Benefits of *Ascophyllum nodosum* extracts in Plant Health and Production: From Genomics to Yield and Quality
Who is Acadian Seaplants…

- Family owned and operated since 1981
- One of Canada’s 50 Best Managed Companies for 15 years
- Over 350 employees located multiple countries:
  - Brazil
  - Canada
  - Chile
  - Colombia
  - Ireland
  - Japan
  - Mexico
  - United States
  - Colombia
  - China
  - India
  - Korea

- Approximately 700 harvesters located in Atlantic Canada, Maine and Ireland
Presentation outline:

• Biostimulants: A fast growing sector of ag products
• Case study: Unique *Ascophyllum nodosum* marine plant extracts (ANE)
• What does the research tell us?
  – Fundamental research on whole-model-plant physiology
  – How plants react to different stresses
  – Implications in abiotic stress management
  – Tracing plant response from genetic regulation to phenotypic expression
• Different application methods (foliar, soil)
• New research on soil health and how we can synergize with microbial communities in the rhizosphere
• Innovation through integration:
  – Synergistic effects from biostimulants with other products, including conventional and biopesticides and pest management programs.
• Take home messages
If you are suffering from Rheumatism, ALWAYS take LA-CAS-KA internally for the Blood and
as SNAKE-OIL LINIMENT externally. When used together we GUARANTEE A CURE in every
instance or MONEY REFUNDED.

If You Are Afflicted With DEAFNESS
Get Our Specially Prepared
PURE Rattlesnake Oil
Biostimulants
Well positioned between Plant Protection Products and Fertilizers
Science and Regulation of Biostimulants:
Outcomes of a scientific review at the request of the European Commission

- “...Biostimulants open up new approaches to the improvement of plant nutrition and plant protection, yet they are not fertilizers nor pesticides...”
- “For some products, like seaweed extracts, this would not be easy as both nutritional effects and defense elicitation effects are described”.
- Focus on peer-review research to support positioning and claims as biostimulant

Patrick du Jardin, Head of Plant Biology Unit of Gembloux Agro-BioTech, University of Liège, 2012

“Biostimulants are a Key Enabling Technology (KET) of sustainable EU agriculture within the circular economy”

- How biostimulants improve the quality and stock of biological materials in agriculture
- How biostimulants foster optimal use of technical materials in agriculture
- Biostimulants can help reduce flow of agricultural inputs and improve use efficiency

Eric Liegeois, Informa Biostimulant Conference, May 2015
The Emerging Landscape of Products – Broad and (Potentially) Confusing

- **Acids**
  - Humic substances
  - Fulvic acids
  - Other organic acids
  - Fatty acids / lipids
  - B Vitamins
  - Inorganic salts
  - Amino acids

- **Other**
  - Humins
  - Polymeric compounds
  - Protein hydrolysates
  - Peptides
  - Nitrogenous compounds
  - Betaines
  - Polyamines

- **Microbials**
  - PGPR’s
  - Mycorrhizae, Trichoderma, other beneficial fungi
  - Rhizobium
  - Complex communities / consortia

- **Extracts**
  - Seaweed / Kelp
  - Carboxyls
  - Phytohormones
  - Laminarin, alginates, other polysaccharides
  - Betaines

- **Allelochemicals**
  - Polyphenols
  - Botanicals

Source: Agricen Sciences’ analysis of market analysts, survey papers on Biostimulants
Acids and Extracts are the Dominant Product Categories (by Market Share)

Percent of Sales (est., 2018)

- Acids: 42%
- Extracts: 33%
- Other: 25%

Sources: MarketsandMarkets Analysis; P. du Jardin analysis of Plant Biostimulants 2015
Biostimulants and Biocontrol
Fruit
- Setting processes
- Fruit size and weight
- Quality
Crouch and van Staden, 1992; Chouliares et al., 1997; Colapietra and Alexander, 2006; Basak, 2008; Chouliares et al., 2009; Ross and Holden, 2010; Loyola and Muñoz, 2011; Paradiković et al., 2011; Khan et al., 2012; Paradiković et al., 2013; El-Hamied et al., 2015.

