



# *Natural Product Producing Microbials for Nematode Control*

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CEO & Founder*



*Innovative Bio-based Products for Pest Management in Agriculture and Water*

# Safe Harbor Statement



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# Marrone Bio Innovations Corporate Overview

## Company Highlights

- Incorporated in June 2006
- **4** commercially available products, **2** add'l approved & **1** submitted for EPA registration
- 110 employees
- Library of **18,000+** proprietary microorganisms screened against a range of
- Wholly-owned, operational fermentation facility in Bangor, Michigan
- Early in our long-term growth curve
- Listed on NASDAQ as MBII August 2, 2013

## Commercial Products Today



## Marquee Partners / Distributors



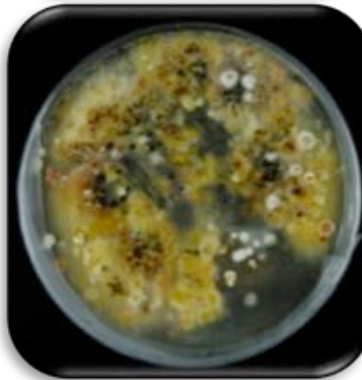
## Robust Pipeline

- Pipeline products: HAVEN™ sun stress anti-transpirant product, two nematicides, a systemic herbicide, a downy mildew fungicide and two biostimulants
- Many more earlier stage candidates across all categories
- 10 US, 20 foreign patents issued; more than 300 patents pending

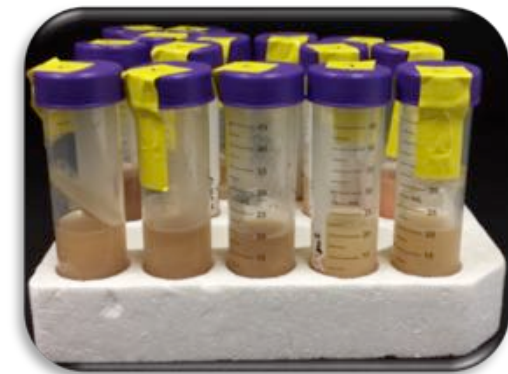
# Discovery: Sourcing and Isolation of Microorganisms



Soil and other types of samples collected, from unique habitats and niches



Individual fungal, bacterial, and actinomycete colonies picked from primary plate

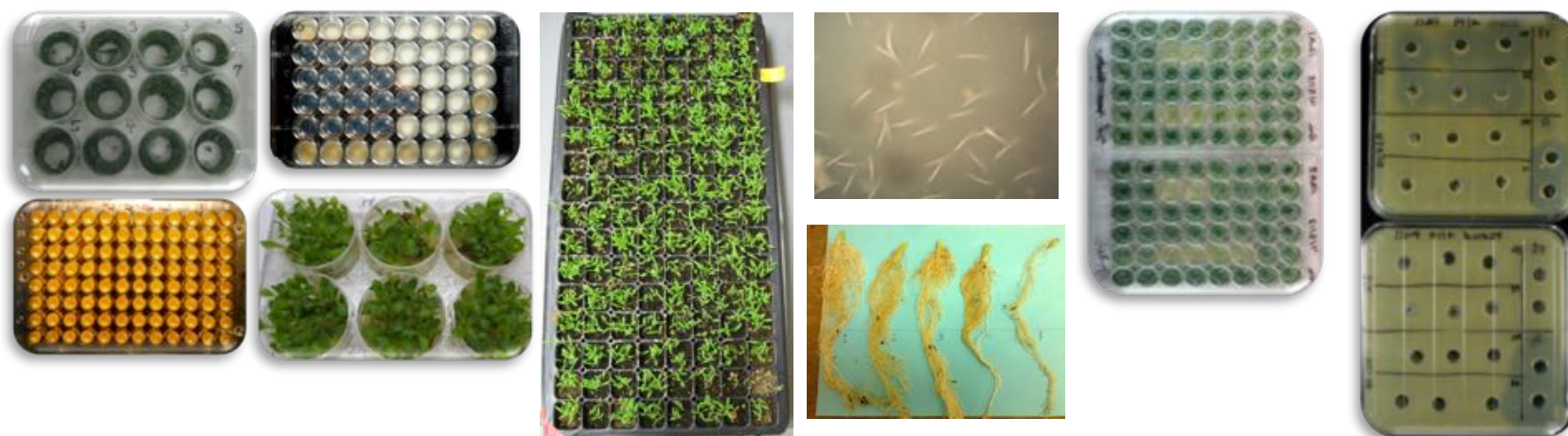


Water extracts of fermentation broths are used for bioassays

# Screening Against Multiple Targets



Target	Insecticide	Fungicide	Herbicide	Nematicide	Algaecide	Bactericide
Primary Screen	<i>Lygus</i> Beet armyworm	<i>Botrytis cinerea</i> <i>Phytophthora capsici</i>	Crabgrass Lettuce	<i>Meloidogyne</i> <i>spp.</i>	<i>Chlamydomonas reinhardtii</i>	<i>Xanthomonas campestris</i> <i>Pseudomonas syringae</i>



- Use a chemical or internal standard for comparison
- Initial hits are fermented a second and third time and tested again to verify activity
- Then tested in biostimulant assays and secondary target assays



# Nematicide Screening Used in Discovery of MBI 302/303

## Primary screen

- *Meloidogyne spp.*
- *In vitro* paralysis of nematode juveniles



## Secondary screen

- Drench 50% whole cell broth in cucumber or tomato test with *Meloidogyne sp.*
- Evaluate gall index.



