# Chapter 3
# The History of the Soil Food Web

Lecture 15 – The History of the Soil Food Web (Part 1)

# Elaine Ingham, B.A., M.S., Ph.D.

## Soil Microbiologist

St. Olaf College, Double Major in Biology and Chemistry
Master's, Texas A&M, Marine Microbiology

Ph.D., Colorado State University, Soil Microbiology
Research Fellow, University of Georgia

Assistant, Associate Professor, Oregon State University (1986 – 2001)

Rodale Institute, Chief Scientist 2011 - 2013

President, Soil Foodweb Inc., 1996 – present
Labs in many places around the world

# Choose the right assessment tool

At Texas A&M, my major professor had been approached by the oyster industry in Galveston Bay.

The oysters were not growing, despite addition of every combination of fertilizer, growth promoters, and nutrient enhancement known to man. Someone suggested that the problem might be biological.

How do you approach this problem?

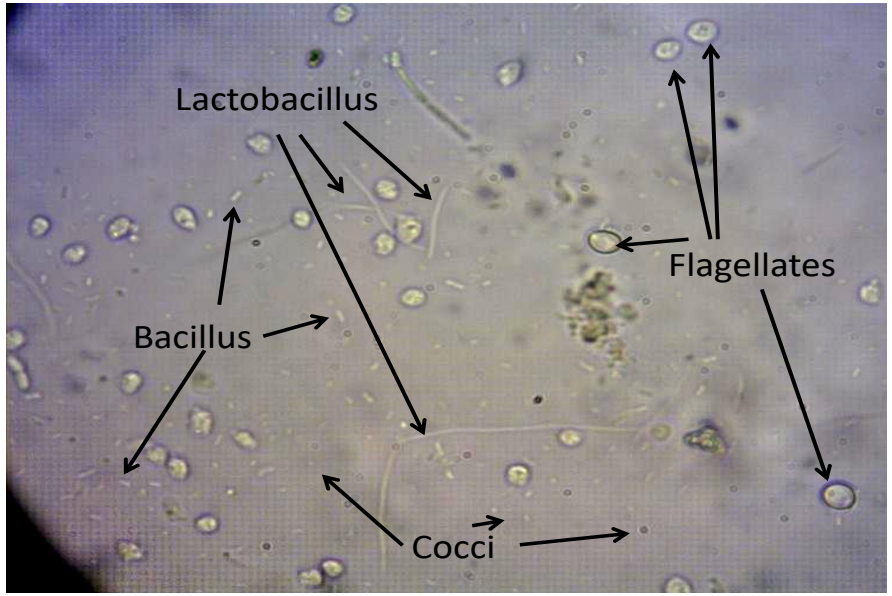
The consensus was to determine what microorganisms were in healthy oyster digestive systems, versus the oysters in the commercial beds.

Using plate counts, no differences were observed.

# Which method is telling the truth?

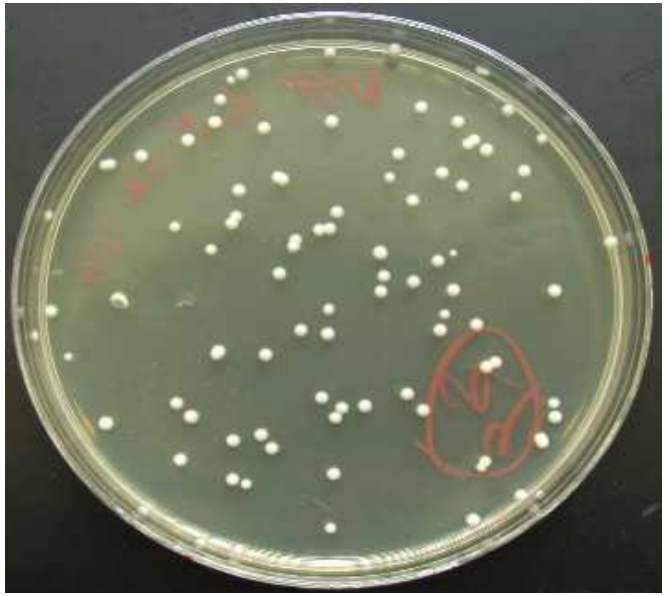
Because I had microscope training, I looked at digestive system samples using a microscope, and determined the following:

Direct Microscopy
1/2000$^{th}$ of a drop (microliter)