Seeds / Seedlings
- Germination
- "Starter effect"
- Overcoming transplant stress
- Priming effect
- Seed quality
Aldworth and van Staden, 1987; Featonby-Smith and van Staden, 1987; Crouch and van Staden, 1992; Russo et al., 1993; Moller and Smith, 1998; Demir et al., 2006; Sivasankari et al., 2006; Farooq et al., 2008; Neily et al., 2010; Kumar and Sahoo, 2011; Matysiaik et al., 2011; Kalaivanan and Venkatesalu, 2012.

Plant
- Plant growth/yield and physiological modulation
- Water/nutrient uptake
- Stress response
Beckett and van Staden, 1990; Beckett et al., 1994; Blunden et al., 1996; Adani, 1998; Mancuso et al., 2006; Zhang and Ervin, 2008; Ross and Holden, 2010; Sangeetha and Thevanathan, 2010; Zhang et al., 2010; Fan et al., 2011; Kumar and Sahoo, 2011; Matysiaik et al., 2011; Paradiković et al., 2011; De Lucia and Vecchietti, 2012; Petrozza et al., 2012; Paradiković et al., 2013; Alam et al., 2014; Petrozza et al., 2014; Saa et al., 2015.

Flowers
- Flowering and sprouting induction
Basak, 2008; Petri et al., 2008; Haweroth et al., 2010; Pereira et al., 2011.

Soil
- Physico-chemical properties
- Development of beneficial soil microorganisms
- Water/nutrient retention
- Overcoming salinity stress
Booth, 1969; Guiry and Blunden, 1991; Temple and Bomke, 1988; Chen et al., 2002; Gulser et al., 2010; Ross and Holden, 2010; García-Martínez et al., 2010; Tejada et al., 2011; Alam et al., 2014.
~80 Articles (just our Acadian team)
Strategic Alliances for R&D
Advancing the understanding of our products through worldwide partnership and communication.
• 30,000 ft² facility comprised of a laboratory, 3 greenhouses and experimental farm
Noted Seaweed Resources for Extract Production

- Ascophyllum nodosum
- Eklonia maxima
- Ochtodes secundiramea
- Laurencia dendroidea
- Durvillaea potatorum
- Enteromorpha intestinalis
- Gelidium pectinutum
- Hypnea musciformis
- Gracilaria textorii
- Sargassum wightii
- Rosenvigea intricate
- Kappaphycus alvarezii
- Ulva lactuca
- Padina gymnospora
- Hypnea musciformis
- Macroystis pyrifera
- Lessonia sp.
- Laminaria sp.
Ascophyllum in the intertidal zone  Ecklonia in the subtidal zone
Ascophyllum Resource Research
Full-time Resource Management
Led by Dr. Raul Ugarte, Resource Scientist,
<table>
<thead>
<tr>
<th>Product name</th>
<th>Seaweed name</th>
<th>Company</th>
<th>Application</th>
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<tbody>
<tr>
<td>Acadian®</td>
<td><em>Ascophyllum nodosum</em></td>
<td>Acadian Agritech</td>
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<td>Emerald RMA</td>
<td>Red marine algae</td>
<td>Dolphin Sea Vegetable Company</td>
<td>Health product</td>
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<td>The Espoma Company</td>
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<td>Inversiones Patagonia S.A.</td>
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<td>Guarantee®</td>
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<tr>
<td>Profert®</td>
<td><em>Durvillea antarctica</em></td>
<td>BASF</td>
<td>Plant biostimulant</td>
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<td>Sea Winner</td>
<td>Unspecified</td>
<td>China Ocean University Product Development Co., Ltd</td>
<td>Plant biostimulant</td>
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<td><em>Ascophyllum nodosum</em></td>
<td>Acadian Agritech</td>
<td>Animal feed</td>
</tr>
</tbody>
</table>
Not a “single chemistry” product

Single mode of action

Multiple potential modes of action
Different Processes, Different Marine Plants, Different products

- Alkaline *Ascophyllum* extracts
- Non-*Ascophyllum* Alkaline extracts
- Green “suspension” products
... result in different NMR profiles...

