Development of Biologicals as Low Input, Sustainable Production Inputs for Fuel and Residue/Food Production

Principal Investigator: Donald L. Smith
Organization: McGill University

Presentation date: May 28, 2022
Location: McGill University

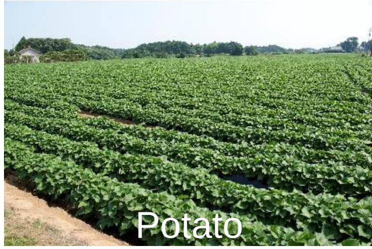
This presentation does not contain any proprietary, confidential, or otherwise restricted information

Acknowledgement

- Government of Canada under the Canadian Agricultural Partnership program
- Biomass Cluster holder: BioFuelNet Canada
- Partners:

Goal Statement

1. For a microbioal consortium already established for controlled environment work, evaluate its ability to improve production of biomass crops, crop residue and food material
2. Bioprospect to find new plant growth promoting phytomicrobiome bacteria and assess their potential
3. Investigate mechanisms of action for the growth promoting bacteria involved
4. Test crops:
    1. Biomass crops – switchgrass, miscanthus
    2. Crop residues – corn, potato



BioMasse Canada

Réseau BioFuelNet Canada

Project Overview

- **Biomass crops** on marginal agricultural lands (>class 3)
  - Little competition with food crops
  - Plants more stressed plus climate change
- Perennial crops – stand can be harvested for 15 - 30 yrs.
- Fuel plus increase carbon/organic matter content of soil
- About 25 Mt biomass
- **Food crop residues**, take about 1/3 yr^{-1}
- About 48 Mt biomass
- PGPR can help deal with stress and increase yield
- Climate change resilience, increase food ***and*** fuel

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

The Problem/Opportunity

- There is a large area of potential land for biomass production in Canada
- Purpose grown biomass would be produced in a more stressful environment (marginal lands)

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Approach: The Problem/Opportunity

- Uncertainties about biomass feedstock supply identified by BioFuelNet as bioeconomy bottleneck

1. Purpose grown biomass and stress
    1. Produced on marginal lands so more often stressed
    2. Climate change leads to more stress more often
    3. Many plant-associated microbes help plants deal with stress
2. Can take about 1/3 of food crop residues each year
    1. Climate change - more stress more often
    2. PGPR increase growth and lead to food ***and*** fuel

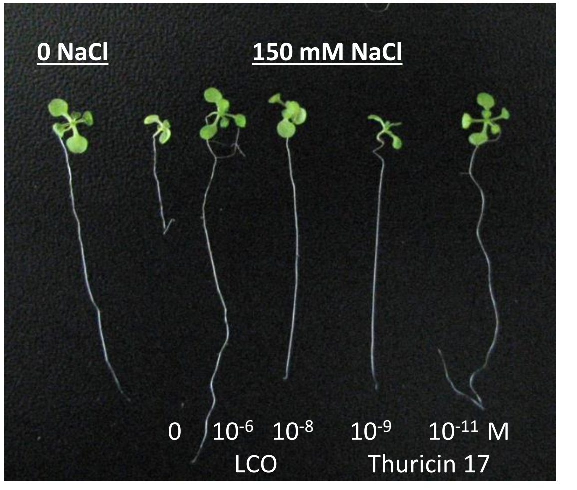
BioMasse Canada

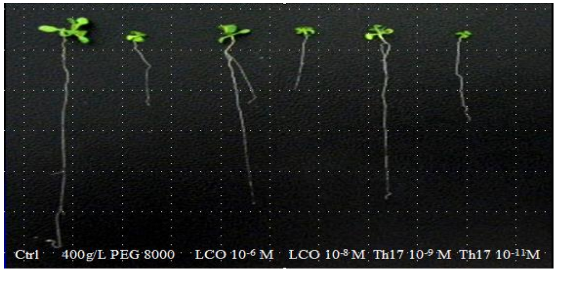
McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Phytomicrobiome and Stress

- Plants, like mammals, have a microbiome
  - Phytomicrobiome
- Help in a wide range of ways
  - Nutrient mobilization
  - Hormone production
  - Disease control
  - Signals
    - Hormones of the holobiont
- Seeds on petri plates with signals
- Control and 150 mM NaCl
- Signals improved growth under stress

