Holistic Nutrients: Research Proposal

July 10

2014

Holistic Nutrients will be located in the City of Corner Brook and serve Newfoundland and the Atlantic Region

Holistic Nutrients

Prepared by: *Andrew Abbass, Owner/Operator*

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1.0 INTRODUCTION

Backgrounder

The Enterprise

Holistic Nutrients is an enterprise developed for Western Newfoundland to supply an organic supplement that acts as a soil inoculant for root development, a growth and bloom stimulant, a biopesticide, biofungicide, and a method for increasing drought tolerance. The enterprise will achieve this aim through controlled breeding of a species of darkling beetle, *Tenebrio moitor.*

The Purpose

To demonstrate the effectiveness of Chitin and Chitin-derived products from *Tenebrio molitor* (common darkling beetle) in complimenting the Newfoundland Agriculture and Forestry Industries, as well as providing for a Global Climate Change Action Plan.

Why *Tenebrio molitor*?

Has the highest total exoskeleton chitin level for of any arthropod. (1) Lives on dry, low-nutrient, agricultural waste material. Proper management of the production process creates a chitin source, a balanced protein source, a concentrated organic fertilizer and a beneficial endophyte source. Different portions of the lifecycle can be used for different purposes. No by-products to properly managed process, negligible carbon footprint. Minimal manual or mechanical processing required. Unlike chitin from shellfish, *Tenebrio molitor*-based chitin does not have to be demineralised.

Research Partner

Memorial University, Grenfell Campus, is uniquely positioned for completing this research. With access to their Environmental Science programme., new Soil Science Labs and excellent researchers this project can be completed entirely within province.

Requirements

To complete this research, Holistic Nutrients has identified funding sources with the necessary capital. Although funding commitments have not been made at this time, options are available through the Green Fund NL and the Department of Natural Resources, Agrifood Division's Growing Forward 2 program. Holistic Nutrients itself will be contributing by providing the core materials required for this research .

2.0 RESEARCH REVIEW

Research Review

- Chitosan, a purified polymer derived from chitin, is used primarily as a natural seed treatment and plant growth enhancer, and as an ecologically friendly biopesticide substance that boosts the innate ability of plants to defend themselves against fungal infections (2)
- Chitosan-active biopesticide represents a new tier of cost-effective biological control of crops for agriculture and horticulture (3)
- Biocontrol mode of action of chitosan elicits natural innate defense responses within plant to resist insects, pathogens, and soil-borne diseases when applied to foliage or the soil (4)
- Chitosan increases photosynthesis, promotes and enhances plant growth, stimulates nutrient uptake, increases germination and sprouting, and boosts plant vigor. When used as seed treatment or seed coating on cotton, corn, seed potatoes, soybeans, sugar beets, tomatoes, wheat and many other seeds, it elicits an innate immunity response in developing roots which destroys parasitic cyst nematodes without harming beneficial nematodes and organisms (5) (6)
- Agricultural applications of chitosan can reduce environmental stress due to drought and soil deficiencies, strengthen seed vitality, improve stand quality, increase yields, and reduce fruit decay of vegetables, fruits and citrus crops (7)
- Horticultural application of chitosan increases blooms and extends the life of cut flowers and Christmas trees (8)
- The US Forest Service has conducted research on chitosan to control pathogens in pine trees (9) (10) and increase resin pitch outflow which resists pine beetle infestation (11)
- Chitin oligosaccharides have been shown to play an important role in defense mechanisms of plants against microbial invasion (12)
- Chitin promotes carrot somatic embryos survival (13)
- Chitin fragments can desensitize the perception system of tomato, which can lead to improvement of the defense mechanism in tomato cells (14)
- Chitin in the form of lipo-chitin can induce the formation of nodule in soybean root (15)
- Chitin boosts the defense systems of rice (16) (17)
- Incorporation of chitin in poultry feed at a level of 0.5 percent decreases the food consumption ratio and increases the body weight by 10-12 percent in comparison with chicks fed on a chitin-free diet (18) (19)
- Fungal endophytes have been shown to be the primary means for plant communication (20) (21)
- Chitin shown to increase forest litter decomposition (22)

