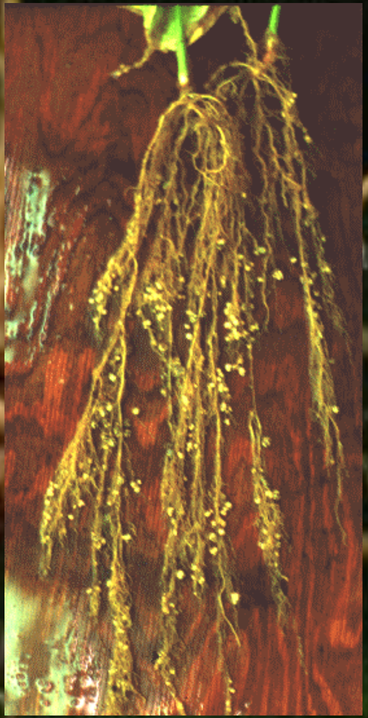
Hormones of the Holobiont: As Biostimulants for Climate Change Resilient Crop Agriculture



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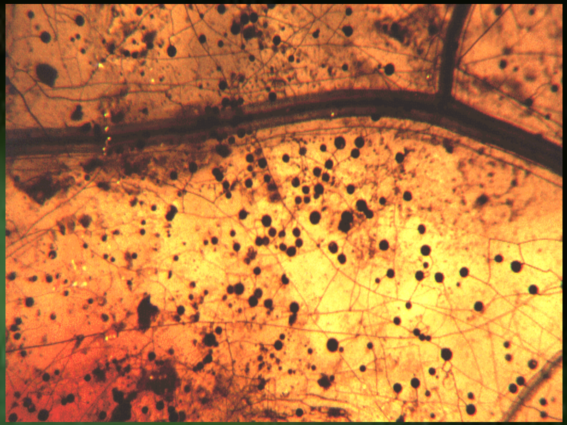
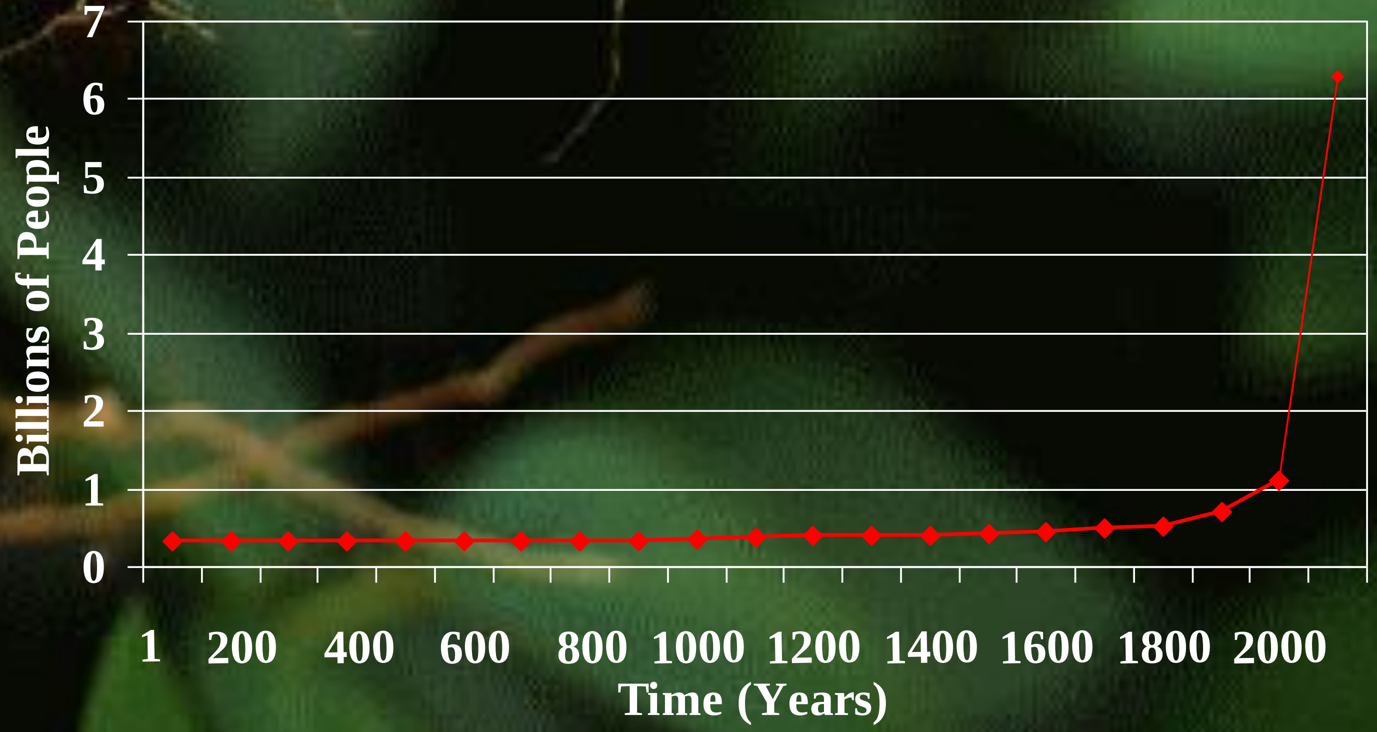


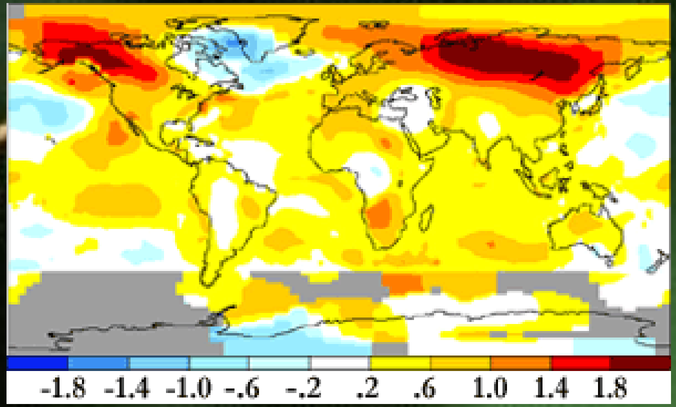
Need for Enhanced Food Production

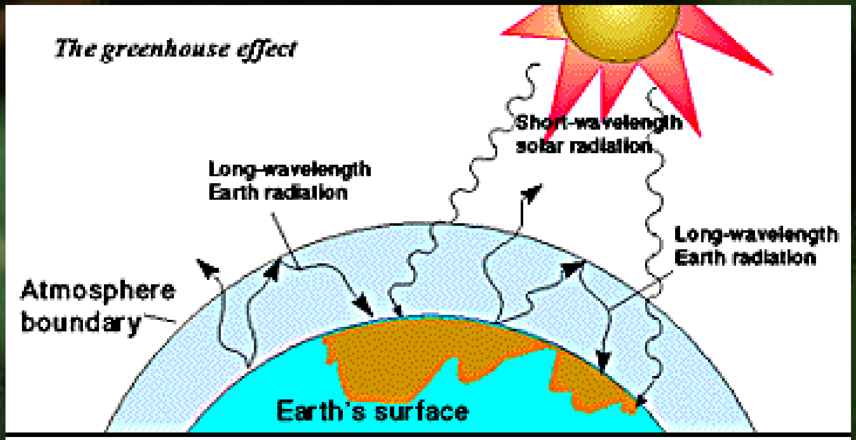
- **Growing global population**
  - **Headed for 9-10 billion**
- **Changes in diet**
  - **More meat**