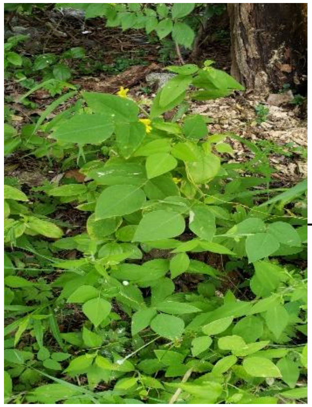




BioMasse Canada

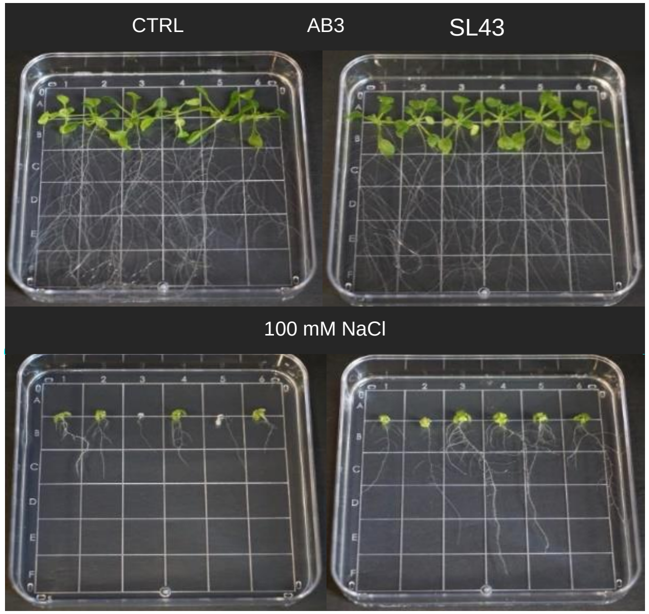
McGill MACDONALD

RÉSEAU BIOFUELNET CANADA



Progress to date – new PGPR

- Strains isolated from roots of an indigenous legume
- 3 with most positive effect on plant growth selected
- One excretes material into medium that enhances stress tolerance
- Exudate enhancing stress tolerance (salt)
- Enhance early plant growth



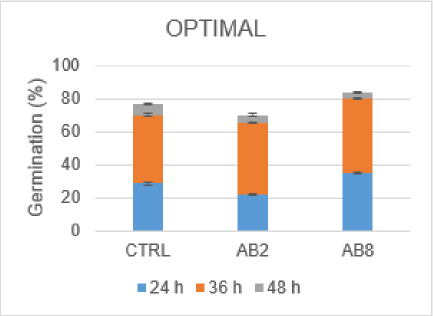
BioMasse Canada

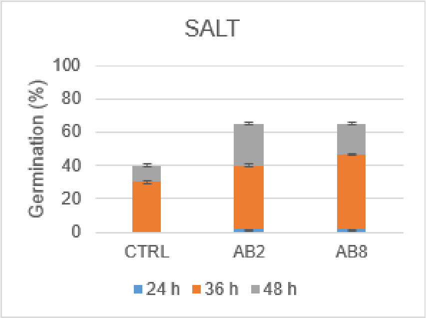
McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

