Microbe Plus™ Kelp -- Additional Lab Analysis Demonstrating Consistent Plant Hormone Content

In 2017 an independent testing laboratory analyzed and compared the plant hormone content of two seaweed-based, liquid fertilizers from Australian companies: Growgreen Microbe Plus™ Kelp and a Leading Global Brand liquid kelp fertilizer. Both samples were analyzed multiple times over the course of three years to validate the consistency of the products regarding their hormone concentrations and the analytical methods utilized by the testing facilities. Resulting data demonstrates consistent plant hormone contents in both products, with Microbe Plus™ Kelp containing a better expanse of hormones.

Microbe Plus™ Kelp is a nutrient-rich seaweed-based fertilizer. A unique method of formulation mimics natural processes to digest and blend high-grade, soluble kelp powders, beneficial microbes, fungi, and nutrients. The resulting Microbe Plus™ Kelp is biologically active, containing plant hormones, beneficial soil microbes, and 100% plant usable nutrients.

The leading Global Brand liquid kelp fertilizer is a 100% liquid seaweed plant conditioner that provides a synergistic range of natural compounds, trace elements, alginates, and carbohydrates. A blend of brown kelps is sustainably sourced from storm-cast materials free from pollutions; the seaweed biomass is then hydrolyzed using an alkaline process (Arioli, Mattner, & Winberg, 2015) to create a complete plant health treatment.

Biostimulant Use in Crop Production

The agriculture industry is continuously looking for ways to improve fertilizer efficiency and increase environmental sustainability. In turn, biostimulant products are gaining credibility as a complementary tool for effective nutrient management.

Biostimulants are products “contain[ing] substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality” (European Biostimulants Industry Council, 2018).

These products are different than traditional fertilizers and crop inputs in a couple of critical ways. They operate through different mechanisms than fertilizers, regardless of the nutrient content; they are different from crop protection products as they increase a plant’s vigor instead of taking direct action against pests or diseases. This makes biostimulants complementary to crop nutrition and protection.

Seaweed Extracts as Fertilizer Sources

Seaweeds and their extracts have been utilized extensively in agriculture because of their positive effect on plant growth and crop yield. As a focus on agricultural sustainability increases, these products help farmers to meet the increasing agricultural demands they are facing while simultaneously increasing sustainability.

Seaweed extracts are inexpensive, naturally occurring, and have substantiated research to document their enhancement of yield and yield attributes of crops when applied exogenously (Panda, Pramanik, & Nayak, 2012). Some of the reported benefits include: strengthening cell walls to prevent insect and fungal attack, promoting budding and flowering, enhancing the ability to tolerate climatic stresses, improving germination rates, improving quality and yield of above ground and root crops, improving root nodulation, and helping to suppress soilborne diseases and nematodes (Khan, 2009; Craigie, 2011; Calvo, 2014).

Microbially active, marine-based fertilizers such as Microbe Plus™ Kelp and the leading global brand are classified as biostimulants. The benefits of using seaweed extracts as fertilizer sources result from them containing a complex mixture of phytohormones, bioactive carbohydrates, plant growth regulators, amino acids, beneficial fungi and bacteria, and numerous plant essential elements and vitamins.

The Role of Phytohormones in Plant Growth

Within the cells of a plant biological processes pertaining to plant growth and development, and plant responses to intrinsic and extrinsic stressors, are regulated by plant hormones (Peleg, and Blumwald, 2011). In the mid-1900s, Thimman designated them as “phytohormones” to differentiate these compounds from the hormones responsible for functions in animals. Phytohormones were defined as “organic compound[s] produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts.”

There are five primary phytohormones that work to regulate gene expression and cellular development: auxins, cytokinins, gibberellins, abscisic acid, and ethylene. Through complex metabolic processes, they may function independently, symbiotically, and sometimes counterintuitively. Research has proven the phytohormones exhibit extensive cross-talk with each other as well as environmental and developmental signaling pathways instead of working in discrete pathways; however, the molecular basis for this regulated behavior remains unclear (Gray, 2004).

Table 1 describes the functions and benefits of the five classical plant hormone types.

Table 1. Phytohormone Types, Functions, and Benefits.