Threat of Climate Change

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



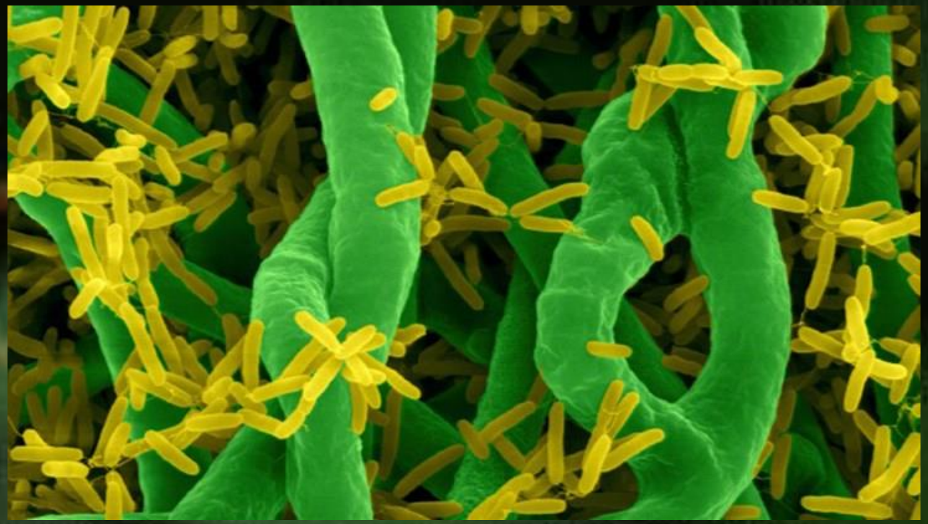


Biomass for Advanced Biofuels and the Bioeconomy

- 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 on more marginal lands so more stressed more often

The Phytomicrobiome

- Plants, like mammals, have a microbiome
  - Phytomicrobiome
- 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

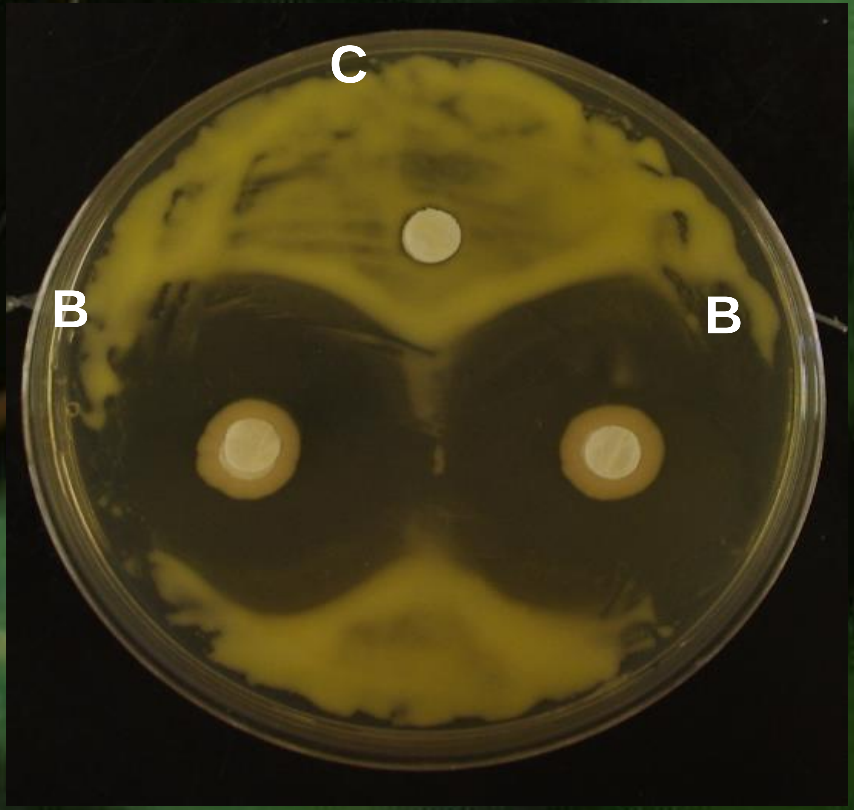


Adapting to Climate Change

- **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**

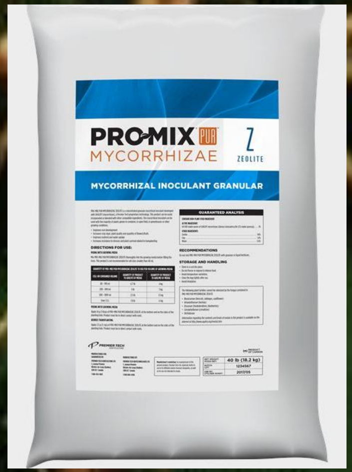
The Approach

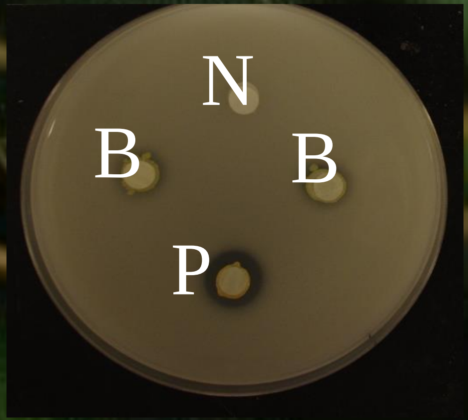
- Biological inputs
  - Addition of small amounts of inexpensive materials that enhance crop growth
    - Reduced costs
  - Enhance stress resistance
    - Both abiotic and biotic
  - Improve yield



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



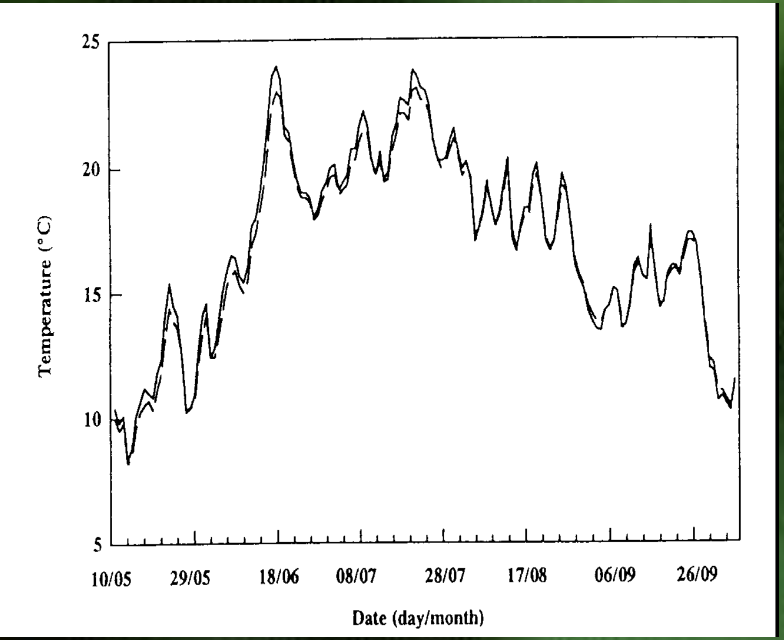


Example Line of Research

- 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 $30^{o}C$
- Production in Quebec since late 1980s

