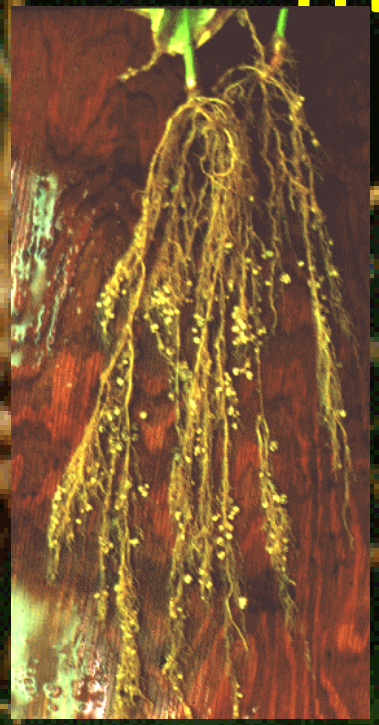
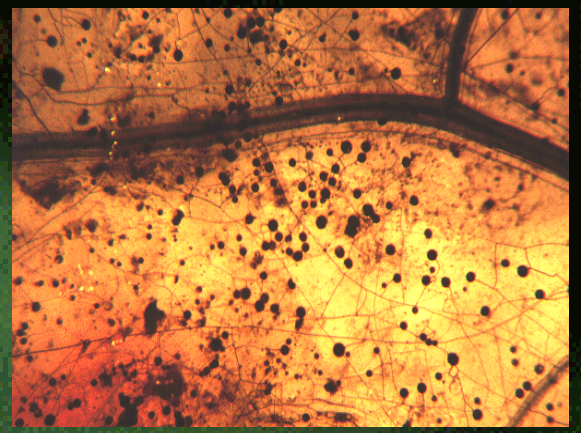
Biostimulants from the phytomicrobiome:

Microbe-to-plant signals as low input crop management technologies

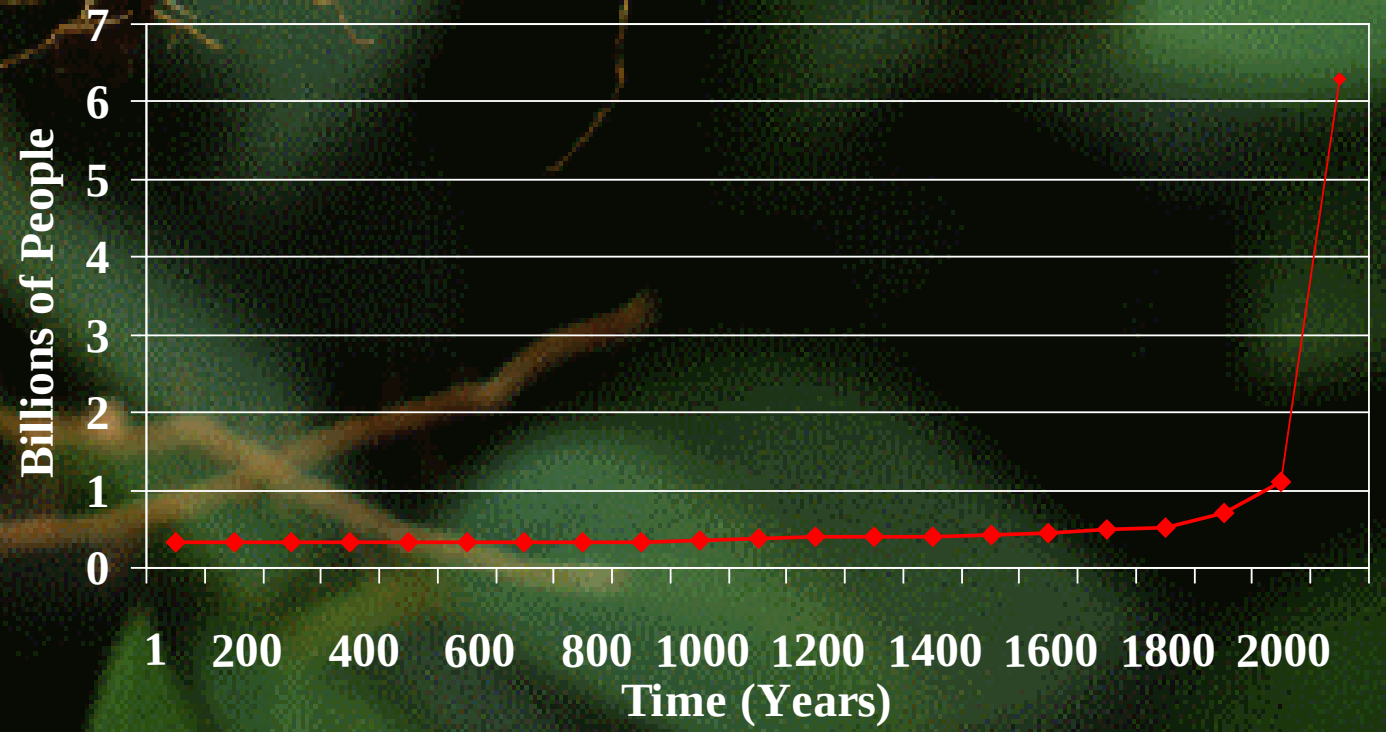


Donald L. Smith
Plant Science
McGill University



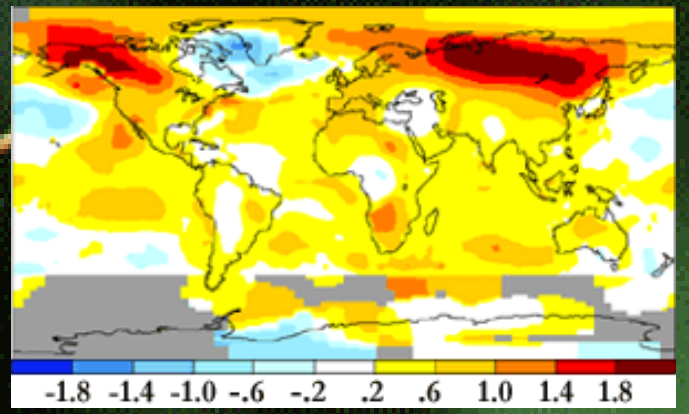
Need for Enhanced Food Production

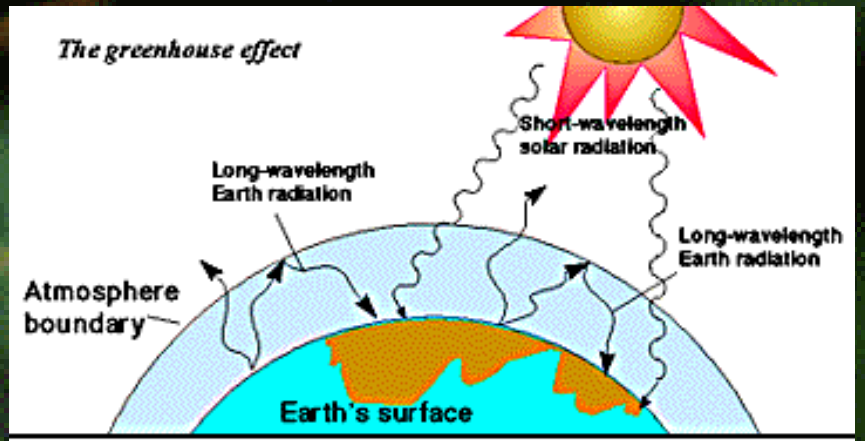
- Growing global population
  - Headed for 9-10 billion
- Changes in diet
  - More meat



Threat of Climate Change

- Melting
  - Mountain glaciers
  - Greenland ice sheet
  - Arctic sea ice
- 5 to 8 ºC warmer in Canada
- Birds return sooner in spring & flowering sooner
- More extreme weather conditions more often
  - Drought
  - Heat
  - Salinity
  - Flooding