## Additional Testing

Spectrum testing:

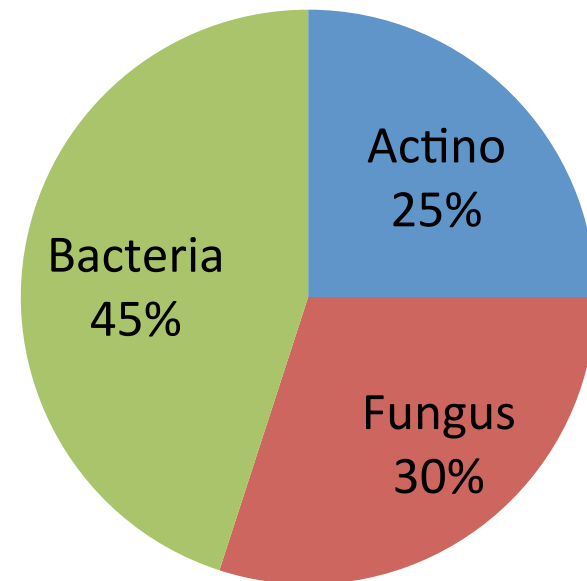
*Heterodera spp.*  
*Meloidogyne spp.*  
*Belonolaimus spp.*  
*Pratylenchus spp.*  
*Hoplolaimus spp.*  
*Helicothylenchus spp.*  
*Criconemoides spp.*  
*Rotylenchus spp.*

- Inhibition of egg hatching
- Inhibition of gall formation, anti-feeding, repellency vs. actual nematode kill

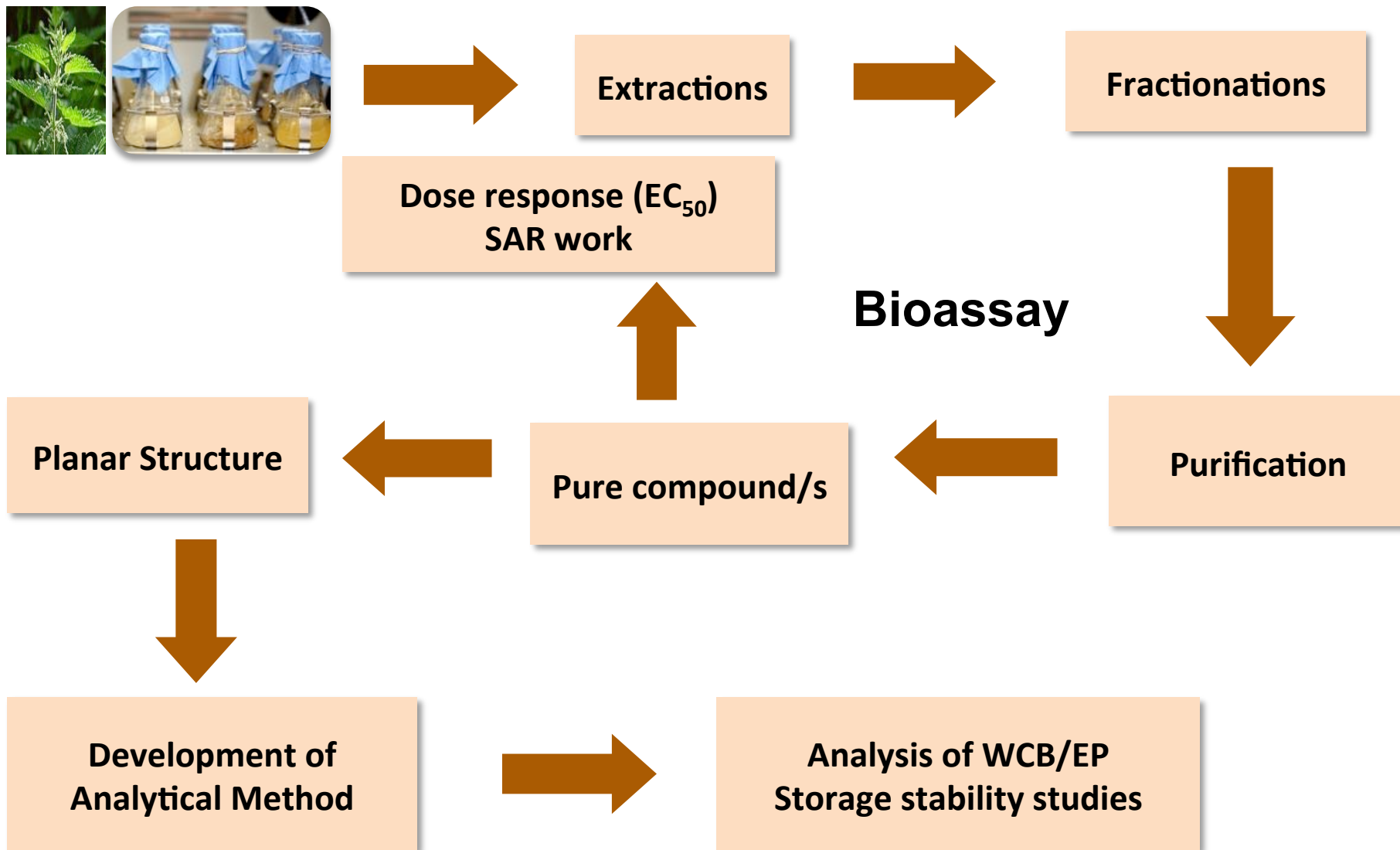
# Nematicidal Screening Has Yielded at Least Two Candidates