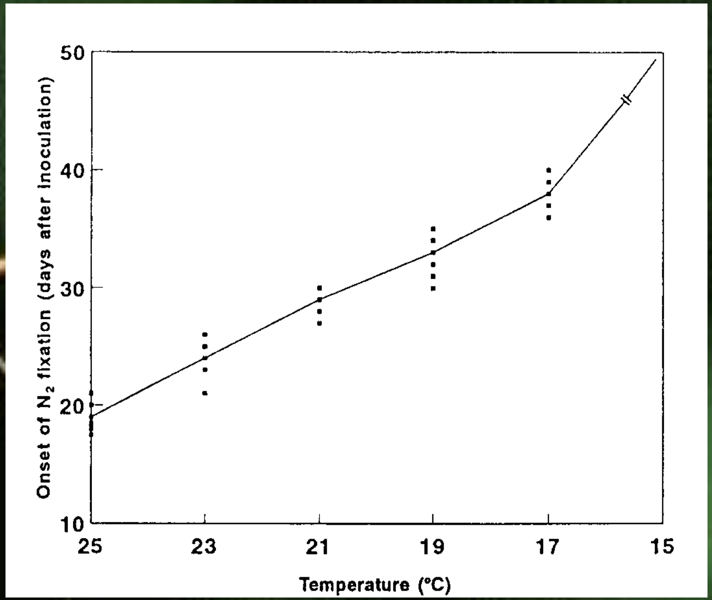
Something New!

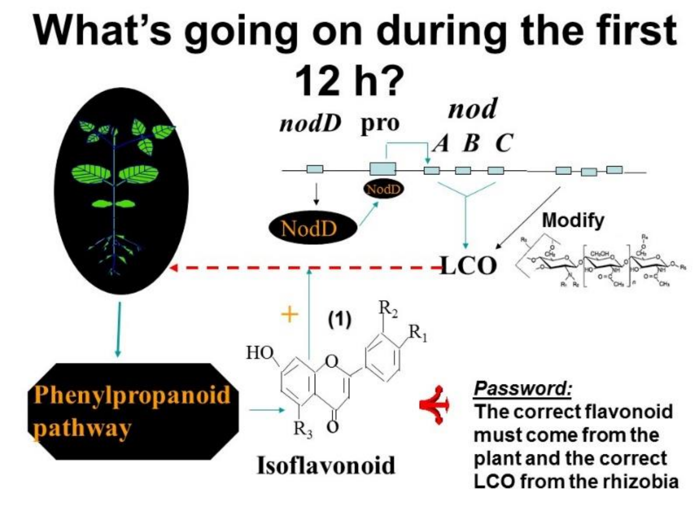
- **We observed that soybean plants, under Quebec field conditions, frequently look pale green for several weeks after the nitrogen in the original seed was expended**
- **After this they "regreened"**
- **We wondered if nodulation was inhibited by low soil temperatures early in the season**