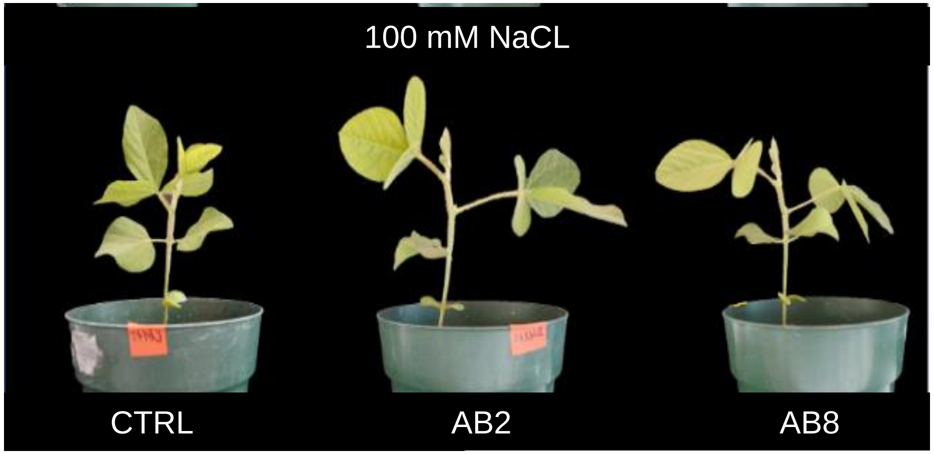
Progress to date – new PGPR

- 2 stimulate plant growth as inocula
- Seed germination





- Soybean growth



- IP filed on these new strains

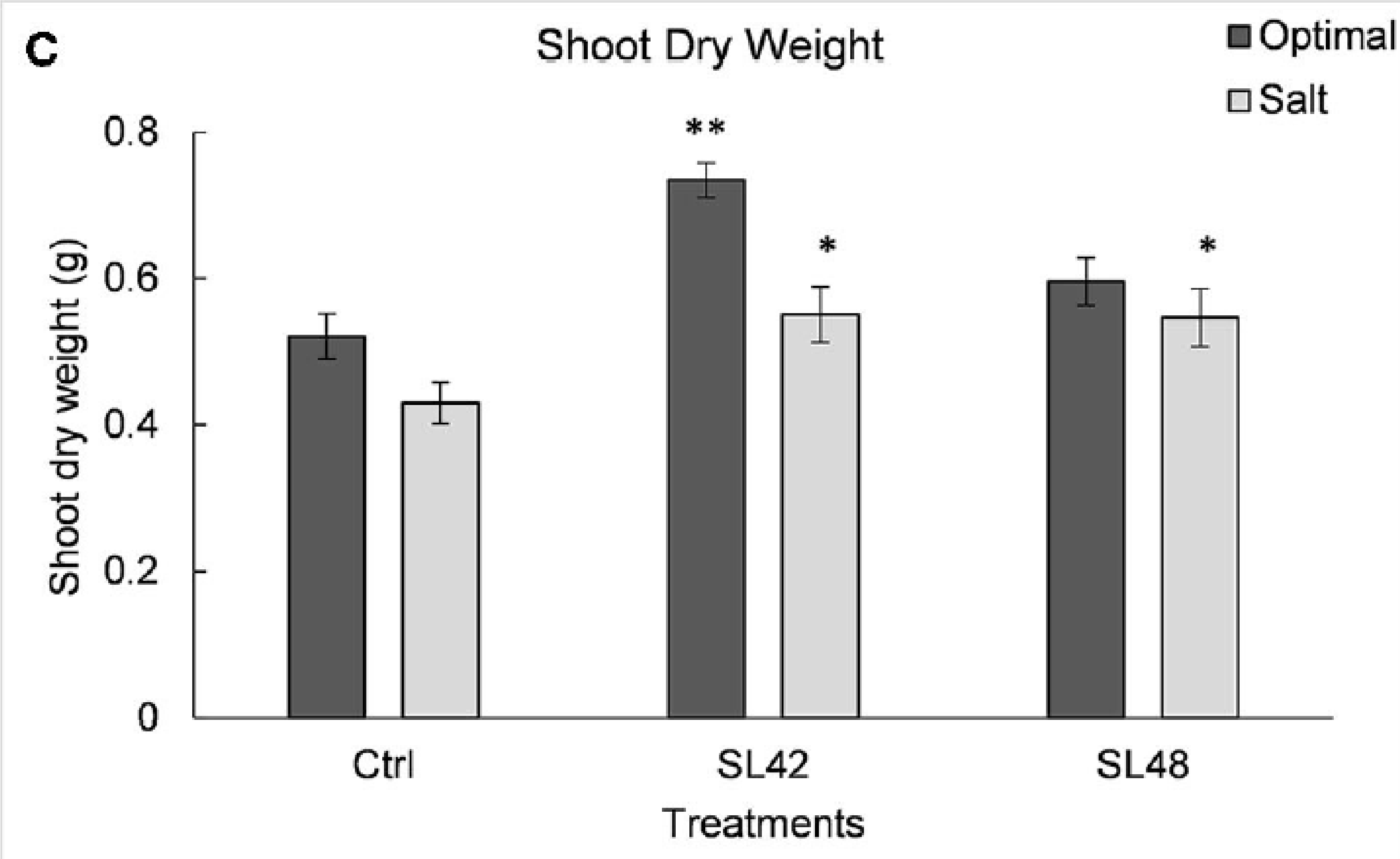
Inoculation and Growth Stimulation

- Two of the most promising strains, *Rhizobium sp.* SL42 and *Hydrogenophaga sp.* SL48 investigated for soybean growth promotion salinity tolerance.
- *Bj*+SL42 resulted in higher shoot biomass than the control, 18% at the vegetative stage, 16% at flowering, 7.5% at pod-filling, and 4.6% at harvest and seed weight was increased by 4.3% under salt stress.
- Grain yield was raised under optimal conditions by 7.4 and 8.1% with treatments *Bj*+SL48 and *Bj*+SL42+SL48, respectively.
- Suggests that inoculation with bacteria from an indigenous legume can induce stress tolerance, improve growth and yield to support sustainability, and encourage broader ecological adaptability of soybean.

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA



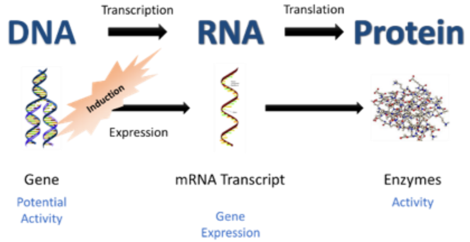
Shoot dry weight measured at 28th DAP under optimal (water) and salt (100 mM NaCl) conditions. The seeds were treated with 10 mM $MgSO_4$ as control or bacterized with strains SL42 and SL48. Values represent mean ± SE (n = 6*$p \leq 0.05$, **$p \leq 0.001$)

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Proteomics too!



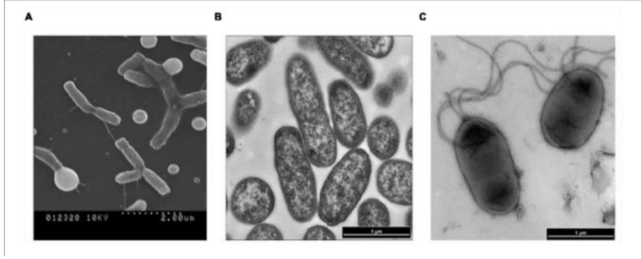
- Soybean plants inoculated with *Rhizobium* sp. SL42 and *Hydrogenophaga* sp. SL48 were more vigorous and salinity tolerant under growth chamber conditions.
- The bacteria triggered multiple signaling pathways that regulated growth (energy metabolism, etc.) and stress tolerance mechanisms, which in turn is a result of beneficial plant-microbe interaction.
- When plants were co-inoculated with *Bradyrhizobium japonicum* 532C inoculation with SL42 and SL48 caused greater growth promotion and stress alleviation, suggesting compatible co-inoculation.
- Under stressful conditions they could be utilized as biostimulants to mitigate stress effects and boost crop productivity.