Phytohormone	*Functions within Plants*	*Benefits*
Auxin	Shoot and cell elongation; apical dominance and various tropisms; stimulates the release of ethylene.	Increase in shoot and root biomass; initiation of secondary roots.
Cytokinins	Stimulate cell division, cell growth, and differentiation; promotes the growth of lateral buds; delay senescence.	Stimulates bud initiation; aids in root growth.
Gibberellins	Breaking bud and seed dormancy; mobilization of storage materials in seed during germination; elongation and bolting of stems; stimulates flowering.	Increased fruit numbers; stalk elongation.
Abscisic Acid (ABA)	Maintains seed and bud dormancy; stimulates closing of stomata; acts as a growth inhibitor.	Improved resiliency to water stress.
Ethylene	Fruit ripening; leaf abscission; sex expression in flowers.	Induces uniform ripening of fruit.

Lab Analysis of Plant Hormone Concentrations

Growgreen Microbe Plus™ Kelp and the leading global brand were analyzed four times throughout 2017, 2018, and 2019 to determine their phytohormone concentration and conclude if they exhibit consistent results across the multiple assays. Samples were analyzed three times (once in each 2017, 2018, and 2019*) by a single analytical laboratory in Canada; in 2019 they were analyzed once by a second lab in New York, providing the fourth set of data for review. (2019* analysis results will be shown separately below, this test was performed in an improved Microbe Plus Kelp recipe, where the levels of auxins were improved, but all other hormones remained as per previous analysis which give us the 4th point when studying consistency in concentrations)

Both fertilizer samples were analyzed for the following hormones and their associated metabolites.

- **Cytokinins**: (*trans*) zeatin-O-glucoside (*t*-ZOG), (*cis*) zeatin-O-glucoside (*c*-ZOG), (*trans*) zeatin (*t*-Z), (*cis*) zeatin (*c*-Z), dihydrozeatin (dhZ), (*trans*) zeatin riboside (*t*-ZR), (*cis*) zeatin riboside (*c*-ZR), dihydrozeatin riboside (dhZR), isopentenyladenine (iP), isopentenyladenosine (iPR), and kinetin (KIN).

- **Abscisic acid**: cis-Abscisic acid (ABA), abscisic acid glucose easter (ABAGE), dihydrophaseic acid (DPA), phaseic acid (PA), 7'-Hydroxyl-abscisic acid (7'OH-ABA), neo-Phaseic acid (neo-PA), and trans-Abscisic acid (t-ABA).

- **Auxins**: indole-3-acetic acid (IAA), N-(indole-3-yl-acetyl)-aspartic acid (IAA-Asp), N-(indole-3-yl-acetyl)-glutamic acid (IAA-Glu), N-(indole-3-yl-acetyl)-alanine (IAA-Ala), N-(indole-3-yl-acetyl)-leucine (IAA-Leu), and indole-3-butyric acid (IBA).

- **Gibberellins**: gibberellin 1 (GA1), gibberellin 3 (GA3), gibberellin 7 (GA7), gibberellin 8 (GA8), gibberellin 9 (GA9), gibberellin 19 (GA19), gibberellin 20 (GA20), gibberellin 24 (GA24), gibberellin 29 (GA29), gibberellin 34 (GA34), gibberellin 44 (GA44), gibberellin 51 (GA51), and gibberellin 53 (GA53).

- **Ethylene**: 1-aminocyclopropane-1-carboxylic acid (ACC).

Both Microbe Plus™ Kelp and the leading global brand were analyzed for ethylene in both 2017 and 2018 but it wasn't measured by either laboratory in the 2019 assays. As ethylene is found in a gaseous state, samples are analyzed instead for ACC which is the direct precursor of ethylene. Not all testing laboratories have methodologies in place to carry out the analysis.

The results display significant differences in both the concentration of plant hormones found in the two products as well as the profile of phytohormones found in each.

Results of Microbe Plus™ Kelp Assays

In 2017, Microbe Plus™ Kelp contained traces of all five of the classical phytohormones; in 2018 the Microbe Plus™ Kelp contained four of five of the phytohormones; 2019 assays again measured all of the plant hormones the sample was tested for (sans ethylene).