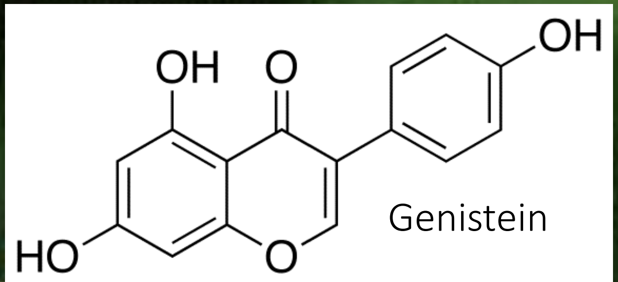
The Problem

- **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**



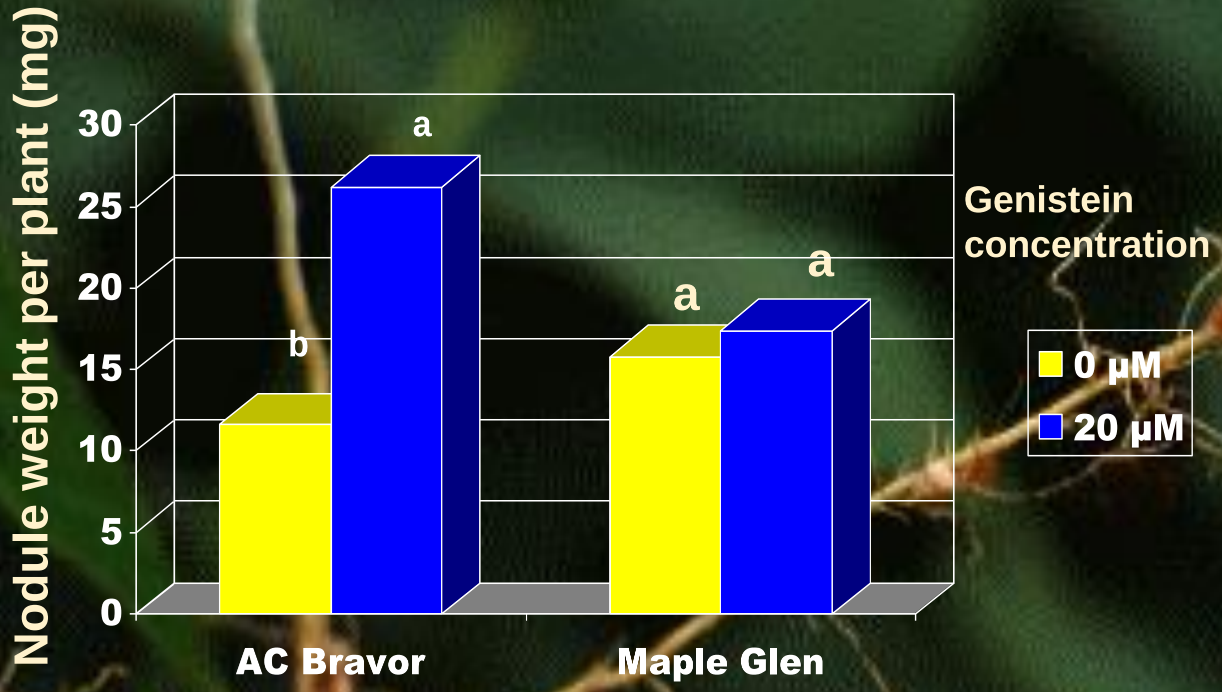


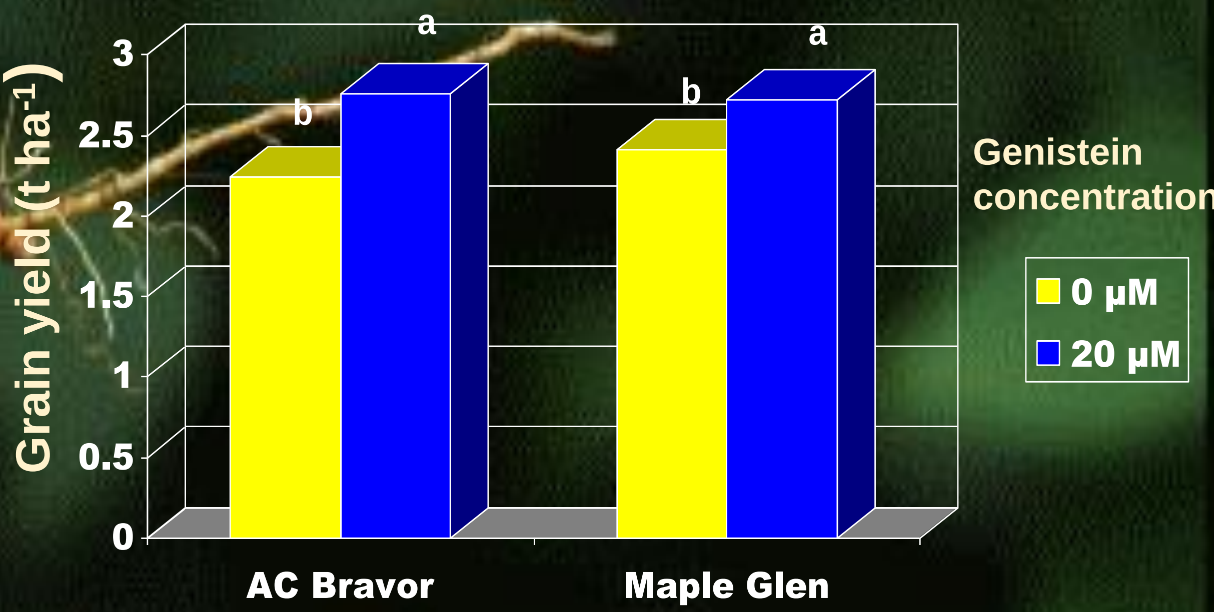
A Solution



- **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



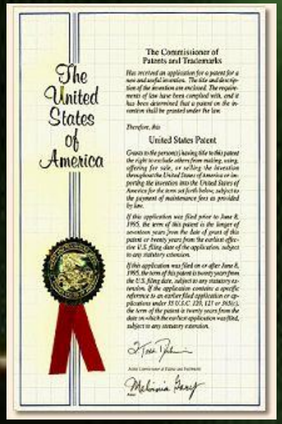


June 17 sampling

- 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

Intellectual Property



- Report of invention
  - To McGill
- Patent search
- Patent filing – Company pays
  - Novelty
  - Utility
  - Cost: $15K Canada-US, $150K for global

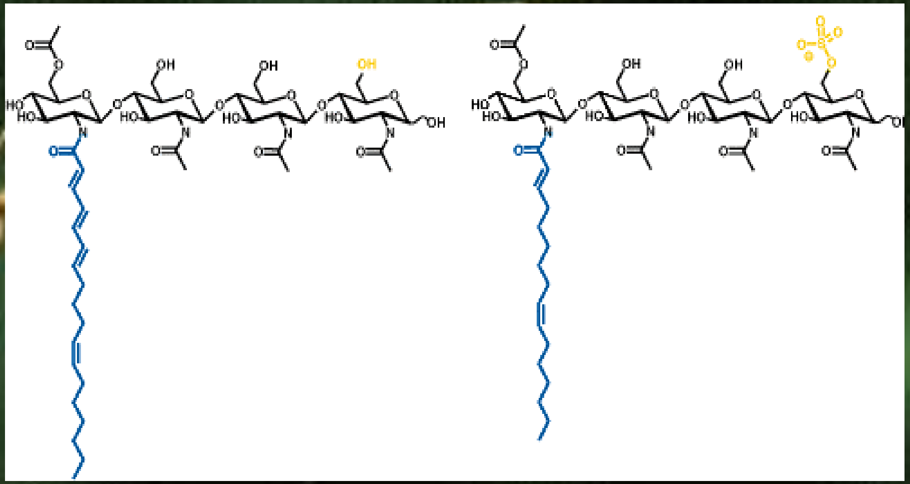
Commercialization

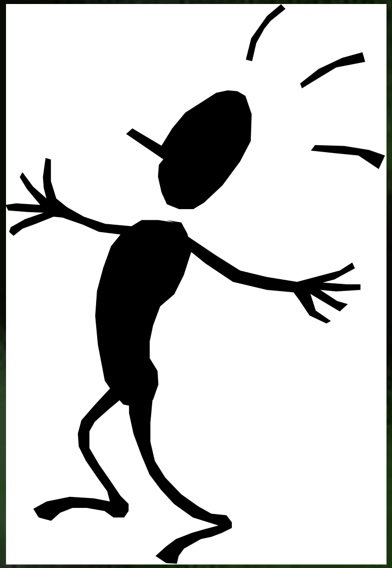
- Established a spin-off company
  - Bios Agriculture Inc.
  - To keep a technician employed post recession
- Spin-offs were quite new at McGill University
  - 5% share, non-dilutable
- The usual things:
  - Venture capital and other investments
  - Regulatory requirements must be satisfied

BIOS AGRICULTURE inc.

Serendipity & Follow Up

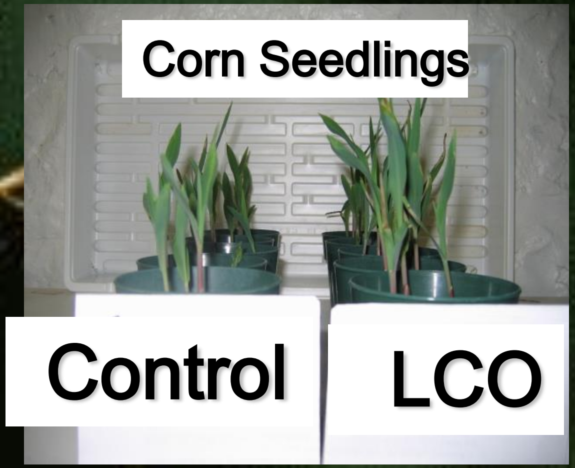
- **Earlier emergence observed at field sites where genistein applied**
- **What did this mean?**
- ***Experiment*:**
  - **seeds in water**
  - **genistein alone**
  - ***Bradyrhizobium japonicum* alone**
  - **genistein and *B. japonicum* together**
  - **only the last treatment accelerated germination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