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Devosia SL43



- Strain appears to be producing a growth stimulating substance
- Strain grown in broth culture then cells removed by centrifugation and filtering
- Resulting cell-free supernatant (CFS) stimulates plant growth
- SL43 CFS and flavonoids were investigated under controlled environment conditions
- They were applied both as seed treatments and foliar sprays

BioMasse Canada

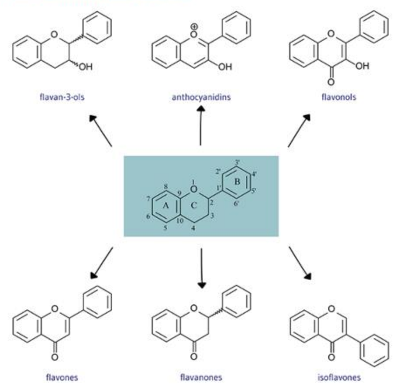
McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Flavonoids

- Play a number of key roles in plants
  - Stress management
  - Allelopathy
  - Mitigate effects of reactive oxygen species (compounds)
  - Plant-to-microbe signalling
- Citrus derived set supplied by industrial partner investigated
- Industrial partner has previous experience in this area

Figure 1. Basic Structures of Flavonoid Subclasses



Methodology (Seed treatment)

- **Seed priming**

Seeds were soaked in the solutions (either flavonoid or CFS) for a specfic time; soybean for **3 h** and canola for **24 h,** followed by drying until the seeds have returned to the initial weight

Canola

- Flavonoids (56 treatments)
  - 10 flavonoid levels (0.5 to 5 μL g^{-1} seed)
  - 4 salt stress levels (125 to 200 mM NaCl)
- CFS (13 treatments)
  - 5 CFS levels (1:50 to 1:1000)
  - 1 salt stress level (150 mM)
- CFS + Flavonoids (13 treatments)
  - 5 mixed levels
  - 1 stress level

Soybean

- Flavoniods (25 treatments)
  - 5 flavonoid levels (1 to 5 μL g^{-1} seed)
  - 3 salt stress levels (80 to 120 mM)
- CFS (13 treatments)
  - 5 CFS levels (1:50 to 1:1000)
  - 1 salt stress level (100 mM)
- CFS + Flavonoids (13 treatments)
  - 5 mix levels
  - 1 stress level

All the treatments were compared with two controls; a primed control (water soaked) and an unprimed control (unsoaked), each for both optimal and stressed growth conditions

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Methodology
(Foliar spray)

- **Foliar Spray**
  - **The experiments were conducted in controlled environmental growth conditions**
  - Crops were sprayed only once throughout the experiment
  - Approximately 1 mL solution (either flavonoid or CFS) was sprayed on each plant
  - Canola was sprayed at the 4-5 leaf stage
  - Soybean was sprayed at 3rd trifoliate

Soybean (treatments)	**Canola (treatments)**
▪ Flavonoids (8 treatments) – 3 flavonoid levels (50, 100 and 150 mL ha^{-1}) – 1 salt stress level (120 mM NaCl) ▪ CFS (8 treatments) – 3 CFS levels (1:100, 1:500 and 1:1000) – 1 salt stress level (120 mM NaCl)	▪ Flavonoids (8 treatments) • 3 flavonoid levels (100, 200 and 300 mL ha^{-1}) • 1 salt stress levels (150 mM NaCl) ▪ CFS (8 treatments) • 3 CFS levels (1:100, 1:500 and 1:1000) • 1 salt stress level (150 mM NaCl)

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Progress to date – biostimulants

- Set of flavonoids provided by industrial partner
- We have been able to show that they also assist with plant stress