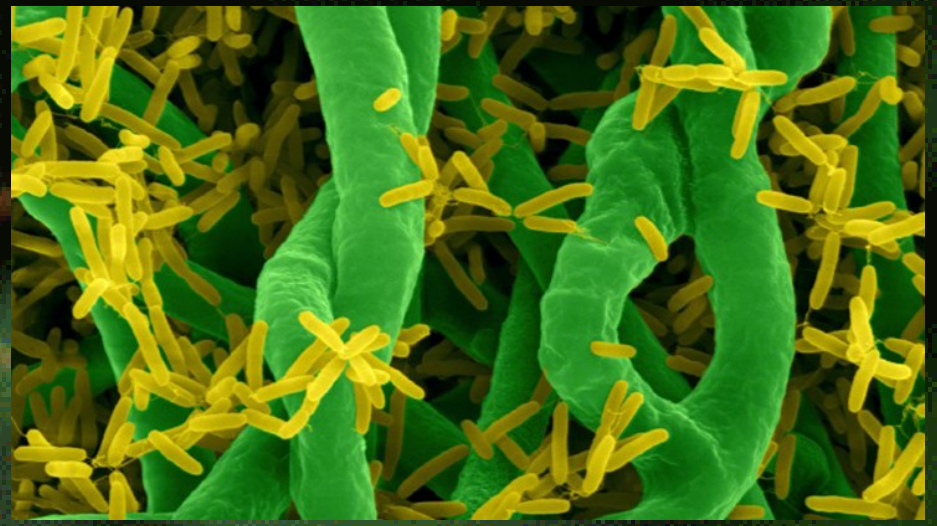


Biomass for Advanced Biofuels and the Bioeconomy

- A key step in the supply chain is reliable, high quality biomass
- Can be:
  - Crop or forestry residues
  - Purpose grown
    - Fast growing grasses
    - Plantation forestry
    - Generally grown on more marginal lands so more often stressed
  - If food crops, more biomass residue and more food
    - Food and fuel, not food versus fuel

The Phytomicrobiome

- Plants, like mammals, have a microbiome
  - Phytomicrobiome
  - Plant + phytomicrobiome is holobiont
- Present through all of terrestrial plant evolution
- Community of microbes associated with all plant parts
  - Roots, in humid soil, have most well developed
- Help in a wide range of ways
  - Nutrient mobilization
  - Hormone production
  - Disease control
  - Signals
    - Hormones of the holobiont

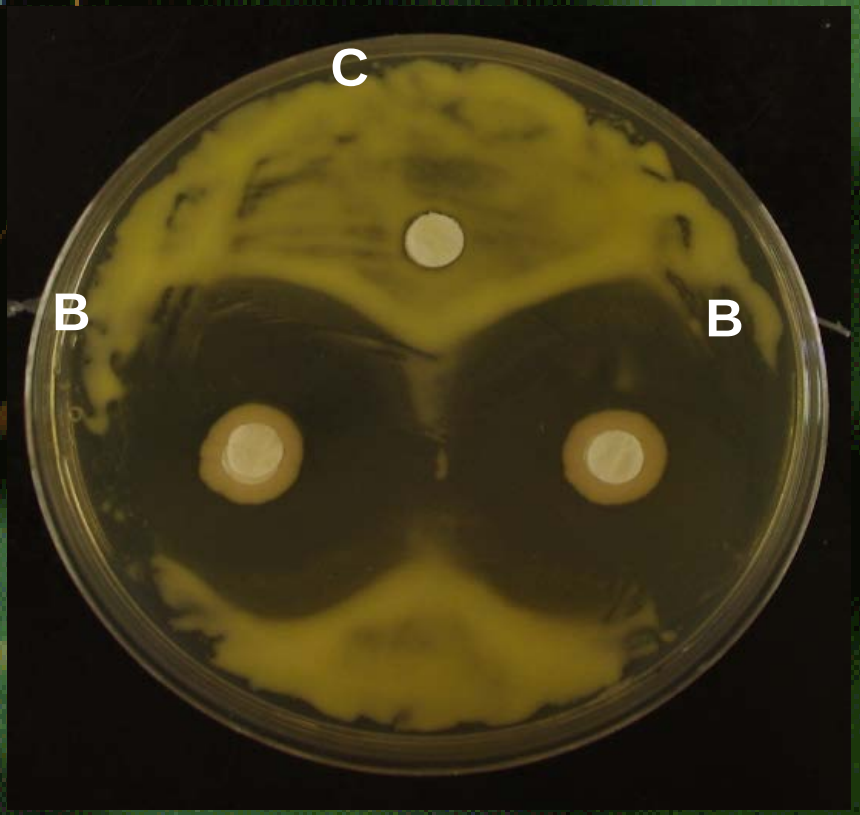


Adapting to Climate Change

- Develop inputs that adapt agriculture to worsening climate change conditions
  - Improve plant stress tolerance
  - Climate change resilient crop production
  - Reduced N_2O and CO_2 emissions

The Approach

- Biological inputs
  - Addition of small amounts of inexpensive materials that enhance crop growth
    - Reduced costs
  - Enhance stress resistance
    - Both abiotic and biotic
  - Improve yield



So Far

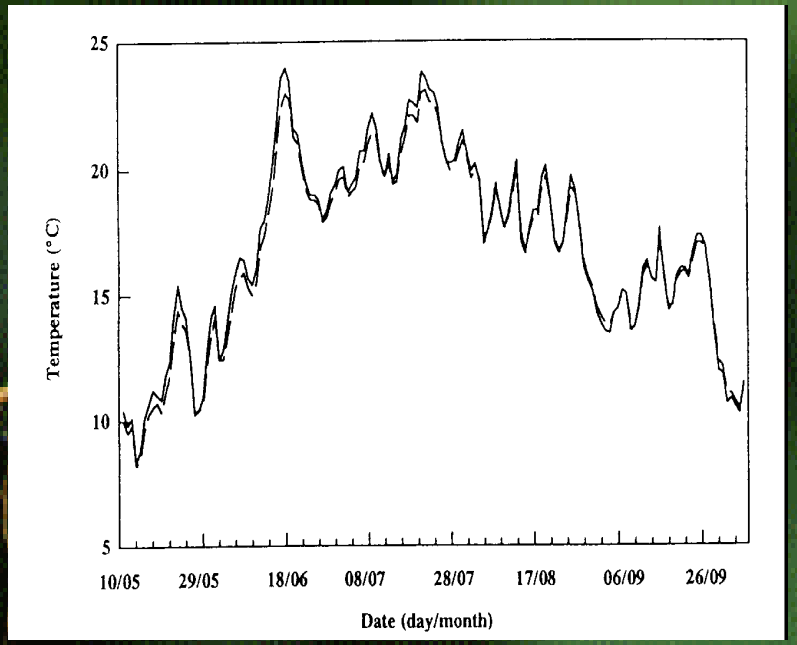
- N_2 fixation inoculants for legumes
- Rhizobia (*Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, etc.)
- Widely used for over a century
- Mycorrhizae for P uptake
- Mobilization of P and Zn

Example Line of Research

- It started with soybean
- Soybean evolved in the tropics and subtropics
- We agriculturalists have carried it further north, and south, ever since
- Its metabolism is adapted to and set up for relatively high temperatures
- The optimum temperature for soybean nodulation was known to be 25 to 30ºC
- Production in Quebec since late 1980s