1000 bacteria, 12 species; protozoa

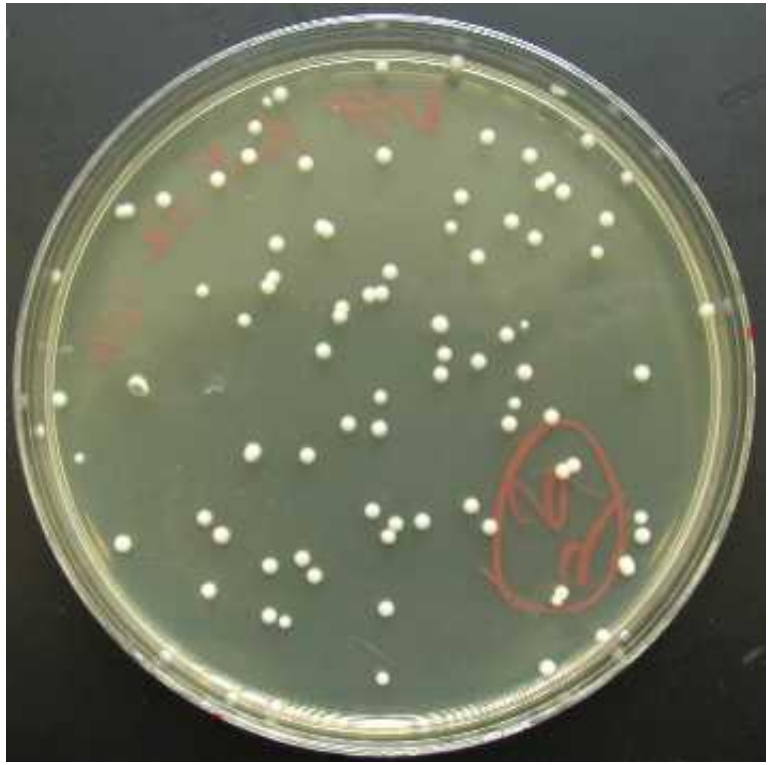
Plate Count using TSA
1 ml of digestive fluid



100 Bacteria CFU; 2 species

# Plate Counts

- A specific mix of foods in the dish
  - Potato dextrose agar (PDA) for “total fungi”
  - Tryptone soy agar (TSA) for “total bacteria”
- Spread sample on surface of medium
- Cover, incubate at constant temperature, humidity, moisture. (Is that normal?)
- Added food allows organisms to grow rapidly from 1 to over a million individuals in 18 hours, and we see the resulting colony.



- The colonies of different species usually look different.
- Oxygen will be used up rapidly when bacteria are growing rapidly in a limited atmosphere. Reduced oxygen conditions select for pathogen growth.

# What was I actually learning from these assessments?

The plate count method was not telling me what I wanted to know. I wasn't asking how many anaerobic, and most likely pathogenic organisms were present in the oyster's digestive system.

Oyster digestive systems aren't typically anaerobic.

I wanted to know if the biology in healthy oysters was different from the biology in sick oysters. And I figured that out using shadowing microscopy.

When we added the right sets of microbes to the digestive systems of the oysters, the oysters grew.

# Assessment Methods

| Knowledge Desired | Name of Method(s) | Description of Methods:<br>Problems with Method |
|---|---|---|
| Organism Biomass | Shadowing Microscopy | Measure length, width or number of individuals separated by general or specific morphological criteria. Compare values over time. Compare to desired ranges based on crops . |
| Organism Activity | $CO_2$ absorption; e.g., Solvita, Haney | $CO_2$ absorbing chemical with a pH indicator is used so color change can be related to $CO_2$ taken up. Other chemical reactions in soil can release $CO_2$. Sampling can alter soil biology. Add food, or not? |
| | Enzymes; e.g., Dehydrogenase, chitinase | Enzymes react with substrate, measure initial and final amounts of substrate. Disturbance reduces numbers and kinds of organisms. |
| Numbers of Individuals of one or more species or genus | Plate Counts | Can organisms grow on the medium used, at the temperature, moisture, humidity used? Useful for specific pathogens, but completely inappropriate for total species or activity. |
| | PLFA | Not all species microbes produce PLFA, so results underestimate actual values. |

# Assessment Methods (cont.)

| Knowledge Desired | Name of Method(s) | Description of Methods:<br>Problems with Method |
|---|---|---|
| Organism numbers | Chloroform fumigation | Fumigation is supposed to kill all the organisms in the sample, but this will not be the case unless the sample is spread very thinly, which means severe disturbance impact on the organisms. All the dead organisms are supposed to now be used by the remaining living organisms (but the chloroform killed everything, so how can living organisms still be present? How much of the total set of organisms weren't killed? Temperature, moisture, amount of organic matter will affect the results as well. Significant controls are needed and are rarely included. |
| Nutrient Cycling | Haney Test | Measure soluble inorganic nitrogen levels at the start and end of an incubation period. Samples incubated in a sealed jar will quite likely become anaerobic, which means inorganic N will be lost as gas when the jar is opened leading to underestimates. |