Control-100mM NaCl

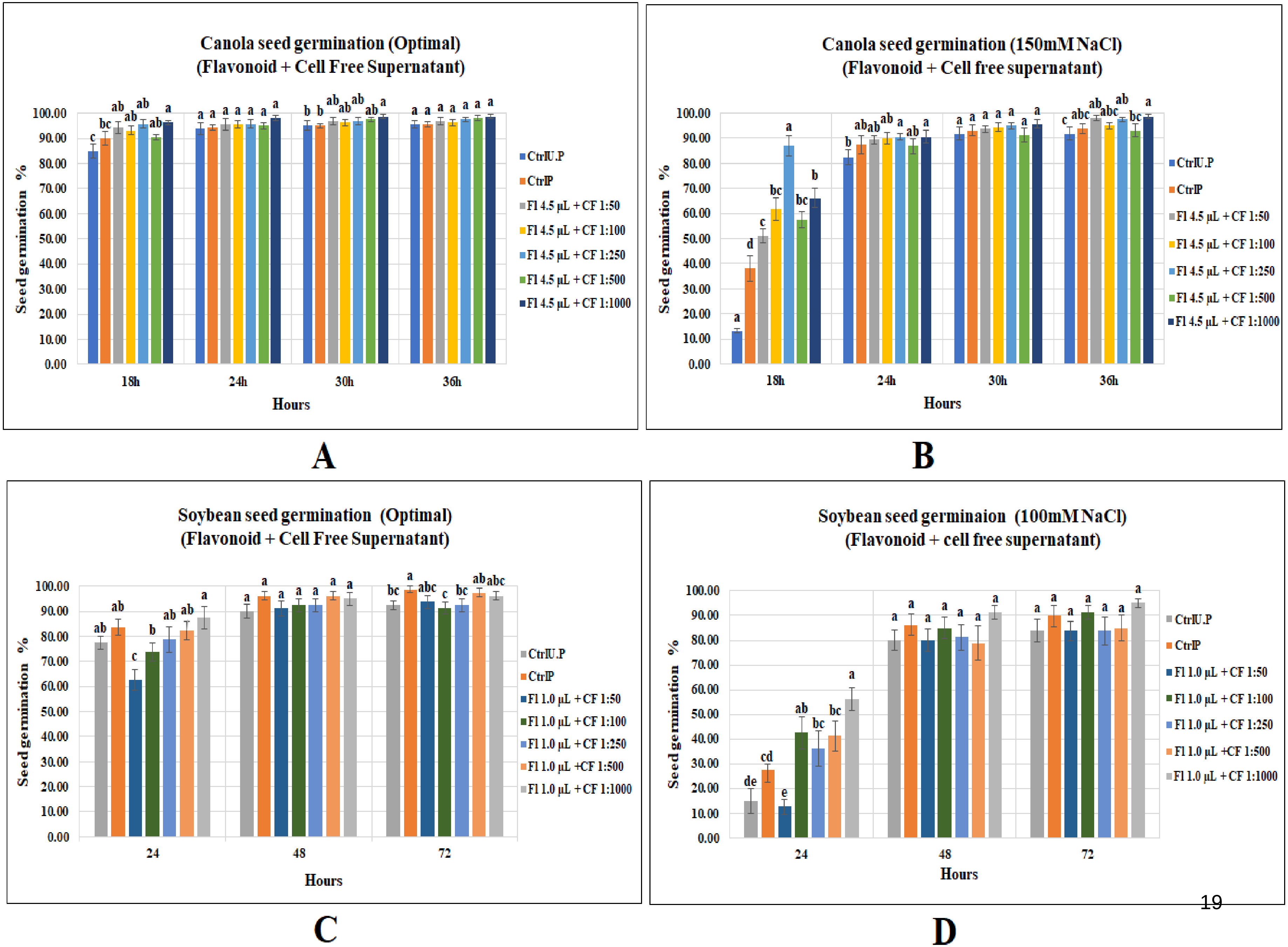
Control-120mM NaCl

Flavonoid +100mM NaCl

Flavonoid +120mM NaCl

Seed germination

- Soybean and canola seed germination
- Seed priming with flavonoids and potential biostimulant activity of the cell free supernatant (CFS; produced by a novel strain of *Devosia sp* – SL43), alone and in combination both under optimal and salt stress.
- Significant increase in canola and soybean seed germination following the application of flavonoids and CFS under both optimal and salt stressed conditions.
- Increases greater under salinity stress
- Use of novel *Devosia* sp. CFS very effective


Canola seed germination (Optimal)
(Flavonoid + Cell Free Supernatant)
Seed germination %
Hours
18h
24h
30h
36h
CtrlU.P
CtrlP
Fl 4.5 µL + CF 1:50
Fl 4.5 µL + CF 1:100
Fl 4.5 µL + CF 1:250
Fl 4.5 µL + CF 1:500
Fl 4.5 µL + CF 1:1000
A
Canola seed germination (150mM NaCl)
(Flavonoid + Cell free supernatant)
Seed germination %
Hours
18h
24h
30h
36h
CtrlU.P
CtrlP
Fl 4.5 µL + CF 1:50
Fl 4.5 µL + CF 1:100
Fl 4.5 µL + CF 1:250
Fl 4.5 µL + CF 1:500
Fl 4.5 µL + CF 1:1000
B
Soybean seed germination (Optimal)
(Flavonoid + Cell Free Supernatant)
Seed germination %
Hours
24
48
72
CtrlU.P
CtrlP
Fl 1.0 µL + CF 1:50
Fl 1.0 µL + CF 1:100
Fl 1.0 µL + CF 1:250
Fl 1.0 µL +CF 1:500
Fl 1.0 µL + CF 1:1000
C
Soybean seed germinaion (100mM NaCl)
(Flavonoid + cell free supernatant)
Seed germination %
Hours
24
48
72
CtrlU.P
CtrlP
Fl 1.0 µL + CF 1:50
Fl 1.0 µL + CF 1:100
Fl 1.0 µL + CF 1:250
Fl 1.0 µL +CF 1:500
Fl 1.0 µL + CF 1:1000
D

Foliar application

- Flavonoids and *Devosia sp* (SL43) CFS containing active compounds as foliar spray to determine growth stimulating effects on canola and soybean, under optimal and salt stress conditions.
- Flavonoids under optimal conditions - soybean growth significantly affected by flavonoids (increases in shoot fresh and dry weight, and leaf area, by 91, 99.5 and 73%, respectively)
- Soybean growth was unaffected by flavonoids under salt stress.
- CFS mitigated negative effects of salinity stress, improved soybean growth, increasing shoot fresh biomass, dry biomass and leaf area by 128, 163 and 194%, respectively.
- Canola less responsive to both biostimulants, as CFS under both salt stress and optimal growth conditions