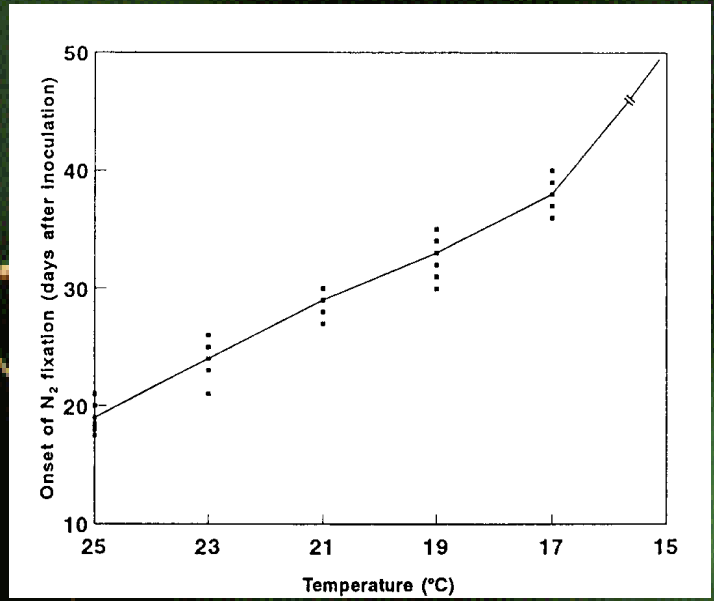
Something New!

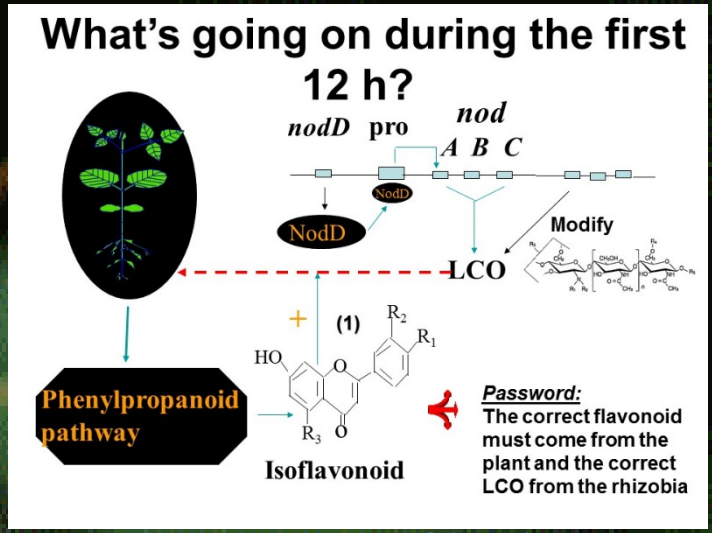
- We observed that soybean plants, under Quebec field conditions, frequently look pale green for several weeks after the nitrogen in the original seed was expended
- After this they “regreened”
- We wondered if nodulation was inhibited by low soil temperatures early in the season



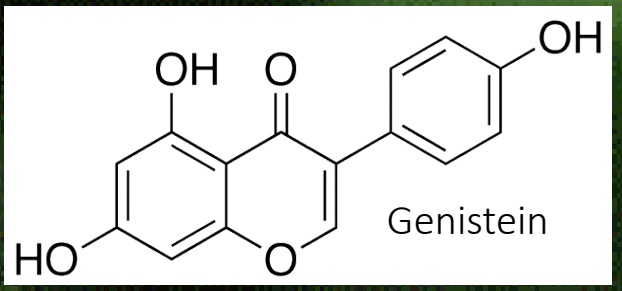
The Problem

- Low root zone temperatures slowed onset of N_2 fixation
  - 1-2 days $^oC^{-1}$ to 17 ^oC
- The cause was disruption in signaling during the first stage of nodulation
  - Symbiont recognition