# Elaine Ingham, B.A., M.S., Ph.D.

## Soil Microbiologist

St. Olaf College, Double Major in Biology and Chemistry
Master's, Texas A&M, Marine Microbiology

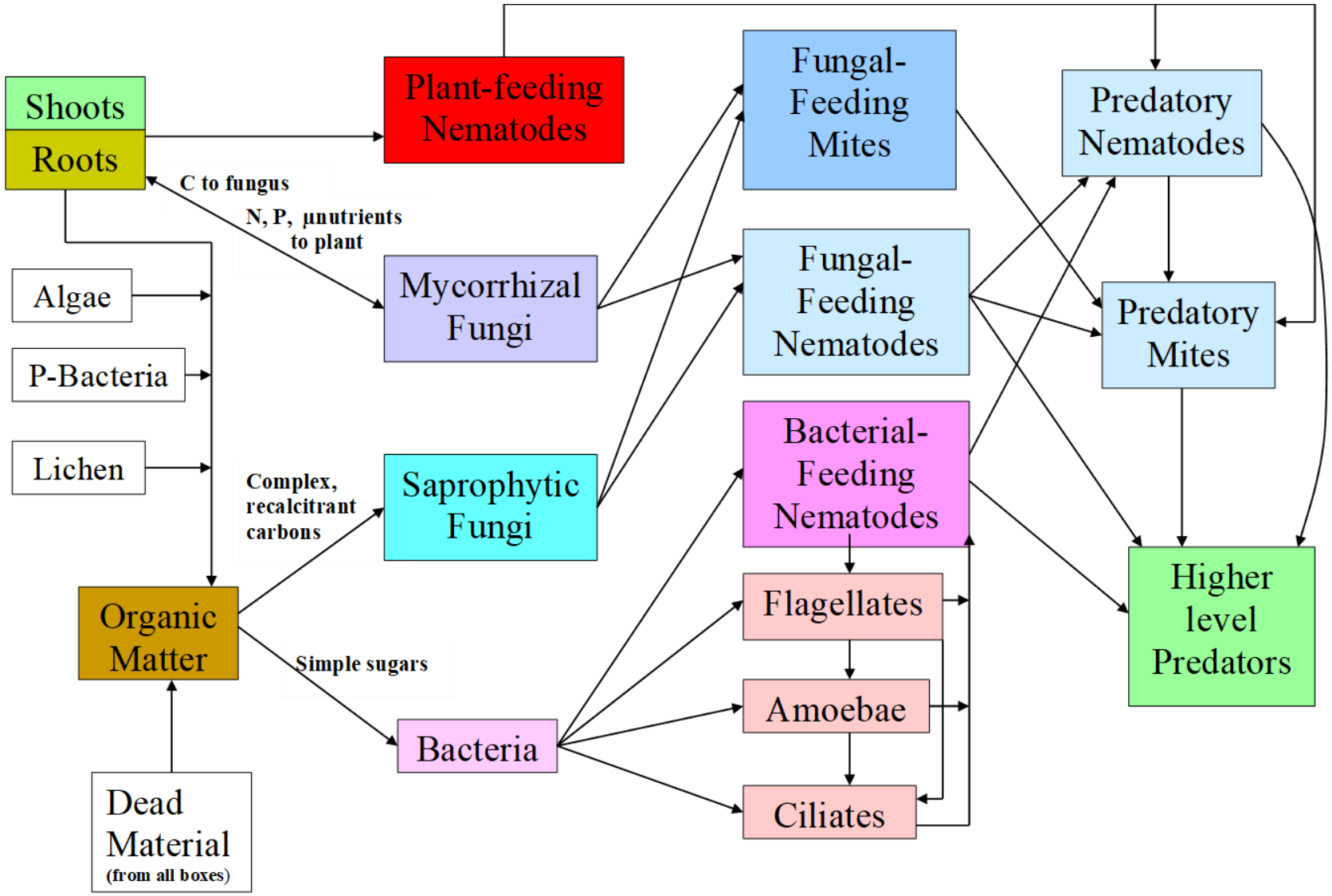
Ph.D., Colorado State University, Soil Microbiology
Research Fellow, University of Georgia

Assistant, Associate Professor, Oregon State University (1986 – 2001)