Acadian *Ascophyllum* Alkaline extract

Sargassum Alkaline Extract

Complex Matrix of Components

Sample Number 13-47
Sample Size 10 mg/ml
Metabolomics can be used to qualify complex mixtures like Acadian

- Uses Nuclear Magnetic Resonance (NMR) spectroscopy to analyze organic compounds
- Can be used to compare products on the basis of biochemical similarity
- Individual peaks and combinations can also be used to identify individual components
Genomics to Phenomics
Research Direction

Genomics
- functions of genes

Transcriptomics
- mRNA expression

Proteomics
- study of proteins

Metabolomics
- chemical fingerprints from cellular processes

Phenomics
- physical and biochemical as plants respond to genetic and environmental influences

Approximate R&D expenditure

Chromosomes
- Genome
- Cell
- DNA
- Genes
- Molecular machine
- Protein

Community of cells
- Living cell

Acadian Plant Health
Sustainably Empowering Plants.

Acadian Plant Health™ is a division of Acadian Seaplants Limited
What we’re NOT talking about
What we ARE talking about

Disease tolerance vs Intolerance

Drought tolerance vs Intolerance
Genes associated with stress resistance in plants

Ethyl acetate sub-fraction of SSEP 1186 Induced Genes
**Ascophyllum** brings change in transcripts..... Microarray reveals

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Gene product</th>
<th>Accession number</th>
<th>Gene product</th>
<th>Accession number</th>
<th>Gene product</th>
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<td>AT1G208 40</td>
<td>Tonoplast (vacuole membrane) located</td>
<td>AT2G436 20</td>
<td>Putative endochitinase</td>
<td>AT4G165 90</td>
<td>Cellulose synthase</td>
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<tr>
<td>AT3G108 70</td>
<td>Leaf morphogenesis</td>
<td>AT3G114 10</td>
<td>Protein phosphatase 2C (PP2C)</td>
<td>AT2G468 30</td>
<td>Response to cadmium ion</td>
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<td>AT1G558 40</td>
<td>Root hair development</td>
<td>AT5G159 50</td>
<td>S-adenosylmethionine decarboxylase</td>
<td>AT5G139 30</td>
<td>Chalcone synthase</td>
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<td>AT3G030 50</td>
<td>Cell wall polysaccharide polymerization</td>
<td>AT2G208 70</td>
<td>Anthocyanin biosynthesis</td>
<td>AT2G313 80</td>
<td>Zn ion binding</td>
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<td>AT1G627 70</td>
<td>Increased root length, fungus resistance</td>
<td>AT2G406 10</td>
<td>Putative expansin</td>
<td>AT5G082 60</td>
<td>Salt stress responsive</td>
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<td>AT4G396 70</td>
<td>Glycolipid transfer protein</td>
<td>AT3G449 90</td>
<td>Xyloglucosyl transferase 8</td>
<td>AT5G557 20</td>
<td>Pectin lyase-like superfamily protein</td>
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<td>AT1G211 20</td>
<td>Putative ATPase</td>
<td>AT4G224 90</td>
<td>RCc3-like protein RCc3 protein</td>
<td>AT1G637 10</td>
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<td>AT3G544 20</td>
<td>Class IV chitinase (CHIV)</td>
<td>AT1G746 70</td>
<td>GAST1-like protein</td>
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</table>

Turn on Your Plants to Optimize Crop Health
Mitigation of chilling stress...

Through influence on cellular osmolytes

- ANE treatments lead to increased proline & glycine betaine
- Genetic studies confirm up-regulation of the proline synthesis genes
- Metabolomics NMR studies indicate chemical shifts corresponding to changes in soluble sugars and fatty acid levels
- Suggests primary mode of freezing tolerance is through priming of plants for osmoprotectant accumulation and altering fatty acid levels (membranes)

Nair et al., 2012. Transcriptional and metabolomic analysis of *Ascophyllum nodosum* mediated freezing tolerance in *Arabidopsis thaliana*. BMC Genomics 13:643
Salinity stress tolerance...