The results for the 2017, 2018 and 2019A samples were all completed by the same lab in Canada; the analyses for cytokinins (Figure 1), abscisic acid (Figure 2), and gibberellins (Figure 4) resulted in similar values across all three years. The 2019A analysis of auxin (Figure 3) by the Canadian lab showered a marked increase in the auxin content of Microbe Plus™ Kelp; this increase was not an error, but rather corresponds to an improvement of the Microbe Plus™ Kelp recipe to increase the auxin content. Ethylene values (Figure 5) stayed consistent the two years they were analyzed (Figure 5).

The second 2019B analysis performed by the New York testing laboratory shows similar results to the three assays completed by the Canadian lab for cytokinins, gibberellins, and abscisic acid in 2017, 2018 and 2019, and similar auxin concentrations to the data collected in 2017 and 2018.

These results demonstrate consistent concentrations in the Microbe Plus™ Kelp product across repeated testing by one lab, and also between different labs.

Results of the Leading Global Brand

In all four years of analysis, the leading global brand did not contain a testable amount of either cytokinin (Figure 1) or abscisic acid (Figure 2). The testing in 2017 determined a slight amount of gibberellins (Figure 4), although negligible at 2 part per billion; gibberellins were undetected the other three times the leading global brand was analyzed.

The leading global brand contained substantially higher concentrations of auxins (over 600 ng/g, Figure 3) and ethylene (ACC over 2900 ng/g, Figure 5) in 2017. Analytical results from 2018 showed markedly lower concentrations of both auxins and ethylene in the leading global brand, albeit still higher concentrations than found in Growgreen Microbe Plus™ Kelp. Both analyses in 2019 saw an increase in auxin concentration but it was still considerably lower than the initial 2017 results.

These results demonstrate fluctuating levels of auxins and ethylene in the liquid kelp fertilizer sample, and a much smaller plant hormone profile as only two of the five phytohormones were consistently detected.

Discussion

There are many benefits to biostimulant use of products such as Microbe Plus™ Kelp and the leading global brand liquid kelp fertilizer, one of them being the addition of plant hormones to the plant or crop being grown.

Supplementing exogenous phytohormones is beneficial to plants because of the functions the plant hormones facilitate within cells. It also significantly reduces the amount of energy a plant employed on the biosynthesis of such hormones -- an asset that may be as important as their functions in the plant. Biosynthesis of phytohormones within root and plant tissues is a highly energy-intensive process. Using biostimulants containing plant hormones in agricultural production allows the benefitting plant to direct the energy it would use for hormone manufacturing towards other metabolic processes and growth. This results in further improvements in yield and corresponding yield attributes.

When comparing products for use, many different aspects of the formulation and resulting (potential) benefits must be examined to determine which is a superior product overall.

Across the results of the four sets of testing of phytohormone concentration in Microbe Plus™ Kelp and the leading global brand there are three key, notable aspects to consider.

1. Microbe Plus™ Kelp has more consistent concentrations of plant hormones in all of the results.

2. An improved recipe of Microbe Plus™ Kelp demonstrates a higher auxin concentration in the formulation.

3. Microbe Plus™ Kelp has a more extensive plant hormone profile than the leading global brand

Consistent Phytohormone Concentration in Microbe Plus™ Kelp

The concentration of cytokinin, abscisic acid, and gibberellin phytohormones found in Growgreen Microbe Plus™ Kelp remains noticeably consistent in all sets of analyses conducted (2017, 2018, 2019A, and 2019B).

The results of the leading global brand had a wider range of variability. Analyses of the present phytohormones showed a more marked degree of variation in the auxin concentrations between the four analyses, and a wider variability in its ethylene concentration between the two samples analyzed in 2017 versus 2018.

These congruent lab results across the four separate analyses validate the uniformity and quality of Growgreen's product.

When working with fertilizers originating from natural elements, it is common to see variations in the components of the finished products because of variability in the raw materials used in manufacturing. While these variations are expected, they are not a desirable trait. A consistent product leads to consistent results from its application to a cropping system. The consistency of Microbe Plus™ Kelp across four sets of analysis establishes it as a dependable, unwavering product.

Table 2 shows the total phytohormone concentration results of the five classic hormones and all metabolites.