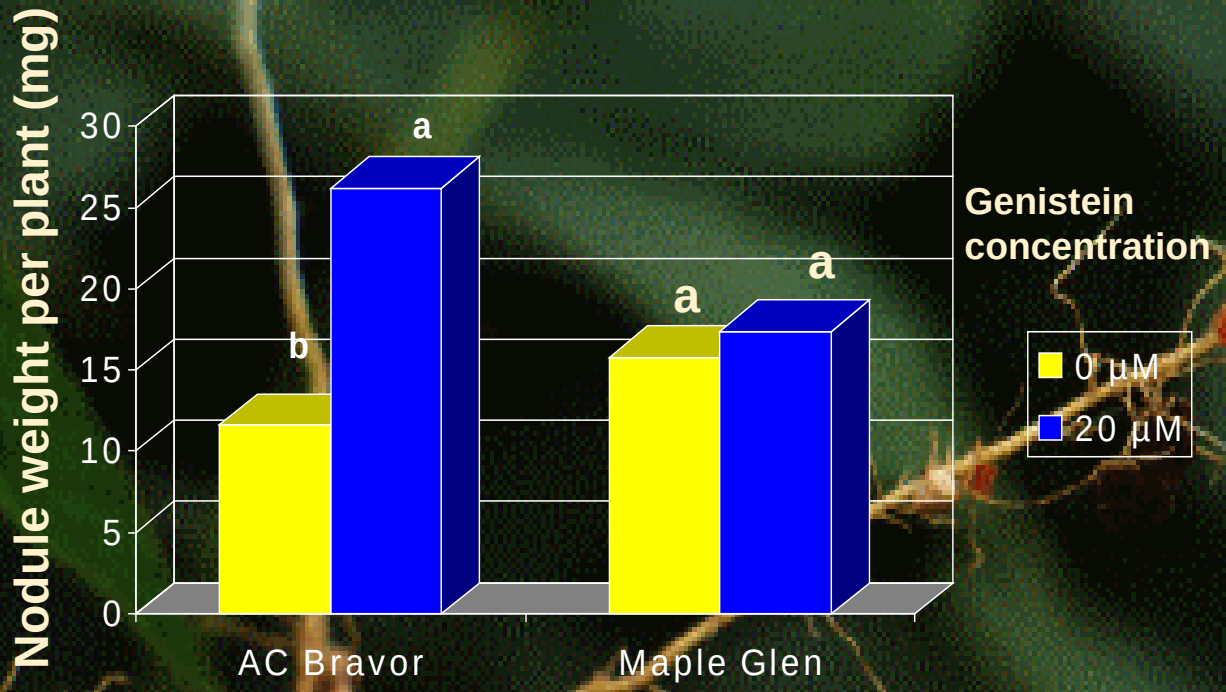


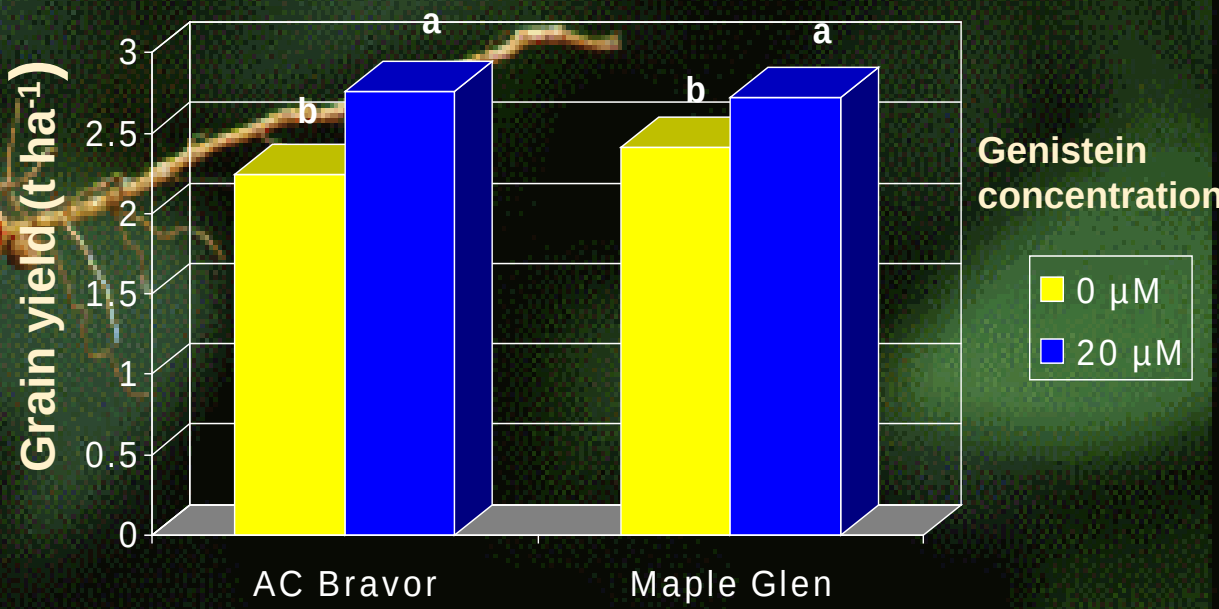
A Solution



- Adding an appropriate isoflavonoid signal (genistein) to *Bradyrhizobium japonicum* cells before application as inoculant switches on the *Nod* genes in the bacterial cells
- The *B. japonicum* cells then began to produce and excrete the return signals
  - Lipo-chitooligosaccharides (LCOs)

N_2 Fixation and Growth - Genistein





June 17 sampling

- N_2 fixation started 4 to 5 days sooner
- Total N fixed without genistein (^{15}N dilution estimate) was 53 kg ha^{-1}, while with genistein it was 95 kg ha^{-1}: 80 % increase.

- Yield increases have been as high as 40 %, with most being in the 10 to 20 % range

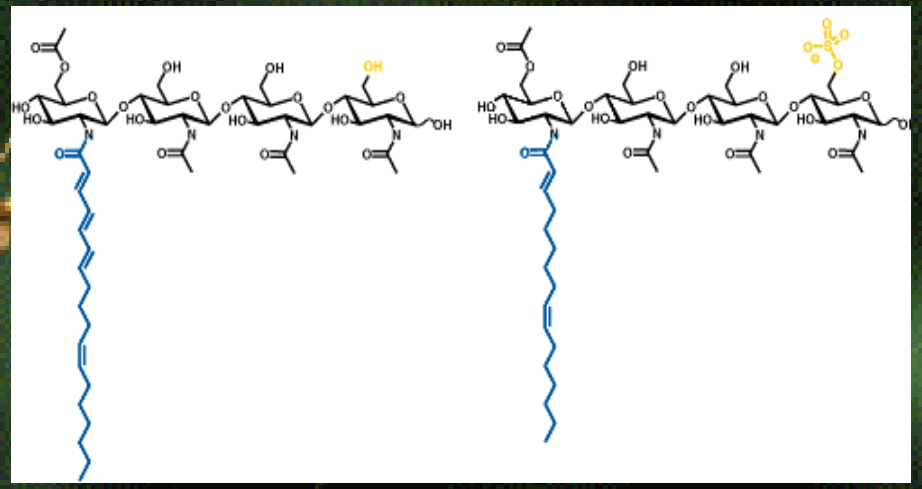
Commercialization

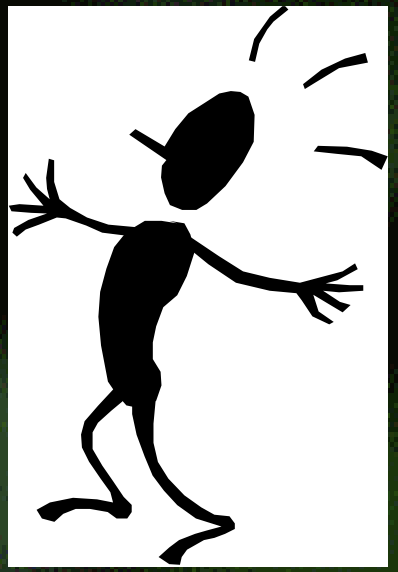
- Established a spin-off company
  - Bios Agriculture Inc.
  - To keep a technician employed post recession
- Spin-offs were quite new at McGill University
  - 5% share, non-dilutable
- The usual things:
  - Venture capital and other investments
  - Regulatory requirements must be satisfied

BIOS AGRICULTURE inc.

Serendipity & Follow Up

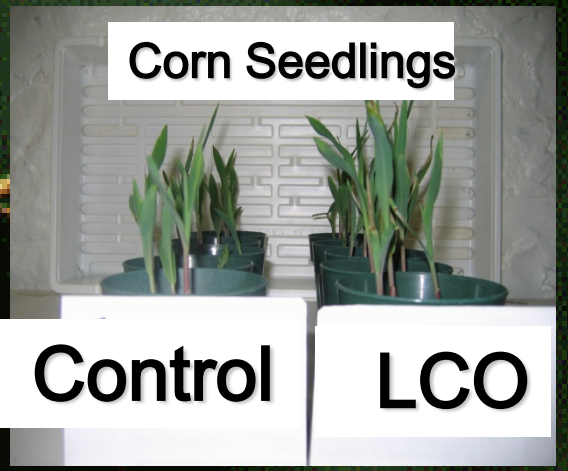
- Earlier emergence observed at field sites where genistein applied
- What did this mean?
- *Experiment*:
  - seeds in water
  - genistein alone
  - *Bradyrhizobium japonicum* alone
  - genistein and *B. japonicum* together
  - only the last treatment accelerated germination - active material was the LCO, the bacteria-to-plant signal