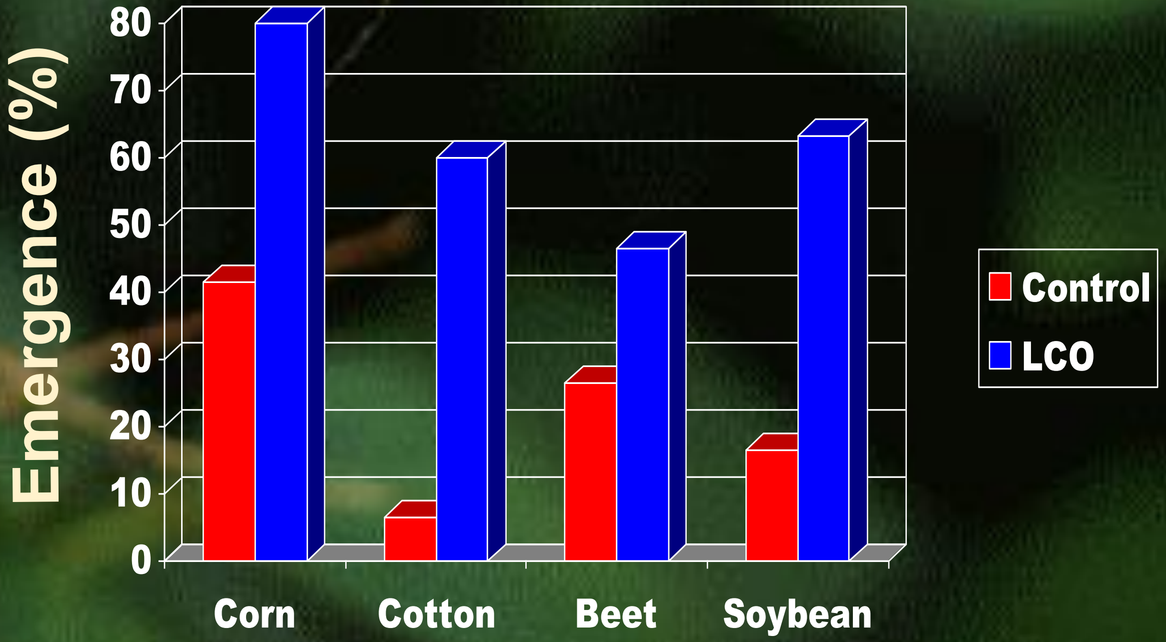




New LCO Activity

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





Commercialization

Optimize
LCO Promoter Technology® for Soybeans

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

AGRIBIOTICS Inc

EMD

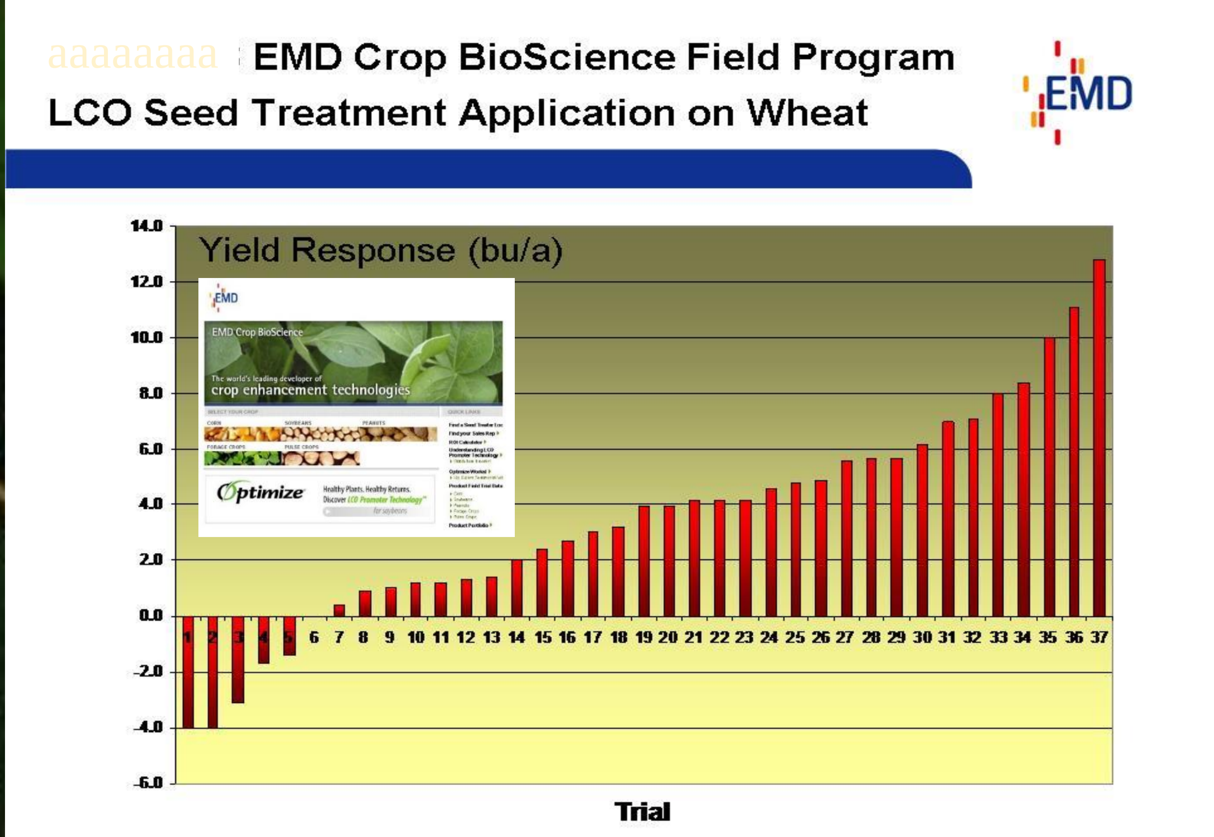
EMD Crop BioScience

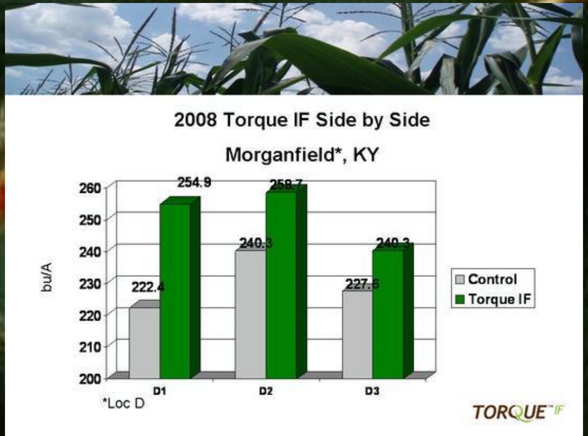
novozymes®
Rethink Tomorrow

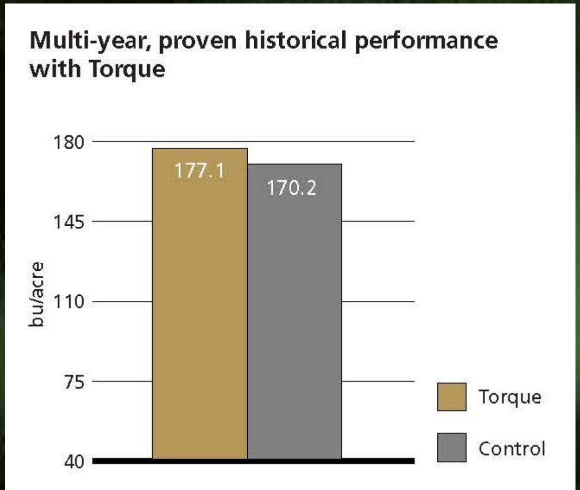
LCOs and Industry

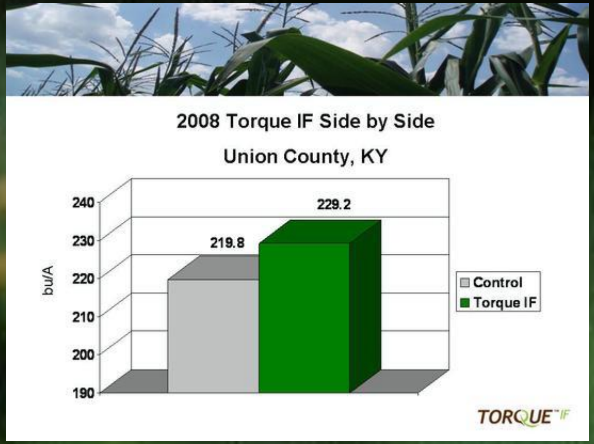
