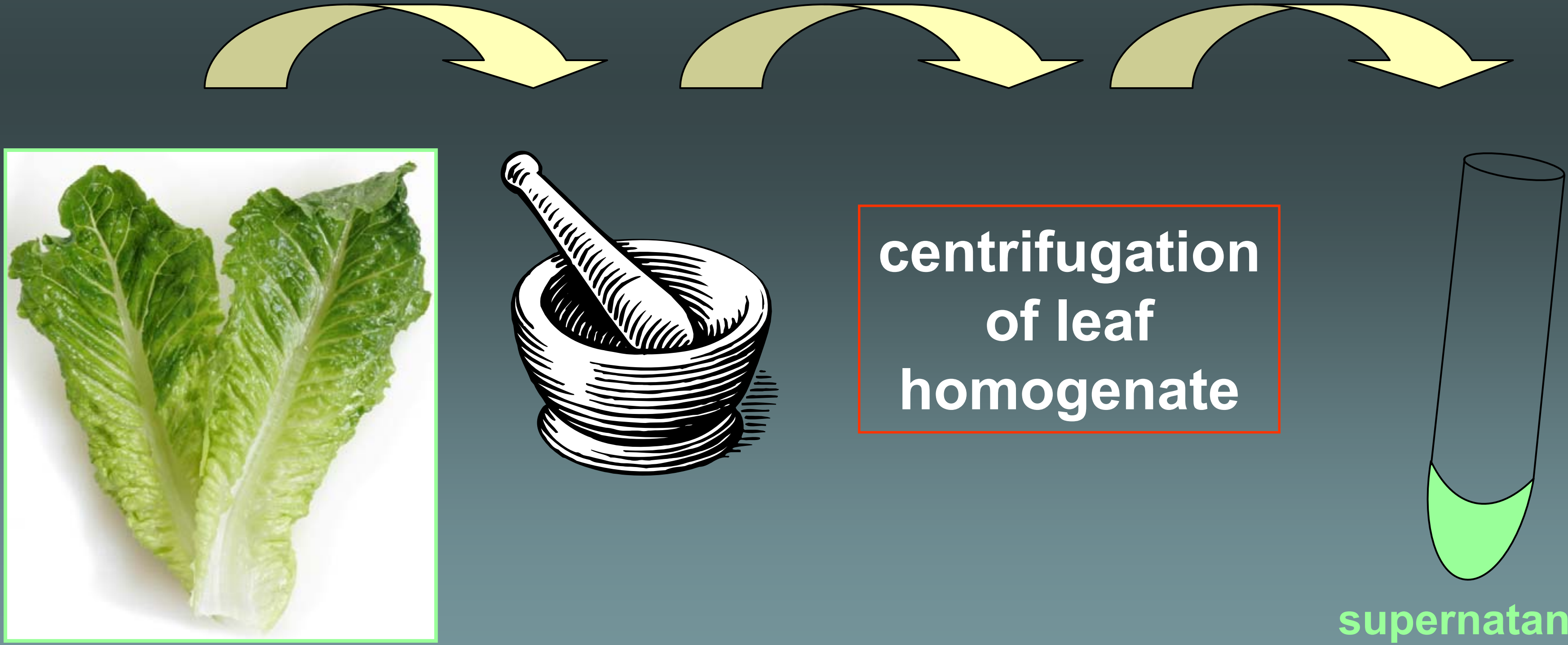
Time of incubation	Bacterial population size	Population increase
h	Log (cells/disc)	
0	2.83A	
2	3.58B	5.62
4	3.87C	11.09
22	7.13D	20090.93

Brandl 2008

Growth of *Ec*O157 on shredded lettuce



Romaine lettuce leaf lysates: A simplified model for the chemical environment in leaf lesions



centrifugation of leaf homogenate

supernatant:
lettuce leaf lysate

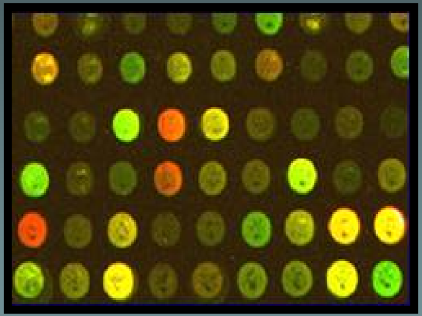
Microarray analysis of *Ec*O157 gene expression in Romaine lettuce leaf lysate