Net Benefits of Introducing Chitin to the Agriculture and Forestry Industry

- Increased drought tolerance of crops & trees (7) (9) (10) (11)
- Increased total yield and decreased decay rates of crops (2) (5) (6) (7) (8)
- Organic methods of suppressing large scale forestry infestations (2) (3) (4) (6) (9) (10) (11)
- Reduced reliance on over application of fertilizer to maintain soil fertility (5) (6) (7)
- Increased pathogen resistance leads to fewer pesticides and fungicides entering the environment and food supply (2) (3) (4) (5) (6) (7) (9) (10) (11) (12) (14) (15) (16) (17)
    - Pesticides reduce biodiversity, reduces nitrogen fixation, contributes to pollinator decline, destroys habitats, threatens endangered species.
    - Pests can develop pesticide resistance, creating new super pests that require stronger chemicals
    - Chlorinated hydrocarbon pesticides dissolve in fats, are not excreted and tend to be stored indefinitely. Biological magnification occurs.
    - Decreasing pesticide usage means beneficial insect populations are not damaged.
    - Decreasing fungicide means plant litter decomposition continues and beneficial fungal networks remain undamaged.
    - Pesticide use has been linked to a variety of diseases and disorders

Potential Benefits to be Verified by Research

- Net Reduction in GHG emission from Agricultural Sources and other uses (page 6)
- Net Increase in GHG sequestration through Forestry and other uses (page 10)

3.0 AGRICULTURE RESEARCH

Verified Methods for Reducing CO_2 & CO_2 Equivalent Footprint

While a large volume of work has been completed pertaining to the specific uses of chitin and chitin-based products for the Agriculture Industry, there's very little research yet that shows how adoption of chitin as a foundation method for biocontrol and yield increase in these industries affects GHG production and carbon sequestration.

Taken as a whole, the Agricultural Industry contributes close to 30% of the world's greenhouse gas emissions. (23)

Suggested Methods for reducing this footprint from existing research:

1. Use a high protein, low carbon footprint insect-based feedstock like Tenebrio molitor larva to compliment the poultry industry. This converts traditional waste products like bran into a viable protein source that also acts to decrease feed consumption and increase total body weight. (18) (19)
2. Reduce the number of grazing animals like cows and sheep to more manageable levels and rely more on poultry, which has a much lower GHG producing potential. (24)
3. Use insect-based fertilizers instead of manure to reduce anaerobic decomposition of manure which leads to GHG production. Soil amendments also replenish symbiotic microbes which allows greater root development over a shorter period in less soil, increasing total yield. (2) (5) (6) (7) (8)
4. Manure from livestock should be mandated to be transferred to biomass generators for energy production during decomposition to capture methane.

Project #1 - Determine Potential for Total GHG Offset

Hypothesis

Increasing soil fertility with insect-based fertilizers reduces the total GHG emissions from over application of manure or industrial fertilizers and allows for increased carbon uptake by fungal endophytes.

Methodology

- Direct crop testing via different application levels of frass and chitinous products in comparison with established methods to determine overall fertilizer requirement to obtain the same yield and how this application affects overall soil fertility.
- Early research has already been demonstrated on small scale with *Antirrhinum* & *Coriander Sativum* (Appendix II)

Expected Results

Collection of more data to allow accurate modelling of the increased C02 uptake in both plants and soil through chitin and endophyte amendment. Data will be used to show how adoption of these strategies can move Agriculture from being a major GHG producer to a neutral or carbon negative industry.

- Net Reduction in greenhouse gases from Agricultural Sources
    - Less Manure usage = Less anaerobic decomposition = Less methane
    - Increased root development means the same crops will grow in less soil through more efficient use of the medium, decreasing the fertilizer requirement = Less nitrogen-based GHG

Industry Partner (Potential)

The Greenhouse Nursery and Gardenstore
Local farms

Project #2 - Networking Crops Through Endophytic Fungal Networks

Hypothesis
The waste of Tenebrio Molitor is not only a source of chitin fragments and fertilizer, it is also a potential vector for both unwanted pathogens and beneficial endophytes. The gut populations of these endophytes fluctuates based on their diet, and so does the population that ends up their waste. By tailoring the *Tenebrio molitor* feedstock to specific food sources that host these beneficial populations, it should be possible to increase the populations of those beneficial endophytes in their waste to act as a crop soil inoculant for specific species and to allow for increased endophyte mediated immune response via fungal networks.

