ADVANCES IN THE USE OF *ASCOPHYLLUM NODOSUM* SEAPLANT EXTRACTS FOR CROP PRODUCTION: LINKING LABORATORY AND FIELD RESEARCH[1]

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ABSTRACT

Marine plant extracts have received increasing attention over the past decade as useful inputs into crop agriculture. Research on the efficacy and mode-of-action of these products is often scattered in the literature with confounding interpretations of results. This has created a need to unravel the "muck and magic" label sometimes associated with this product group. However, there is increasing interest in incorporating at least some "organic" or "soft chemistry" products into conventional agriculture. Together with researchers at the National Research Council, the Nova Scotia Agricultural College, Agriculture and Agri-Food Canada, and several private research companies, Acadian Seaplants is attempting to bridge the informational gap between chemistry and product action. Research into specific active ingredients using technologies such as HPLC, MS-MS and NMR is being used to differentiate between related products and different processing methods. The use of bioassay techniques such as plantlet early growth bioassays, hypocotyl elongation and root assessments complement the analytical research and help identify specific plant responses. Of course, synergisms and/or antagonisms between compounds within products can make the interpretation of these results and interactions difficult. Using these laboratory and growth assays together provides a more complete picture of how these products can be used as a tool in plant agriculture. The strengths and limitations to this research will be discussed.

INTRODUCTION

Seaweeds and marine plants constitute an integral part of the coastal ecology and landscape. The brown algae *Ascophyllum nodosum* (commonly known as Norwegian Kelp) grows in abundance within the intertidal zone, along the North Atlantic Coastline. For centuries, agricultural areas close to these coastal zones have utilized seaweeds as a valuable source of organic matter and fertilizers for various soil types and for many different fruit and vegetable crops.

Today, seaweed meals and soil amendments are available in ready-to-apply dry form for use in crop soils and home gardens alike. Moreover, high quality liquid and powder seaweed extract products for use in foliar or soil applications can be found in pure form, or in recipe formulations with or without other added ingredients ranging from traditional (e.g. fertilizers, pesticides, etc.) to nontraditional products (e.g. humates, fish hydrolysates, etc.). Due to its natural abundance and history, *Ascophyllum nodosum* extracts are arguably the most widely used and researched seaweed species in agriculture (Senn, 1987).

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Nova Scotia, Canada, is home to some of the richest *Ascophyllum* beds in the world. Prudent stewardship of these beds through joint research with government and university scientists ensures continued sustainable seaweed harvests from one year to the next. Several recognized "Expert Centers" are also focusing efforts on seaweed research including the National Research Council-Institute for Marine Biosciences (NRC-IMB), Agriculture and Agri-Food Canada (AAFC), the Nova Scotia Agricultural College (NSAC), Dalhousie University and Acadia University.

By working with the Federal government through the Atlantic Canada Opportunities Agency (and the Atlantic Innovation Fund), an agency whose mandate is to support commercial development of new technologies, several long-term projects have been initiated to investigate chemical, biological and genetic influences from marine-plant products on plant growth.

APPLICATION METHODS, RATES AND TIMINGS

Current users of seaweed extracts usually treat crops with either, or both, foliar and soil-run applications. Seed or seedling treatments may also promote early plant establishment and stress tolerance. Soil applications and root dipping are also practiced by some growers and can be incorporated into irrigation, fertigation (drip or drip-tape) or furrow-run programs applied with other foliar or soil products (e.g. fungicides, herbicides).

Differing application rates and timings are often used over a wide range of temperate, tropical and subtropical food crops, as well as turfgrass and ornamental species. Rates and timings for various seaweed extract products can be crop specific and result in different responses affecting yield and/or quality. However, application rates most often range from about 0.2 to 1.5 kg of dry matter product per hectare (0.1-1 lbs/acre) per application. Liquid products are generally applied in rates from 500 ml to up to 10L/ha (0.25-5 quarts/acre). In many cases, early applications of either liquid or soluble powder extracts are beneficial in helping crops deal with early season temperature and disease stresses while helping to maximize yield potential. Other timings and multi-treatment programs can benefit flowering, fruit set or crop quality.