Lettuce lysate + ***Ec*O157:H7**

15 and 30 min-incubation

***Ec*O157:H7 RNA extraction**

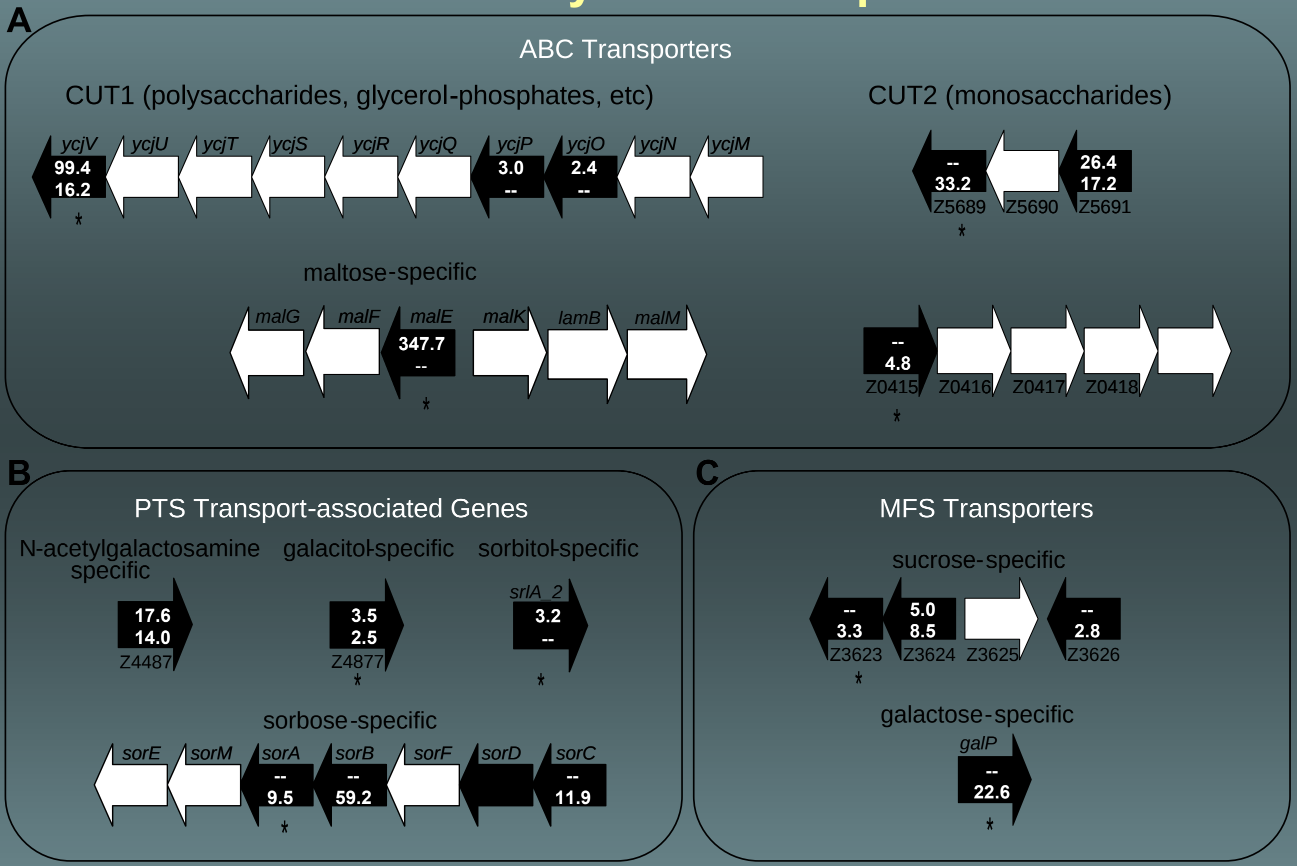
Microarray hybridization



Spots=5,000 K12+O157:H7 genes

Upregulation of nutrient uptake genes

Carbohydrate Transport

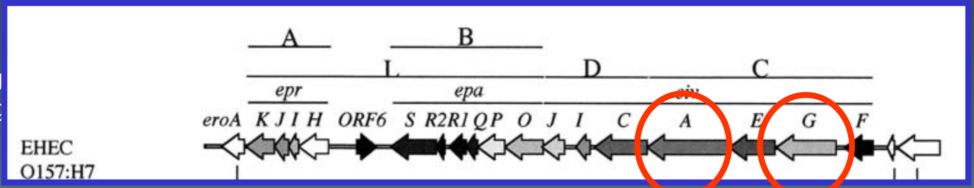


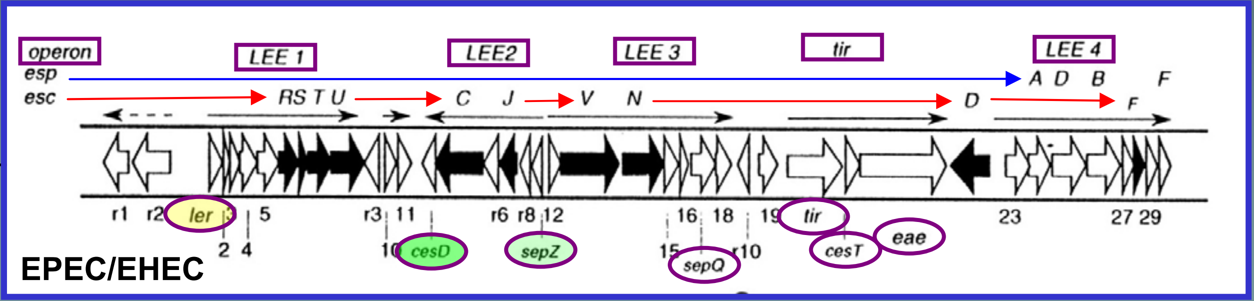
Kyle *et al.* 2010

Induction of virulence genes in lettuce lysates

Gene Name	15 min Fold Increase	30 min Fold Increase	Gene Function
etpI		6.7	type II secretion protein
etpM	2.6		type II secretion protein
ppdD	2.2		prelipin peptidase dependent protein
yadM	3.4	5.7	putative fimbrial protein
fhiA	6.8	11.3	flagellar biosynthesis
ybgP	12.8	7.4	putative chaperone
ycbQ	5.2		putative fimbrial-like protein
Z1291	3.0		hypothetical protein
Z1536		3.4	putative usher protein
Z2361		8.1	protein C Serine peptidase. MEROPS family S49
Z2506		10.8	partial putative outer membrane channel protein
fliJ	4.6		flagellar protein
Z3097	3.7	3.8	ATP-dependent Clp protease proteolytic subunit ClpP
yehB	5.5	7.9	putative outer membrane protein
ccmD		13.2	heme exporter protein C
ppdB	6.7	7.9	prepilin peptidase dependent protein B
Z4180		20.8	putative lipoprotein of type III secretion apparatus
Z4187		16.5	type III secretionapparatus protein
Z4189	6.4		component of typeIII secretion
Z4195	10.8	14.5	type III secretion apparatus protein, *eivA* homolog
Z4197		11.0	type III secretion apparatus protein, *eivG* homolog
yraH		2.2	putative fimbrial-like protein
sepQ	3.4	7.4	sepQ
escJ		6.2	escJ
escC	18.1	22.1	escC
escU	10.0	10.0	escU
escR	16.8	50.7	escR
Z5221		3.5	putative fimbrial protein
Z5225	9.9	12.3	putative major fimbrial unit
fimI	2.7		fimbrial protein
fimF	4.1		fimbrial morphology