	Microbe Plus™ Kelp			**Leading global brand**		
	2017	*2018*	*2019*	*2017*	*2018*	*2019*
Cytokinins	90	77	82	0	0	0
Abscisic Acid	158	162	145	0	0	0
Auxins	20	21	17	607	335	486
Gibberellins	4	0	3	2	0	0
Ethylene	34	42	n/a	2918	812	n/a

Table 2. Phytohormone Concentrations (ng/g FW) in Growgreen Microbe Plus™ Kelp and a the leading global brand. Samples 2017 and 2018 were analysed by a laboratory in Canada; sample 2019 was analysed by a laboratory in New York, USA.

Improved Auxin Concentration in Microbe Plus™ Kelp

Auxin analysis by the Canadian testing lab in 2017 and 2018 and the 2019B analysis by the New York testing laboratory showed significantly lower levels in Microbe Plus™ Kelp versus the leading global brand. However, the 2019 analysis by the Canadian lab measured the auxin concentration of Microbe Plus™ Kelp.

This increase in auxin level of the 2019 corresponds with an improvement in the formulation of Microbe Plus™ Kelp to enhance auxin levels.

Higher auxin levels lead to an increase in associated gains demonstrated with auxin supplementation through biostimulant use. An elevated auxin level is a positive attribute for Microbe Plus™ Kelp, adding to its claims of being a well-rounded, excellent soil conditioning amendment and organic fertilizer. A higher concentration results in increased benefits seen after crop application.

Table 3 shows the total phytohormone concentration results of the Microbe Plus™ Kelp improved recipe vs the leading global brand.

	M+Kelp *2019*	**Leading global brand** *2019*
Cytokinins	85	0
Abscisic Acid	103	0
Auxins	723	423
Gibberellins	11	0
Ethylene	n/a	n/a

Table 3. Phytohormone Concentrations (ng/g FW) in Growgreen Microbe Plus™ Kelp and the leading global brand. Samples were analysed by a laboratory in Canada.

Differences in Plant Hormone Profiles

When comparing all four sets of phytohormone analysis, there is a clear empirical demonstration that the leading global brand liquid kelp fertilizer has a much narrower plant hormone profile than Microbe Plus™ Kelp. It does not contain all five, or even the majority, of

the phytohormones available in Microbe Plus™ Kelp's formulation; the profile only consists of auxins and ethylene. Microbe Plus™ Kelp contains all of the phytohormones tested for in all but one set of results.

This lack of all plant hormones in the leading global brand severely limits the scope of the product and the benefits seen from soil or foliar application. The symbiotic and sometimes counterintuitive relationships between the plant hormone types are adversely affected when any of the types of hormones are lacking.

Conclusion

Growgreen Microbe Plus™ Kelp exhibits consistent plant phytohormone concentrations across four separate incidents of analyses, completed by two different testing laboratories. The commercial grade product from the leading global brand shows more variable results when subjected to the same analysis. Natural products have the tendency to fluctuate in their ingredient concentrates because of the variability in the raw materials used. Consistency across product analysis demonstrates a high-quality, reliable fertilizer.

The completed laboratory analysis of the two similar products validates Microbe Plus™ Kelp contains a broader spectrum of phytohormones than the leading global brand. This well-balanced biostimulant provides agricultural producers with an amendment proven to bolster a wider range of both soil and plant benefits.

References

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Appendix

2019A refers to the analysis performed in Canada (where the new M+Kelp was analysed)
2019B refers to the analysis performed in New York (to show consistency in results from a different lab)