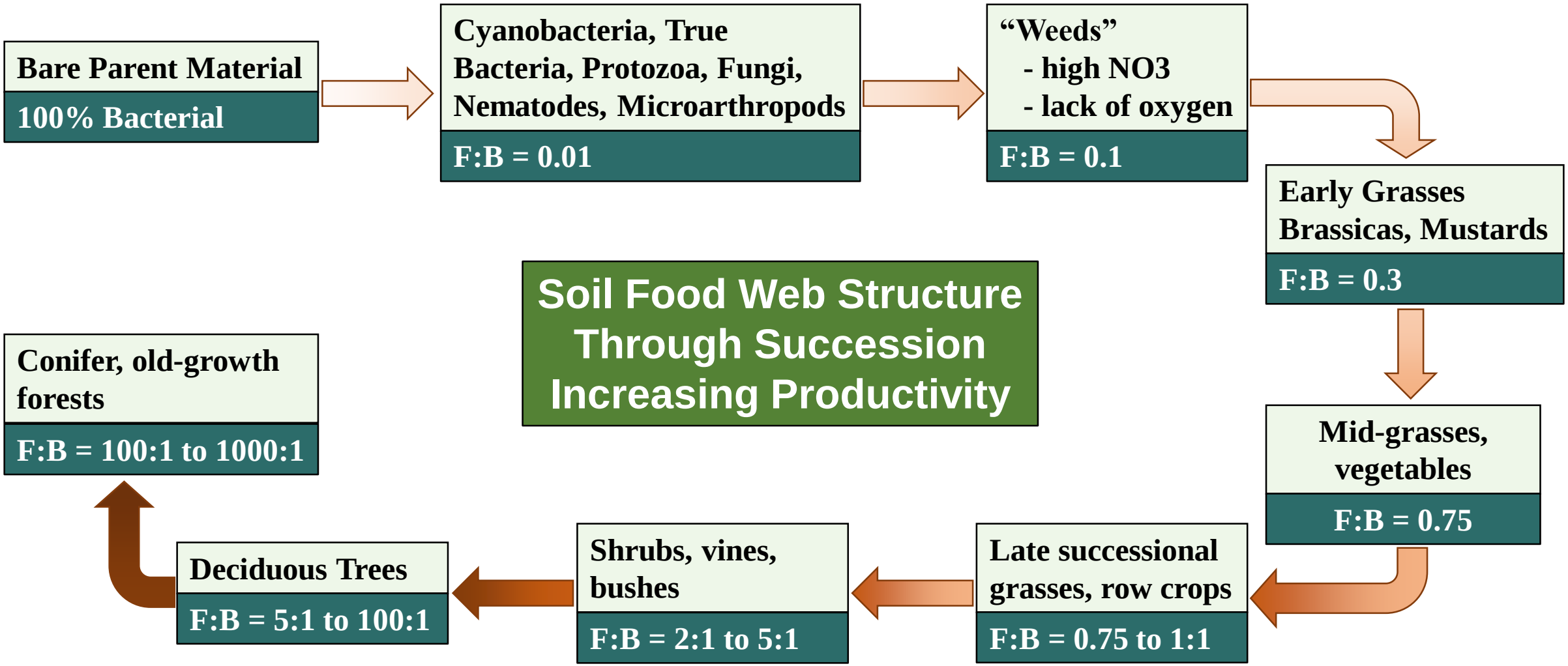
Rodale Institute, Chief Scientist 2011 - 2013

President, Soil Foodweb Inc., 1996 – present
Labs in many places around the world

# The Soil Food Web in Lawns, Vegetable, and Row Crops Systems



# What does your plant need?



# Biological Counts

| Production Gradient | AB | TB | AF | TF | Diam-eter | Protozoan Numbers /g | | | Nema-todes | INDEX |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (µg/g) | | | (µm) | F | A | C | (#/g) | |
| Weeds | 56 | 147 | 11 | 64 | 2 | 6,400 | 6,400 | 51 | 7 | 1 |
| Garden | 78 | 144 | 3 | 19 | 2 | 51280 | 55400 | 1001 | 7.0 | 4 |
| Chem Pas | 44 | 127 | 13 | 55 | 2.5 | 5475 | 4242 | 33 | 2 | 5 |
| Pasture | 84 | 117 | 23 | 83 | 2.5 | 16178 | 6715 | 417 | 5 | 8 |
| Clearcut | 17 | 124 | 16 | 73 | 3 | 1819 | 5325 | 7 | 1 | 15 |
| OgGarden | 81 | 180 | 30 | 47 | 2.5 | 5787 | 5356 | 73 | 16 | 17 |
| O Potato | 94 | 229 | 10 | 237 | 2.5 | 7309 | 21998 | 5665 | 11 | 19 |
| Strawberry | 340 | 531 | 22 | 702 | 2.5 | 27070 | 27070 | 1123 | 1 | 22 |
| YoungFir | 165 | 245 | 29 | 1275 | 2.5 | 18 | 7489 | 0 | 18 | 23 |
| Oldgrowth | 194 | 458 | 79 | 2946 | 3 | 126 | 77716 | 0 | 24 | 25 |
| Variation | 17% | | | | | | 20% | | 8% | |