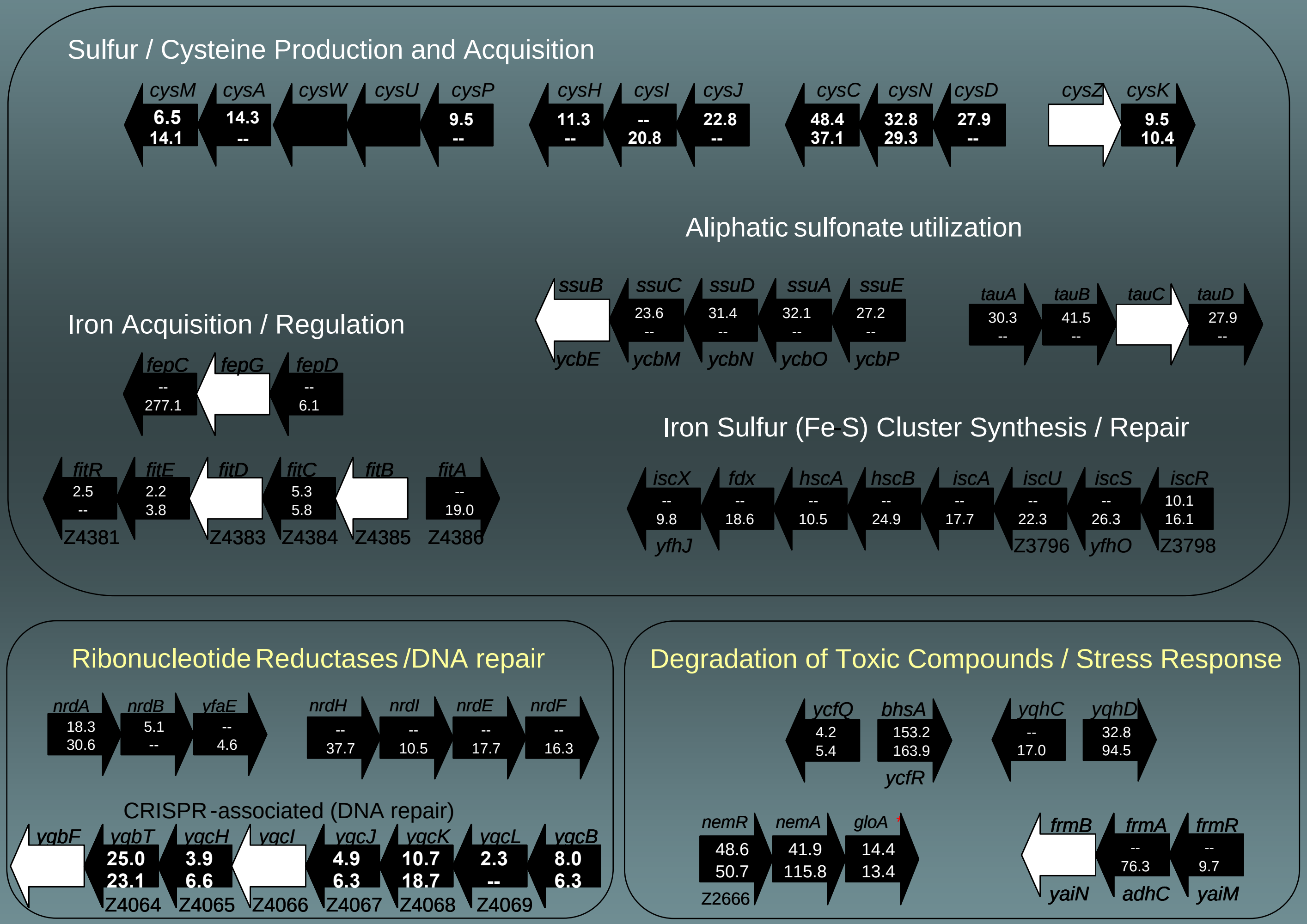
What is the effect of the plant environment on the infectious dose of the pathogen?





Kyle *et al.* 2010

Upregulation of numerous stress-response genes



Oxidative Stress - Related Operons

Sulfur / Cysteine Production and Acquisition

cysM 6.5 14.1
cysA 14.3 --
cysW
cysU
cysP 9.5 --

cysH 11.3 --
cysI -- 20.8
cysJ 22.8 --

cysC 48.4 37.1
cysN 32.8 29.3
cysD 27.9 --

cysZ
cysK 9.5 10.4

Aliphatic sulfonate utilization

ssuB *ycbE*
ssuC 23.6 -- *ycbM*
ssuD 31.4 -- *ycbN*
ssuA 32.1 -- *ycbO*
ssuE 27.2 -- *ycbP*

tauA 30.3 --
tauB 41.5 --
tauC
tauD 27.9 --

Iron Acquisition / Regulation

fepC -- 277.1
fepG
fepD -- 6.1

fitR 2.5 -- Z4381
fitE 2.2 3.8
fitD Z4383
fitC 5.3 5.8 Z4384
fitB Z4385
fitA -- 19.0 Z4386

Iron Sulfur (Fe-S) Cluster Synthesis / Repair

iscX -- 9.8 *yfhJ*
fdx -- 18.6
hscA -- 10.5
hscB -- 24.9
iscA -- 17.7
iscU -- 22.3 Z3796
iscS -- 26.3 *yfhO*
iscR 10.1 16.1 Z3798

Ribonucleotide Reductases /DNA repair

nrdA 18.3 30.6
nrdB 5.1 --
yfaE -- 4.6

nrdH -- 37.7
nrdI -- 10.5
nrdE -- 17.7
nrdF -- 16.3

CRISPR-associated (DNA repair)

ygbF
ygbT 25.0 23.1 Z4064
ygcH 3.9 6.6 Z4065
ygcI Z4066
ygcJ 4.9 6.3 Z4067
ygcK 10.7 18.7 Z4068
ygcL 2.3 -- Z4069
ygcB 8.0 6.3