<table>
<thead>
<tr>
<th>Arabidopsis</th>
<th>Peppers</th>
<th>Lettuce</th>
<th>Tomato</th>
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<tr>
<td>- NaCl</td>
<td>Fertilizer + Salt</td>
<td>Fertilizer + Salt + ANE</td>
<td></td>
</tr>
<tr>
<td>+ NaCl</td>
<td>0.5g/L + NaCl</td>
<td>+ Salt</td>
<td></td>
</tr>
<tr>
<td>1.0g/L + NaCl</td>
<td>150mM NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150mM NaCl</td>
<td>Fertilizer + Salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Salt + ANE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control: + Salt + ANE
Salinity Damage: Strawberry

Saline Soil, Fertilizer only

Fertilizer + ANE

salt burn
Strawberry Trial: Ventura, California

Control

ANE Treated

Acadian Plant Health™ is a division of Acadian Seaplants Limited
Chart 10: Acadian on Strawberries - Ventura County, Winter 2007 - Cumulative Marketable Production Net Return by Pick Day

Break even point approximately $18,000
Improved Growth Under Stress

Photo: By Katy Griesoschewski. Soybean water stress study at Dr. James S. Craigie Research Center, Canada
Electrolyte Leakage

Percentage Electrolyte Leakage After Water Stress

Katy Griegoschewski soybean water stress study Dr. James S. Craigie Research Center, Canada

Acadian Plant Health™ is a division of Acadian Seaplants Limited
Plant Temperature under Water Stress

Photo: By Katy Griegoschewski and Alex Martynenko. Soybean water stress infrared study Dr. James S. Craigie Research Center, Canada
Transgenic GUS marker gene
Cytokininin Bioassay

Transgenic Plants will respond to the presence of Cytokininin by producing an enzyme that creates a blue color. This demonstrates that Acadian Extract induces Cytokininin-like activity.
Objectives

- Examine seaweed extracts effects on endogenous PGR biosynthetic pathway
- Examine the induction of these pathways to better determine “how seaweed extracts work”

Wally et al., 2013. Regulation of Phytohormone Biosynthesis and Accumulation in Arabidopsis Following Treatment with Commercial Extract from the Marine Macroalga Ascophyllum nodosum. JPGR 32(2) 324-339
Visualized using transgenic *Arabidopsis*

- ANE activates CK responsive gene promoter ARR5 →
- This drives GUS expression →
- This leads to blue staining in areas where cytokinin is sensed in the plant

*Wally et al., 2013. Regulation of Phytohormone Biosynthesis and Accumulation in Arabidopsis Following Treatment with Commercial Extract from the Marine Macroalga Ascophyllum nodosum. JPGR 32(2) 324-339*
Modification of PGH levels

Wally et al., 2013. Regulation of Phytohormone Biosynthesis and Accumulation in Arabidopsis Following Treatment with Commercial Extract from the Marine Macroalga Ascophyllum nodosum. JPGR 32(2) 324-339
...via up-regulation of synthesis genes...

Wally et al., 2013. Regulation of Phytohormone Biosynthesis and Accumulation in Arabidopsis Following Treatment with Commercial Extract from the Marine Macroalga Ascophyllum nodosum. JPGR 32(2) 324-339
Alternaria infection levels

Dr. Jayaraman Jayaraj (Simon Fraser University)
Effect of elicitors on *Alternaria* infection in Carrot

- Elicitors applied 6 h before inoculation, 10 and 20 days after inoculation on 2 month old carrot plants
- Conidial suspensions (1x10^6 conidia/ml) of *Alternaria radicina* and *Botrytis cinerea* were used for inoculation
- Disease severity rated 10/12 and 25 days after inoculation PDI calculated based a 6 (1-6) point disease rating scale

**LSD (p=0.05)= 1.34**
Evaluation of *Ascophyllum* extract for suppression of *Fusarium oxysporum* on greenhouse cucumber (0.5% extract)

**Fig. 6a**
Chitinase activity (in units NAG/mg protein) over time (hours) for different treatments: Control, Spray, Drench, Spray+Drench.

**Fig. 6b**
β-1, 3 Glucanase activity (in units mg protein) over time (hours) for different treatments: Control, Spray, Drench, Spray+Drench.

**Fig. 6c**
Peroxidase activity (in units units/mg protein) over time (hours) for different treatments: Control, Spray, Drench, Spray+Drench.

**Fig. 6d**
Polyphenol oxidase activity (in units units/mg protein) over time (hours) for different treatments: Control, Spray, Drench, Spray+Drench.