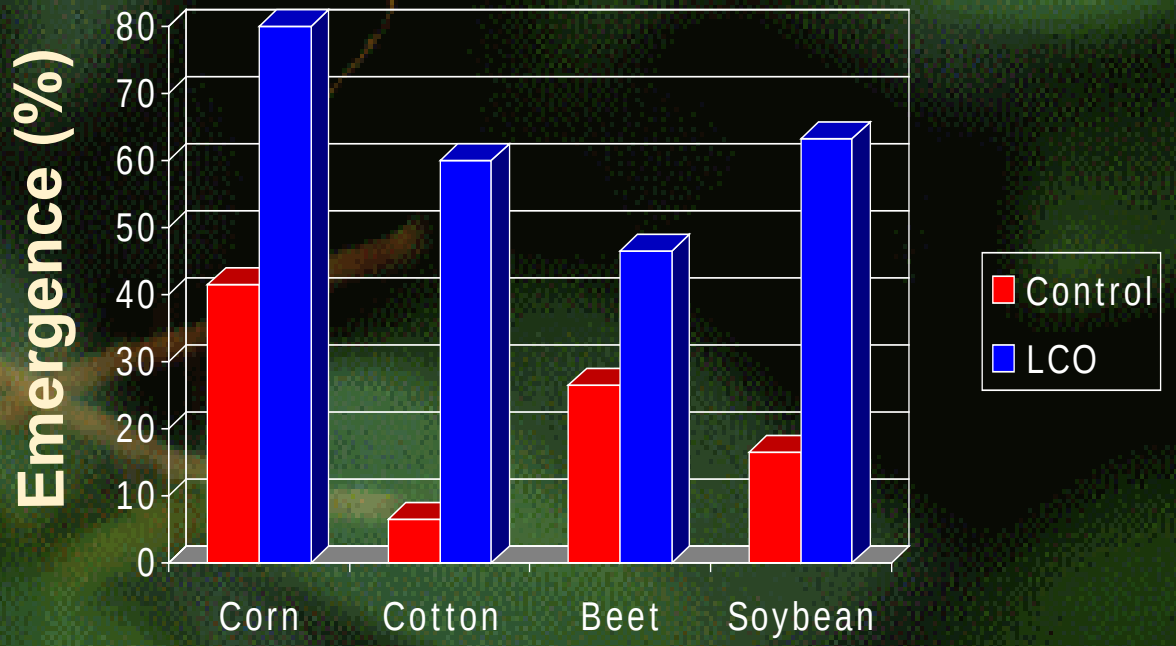




New LCO Activity

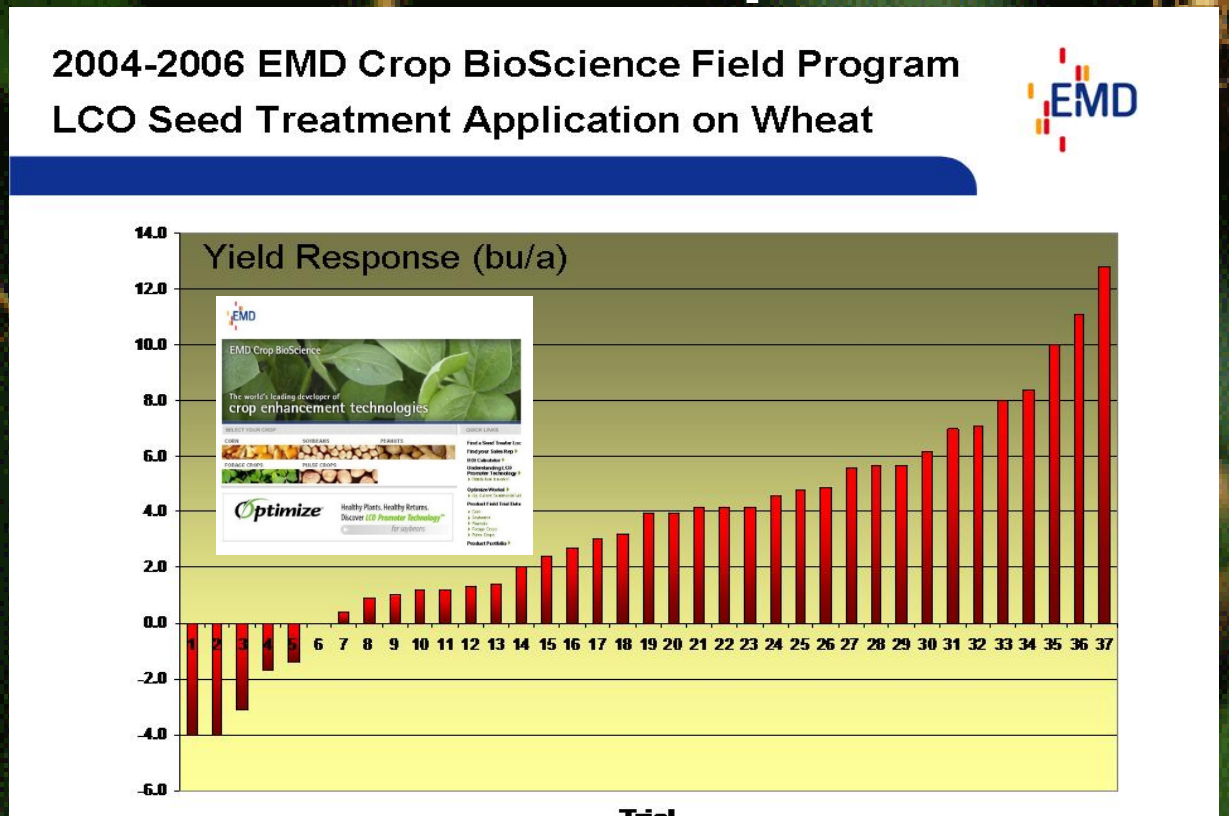
- We also found that the return signals (lipo-chitooligosaccharides) also directly stimulate growth of non-legume plants, and this can increase yields

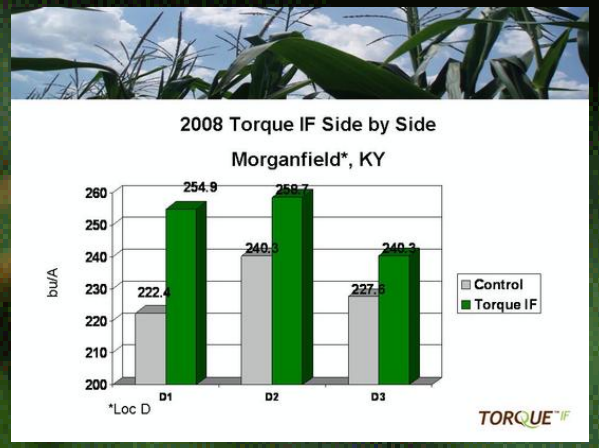


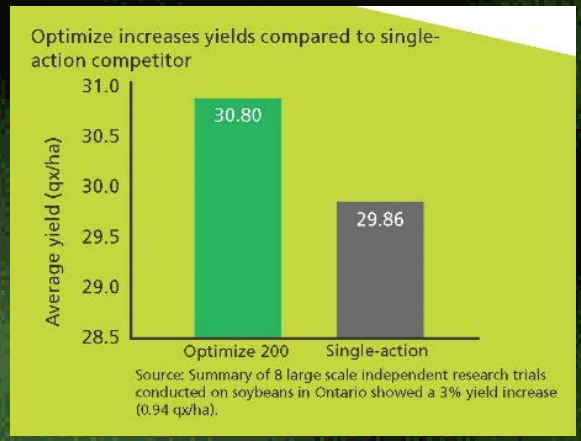


Commercialization

- Bios Agricluture eventually taken up by Agribiotics (also a Canadian Company)
  - Agribiotics was purchased by EMD
  - EMD became part of Novozymes







Jasmonates – An Alternative

- Flavonoids have two problems
  - Extremely expensive (up to $1000 per mg)
  - Damaging to rhizobial cells (20 µM slows growth)
- A paper suggested that jasmonic acid might also activate *nod* genes
  - Much less expensive
  - Not damaging to rhizobial cells
  - Generally involved in stress related signaling
    - Biotic and abiotic stresses
    - In this case, signal to the microbe that the plant is stressed and the microbe responds with a signal to manage stress
- Both jasmonates and flavonoids stress realted in plant

Findings

- Jasmonates induced the expression of *nod* genes in *B. japonicum*
- Also induce return LCO signal production
- However, the strain specificity is very different from isoflavonoids
- Accelerates nodulation at low root zone temperature
- Commercialized