Degradation of Toxic Compounds / Stress Response

ycfQ 4.2 5.4
bhsA 153.2 163.9 *ycfR*

yqhC -- 17.0
yqhD 32.8 94.5

nemR 48.6 50.7 Z2666
nemA 41.9 115.8
gloA * 14.4 13.4

frmB *yaiN*
frmA -- 76.3 *adhC*
frmR -- 9.7 *yaiM*

Kyle *et al.* 2010

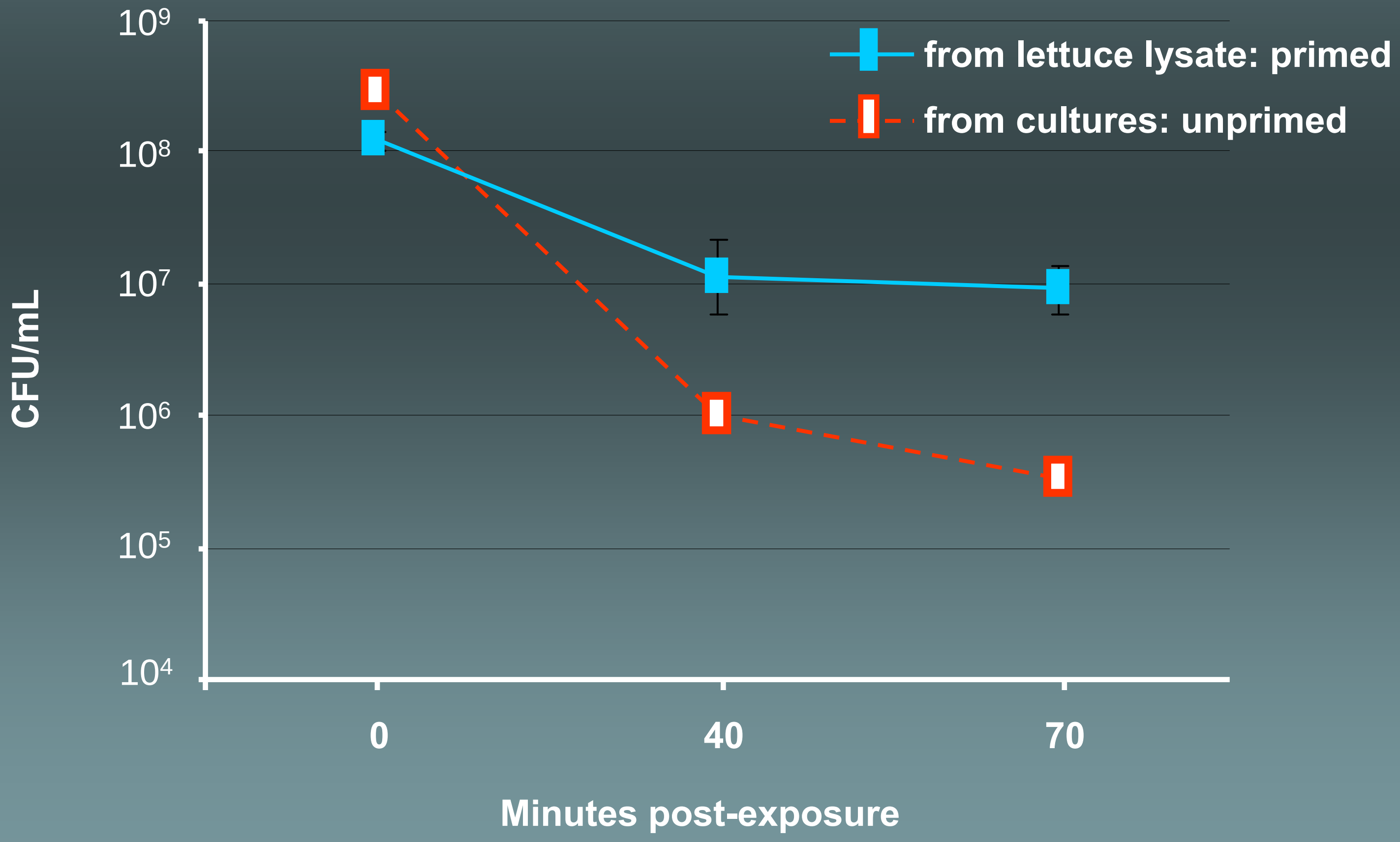
OxyR regulon

- *ahpC*
- *ahpF*
- *dps*
- *grxA*
- *trxC*
- *ybjM*

Antimicrobial stress and detoxification

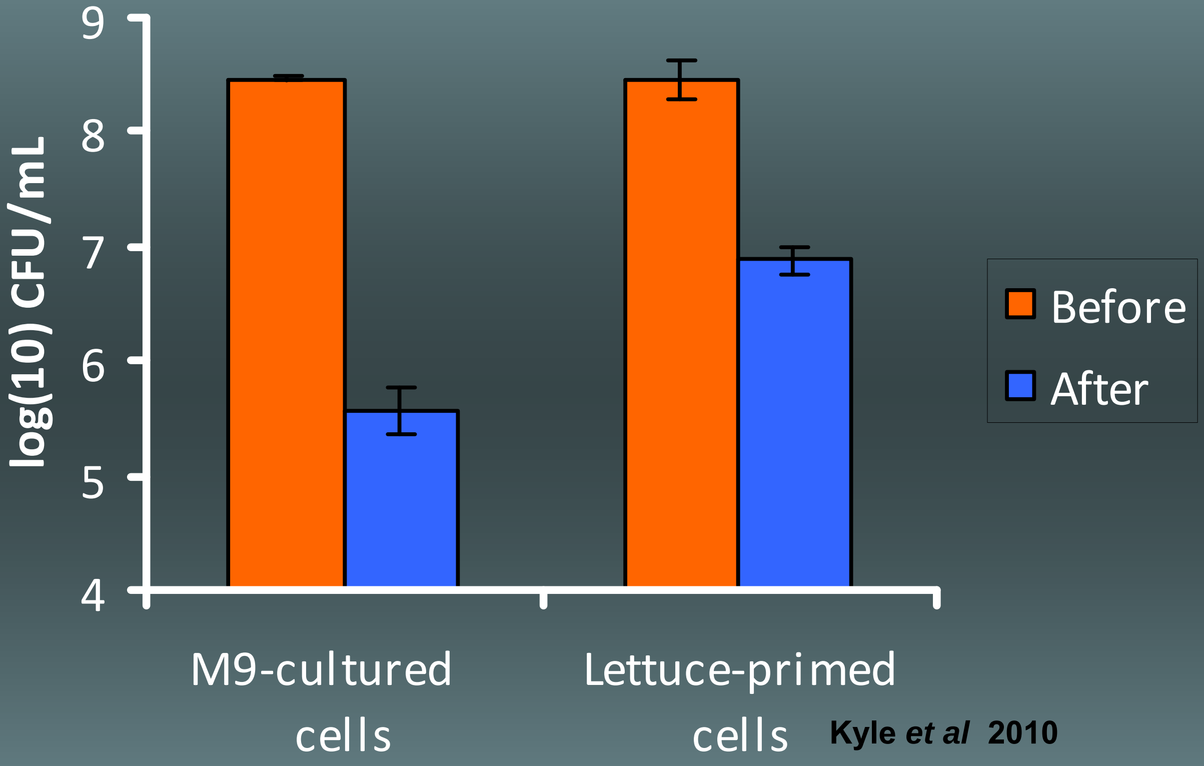
- *gatA*
- *hemB*
- *inaA*
- *marA*
- *marR*
- *ribA*
- *yfaE*
- *emrA*
- *emrD*
- *gloA*
- *nemA*