Hit type	Total # Hits	Hit Rate	One hit per...	What has been screened?
Nematicidal	117	1.17 %	85	7,500

- Screen stats as of Dec 31, 2013
- New species of *Flavobacterium* (MBI-302)
- New strain of *Bacillus megaterium* (MBI 303)
- Several more hits not yet fully characterized



# Natural Product Chemistry





# MBI 302 Bio-Nematicide

(*Flavobacterium* sp. H492 – novel species)



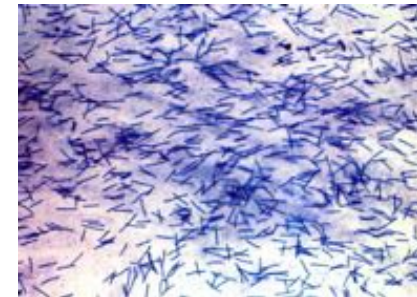
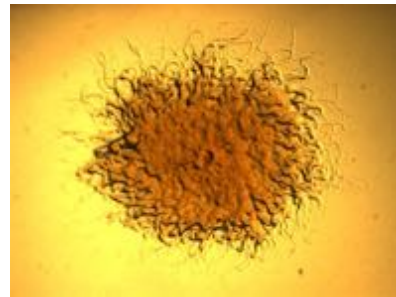
- Wide-spectrum nematicidal activity against plant parasitic nematodes, such as *Belonolaimus longicaudatus*, *Heterodera glycines*, *Meloidogyne incognita*, *Pratylenchus* sp., *Rotylenchulus reniformis*.
- Activity observed in whole cell broth, cell pellet, and supernatant
- Identified a polyketide compound in part responsible for the nematicidal activity
- Full EPA package, including toxicology prepared – just needs formal species ID of the microbe
- We are looking for development partners



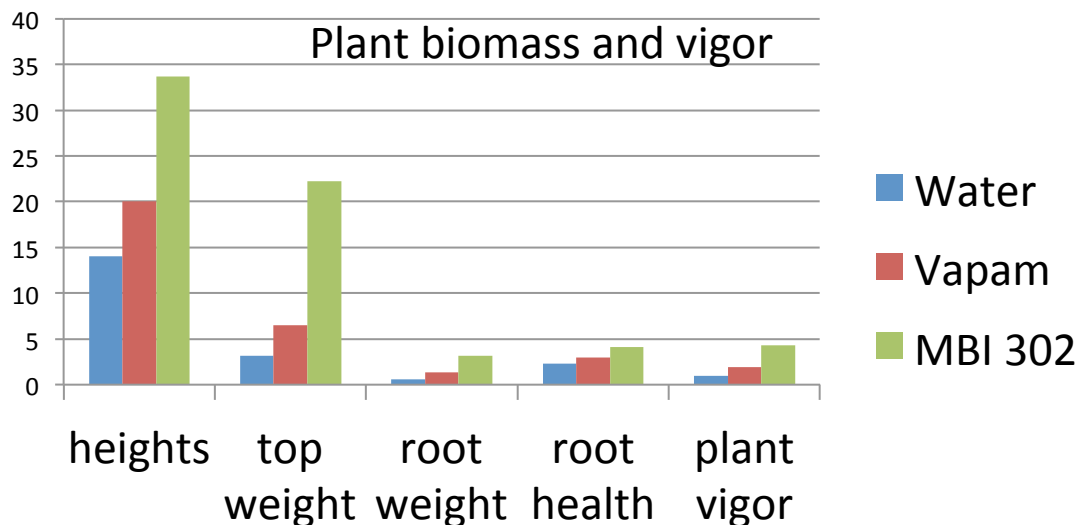
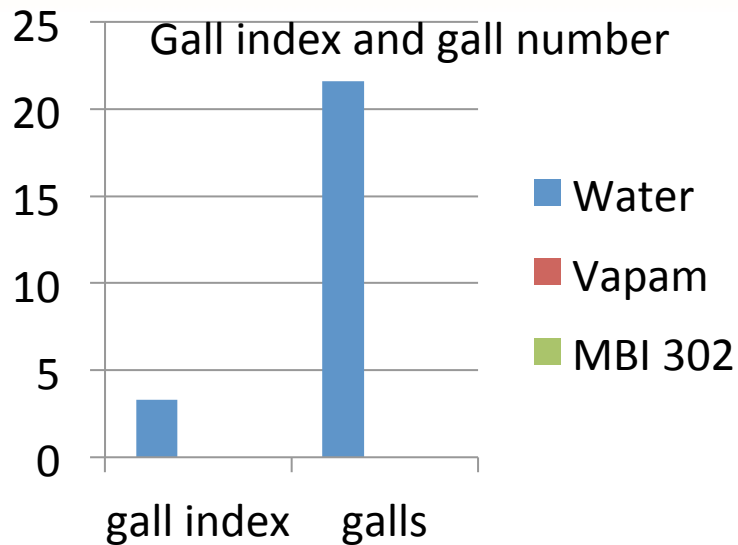
# The Genus *Flavobacterium*