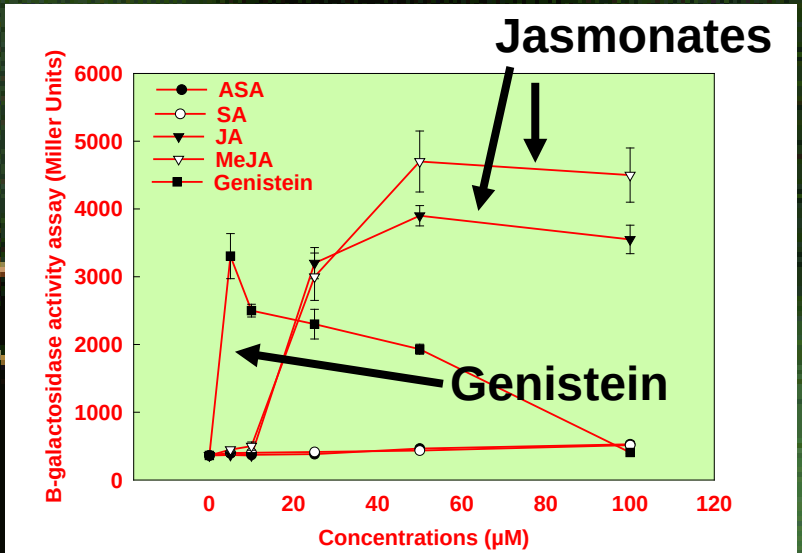
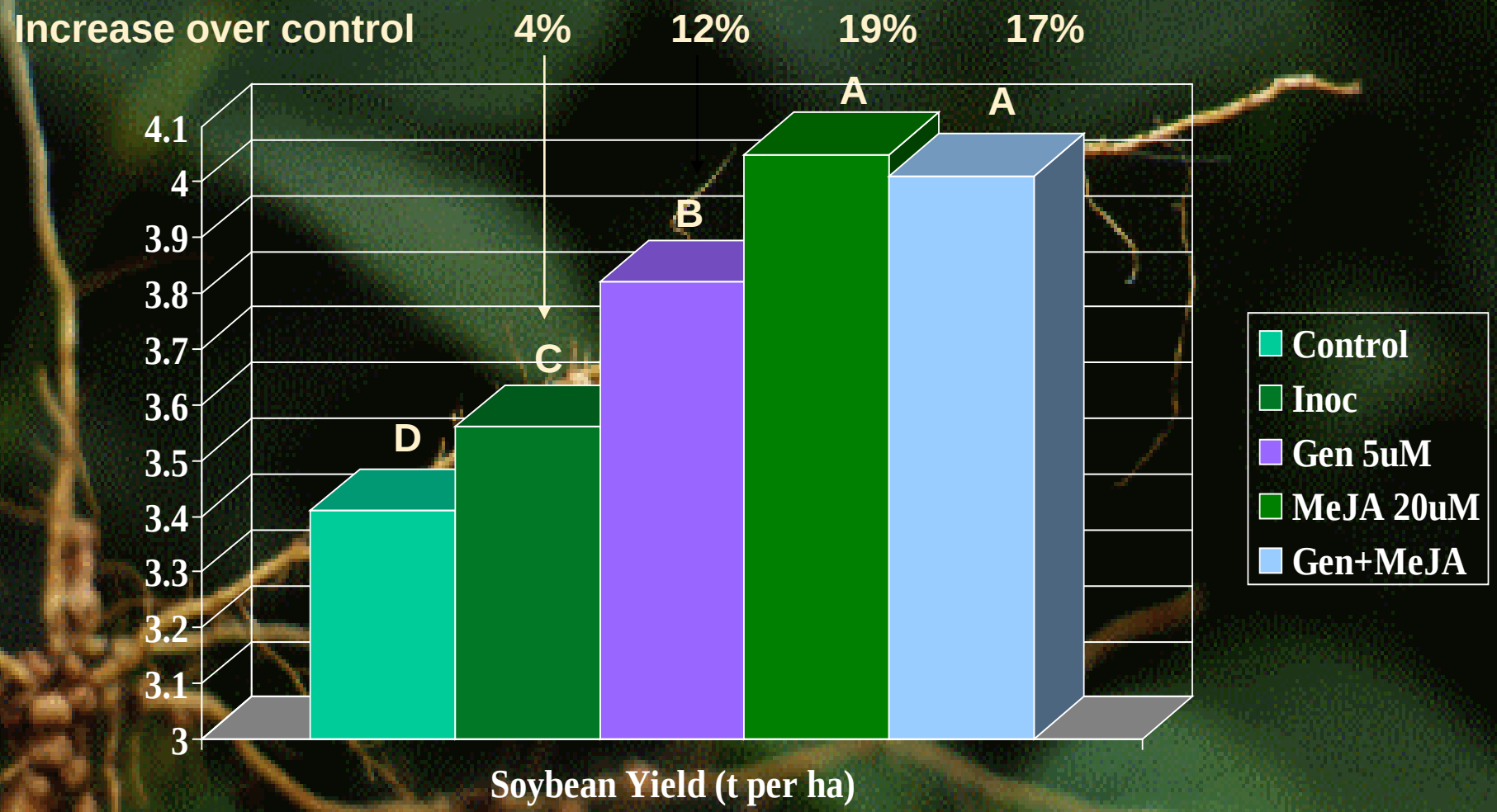


Figure 1: Effect of various concentrations of genistein, JA, MeJA, SA and ASA on the induction of *nod* genes in *Bradyrhizobium japonicum* strain USDA3 as measured by B-galactosidase activity (Miller Units).

BASF
The Chemical Company

BECKER UNDERWOOD
Inventing the Future

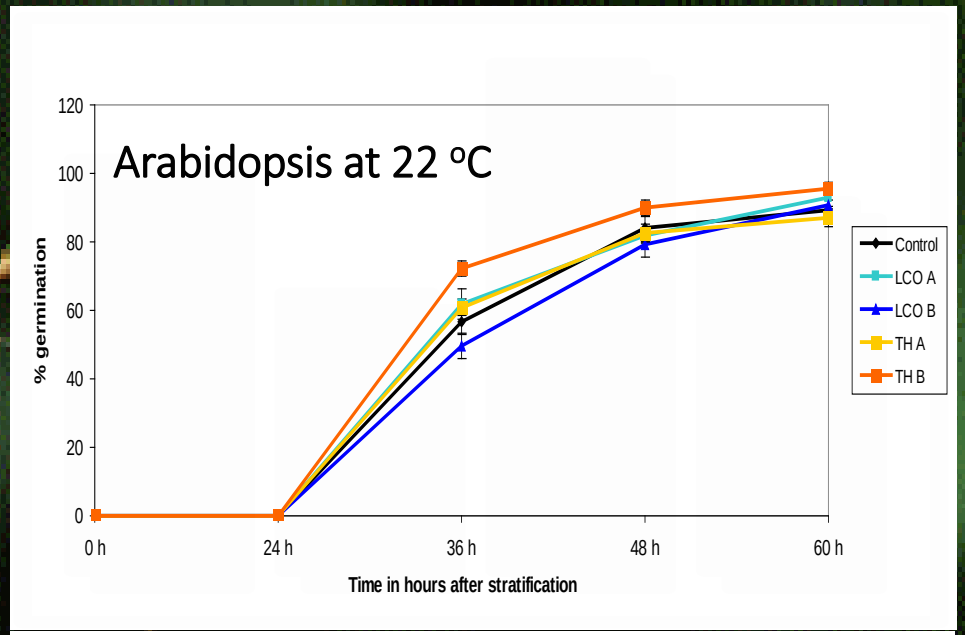
Effect of Methyl Jasmonate on Yield

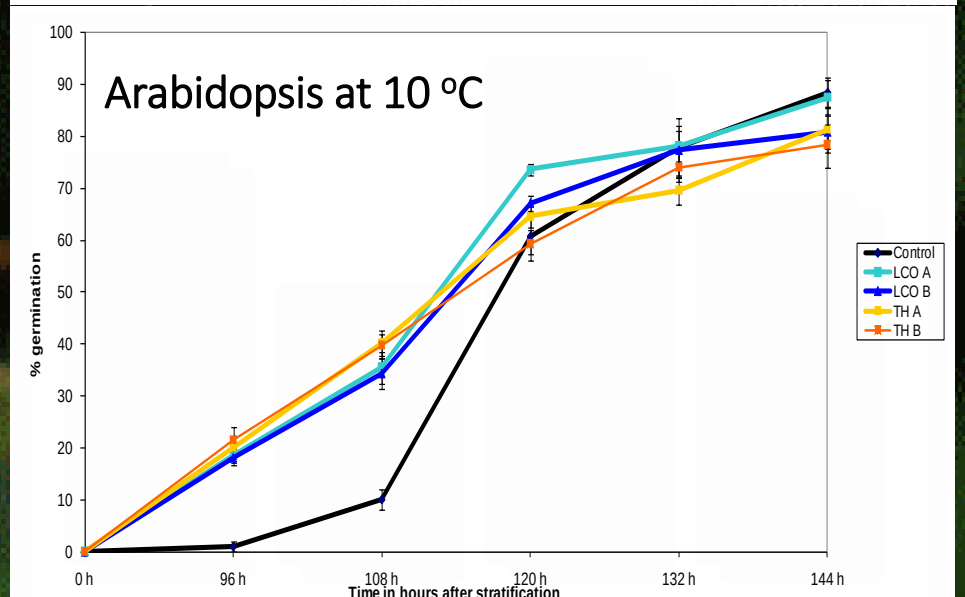


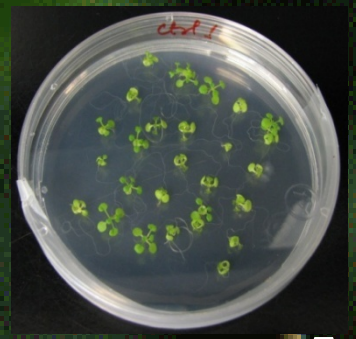
Two sites, two strains at each, this is typical data – strain 532C at a sandy-loam site.