Growth in lettuce lysate increases tolerance of *Ec*O157:H7 to H_2O_2



Kyle *et al.* 2010

Exposure to lettuce lysate increases tolerance of *Ec*O157 to chlorine



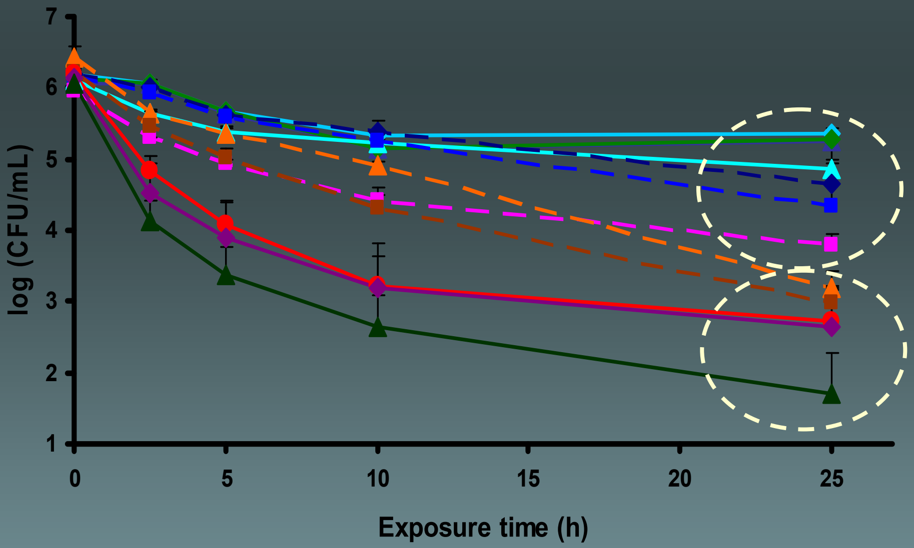
Kyle *et al* 2010

We need to better understand the physiology of human pathogens in/on plants and what plant factors weaken or strengthen them in order to design more effective control/decontamination strategies → **Hurdle technologies?**

Stress tolerance of 12 *Ec*O157 strains related to the spinach-linked outbreak in 2006

– All strains have same PFGE pattern

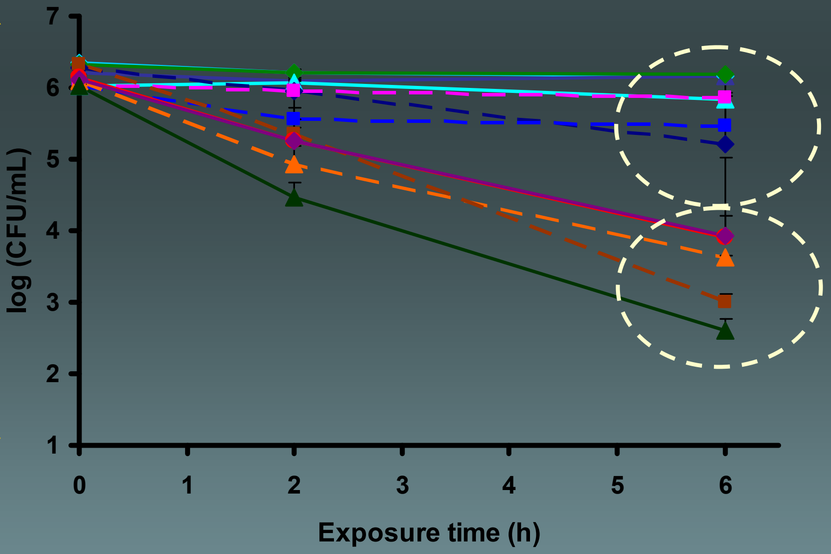
Survival to osmotic stress



Environmental

Clinical

Survival to acid stress



Parker *et al*. 2012

Interactions with plant microbes

Variable effect of plant-associated fungi

Wade and Beuchat

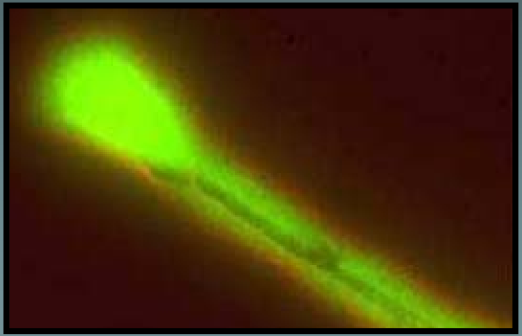
***Alternaria* & *Cladosporium*:**
↗ pH of tomato → ↗ *Salmonella*