- Gram negative, rods, most are catalase positive, strictly aerobic.
- Widely distributed in nature: fresh to salt water, soils, microbial mats, fish, algae, microbial mats.
- Prefer cool to cold environments and are psychrotolerant
- Biotechnological applications due to cold active enzymes
- Strict aerobic metabolism, oxidase positive
- Some species are known fish pathogens (salmon and rainbow trout):
  - *F. columnare*
  - *F. branchiophilum*
  - *F. hydatis*
  - *F. johnsoniae* → opportunistic “soft rot” in plants
  - *F. psychrophilum*
  - *F. succinicans*
- Normal bacterial flora in fish surface, gills, guts and eggs.

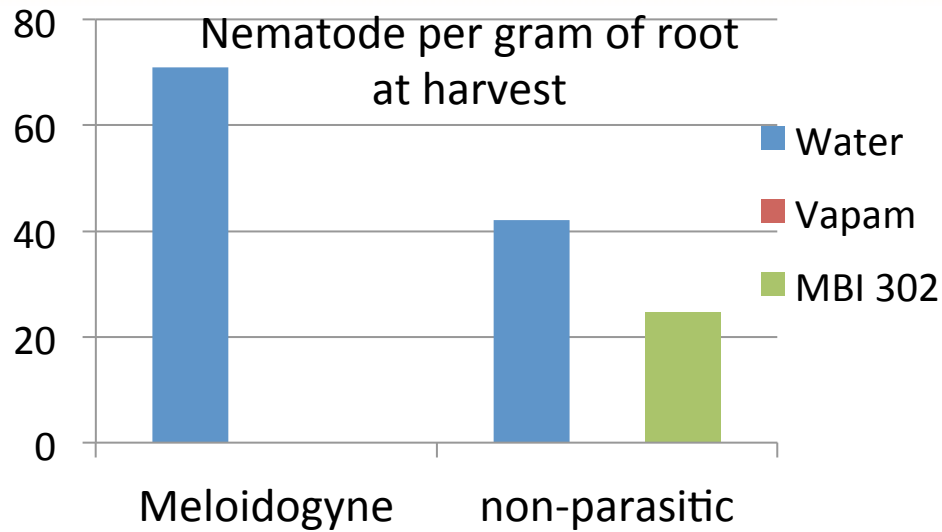


# MBI-302 *Meloidoyne incognita* (Syntech tests) (whole cell broth)



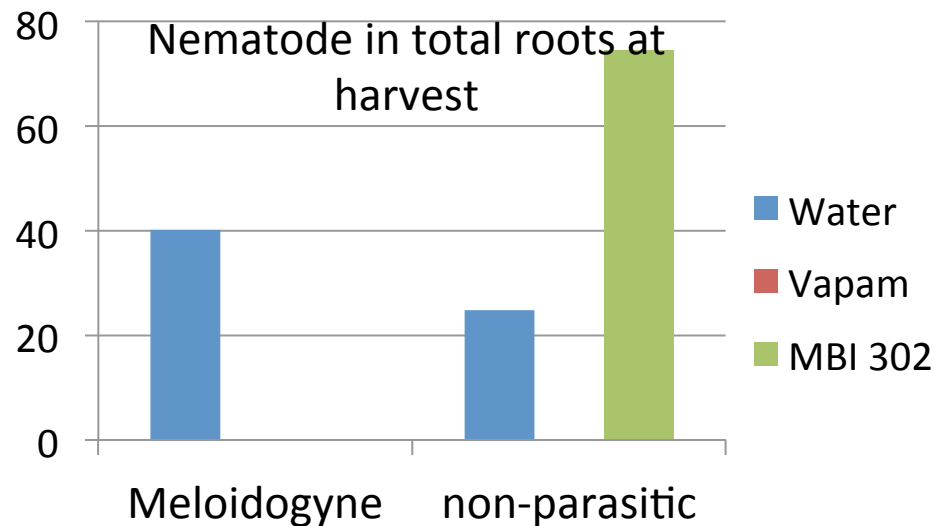
- **MBI-302 reduces RKN galls and also enhances plant growth & vigor**
- **Similar results with lesion, sting, soybean cyst and reniform, confirming broad spectrum vs. pest nematodes**