Methodology

- Assay population of bacteria and fungus to ensure absence of pathogens in initial breeding stock. Stock has been obtained from a commercial source and bred in a clean environment on heat-treated substrate for over 2 years.
- Determine how their gut flora population fluctuates based on organic moisture feed stock. (E.g.: Carrots vs. potatoes vs. apples vs. tomatoes)
- Test the specific strains against their source and controls in sterile soil conditions to determine efficacy of tailored inoculants
- Test the specific strains in crop settings to determine efficacy of endophyte-mediated immune response through undergrowth fungal networks via induction of insect predators to portions of test crop

Expected Results
Research has shown that endophytes are ubiquitous and necessary for plant health. Lack beneficial endophytes increases the likelihood of colonization by pathogenic bacteria and fungus.

Research has also shown that endophytic fungal networks allow crops to communicate the presence of pests soil based on a shared immune response. (20) (21) By employing this effect through crop soil inoculation of these beneficial, it should be possible to ensure crops are properly colonized by these endophytes as well as providing a method for networking these crops together on a large scale through fungal networks. This should create a unified immune response that would further reduce the possibility of pest infestation. When one crop is attacked, the remainder are forewarned via these endophyte networks.

Industry Partner (Potential)
Holistic Nutrients
Local farms

Project #3 - Increased Nectar Secretion through Immune Stimulation

Hypothesis

Pollinators are one of the most important insects to modern agriculture. Modern use of pesticides has shown to be incredibly damaging to these vital species. Bloom stimulation properties of chitinous products through immune stimulation represents a possible route for increasing honey production while still maintaining biocontrol. Increase bloom production and overall plant vitality should increase the flower nectar available to pollinators, increasing final harvest of honey from commercial beehives above that of the control.

Methodology

- Test chitinous products against flower strains to determine effect on nectar production.

Expected Results

Bloom stimulation effects of chitinous products should increase total honey yield while maintaining pollinator vitality for commercial producers.

Industry Partner (Potential)

Newfoundland Bee Keepers

4.0 FORESTRY RESEARCH

Project #1 - Determine Potential for Total GHG Offset

Hypothesis
Stimulating seed production through use of chitinous products on boreal stands should increase CO_2 uptake via cone production and an increased rate of soil decomposition and carbon storage in ectomycorrhizal networks. (22)

Methodology

- Test chitinous products on tree stands to determine potential for use as a bloom stimulant

Expected Results
Collection of more data to allow accurate modelling of the increased C02 uptake in both trees and soil through bloom & ectomycorrhizal stimulation. Data will be used to show how adoption of these strategies can economically increase the rate of CO2 sequestration by trees and their associated ectomycorrhizal networks for use as a tool for large-scale, long-term sequestration of CO2 while improving stand health at the same time.

- 1 tree produces 500 cones @ 1g/cone (dried) - 500g
- Estimate minimum stand density of - 0.5 trees/m^2 (1 tree/2m^2)
- # number of trees per km^2 - 0.5trees/m^2 x 1000000m^2 = 500000 trees
- Sequestration per km^2 = .5kg/tree x 500000 trees = 250 tonnes

Initial estimates based of 500g per tree are quite low and only consider the total weight of dry seed production, not the additional growth that would take place on the forest floor or the increase in biomass of smaller animals that feed on this stimulated growth.

The CO2 sequestration project would also have to be much larger to have a global effect. Boreal forests cover about 16 million square kilometers. If only half of that area is used for this project and only half that is used each year, that's 4 million square kilometers at 250 tons (lowball estimate) per square km, or about 1 billion tons of CO2 per year. It's only about 3 percent of the total global production, but the half kilo per 2m^2 may also be somewhat on the small side.

Industry Partner (Potential)
NL Department of Natural Resources, Forestry Division

Project #1 - Natural Biocontrol Method

Hypothesis

Overuse of pesticides in Forestry destroys not only pathogenic insects, but also beneficial insects that keep these populations in check and those that consume forest floor detritus to return the litter to the soil. Using chitinous products to enhance stand immune response against infestation is a more environmentally friendly method of dealing with these insect imbalances and reduces the possibility of a catastrophic disruption of the soil biome through mass pesticide application.