***Alternaria, Cladosporium, Geotrichum* & *Penicillium*:**
No growth promotion of *Salmonella* on cantaloupe rind

Riordan *et al*

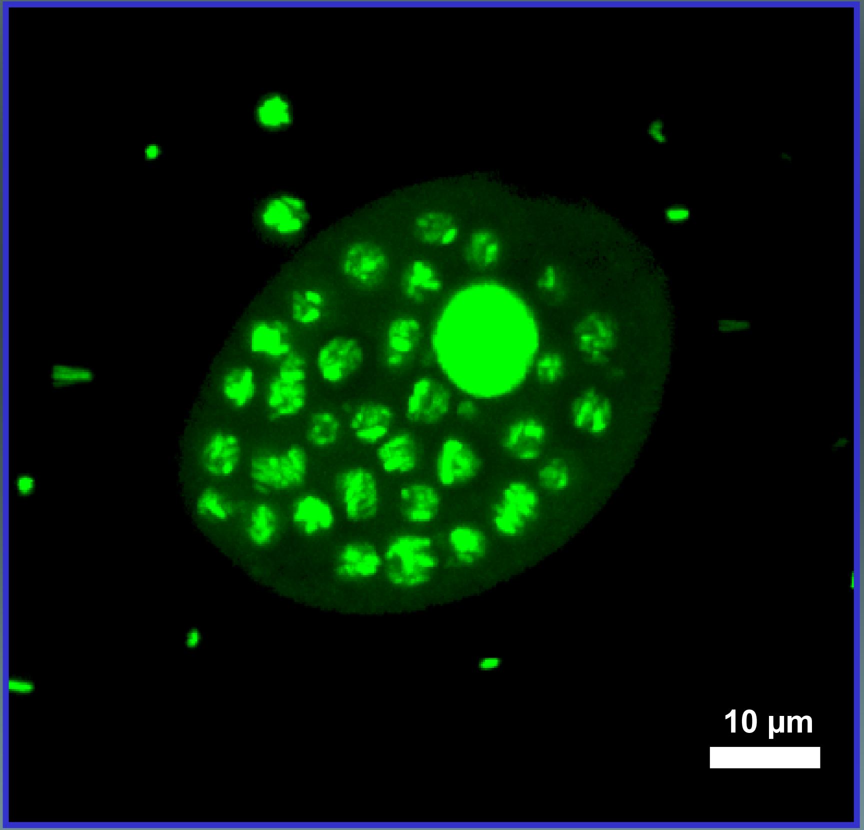
***Glomerella:* ↗ pH of apple → ↗ *Ec*O157**
***Penicillium:* ↙ pH of apple → ↙ *Ec*O157**

Aspergillus niger* & *Salmonella

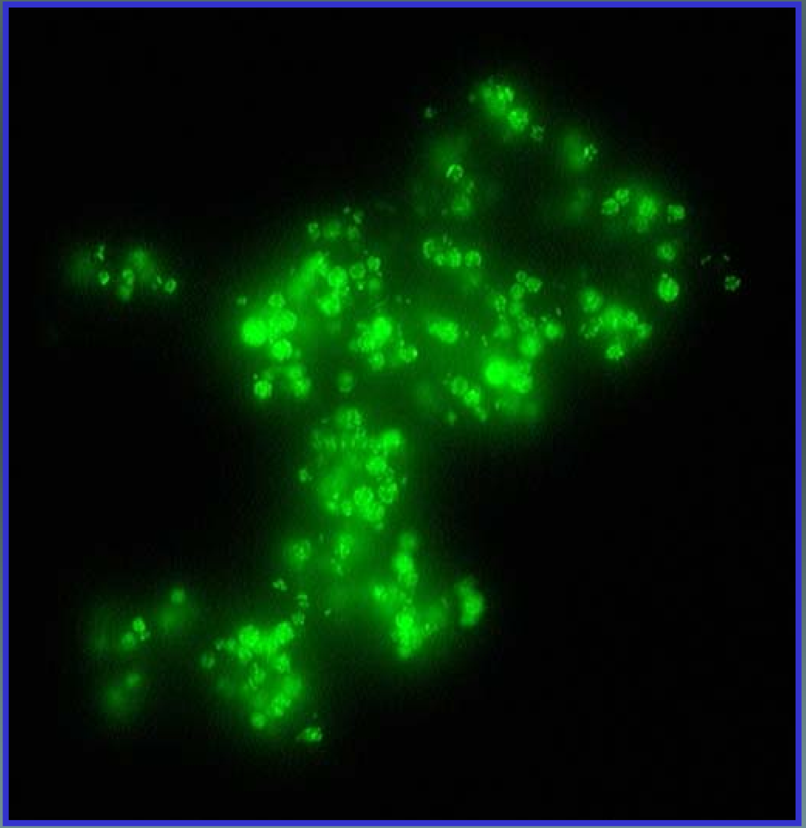


Brandl *et al* 2011

Syto 9 DNA staining of *Tetrahymena* and its fecal pellets after grazing on *Salmonella*