# MBI-302 *Meloidoyne incognita* (Syntech tests) (whole cell broth)



**MBI-302 reduces RKN in roots but has low effect on beneficial nematodes**

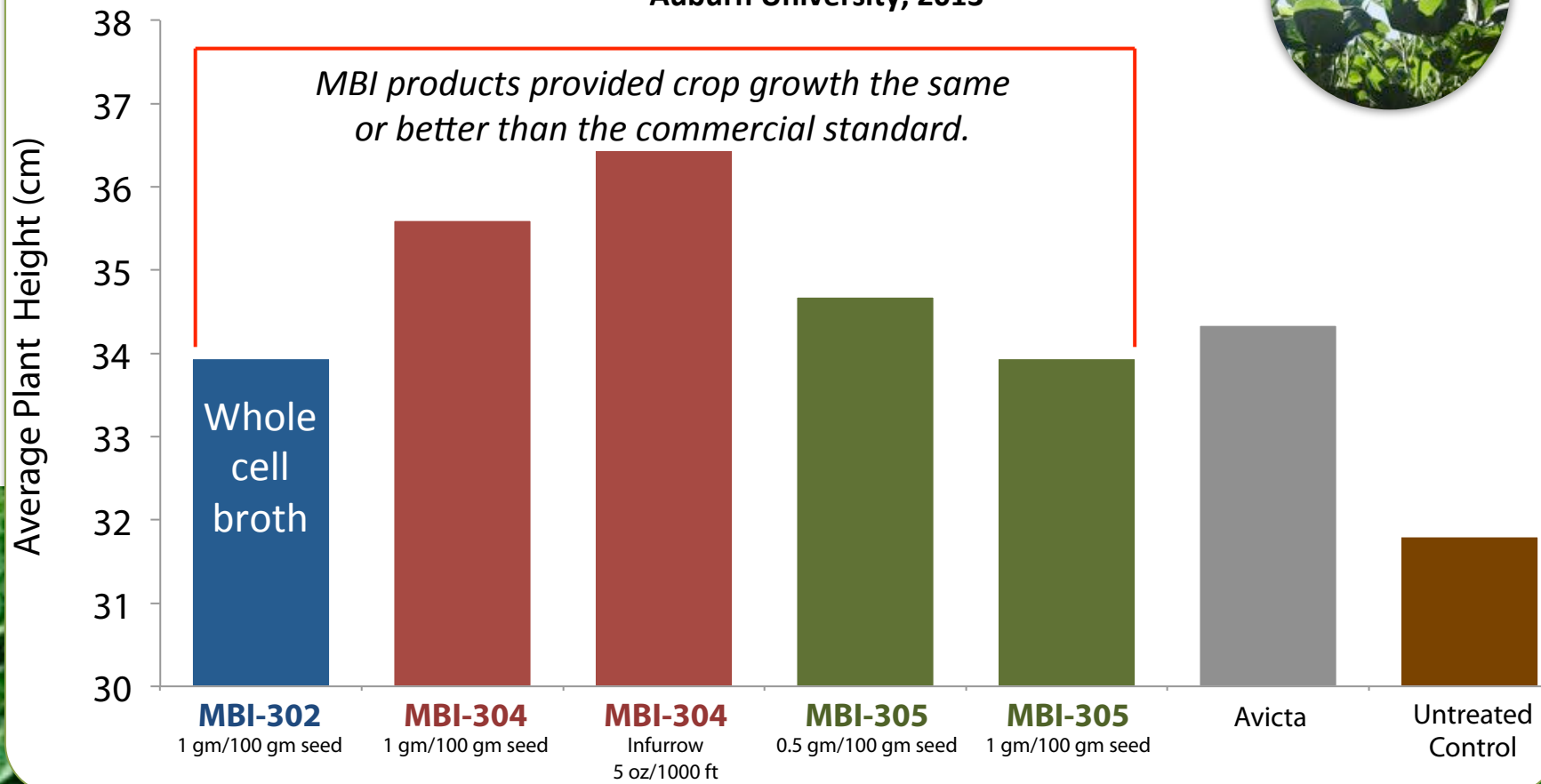
Cucumber



# Soybean Seed Treatments – Plant Height



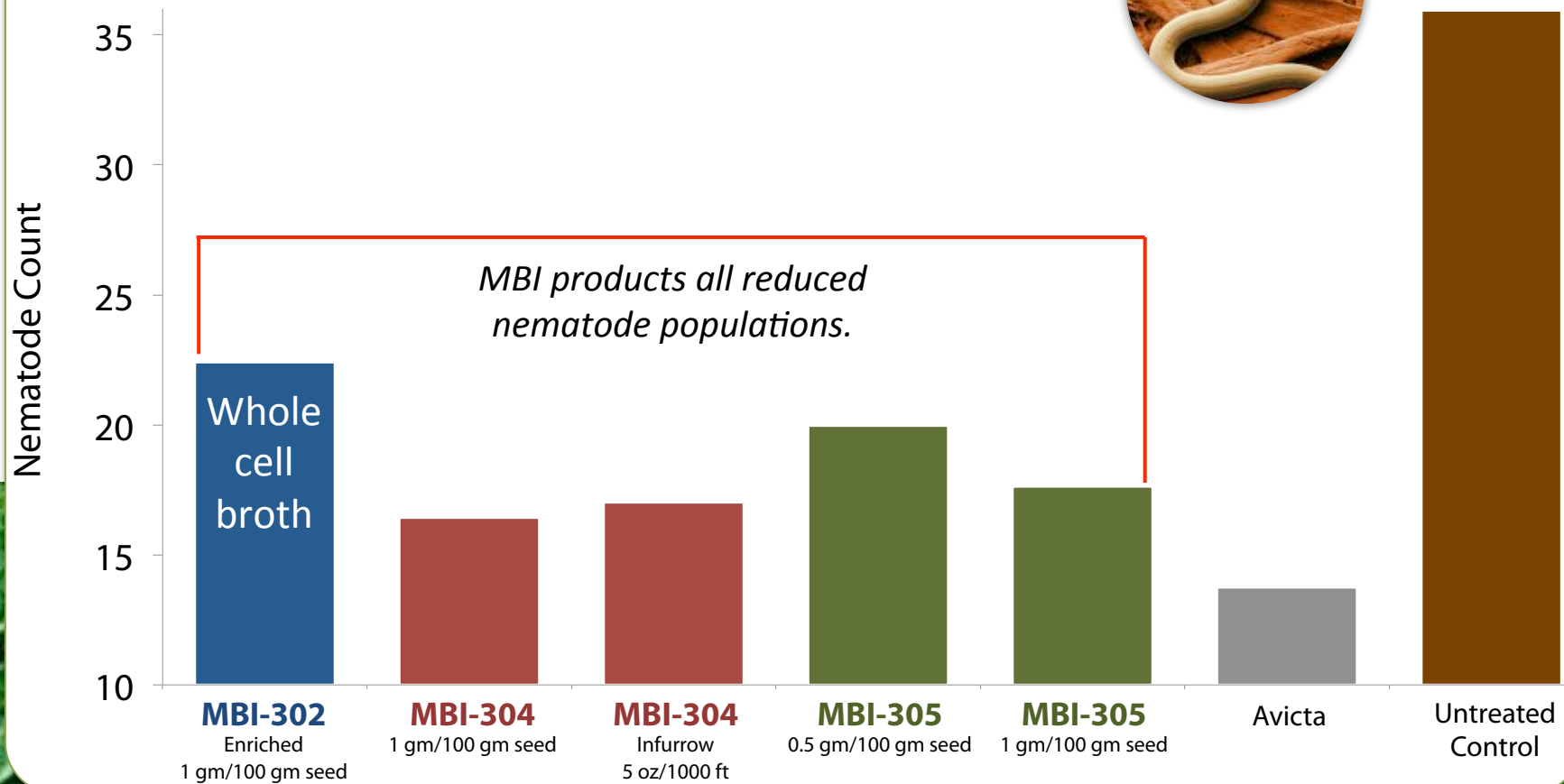
**Soybean Cyst Nematode Control**  
*(Heterodera glycines)*  
**Average Plant Height (cm)**  
**Auburn University, 2013**



- Treatments applied at planting on Jun 3.  
- Treatments evaluated on July 30.

# Soybean Seed Treatments – Nematode Counts

Soybean Cyst Nematode Control  
(*Heterodera glycines*)  
Nematode Count  
Auburn University, 2013