Sponsored government, university and private field trials in dozens of regions and countries also helps meet demand for crop-specific data. However, to further examine the modes-of-action of these complex extracts, we are focusing on their unique chemical profiles as well as specific effects on plant expression after treatment. Working with *Ascophyllum nodosum* marine algae, Acadian Seaplants is conducting a long-term study investigating fractions from raw algae as well as from algal extracts, and their related effects on plant growth, development and physiology.

We are currently examining active ingredients (e.g. plant hormones, carbohydrates, organic fractions) derived from marine algae and exploring the molecular basis for product action in addition to developing new bioassay procedures for rapid bioactivity testing. This research may lead to the development of new product formulations that help plants fortify against different regional biotic (e.g. disease or insects) or abiotic (e.g. drought, salinity, temperature) stresses.

The final step in the research pipeline is to transfer the understanding of these technologies to the grower community. To this end, there are several aspects or "approaches" we are taking to fully explore these products. Combined, these approaches should provide a comprehensive understanding of how these products can be used most effectively.

EXTRACT PROFILES APPROACH

With the help of Drs. John Walter and James Craigie at the NRC-IMB research facilities in Halifax, Nova Scotia, a series of chemical profiles of *Ascophyllum* products was produced. Using Nuclear Magnetic Resonance Imaging and Principle Components Analysis, results demonstrate that some extract products are remarkably consistent despite originating from different batches, different locales or from one season to the next. A series of 36 samples of Acadian Seaplants *Ascophyllum nodosum* extract exhibited very similar profiles as shown by the concentration of data points in Figure 1. Outlying points represent samples from different commercial sources, processing methods or formulations.

Although these comparisons do not identify bioactivities, they do provide a footprint for identifying specific products. This tool can be extremely valuable for registration requirements and provides confidence to the marketplace regarding product consistency and origin.

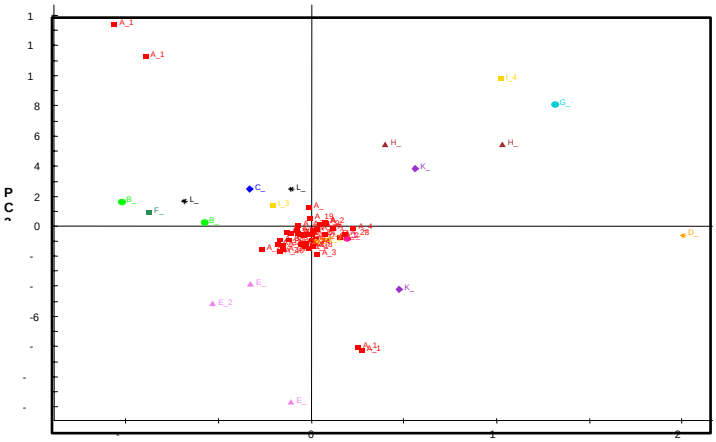


Figure 1. Nuclear Magnetic Resonance (NMR) Principle Components Analysis graph of 36 ASL extract samples as well as a number of other marine-plant extract samples (Craigie et al., 2007).

In addition, a High Pressure Liquid Chromotograph of the extracts indicates a unique profile of the carbohydrate fraction (Figure 2). This carbohydrate profile includes larger polysaccharides as well as smaller oligosaccharides. Different extraction techniques (high pressure, high temperature, etc.) can alter this profile thereby providing a different product-specific profile.