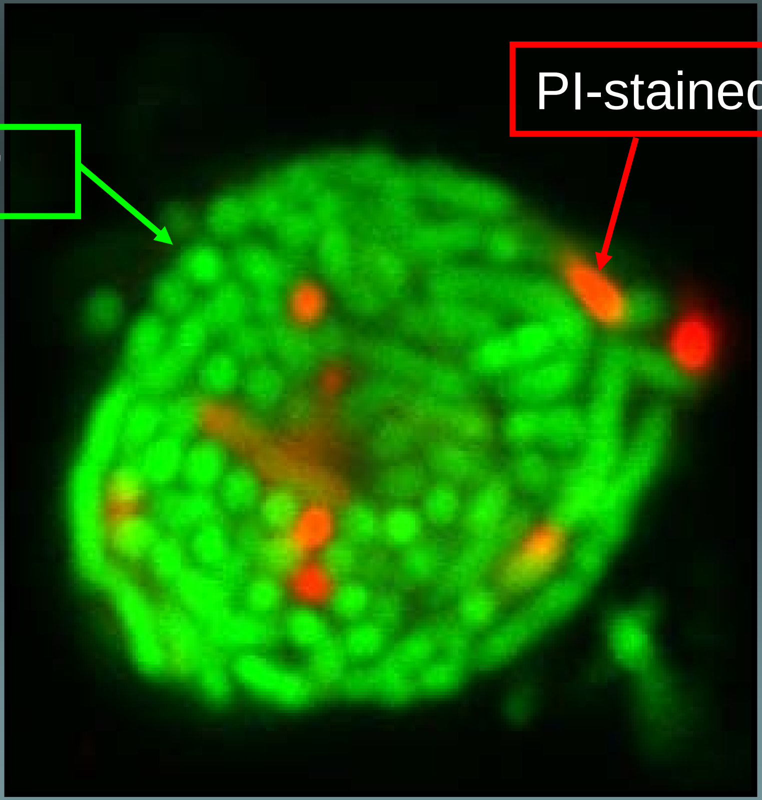
Brandl *et al.* 2005



Rehfuss *et al*. 2011

Use of Propidium Iodide stain to test cell viability within pellets

Pellet released by *Tetrahymena* and containing GFP-*Salmonella* cells

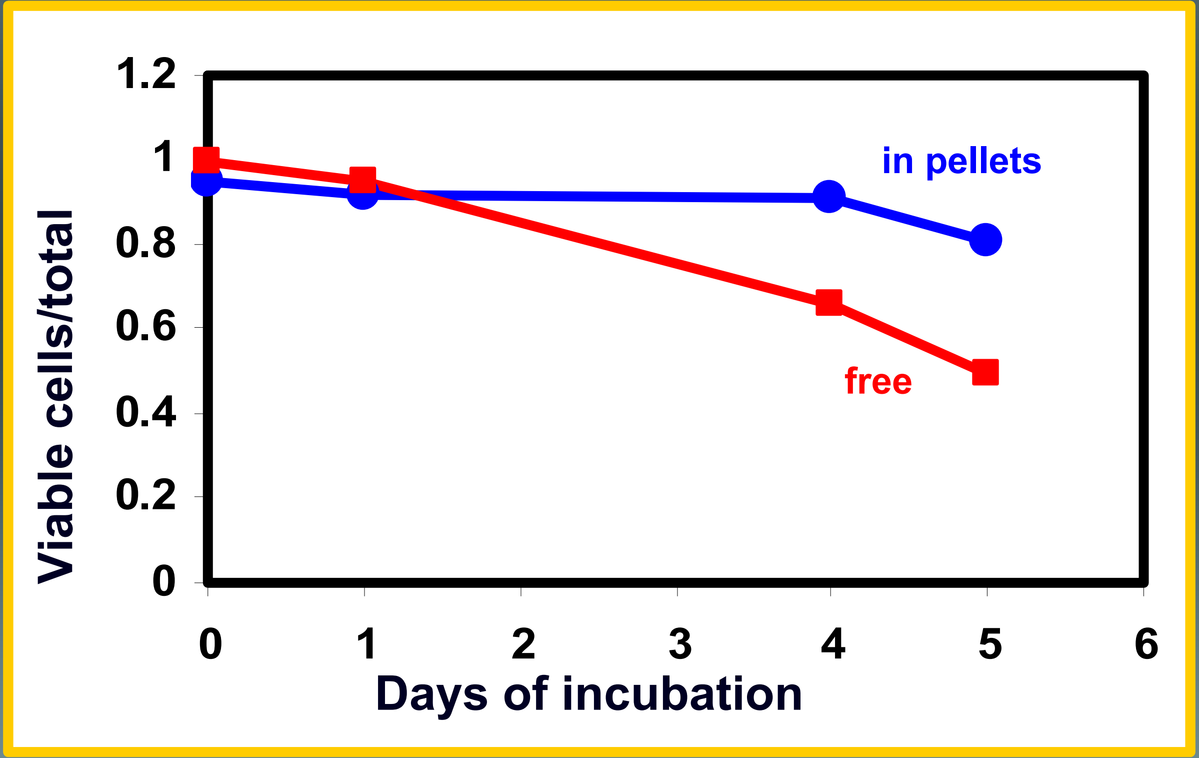


GFP-cell = “viable cell”

PI-stained cell = “dead cell”

Brandl *et al.* 2005

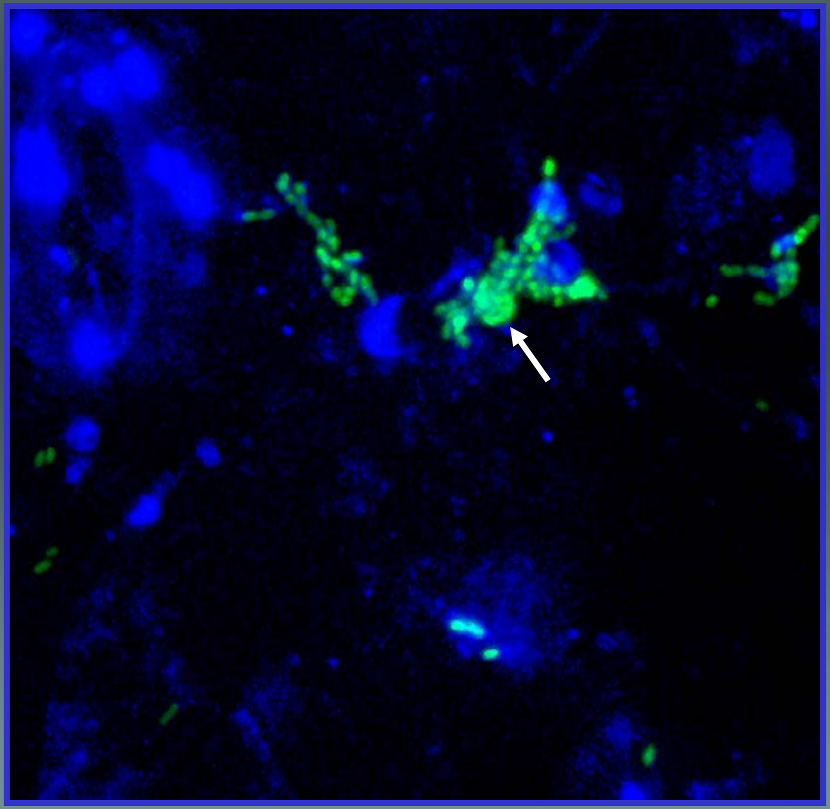
Salmonella survives at higher rates in *Tetrahymena* pellets than as free cells in suspension