EMD Crop BioScience

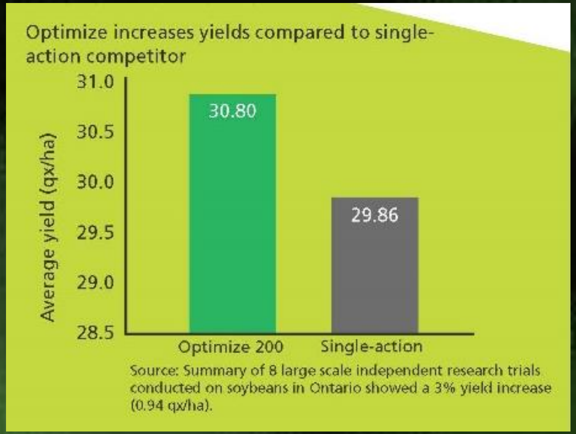
novozymes
Rethink Tomorrow











Jasmonates – An Alternative

Jasmonic Acid

- 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**

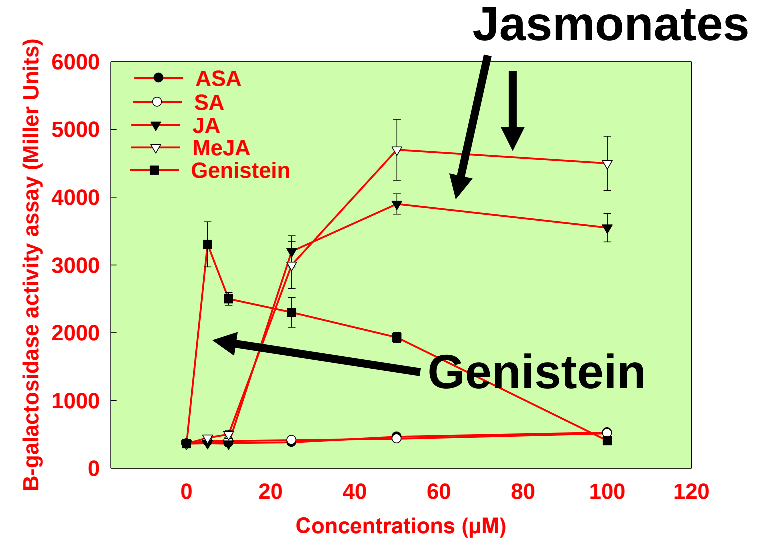
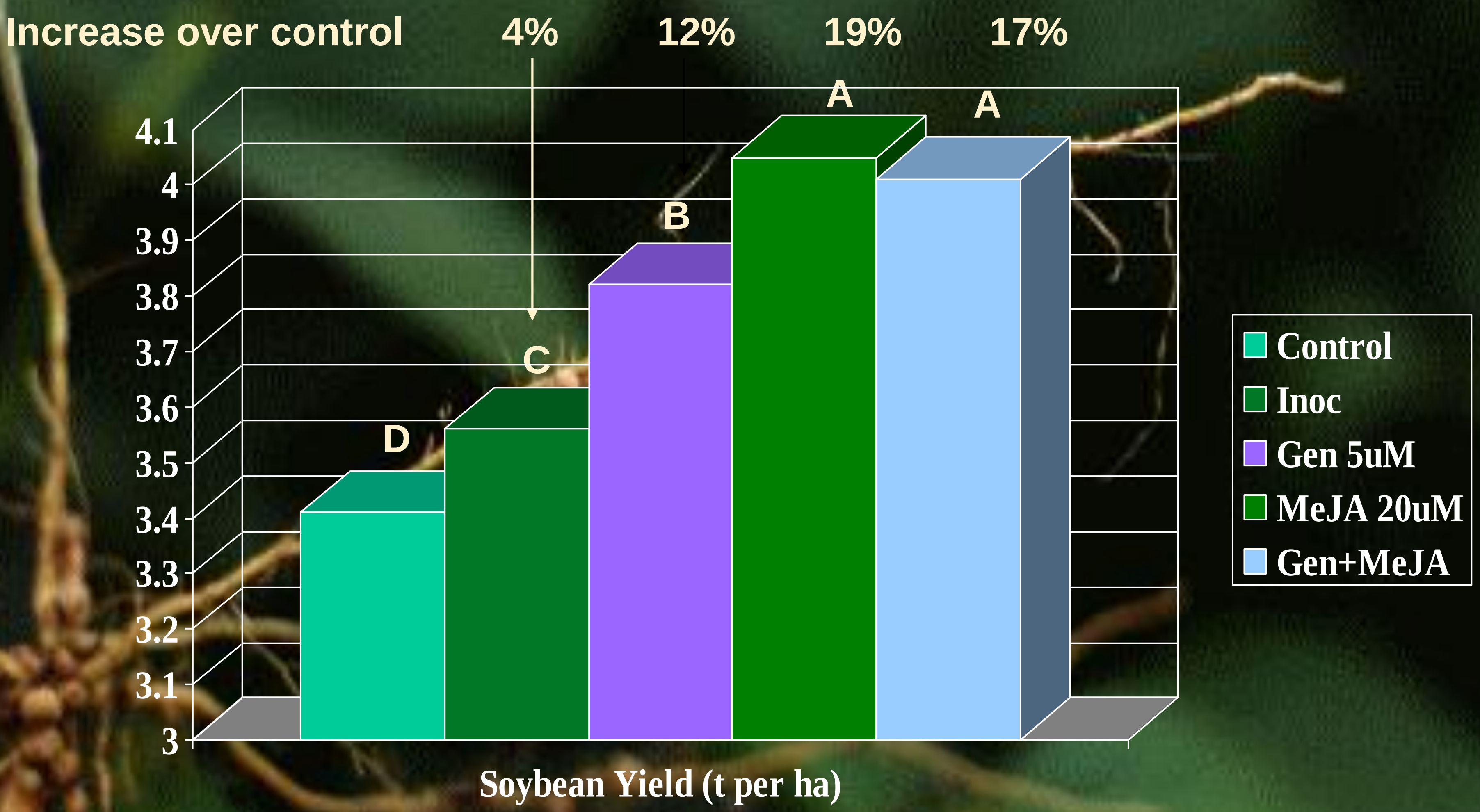


Figure 1: Effect of various concentrations of genistein, JA, MeJA, SA and ASA on the induction of *nod* genes in *Bradyrhizobium japonicum* strain USDA3 as measured by B-galactosidase activity (Miller Units).