Figure 1.Cytokinin content in Microbe Plus™ Kelp and Leading Global Brand

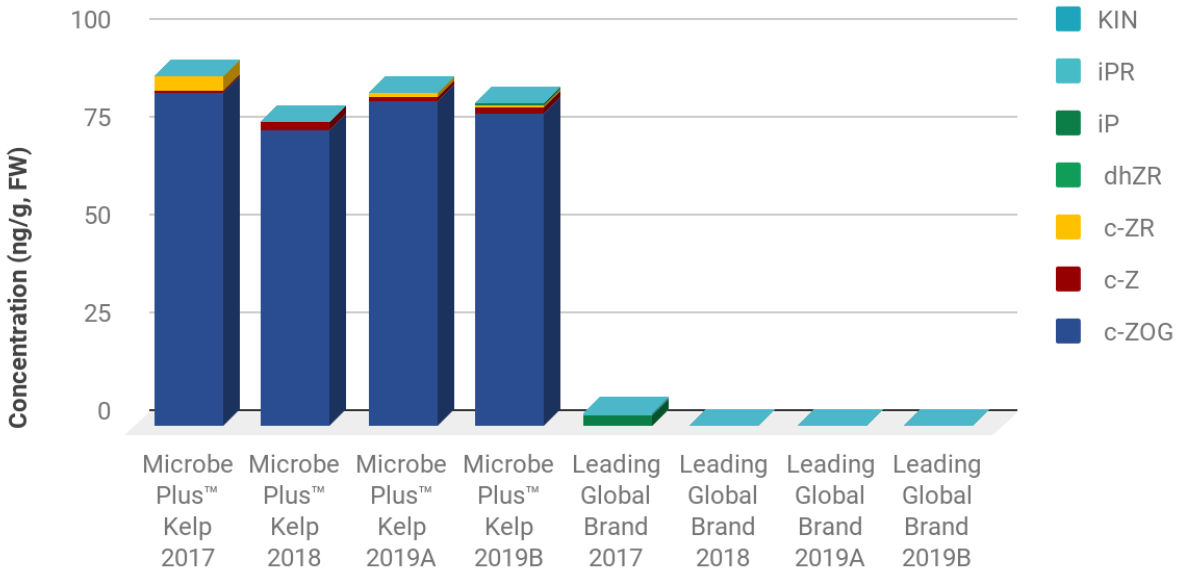

Figure 2. Abscisic acid content in Microbe Plus™ Kelp and Leading Global Brand

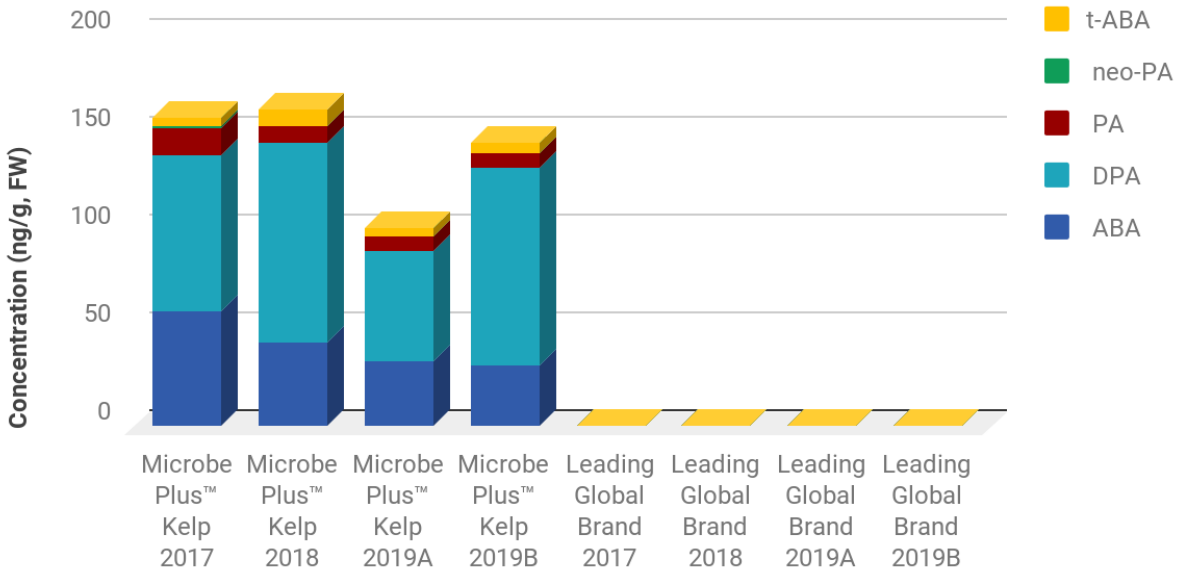