Methodology

- Test chitinous products against tree stands to determine efficacy immune response against native Newfoundland Forestry pest species (spruce budworm, hemlock looper, etc)

Expected Results

Chitinous products should allow for less invasive methods of biocontrol than pesticides in forestry applications with should also minimize the effects on the health of the soil biome.

Industry Partner (Potential)

NL Department of Natural Resources, Forestry Division

5.0 CONCLUSION

While there has been extensive research over the last three decades demonstrating the effectiveness of chitin-based products, the Agriculture and Forestry industries have been slow in moving this research towards large scale adoption.

The use of chitinous-based products in these major industries has the potential to revolutionize their carbon footprint while at the same time eliminating the levels of toxic chemicals that are being pumped into the environment by these industries.

With the evidence of manmade climate change mounting, completing this research and implementing this movement towards organic biocontrol on a global scale isn't just a healthier and economically viable alternative, it's essential to global sustainability.

Thank you,

Andrew Abbass
July 10th, 2014

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APPENDICES

APPENDIX I – AVERAGE CHITIN CONCENTRATION

Table 1 Samples analysed in the course of this study and results of the colorimetric assay

Sample	Age*	Locality†	Results of colorimetric assay (dry mass basis): Amount used/mg	Chitin/mg	Chitin‡ (%)
Cockroach (*Periplaneta americana*)§	Recent	UK (Bristol)	0.102	0.020	19.25[b]
Shrimp (*Crangon crangon*)§	Recent	Scotland (North Sea)	0.110	0.037	33.96[b]
Beetle (*Tenebrio molitor*)§	Recent	UK (Bristol)	0.100	0.039	39.47[b]
Crayfish (*Pacifastacus*) claw (no HCl)	Recent	UK (Gillingham)	0.400	0.049	12.22[a]
Crayfish (*Pacifastacus*) thorax (no HCl)	Recent	UK (Gillingham)	0.415	0.115	27.66[a]
Crayfish (*Pacifastacus*) claw	Recent	UK (Gillingham)	0.290	>0.131	>45.3[a]
Mantis shrimp (*Neogonodactylus oerstedii*)§	Recent	Florida (Fort Pierce)	0.108	0.036	33.67[c]
Mantis shrimp decayed for 1 week	Recent	Florida (Fort Pierce)	0.105	0.032	30.22[b]
Mantis shrimp decayed for 2 weeks	Recent	Florida (Fort Pierce)	0.110	0.015	13.89[b]
Mantis shrimp decayed for 8 weeks	Recent	Florida (Fort Pierce)	0.105	0.008	7.18[b]
Beetle	Ho (~1.6 ka)	N. Ireland (Thornog Bog)	0.088	0.032	36.54[c]
Beetle (Scarabaeidae)	Ple (~25–28 ka)	UK (Woolpack Farm)	0.100	0.031	31.46[c]
Beetle	Ple (~25–28 ka)	UK (Welland Bank)	0.097	0.025	26.00[c]
Beetle (*Olophrum boreale*)	Ple (<10.8 ka)	Canada (West Mabou)	0.110	0.036	32.54[a]
Beetle (*O. boreale* and *O. rotundicolle*)	Ple (<10.8 ka)	Canada (Benacadie)	0.125	0.027	21.97[a]
Beetle (*O. rotundicolle*)	Ple (~11.8 ka)	Canada (Hirtles)	0.145	0.033	22.63[a]
Beetle (*O. rotundicolle*)	Ple (33–40 ka)	Canada (Lynn Canyon)	0.037	0.009	23.03[e]
Beetle (*O. rotundicolle*)	Ple (~80 ka)	Canada (Moose Point)	0.160	0.053	33.15[a]
Beetle (Tenebrionidae)§	Ple (20 ka)	USA (La Brea, CA)	0.210	0.047	22.62[c]
Shrimp *Astacus* sp. claw 1085**	Pli (~3 Ma)	Germany (Willershausen)	0.220	0.011	4.96[a]
Shrimp *Astacus* sp. claw after HCl 1085**	Pli (~3 Ma)	Germany (Willershausen)	0.190	0.035	18.49[a]
Weevil 31.209–60/30a**	Pli (~3 Ma)	Germany (Willershausen)	0.240	0.091	37.90[a]
Orthoptera 19.466**	Pli (~3 Ma)	Germany (Willershausen)	0.350	0.007	2.06[d]
Insect indet. 31.208 46/34b**	Pli (~3 Ma)	Germany (Willershausen)	0.175	0.009	5.30[d]
Arthropod exuviae #23.589**	Pli (~3 Ma)	Germany (Willershausen)	1.000	0.006	0.57[b]
Beetle	Mio (~4–6 Ma)	France (St. Bauzile)	0.070	0.009	12.70[b]
Carcinoplax prisca	Pli (2.5–4 Ma)	Japan (Miyazaki)	0.209	0.008	4.12[c]
Cancer minutoserratus	Pli (4 Ma)	Japan (Miyagi)	0.195	0.006	2.90[c]
Galene bispinosus	Pli (2–4 Ma)	Australia	0.208	0.014	6.72[c]
Majidae gen. et sp. indet	Mio (5–15 Ma)	Japan (Akita)	0.206	0.006	2.93[c]
Xanthidae (?) gen. et sp. indet	Mio (15 Ma)	Japan (Gifu)	0.205	0.006	2.83[c]
Portunidae gen. et sp. indet	Mio (15–20 Ma)	Japan (Saitama)	0.202	0.007	3.35[c]
Carcinoplax sp.	Mio (15–20 Ma)	Japan (Toyama)	0.210	0.005	2.43[c]
Callianopsis sp.	Eoc (35–40 Ma)	Japan (Hokkaido)	0.198	0.007	3.42[c]
Callianassa faujasi§¶	K (65–72 Ma)	Netherlands (Maastricht)	2.000	0.015	0.73[d]
Zanthopsis bispinosus§¶	Eoc (40–53 Ma)	Belgium (Ypres)	2.000	0.006	0.32[d]
Pseudocarcinus burtini§¶	Oli (24–34 Ma)	Belgium (Uccle)	0.120	0.002	1.90[d]
Coeloma¶	Oli (24–34 Ma)	?	2.040	0.008	0.37[d]