BASF The Chemical Company

BECKER UNDERWOOD Inventing the Future

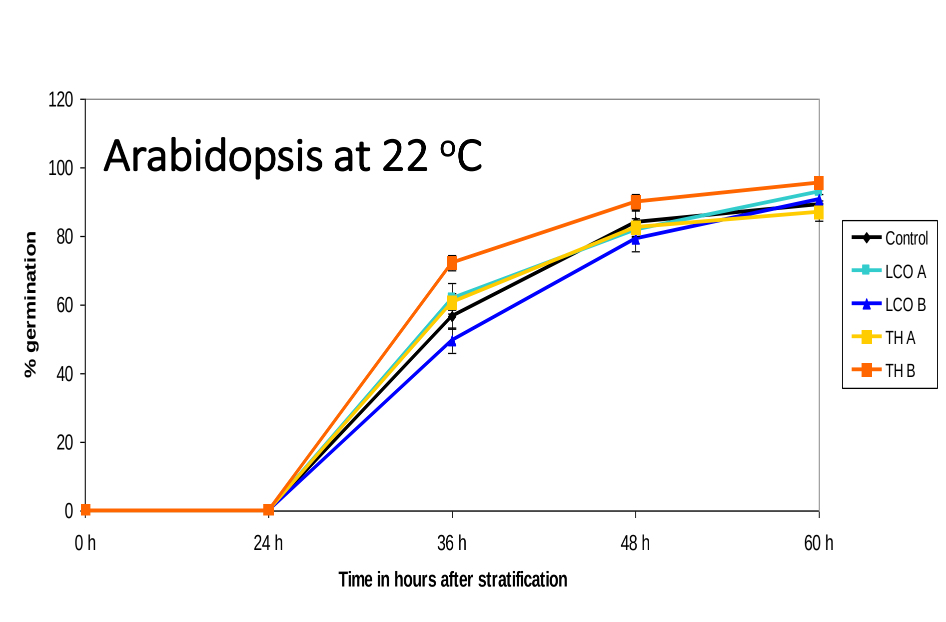
Effect of Methyl Jasmonate on Yield

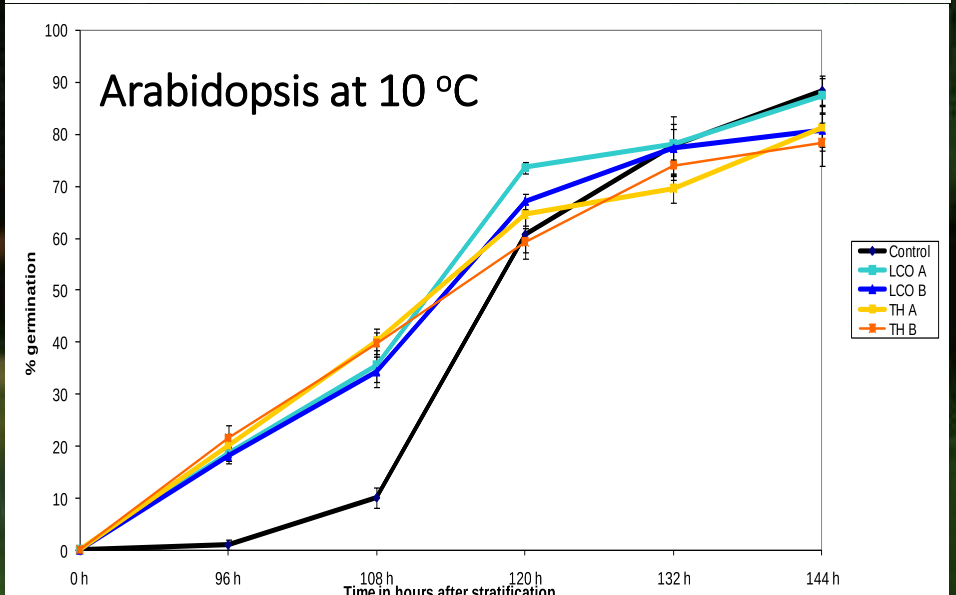


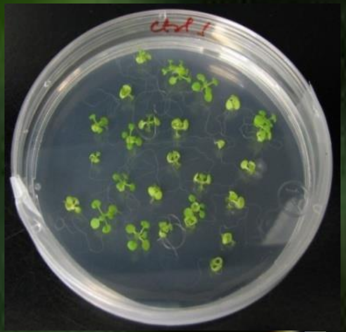
Two sites, two strains at each, this is typical data – strain 532C at a sandy-loam site.