Environment - Temperature and Response

- In the lab responses could be variable and frustrating
- In the field responses were strongest in cold springs on heavy soils
- Over the last few years have shown that low temperatures make the lab results clearer & consistent

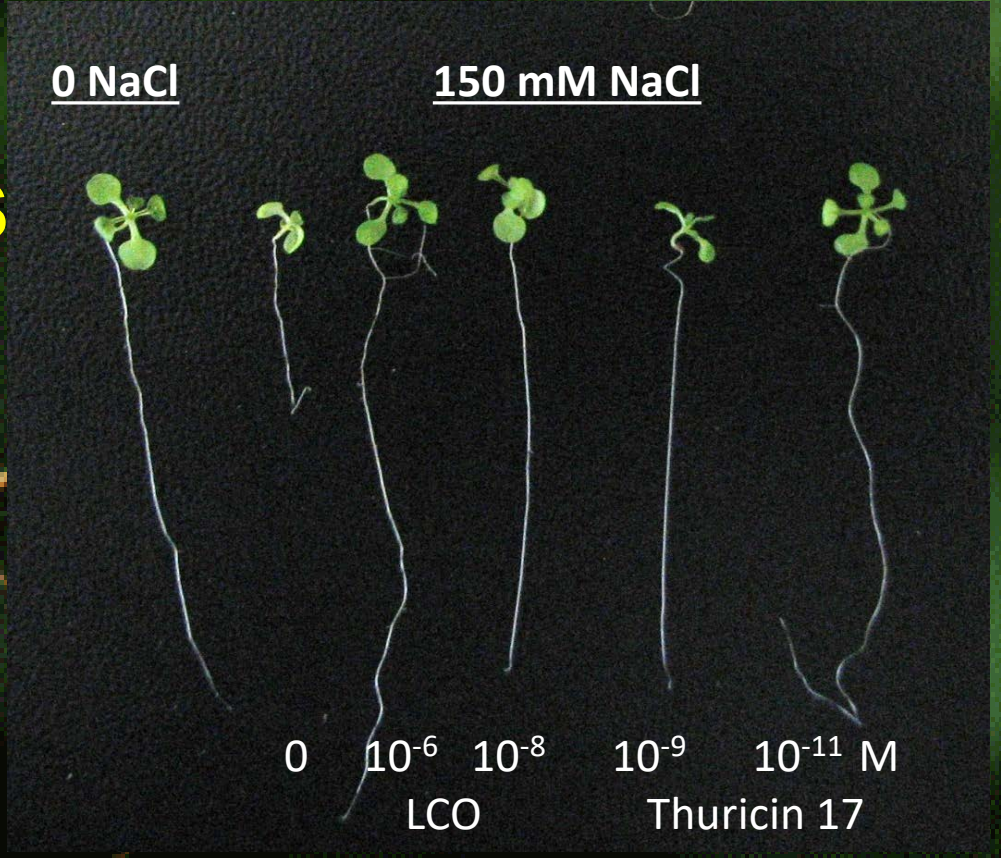


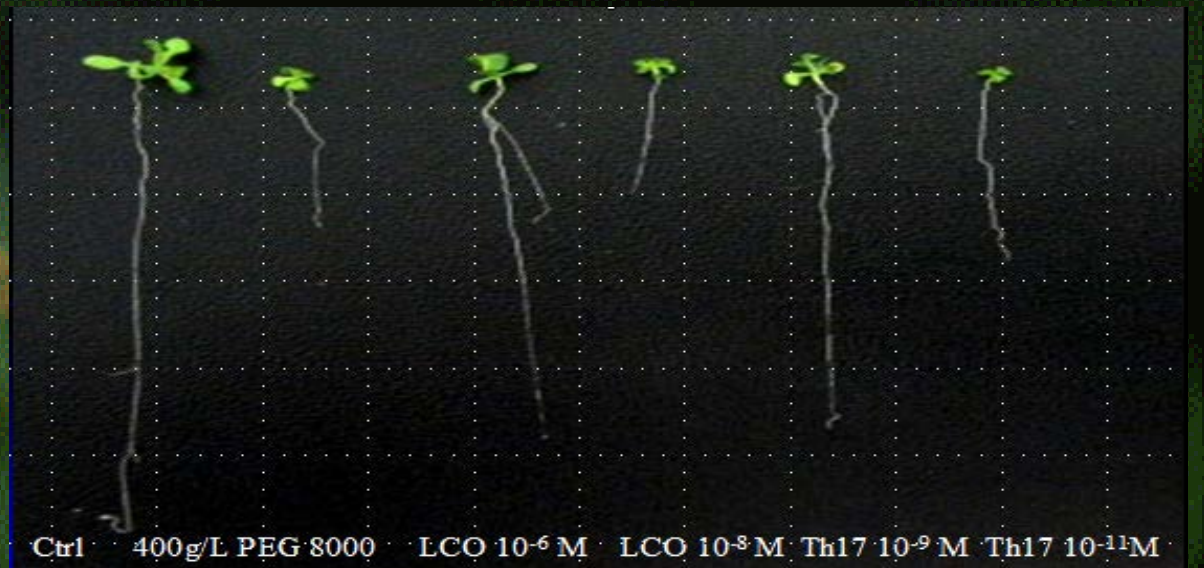




Salt & Drought Stress

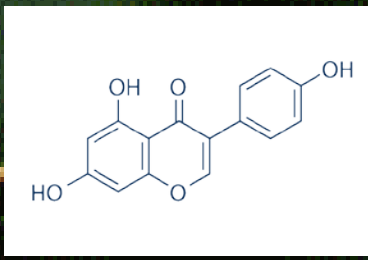
- Arabidopsis
- Seeds on petri plates with signals
- Control and 150 mM NaCl
- Signals improved growth under salt stress

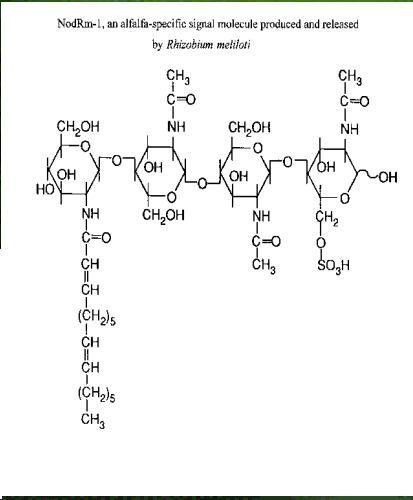




Hormones of the Holobiont

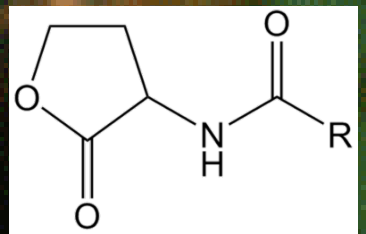
- Effective at very low concentrations
- Regulate plant activity
  - Large changes in gene espression, protein production, hormone profile
- Regulate other microbes
- Powerful plant productivity effects
- Inexpensive to apply
- Low environmental impact
  - Small amount
  - Already produced in environment
- Very poorly understood
- Enormous potential
  - New network?
  - > 10 strains in lab now





Phytomicrobiome Potential

- Wide range of species in the phytomicrobiome
- Most powerful control factor is the plant
  - Development
  - Stress
  - Nutrition
- Are key to plant growth and productivity
- Management
  - Inoculation
  - Hub species
  - Signals
    - Microbe-to-plant
    - Plant-to-microbe
    - Microbe-to-microbe



Development of Biologicals - Who?