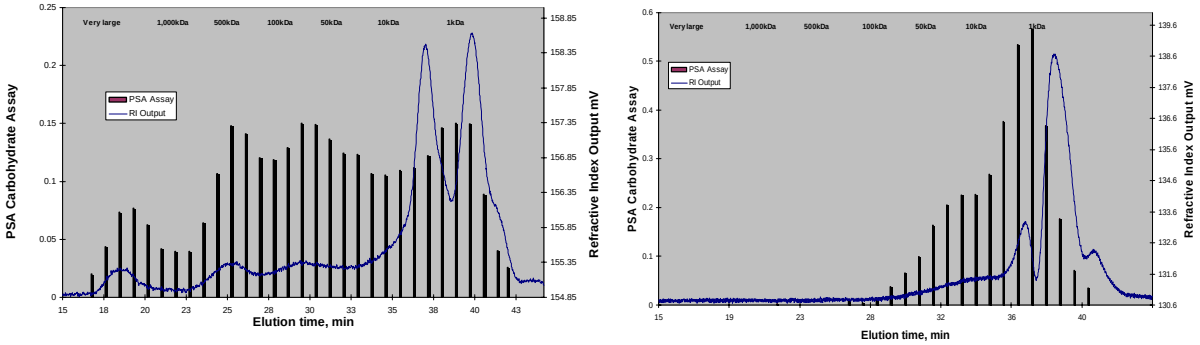


Figure 2. Carbohydrate HPLC profile of ASL- *Ascophyllum nodosum* (L) and high-temperature high-pressure treated (R) marine-plant extracts showing the distribution of large and small saccharide fractions (Figure courtesy D. Hiltz, 2007)

ACTIVE-INGREDIENT APPROACH

Effects of *Ascophyllum* extracts are often attributed to the plant hormone family, cytokinins, other active ingredients exhibiting cytokinin-like effects (termed cytokinin-equivalent activity), or both. Active ingredients derived from seaweeds have been reviewed previously (Jameson, 1993). Researchers at the NRC are qualifying and quantifying the plant hormone fraction as well as a number of other compounds previously identified as being active in plant defense mechanisms and plant protection. Among these, the betaine (Table 1; Blunden, 2003) and polyphenolic compounds can have wide-ranging effects on plant development.

Table 1. Concentration of glycine betaine (GB), aminobutyric acid betaine (ABAB) and aminovaleric acid betaine (AVAB) from *Ascophyllum nodosum* seaweed and seaweed extracts.

Sample	**GB** (ppm)	**ABAB** (ppm)	**AVAB** (ppm)
Ascophyllum nodosum	20-60	250-350	60-120
Acadian Extract	30-60	130-260	100-200

Reference: ; Craft et al., 2007; Hiltz et al., 2007

THE MOLECULAR BIOLOGY APPROACH

One area of considerable interest focuses on *how* marine plants stimulate improved crop hardiness in the face of various stresses. From an analytical standpoint, we can identify the active ingredients. However, a more fundamental approach would be to identify how these products up-regulate and down-regulate specific genes and lead to specific (or general) plant responses. Past research has included an examination of proteins and antioxidants produced by the plant in response to these treatments (Ayad et al, 1997; Coelho et al., 1997; Lizzi et al., 1998; Norrie and Hiltz, 1999; Schmidt and Zhang. 1997). Dr. Balakrishnan Prithiviraj, Industry-sponsored research chair in Molecular Biology and Stress Physiology at the NSAC, is investigating these responses. The Prithiviraj team is examining seaweed extract effects on molecular and cellular responses in *Arabadopsis* and other plants, using micro-array techniques and several bioassays. They have identified over 250 up-regulated genes and 150 genes down-regulated in response to *Ascophyllum* extracts (or fractions; Prithiviraj, personal communication).

BIOASSAY APPROACH

A number of bioassays are used to evaluate extracts, extract fractions and other compounds from raw marine algae. Working in conjunction with Dr. Mark Hodges at AAFC, as well as Dr. Dave Christie at Acadia University, several bioassays were developed using chlorophyll fluorescence technologies and different seedling trials, including the Lettuce Hypocotyl Elongation Bioassay (Figure 3).