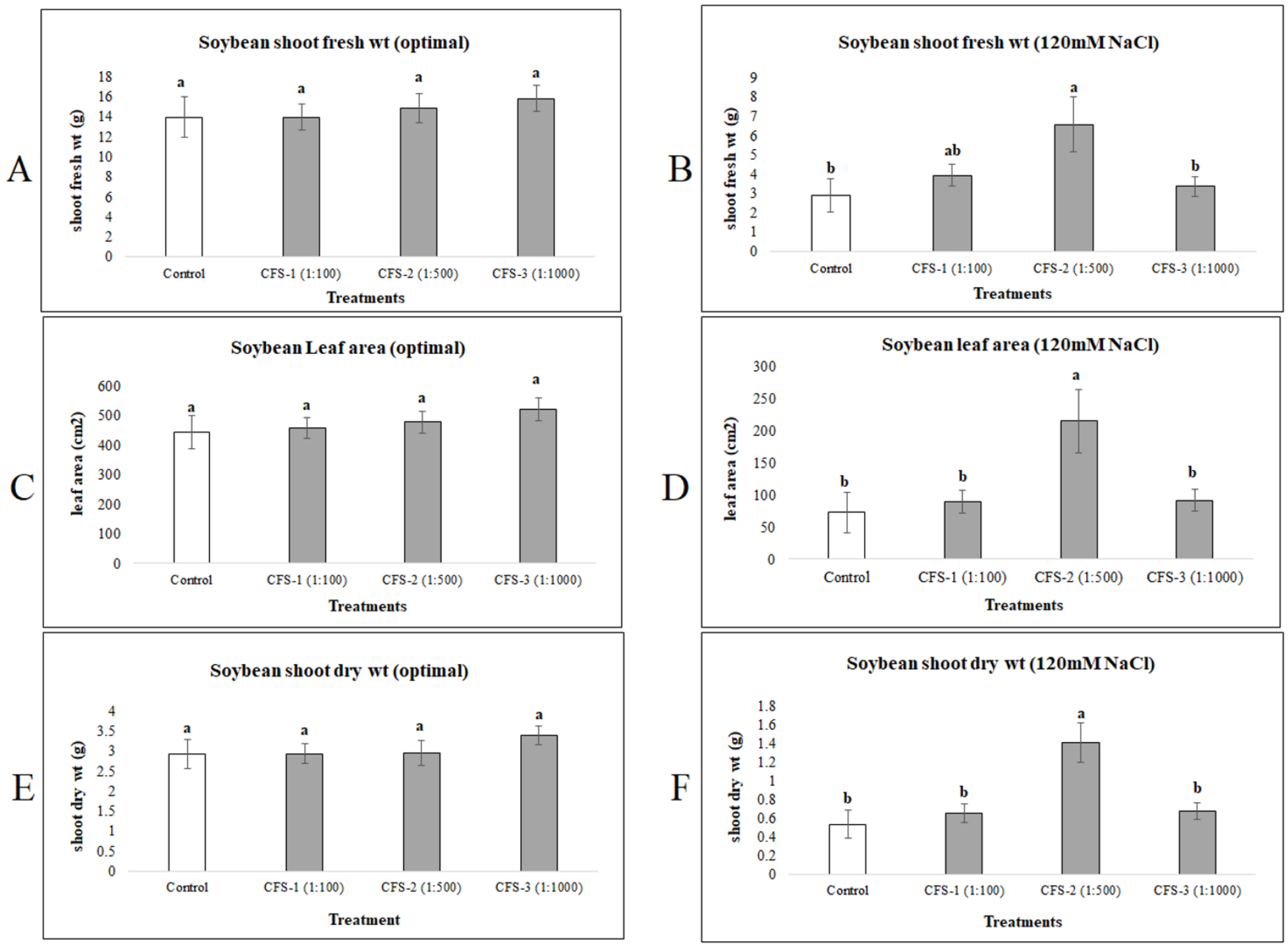


Figure 2: Soybean above ground biomass affected by CFS **(A)** shoot fresh wt - optimal **(B)** shoot fresh wt – 120 mM NaCl **(C)** shoot dry wt - optimal **(D)** shoot dry wt – 120 mM NaCl **(E)** leaf area - optimal **(F)** leaf area – 120 mM NaCl

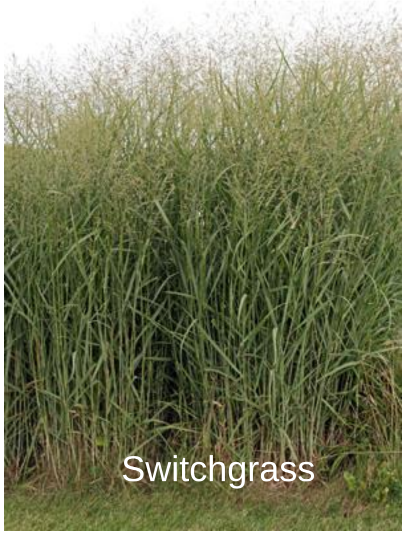
BioMasse Canada

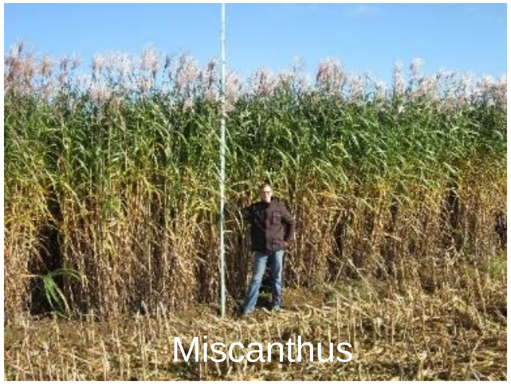
McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Progress to date – biomass crop field trials

- Trials planted in 2019
  - Switchgrass
  - Miscanthus
- Each grass treated with the various microbes
  - Consortium
  - Each of new ones





BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Effects

- Effects for established stands
- Spray application enhancements occurred about half the time
  - As with other crops, probable interaction with environment and stress
- Levels of increase varied
  - A few percentage points to 20 %
- If making crop more stress tolerant could be very good for enhancing yields on marginal lands
- Could be applied to food crops on less severely marginal lands and cause crops produced on those lands to have commercially acceptable yields
  - Less challenging marginal lands no longer "marginal" with this technology