Figure 3. Auxin content in Microbe Plus™ Kelp and Leading Global Brand

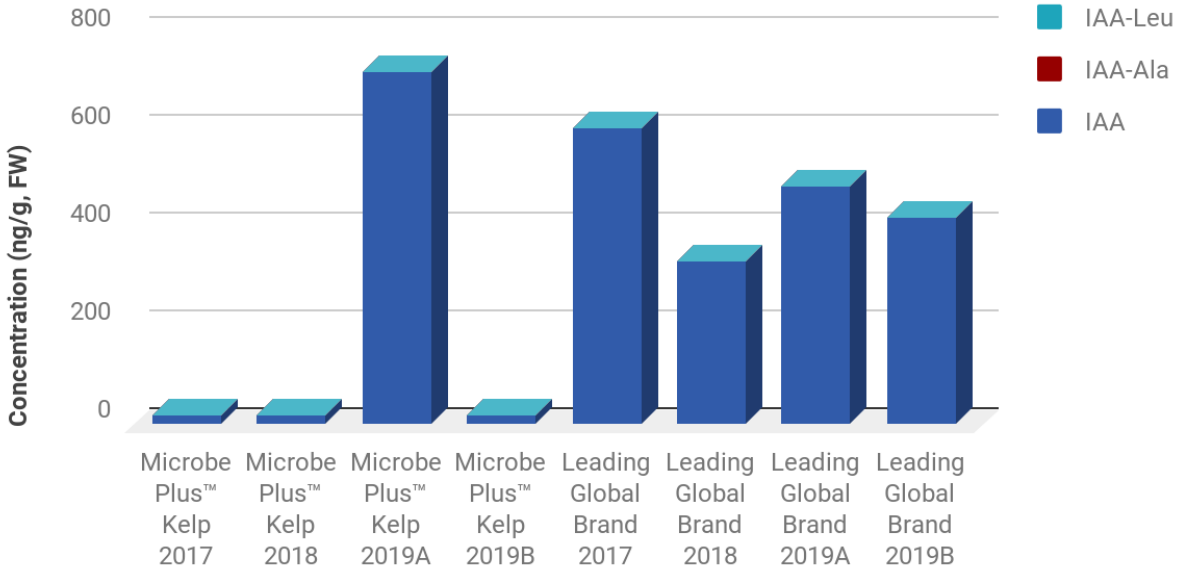

Figure 4. Gibberellin content in Microbe Plus™ Kelp and Leading Global Brand

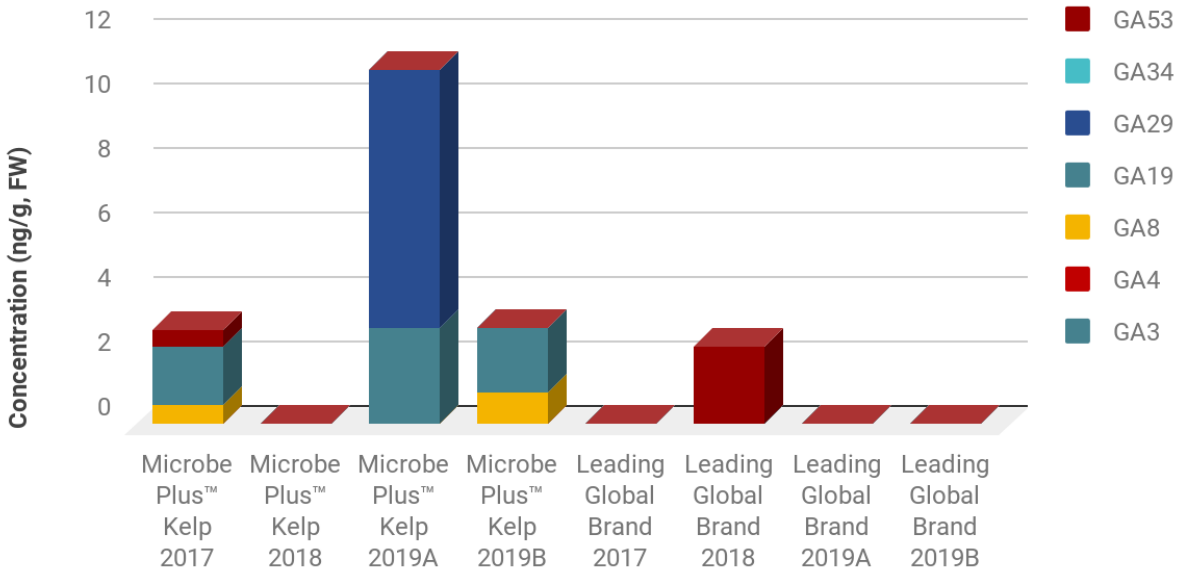

Figure 5. Ethylene content in Microbe Plus™ Kelp and Leading Global Brand