**Fig. 6e**
Phenylalanine ammonia lyase activity (in units units/mg protein) over time (hours) for different treatments: Control, Spray, Drench, Spray+Drench.

**Fig. 6f**
Lipoxigenase activity (in units units/mg protein) over time (hours) for different treatments: Control, Spray, Drench, Spray+Drench.

Fig. 6. Defense enzyme activities in cucumber plants treated with Stimplex (0.5%).

a. chitinase; b. β-1,3-glucanase; c. peroxidase; d. polyphenol oxidase; e. phenylalanine ammonia lyase; f. lipoxigenase. Vertical bars indicate mean±SE. Data are means of three replicates.
Total phenolic levels

6-methoxymellin levels

Phenolics

Phytoalexins
Generalized Recommendations – Application Timings and Targets:

Applications to foliage

Seed

Transplant

Two applications 4 weeks apart

Applications to Roots

When needed mixed with fungicides, insecticides, nutrients and other inputs

Post-harvest

(Soak)

(Treat / Soak)
Wine Grape Treatments

• Rate: 3-4 L/ha
• Treatment timings:
  • 10-15 cm inch shoot growth: foliar
  • One week after previous application: foliar
  • 20-25 cm shoot growth: foliar
  • 5 days pre-bloom: foliar
  • ‘BB’ sized berries (2-3 mm): foliar
  • Veraison: foliar
  • Post harvest foliar/soil treatment was applied.
In this three year Pinot Noir wine grape trial bunches treated with Acadian are visually longer and have more berries set.
Acadian Extract on Desirable Yield

The average increase of berries per bunch is 14.6%.

Bunch weight was increased an average of 14.5% over 10 trials.
"We speak a lot of the importance of sustainable food systems for healthy lives. Well, it starts with soils."

José Graziano da Silva, FAO Director-General
Plant-microbe interactions

Epiphytic (phyloplane) microbes

Leaf pathogens (e.g. *Alternaria brassicicola*)

Induced systemic resistance & systemic acquired resistance

Carbon root exudates attract heterotrophic microbes

Root pathogens (e.g. *Fusarium oxysporum*)

Plant growth promoting rhizobacteria and fungi

Microbe-induced priming

Mycorrhizae

Endocytosis of microbes via root hairs

*N* fixing bacteria

Uptake of organic C, N & P from soil organic matter (e.g. from lyzed microbial cells)

Increased or reduced nutrient availability (e.g. P solubilizing microbes, siderophores)

Secreted enzymes compete for nutrients
Field study

EFFECT OF SSEP ON GROWTH OF STRAWBERRY UNDER FIELD AND GREENHOUSE CONDITIONS
Fig. 7: Effects of SSEP on microbial colony counts

![Bar chart showing effects of SSEP on microbial colony counts in greenhouse study and field study.

Fig. 8: Effects of SSEP on average well colour development (AWCD)

![Bar chart showing effects of SSEP on AWCD in greenhouse study and field study.]
Effects of tri-weekly applications of SSEP on microbial respiration (carrot, Maverick)

Note: All rates had greater microbial respiration when applied once every three weeks.
To investigate the interaction of *Ascophyllum* extracts in amended soils for high value horticultural crops: Tomatoes and Peppers

**Root colonization**

Evidence of mycorrhizal association

Evidence of arbuscular mycorrhizal colonization
Photos at harvest of tomato (March 24th)

**With Plants**
- Seaweed extract plus fertilizer
- Seaweed extract alone

**Without Plants**
- Seaweed extract plus fertilizer
- Seaweed extract alone
Preliminary results of Pepper’s Experiment

Pepper fruits number

<table>
<thead>
<tr>
<th>Fruits number per plant</th>
<th>F+</th>
<th>p&lt;0.001</th>
<th>F-</th>
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<tbody>
<tr>
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<td>b</td>
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Pepper shoots fresh weight

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<tr>
<th>Shoots fresh weight (g)</th>
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<th>F-</th>
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Pepper roots fresh weight

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Pepper shoots dry weight

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Photos at harvest of pepper (May 3rd)