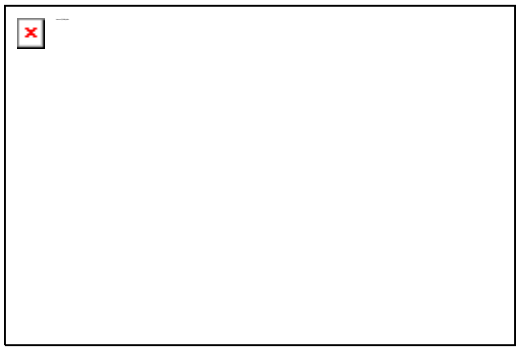


Figure 3. Bar graph illustrating lettuce seedling hypocotyls extension with increasing concentration of ASL extract (Courtesy Dr. D. Christie)

Other bioassay tests such as the Mung Bean Root Proliferation Bioassay (Figure 4) have shown considerable promise as an indicator for early root development.

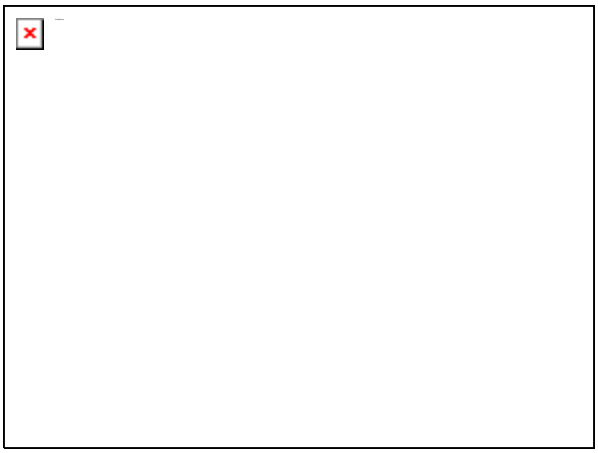
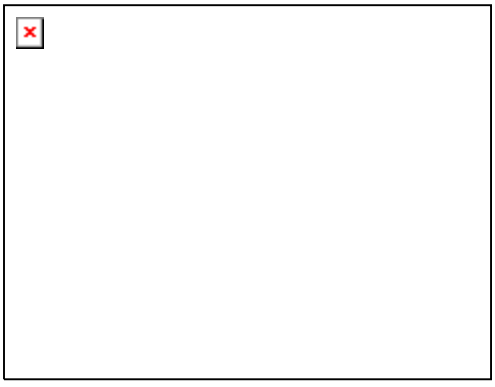


Figure 4. Mung Bean root development after treatment with distilled water, (L), treatment with a modified Long Ashton Nutrient Solution (C), and *Ascophyllum nodosum* seaweed extract (R) shows a "bearded-root" effect on treated plants (Courtesy Dr. D. Christie).

FIELD TESTING APPROACH

Although we continue to gather information on product constituents, active ingredients and molecular effects, the bottom-line indicator for product action is in field performance. We need to ensure that *Ascophyllum* products provide predictable and consistent benefits when used in various crop production systems around the world. Most of our research has focused on high value horticultural crops and less so on broad-acre or field crops. Crops that consistently respond favorably to *Ascophyllum nodosum* exracts include grapes (Norrie et al., 2002; Norrie and Keathley, 2006) and members of the Solanaceae family (e.g. tomatoes, peppers, potatoes, eggplant, tobacco; Figure 4). We are still unsure of why these crops respond so positively. The challenge is then to relate growth and development effects back to the molecular signals responsible for the response.

Figure 4. Effects of *Ascophyllum nodosum* marine-plant extract on sizing and color distribution in Pinot Noir wine grapes (L) and bell peppers (R) (Courtesy Acadian Seaplants Limited).



CUSTOMER-CONFIDENCE APPROACH

Ascophyllum nodosum marine-plant extracts were for many years grouped with "snake oil" products. This image often presented a difficult obstacle to impartial and objective research. From this brief overview, we hope to provid some insight into our R&D program and our overall objectives (both general and specific). Clearly, we are beginning to build a rational, scientific case for the use of these products in plant agriculture via substantive research. We are able to do this through the efforts of many dedicated scientists and are fortunate to have an extensive brain-trust to draw from within Nova Scotia. We feel this is the best way to maximize confidence in our research program and provide objective, independent, best-use recommendations to formulators and end-users. In short, we want growers to have complete confidence into how to most effectively use *Ascophyllum* marine-plant products to improve yields and crop quality.

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