# Do genetically engineered *E.coli* have ecological effects?

**Assistant, Associate Professor,**

**Oregon State University**

IGPA at the USEPA in Corvallis

1. What is an ecological effect?
2. Decided on Fed Regs: FIFRA and TosCA to define ecological effect.
3. FIFRA: add the bacteria to: fish food, duck food, and shrimp food.
4. TosCA: Measure a battery of toxic chemicals with no added GEM, and with added GEM.
5. Why would there be an effect on these things?

So, no effects were observed!

# Conclusion: Not so fast!

USDA – APHIS decided, on the basis of tests like this, that:

**GEO's are no greater risk than the parent -** so Monsanto says

"It has been proven that testing of new GEO's are not necessary."

Which, of course, is **NOT TRUE!**

Every single novel genetic engineering event MUST be tested, since every event produces something different. Cannot generalize that one genetic change is the same as all genetic change.

# The Story of Klebsiella planticola

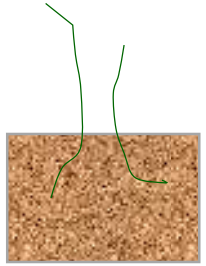
In order to have data to make the point that their testing criteria were silly, one of my graduate students, Michael Holmes, decided to search out a bacterial species that normally lives in soil, but had been genetically engineered.

He found *Klebsiella planticola*

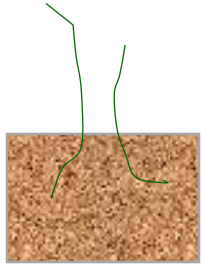
1. Decomposes green plant material and exudates and exists in the root systems of ALL PLANTS.
2. Engineer alcohol production into the bacteria, then you can make alcohol from all plant residues.
3. Instead of field burning, remove residues to container on-farm, add this GEM, produce alcohol, sell it.
4. Remaining material in the container could be spread on field as fertilizer.
5. WIN –WIN – WIN, right?

# Experiment done by Mike Holmes

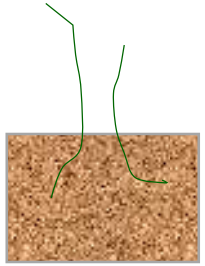
Sieved, mixed soil added to soil microcosms



Just water



Parent *Kp*



GEM *Kp*

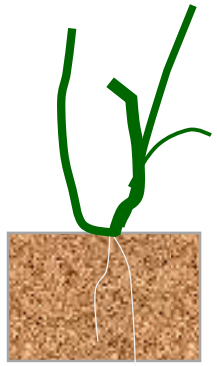
Used the same amount of water as control

Wheat seedling planted in each microcosm

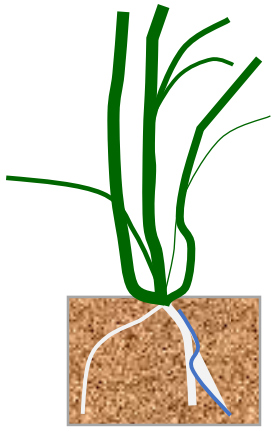
Placed in incubator, moved daily to make certain no incubator bias.

# Published: M. Holmes et al. (Applied Soil Ecol., 1999)

A week later:



Just water



Parent *Kp*

GEM *Kp*

Alcohol is one of the most plant toxic materials known

# United Nations Biosafety Protocol meetings Madrid, 1995

Presented Dr. Holmes' data to the United Nations Biosafety Protocol meetings in Madrid in 1995 and prevented the US delegation from deleting the Biosafety protocol.

On returning to Oregon State University, the "quality", "validity" and "repeatability" of my science was questioned.

Until that point, none of my publications, none of my scientific methods were never questioned or held "suspect".

When I had the audacity to suggest GMO's could be dangerous, and showed that was exactly the case, then my science became suspect.

When my research might require bio-tech companies to actually test their products, then I was suspect.

# Soil Foodweb Inc.

Because of the attack on my reputation and the harassment from Oregon State University

I started Soil Foodweb Inc. in 1996

Work with growers all over the world

Experience with all types of ecosystems

Tropical to Polar

Experience with all agricultural and landscape situations

We will go over examples of some of these systems, from small to large scale, natural landscape to agriculture and everything in-between