BioMasse Canada · McGill Macdonald · Réseau BioFuelNet Canada

Fertilizer as a Carrier

- Strains (commercial consortium) applied with chemical fertilizer
  - Challenging due to salt levels
- Seed early and late on heavy, medium and light soils
- Testing showed strains survived
- The recommended inoculation rate seemed most effective
- Caused meaningful and statistically significant increases in yields of both potato and corn
- Effects varied, suggesting interaction with environmental conditions
- Ulysse consortium evaluated

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Progress to date – corn field trials

- Corn and potato were grown in 2018, 2019, 2020 and 2021 to examine
  - Samples and data being processed now, papers started
- Microbial consortium (a group of 5 *Bacillus* strains), at various concentrations (1x recommended and 2x) and two seeding dates (recommended and late), 3 soil types
- **Key findings for corn** –
  - Biomass production was increased –
    - on clay soil **15%**
    - on clay loam soil **20%**
    - on sandy loam soil **13%**
  - Both grain yield and starch content were significantly increased by microbial inoculation in corn


Corn

Relevance

- This research is demonstrating the impact of specific sets of PGPR and the overall potential of the phytomicrobiome for increasing available biomass for the bioeconomy
- This will help remove the greatest bottleneck in the bioeconomy and help make the bioeconomy more climate change resiliant
- There is large potential with regard to further development of the current technologies and development of others through exploitation of the phytomicrobiome

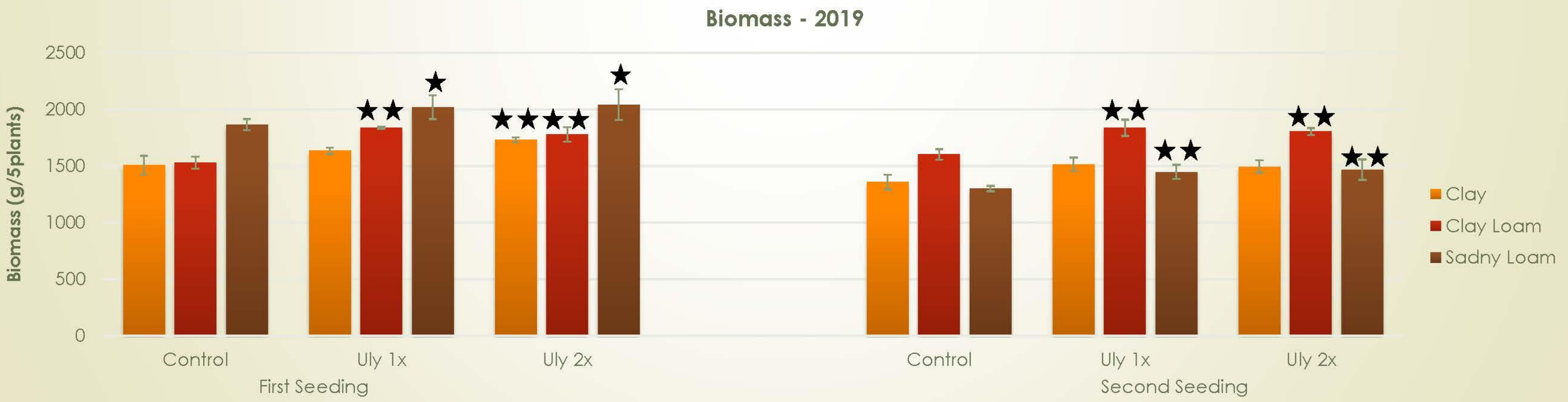
Progress to date – potato field trials

- Consortium at seeding, at hilling, at both times
- Nutritional quality assessed (starch, protein, ascorbic acid and phenolics) in tubers
- Greatest increase was for treatments to which the consortium was applied at seeding
- Inoculation increased in-season biomass **27.9%,** tuber yield by **20%**