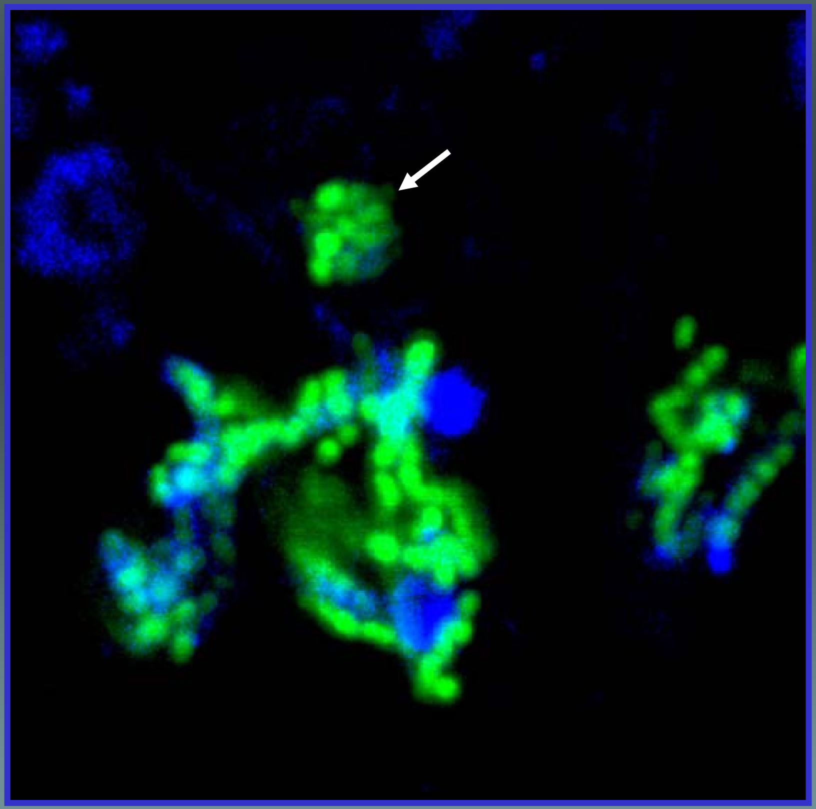


Slopes of regressions significantly different at *P* <0.0001

Brandl *et al.* 2005

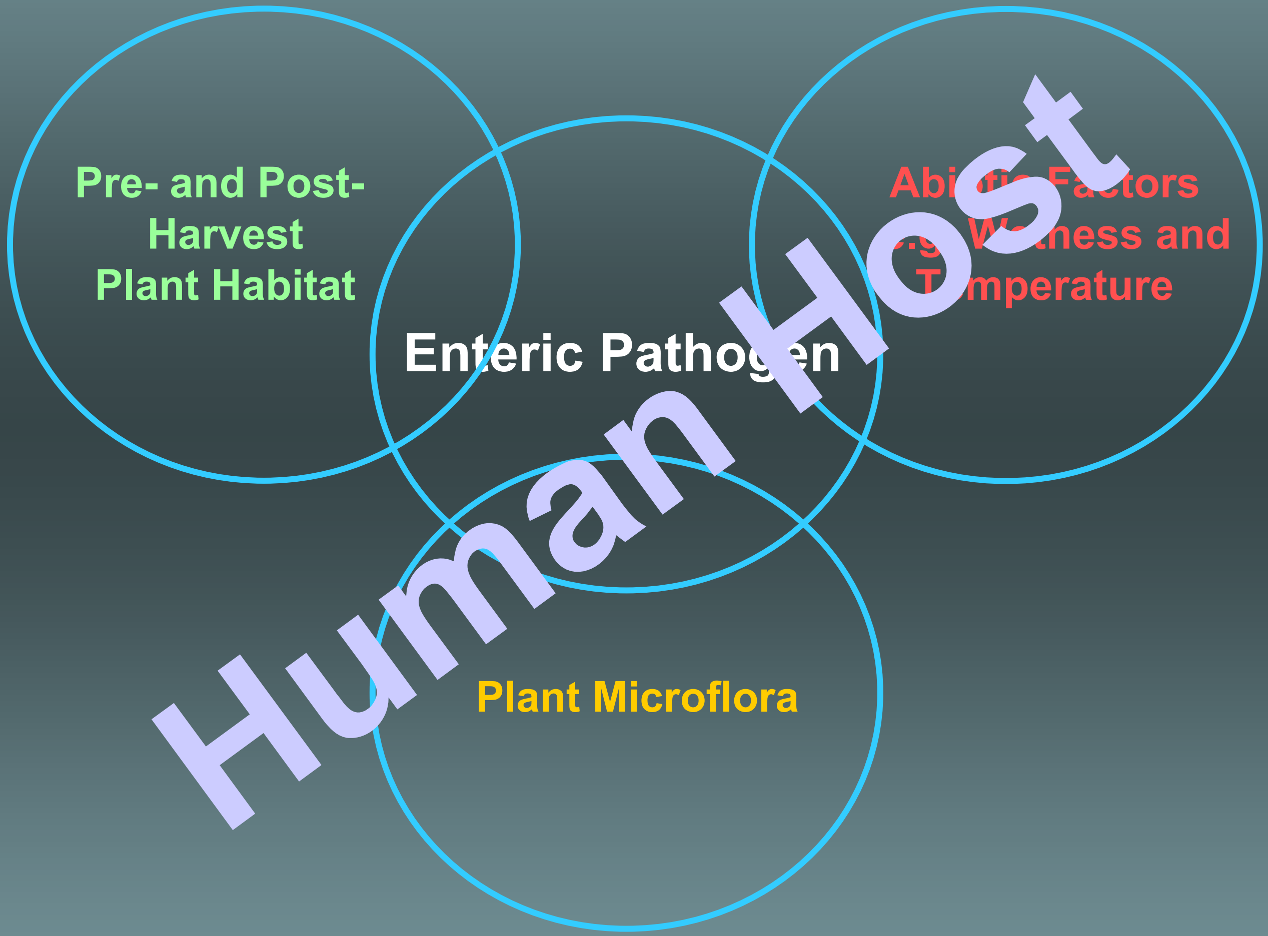
Salmonella-containing pellets produced by *Tetrahymena* on plants





Gourabathini *et al*. 2008

The Perfect Storm



Pre- and Post-Harvest
Plant Habitat

Abiotic Factors
e.g. Wetness and
Temperature

Enteric Pathogen

Human Host

Plant Microflora

Thank you
(and eat your veggies!)

Photo by P. Greb