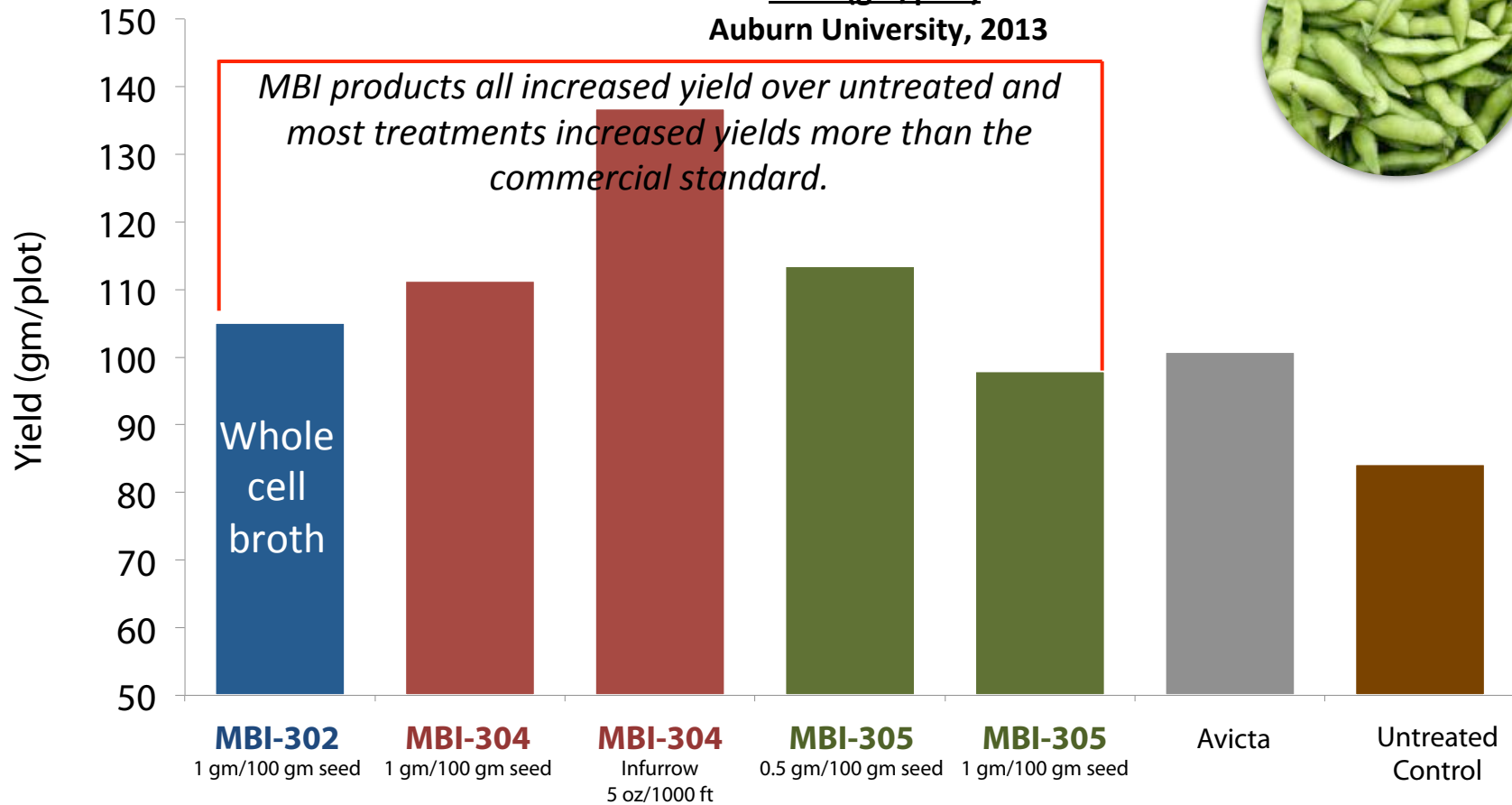
- Treatments applied at planting on Jun 3.  
- Treatments evaluated on July 30.



# Soybean Seed Treatments – Yield



Soybean Cyst Nematode  
(*Heterodera glycines*)  
Yield (gm/plot)  
Auburn University, 2013



- Treatments applied on Jun 3.  
- Yield evaluated on Oct 8.

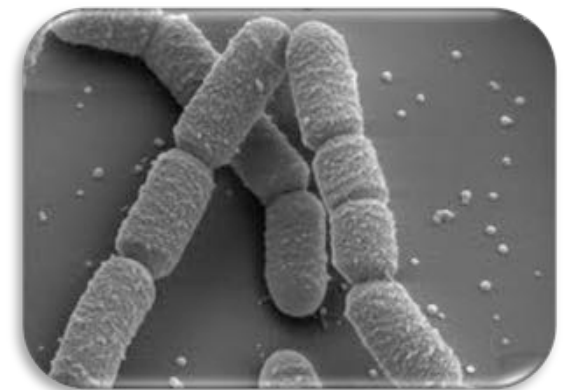
## *Bacillus megaterium* (MBI-303) with nematicidal activity

- Discovered in our routine screening in the primary screen against an in vitro paralysis test
- Confirmed on *Meloidogyne hapla* and *incognita*
- MBI 303 tested in pots with good activity
- Toxicology package generally completed – no issues
- Chemistry partially characterized
- Fermentation improvements have improved efficacy compared to 302, which was superior in initial testing