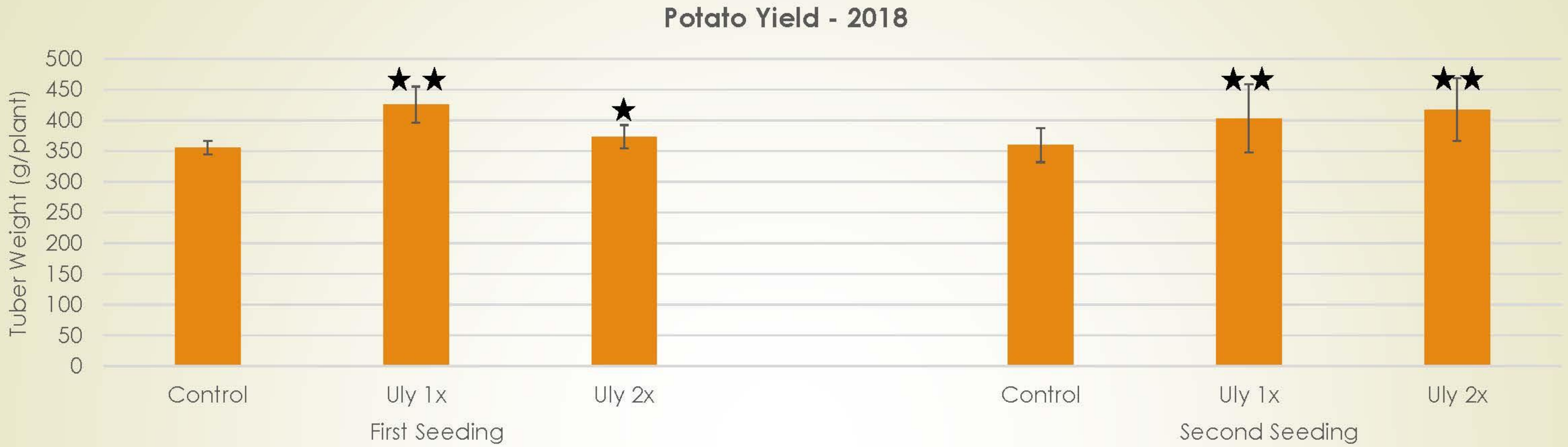
BioMasse Canada

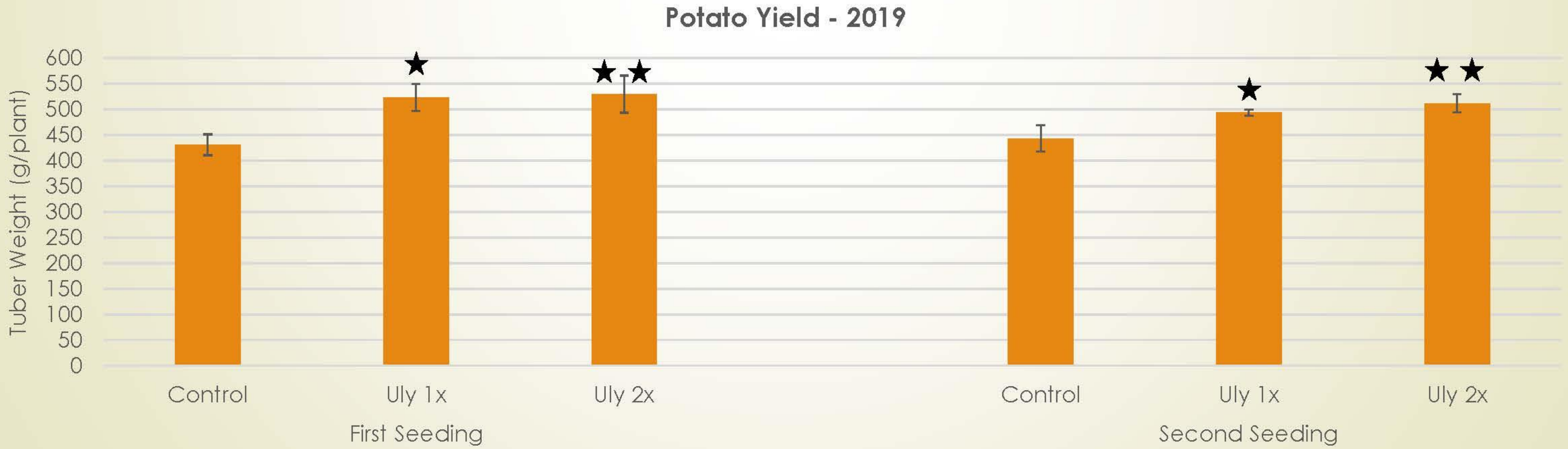
RÉSEAU BIOFUELNET CANADA

Corn Effects



Potato Effects





Relevance to the industry

- Evaluated consortium shows clear benefits to production of both biomass (crop residues) and food
  - Strongly enhances market for this consortium
  - Promotes use of biologicals in general
- Research on novel plant growth promoting strains shows clear benefits; they have been patented
  - The supernatant material is being evaluated in a commercial context and is showing promise
  - The company is developing a product based on this
- PGPR or their products enhance biomass production in a sustainable way and so strengthens the underpinning of the Canadian bioeconomy

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Critical Success Factors

- Success Factors:
  - Technical: Can new strains be developed for application to crops in a commercial setting?
  - Market: Essentially all crop production, but particularly so in areas where stress could be more common
  - Business: Growth and development of companies focused on development of plant-microbe interactions – both strains and signals
- Potential challenges:
  - Technical: Screening for strains and signal compounds they produce
  - Technical: Scale up and commercial development
  - Technical: Develop demand from the bioeconomy, including both biomass and food sectors

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Critical Success Factors - Continued

- Success of the project will:
  - Demonstrate the economic and agronomic viability of phytomicrobiome members in developing sustainable and climate change resilient crop production
  - Provide new revenue streams from under utilized, marginal lands and for crop residues, for agricultural producers.

BioMasse Canada

RÉSEAU BIOFUELNET CANADA

Future Work

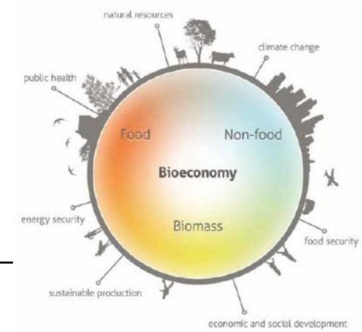
- Continue to isolate and evaluate new strains and the compounds (biostimulants) they produce, and combine with other biostimulants
- Collect further data on strains already under investigation, and signal compounds they produce
- Collect additional data regarding PGPR and biostimulant effects on biomass crops
- Evaluate PGPR and PGPR signals with flavonoids
- Collect data on existing microbial materials (consortium) and new strains through to 2023

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

Summary



- An established consortium is showing promise outside the greenhouse
- New strains are isolated and are showing substantial promise
- Evaluation of other biostimulants is ongoing and also showing promise
- This approach shows the potential to enhance the production of biomass from both dedicated biomass crops and food crops, facilitating establishment and growth of the Canadian bioeconomy

BioMasse Canada

McGill MACDONALD

RÉSEAU BIOFUELNET CANADA

The End!

Questions?