- Scope is large
- Collaboration
  - Basic research labs
    - Academic
    - Government
  - Industry laboratories

novozymes®
Rethink Tomorrow

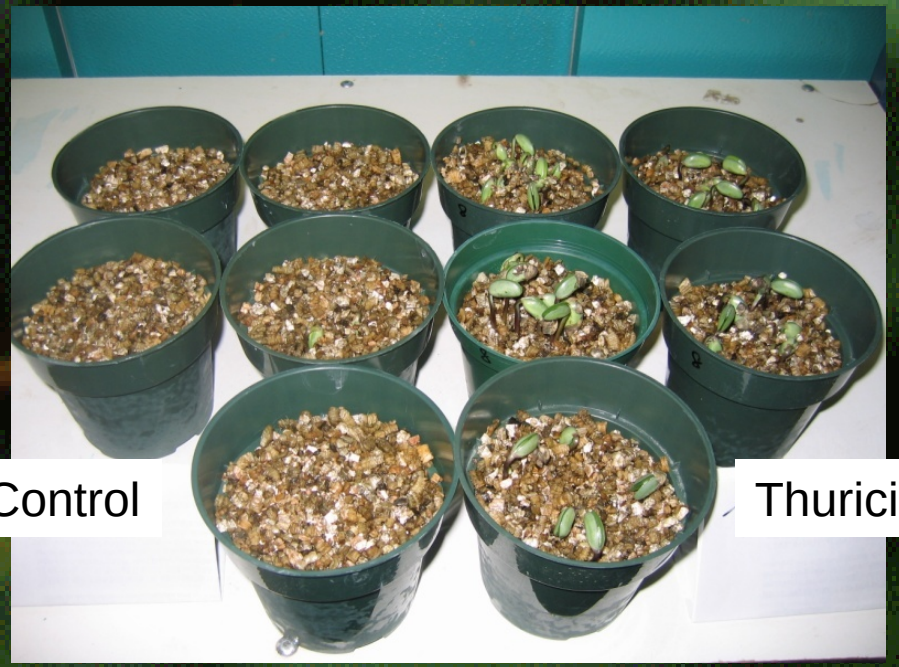
BASF
The Chemical Company

McGill
UNIVERSITY

The Approach

- Sample plant-associated microbes
  - The phytomicrobiome
- Efficient / rapid screening to identify microbes of interest
- Biological assessments
- Agronomic assessments

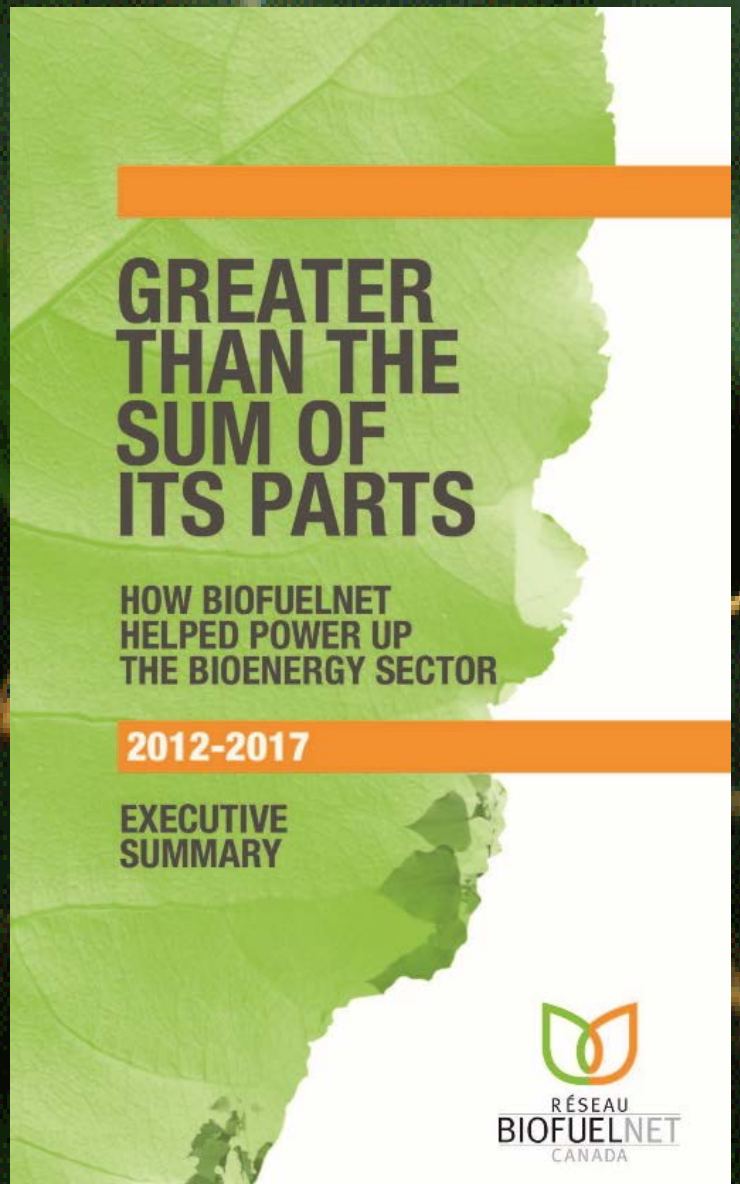
KEM GLOBAL
RENEWABLE · NATURAL · ORGANIC



Industrial Collaborators

- Synagri
- EVL
- BASF
- RavenQuest
- Ulysse
- Agroworld Natural Products
- Inocucor
- Axter

BioFuelNet - NCE



IMPACT BY THE NUMBERS

$25 MILLION
NCE

$21 MILLION
INVESTED BY STAKEHOLDERS

713
HQPS TRAINED

230
RESEARCHERS ENGAGED

29
UNIVERSITY PARTNERS

127
INDUSTRY PARTNERS

38
PRODUCTS AND INNOVATIONS

22
LICENSING AGREEMENTS

5
POLICY BRIEFS

5
SPIN-OFF COMPANIES

970+
PUBLICATIONS

4,500+
CITATIONS

108,000
WEBSITE VISITS IN 2016

6,550
VIEWS OF 24 YOUTUBE PRESENTATIONS

3,500+
SOCIAL MEDIA FOLLOWERS

Acknowledgements:

- **Past and Present Students**
  - Dr. Feng Zhang
  - Dr. Narjes Dashti
  - Dr. Pan Bo
  - Dr. Yuming Bai
  - Elizabeth Gray
  - Dr. Fazli Mabood
  - Keldeagh Lindsay
  - Dr. Sowmya Subramanian
  - Dr. Xiaomin Zhou
  - Nan Wang
- **PDFs & Research Associates**
  - Dr. Xiaomin Zhou
  - Dr. Balakrishna Prithiviraj
  - Dr. Alfred Souleimanov
  - Dr. Kung Dong Lee
  - Dr. Woo Jung
  - Mike Lewis
  - Stewart Leibovitch

ALSO: Trevor Charles at U Waterloo

Macdonald Campus of McGill University
Faculty of Agricultural & Environmental Sciences

The End

Thank You!