Root system of pepper

without fertilization  with fertilization

without fertilization  with fertilization
Beta-diversity | Pepper | planted

Results of the permanova

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<th>Df</th>
<th>SumsOfSqs</th>
<th>MeanSqs</th>
<th>F.Model</th>
<th>R2</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fertilized</strong></td>
<td>1</td>
<td>0.6691</td>
<td>0.66912</td>
<td>4.0584</td>
<td>0.08107</td>
<td>0.001 ***</td>
</tr>
<tr>
<td><strong>Residuals</strong></td>
<td>46</td>
<td>7.5841</td>
<td>0.16487</td>
<td></td>
<td></td>
<td>0.91893</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>47</td>
<td>8.2532</td>
<td></td>
<td></td>
<td></td>
<td>1.00000</td>
</tr>
</tbody>
</table>

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Integrate to Innovate?
Blueberry Protocol

• 18 rows (i.e., experimental units),
• six rows of each of the three varieties
• split into three replicated blocks of two rows each.
• In each replication, a control row received a fertility management plan designed to stimulate root regrowth with the following:
  – Ammonium polyphosphate solution (10-34-0) at 30 L/acre,
  – Phosphites (Phostrol®) at 2 L/acre,
  – Humic acid-based fertilizer product (Black Label® Zn) at 20 L/acre
  – Supplemental boron (Borosol® 10) at 1 L/acre; t

• Treated rows received application of these same products with the addition of Stella Maris™ at 2 L/acre.
Figure 1. First pick fruit yield (+/- SE) in response to Stella Maris™ application for three varieties of highbush blueberry in the Fraser Valley in 2016. Bars connected by the same letter are not statistically different (p < 0.05).
Figure 5. First pick fruit weight (± SE) in response to Stella Maris™ application for three varieties of highbush blueberry in the Fraser Valley in 2016. Bars connected by the same letter are not statistically different (p < 0.05).
Efecto de extractos de *Ascophyllum nodosum* sobre *Fusarium* Crown Rot en Sandía

Means followed by the same letter are not significantly different, P=.05, orthogonal contrasts.
## Fungicides used to control *Sclerotinia* disease spread

<table>
<thead>
<tr>
<th>#</th>
<th>Fungicide</th>
<th>Application per acre</th>
<th>Active ingredient</th>
<th>Content of a.i</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lance</td>
<td>142 g</td>
<td>Boscalid</td>
<td>70%</td>
<td>Systemic</td>
</tr>
<tr>
<td>2</td>
<td>Proline</td>
<td>126 - 147 mL</td>
<td>Prothioconazole</td>
<td>480 g / L</td>
<td>Systemic</td>
</tr>
<tr>
<td>3</td>
<td>Quadris</td>
<td>280 to 400 mL</td>
<td>Azoxystrobin</td>
<td>250 g / L</td>
<td>Systemic</td>
</tr>
</tbody>
</table>

Registered fungicides for use against Sclerotinia stem rot, as given in the crop protection guide – Province of Manitoba
Values represent Mean ± Standard error, n=10
Biostimulant Thinking Points

• Biostimulants will continue to play a larger part in the future of agriculture in both soil and foliar applications
• EBIC is championing biostimulants in Europe
  – Focus on abiotic stress, nutrient uptake and microbials
• USBC also establishing similar norms for US industry
• Joint efforts to represent Biocontrol and Biostimulant interests/legislation (EBIC/IMBA; USBC/BPIA)
• There are many biostimulant products touting related benefits
• Companies are looking towards “integrated” solutions to be “innovative”.
• The fun is in identifying the benefits, synergies, antagonisms and new uses for biostimulants in agriculture.
Ascophyllum in Agriculture
Take Home Messages

- Ascophyllum nodosum extract provides general biostimulant effects
- Identifying active ingredients in Ascophyllum extracts
  - Examination of OMICS research to monitor changes in gene expression and physiology
  - Focus on abiotic and biotic challenges
- Connecting molecular activities with specific plant responses
  - In controlled bioassays
  - In the field
- Typical applications are multiple use depending on crop programs
  - Foliar and/or soil applications
- Delivering best-use application information to growers and end-users on ongoing basis to maximize ROI
Thank you

Tak
Grazie
Obrigado
Gracias
Merci