* Key to abbreviations of geological periods: Ho, Holocene; Ple, Pleistocene; Pli, Pliocene; Mio, Miocene; Oli, Oligocene; Eoc, Eocene; K, Cretaceous. † Names in parentheses indicate town, city or prefecture in the case of Japanese sites. ‡ [a] Indicates samples the chitin content of which was estimated based on the calibration curve in Fig. 1; [b] indicates samples estimated on the calibration curve $y = 6.6542x + 0.0141$, $r^2 = 0.9465$; [c] indicates samples estimated based on the calibration curve $y = 1.8023x - 0.0062$, $r^2 = 0.9781$; [d] indicates samples estimated based on the calibration curve $y = 10.109x - 0.0194$, $r^2 = 0.9968$; [e] indicates samples estimated based on the calibration curve $y = 12.217x - 0.0244$, $r^2 = 0.9529$. § Samples analysed at least in duplicate. ¶ Denotes samples originally investigated by Brumioul and Voss-Foucart.[16] ** University of Göttingen (1.085 IMPGÖ52).

Source: (1)

APPENDIX II – SAMPLE RESULTS OF SMALL SCALE RESEARCH

(Central 2 pods inoculated, remaining 5 control)
Species: *Cilantro sativum*

Results: Seeds germinated in inoculated soil showed decreased germination rates but much higher root development over same time period as control. However, as both inoculated tray and control were fed from the same water source, transmission of endophytes and micronutrients between trays likely occurred.

(Left tray inoculated via cross-hatched pattern, Right tray control)
Species: Dwarf *Antirrhinum* (Snapdragon) mix

Results: Seeds germinated in inoculated soil showed decreased germination rates versus control tray as with the *Cilantro sativum*. However, final product is blooming and healthy while the control tray is suffering from severe nutrient deficiencies. Major of germinated and flowering plants in inoculated tray are only experiencing trace endophyte and micronutrient exposure, yet it is enough of an amendment to allow full blooming in an inch of soil.