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**

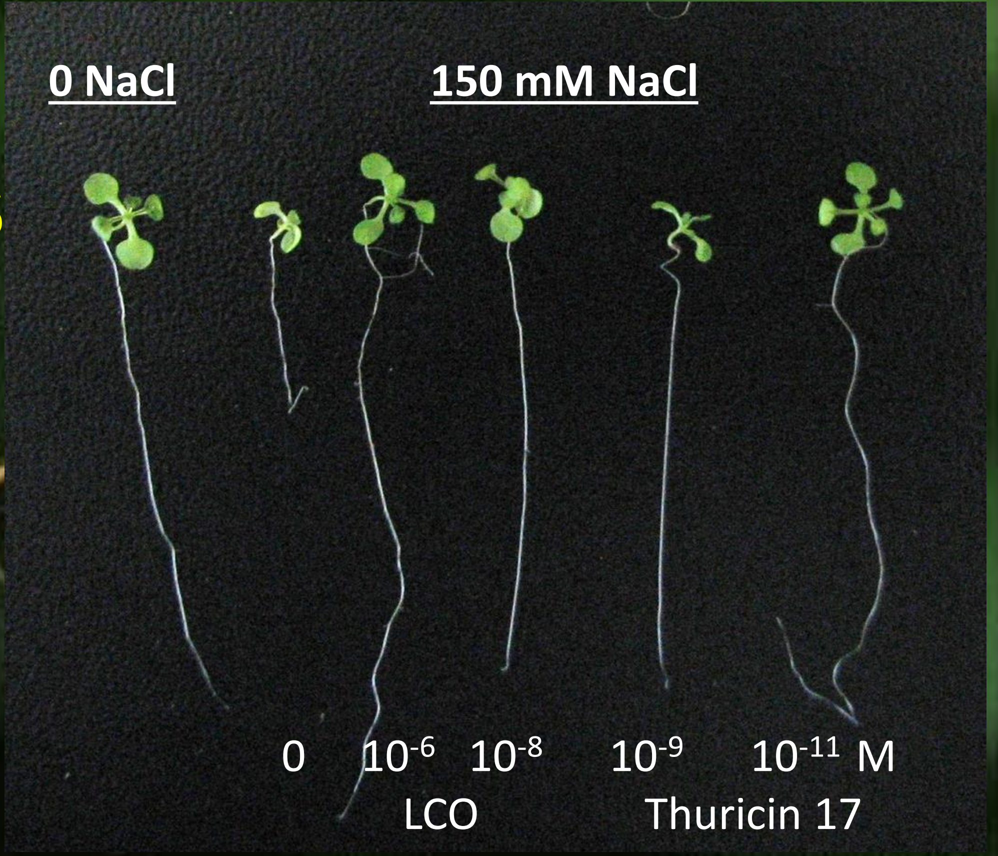


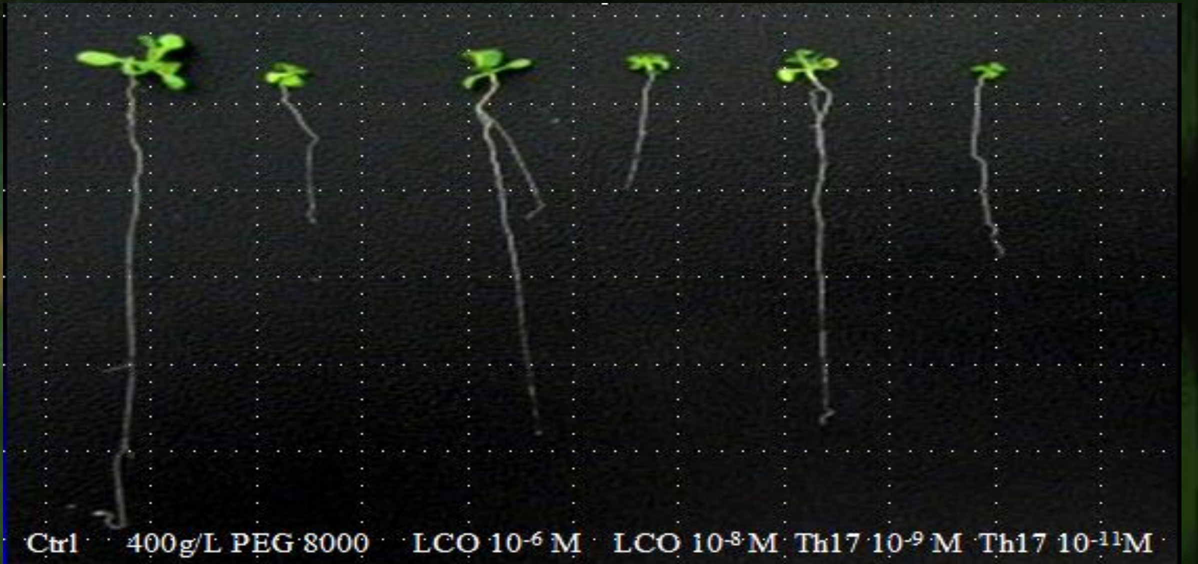




Salt & Drought Stress

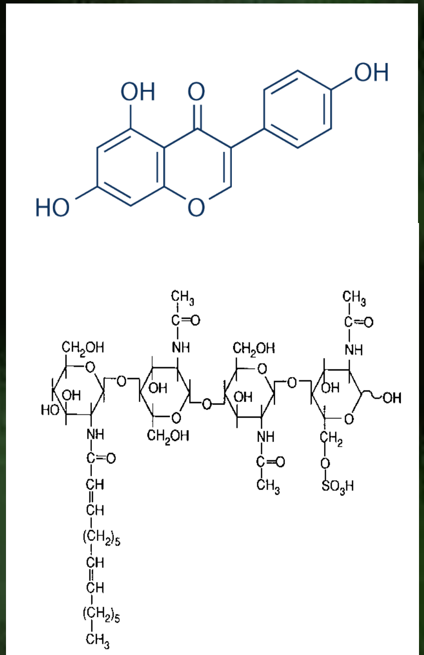
- Arabidopsis
- Seeds on petri plates with signals
- Control and 150 mM NaCl
- Signals improved growth under salt stress





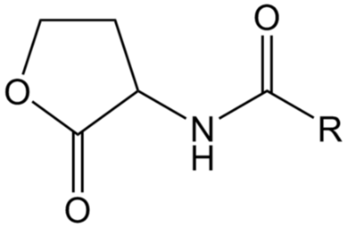
Hormones of the Holobiont

- Effective at very low concentrations
- Regulate plant activity
  - Large changes in gene espression, protein production, hormone profile
- Regulate other microbes
- Powerful way to affect plant growth and productivity
- 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

Agriculture and Agri-Food Canada

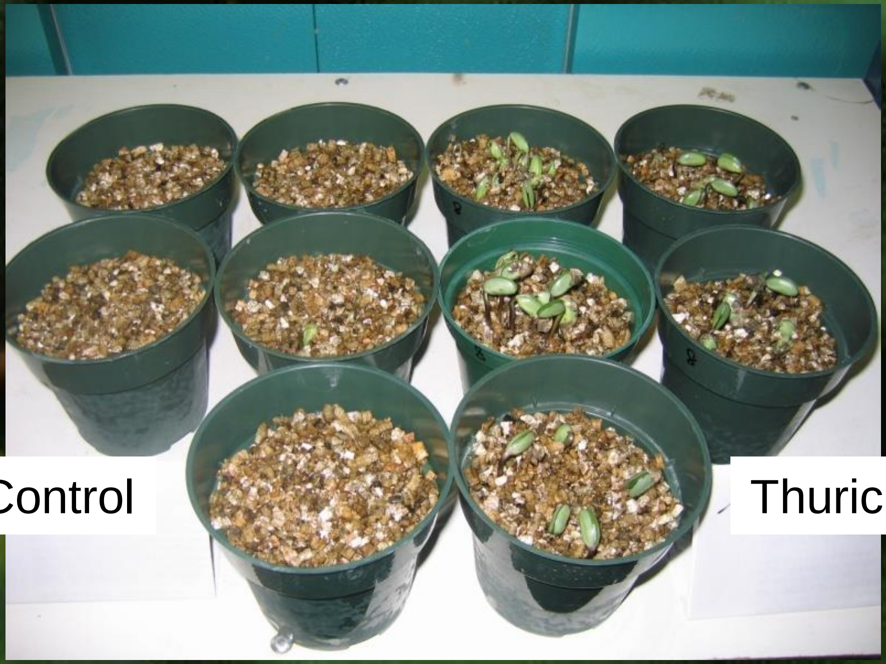
novozymes Rethink Tomorrow

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

Finding & Screening the Microbes

- Collect microbes that can be cultured
  - Crop plants
  - Specific resilient species
  - Range of habitats
- Efficient/rapid screening for early growth response (controlled environment)
  - Seed germination
  - Seedling emergence
  - Early seedling growth
  - Photosynthetic activity
  - Gene and protein expression
- Single strains and consortia

Agronomy

- Agronomic assessments
  - Field experiments
    - Range of soils and climates
    - Planting dates
    - Over a reasonable geographic area
    - Reduced inputs allowing full yield
- Regulatory process
- Commercialization

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The End

Thank You!