# Bacillus megaterium

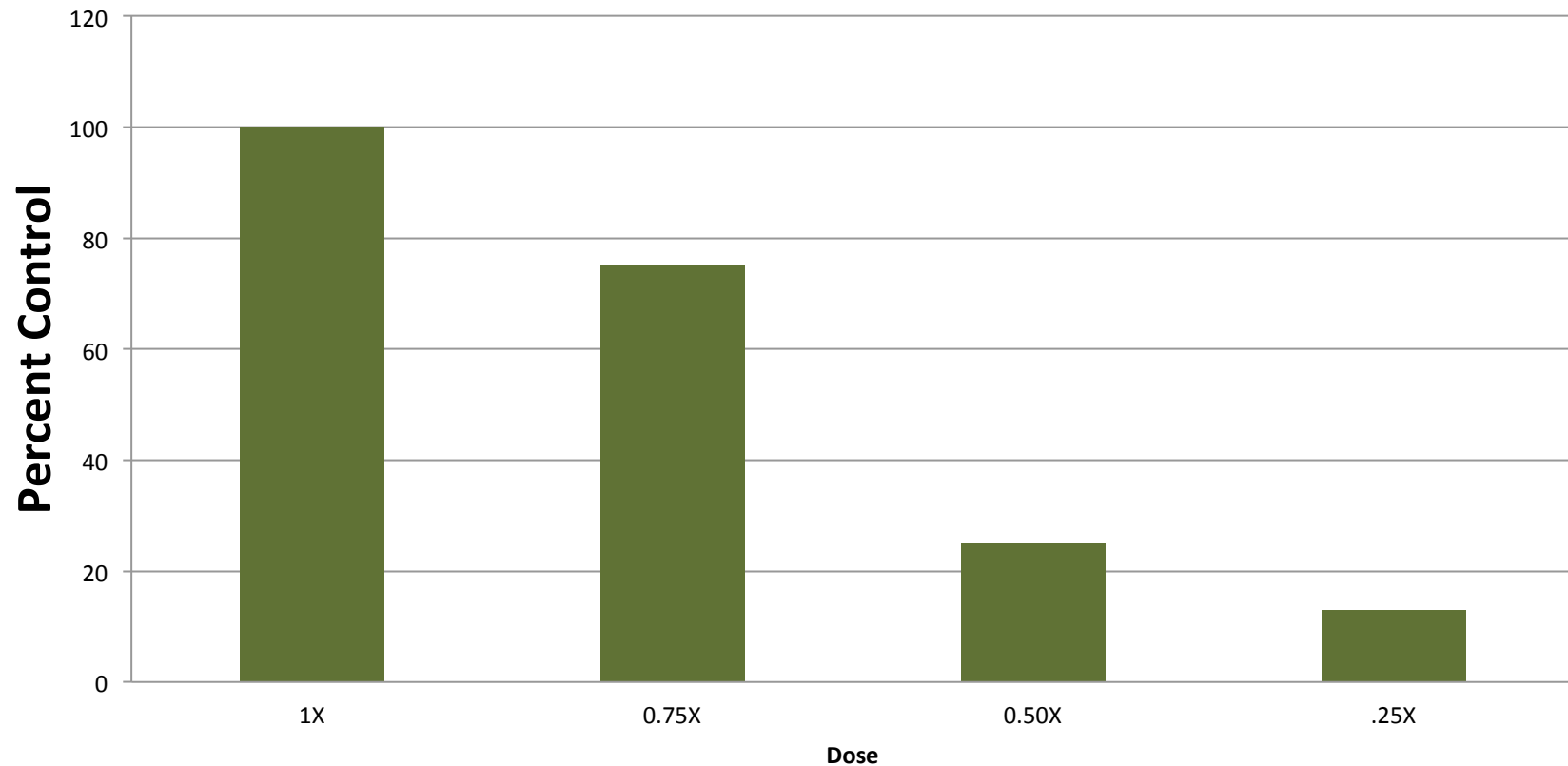


- Rod shaped, Gram-positive, endospore forming, motile by flagella
- One of the largest bacteria found in soil, widely spread in nature
- Considered non-pathogenic
- Type strain genome has been sequenced
- Biotechnological applications:
  - Cloning and other lab applications
  - Production of proteins: amylases
  - Applications to the production of penicillin
- Biofungicidal activity is well documented, as well as root colonization
- Reported cases of plant pathogenicity from a subtaxa:
  - *Bacillus megaterium* pv. *cerealis* (in wheat), 1982 report.
- Reported nematode control activity:
  - *Heterodera schachtii* (Neipp and Becker, 1999)
  - *Meloidogyne graminicola* (Padgham and Sikora, 2007)
  - *Meloidogyne incognita* (El-Hadad et al, 2011)



# MBI-303 Whole Cell Broth Effect on Root Knot Nematode

**MBI-303 Dose Response *in vitro* Activity against *M. hapla***



Fermentation improvements reduce dose of whole cell broth needed

# Effects of MBI-303 supernatant on *M. hapla* in cucumber in a greenhouse pot assay

Treatment	Plant Vigor	Root Gall Index	Nematode Population
H491	9 ± 1 a	2 ± 1 b	2233 ± 924 b
Avid (1%)	0 ± 0 b	0 ± 0 c	0 ± 0 c
Water	1 ± 1 ab	8 ± 1 a	5467 ± 611 a

Means followed by the same letter in the same column are not different, according to Fisher's least significant difference at  $P \leq 0.05$ .

- **Efficacy has improved with fermentation optimization so now in the lab it may be equal to or better than 302**

# Effects of MBI-303 supernatant on *M. hapla* in tomato in a greenhouse pot assay.



Treatment	Shoot Height (cm)	Shoot Weight (g)	Root Weight (g)	Root Gall Index	Nematode Number
H491	26 ±1 a <sup>z</sup>	17 ±3 b	4.3 ±2	2 ±1	140 b
Avid (1%)	8 ±8 b	1 ±1 c	0.4 ±1	0 ±0	0 c
Water	28 ±6 a	14±4 b	4.2 ±1	2 ±1	325 a

Means followed by the same letter in the same column are not different, according to Fisher's least significant difference at  $P \leq 0.05$ .

- **Efficacy has improved with fermentation optimization so now in the lab it may be equal to or better than 302**





# QUESTIONS?

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