EVALUATING THE IMPACT OF BIOSTIMULANTS ON BLUEBERRY GROWTH AND SOIL BIOLOGICAL HEALTH IN A GREENHOUSE STUDY

by

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ABSTRACT

The drive to adopt sustainable practices in agriculture has led to the use of products that are collectively called biostimulants. However, the efficacy of these products has not yet been well established. A nine-month greenhouse study was conducted to evaluate the impact of eight biostimulants on growth of four blueberry cultivars and soil biological health. None of the plant growth and biomass parameters were significantly affected by treatment. Similarly, no significant difference was detected in leaf pigment indicators between the Control and biostimulants, with few exceptions. For example, the biostimulant Terra Grow resulted in significant higher chlorophyll than the Control with the Brightwell cultivar in February. The biostimulants did not significantly affect the soil biological health parameters either except for the abundance of ammonia-oxidizing bacteria and archaea. While biostimulants are often described as being beneficial to plants and soil health, it was not consistently evident with products in this study.

INDEX WORDS: *Vaccinium spp*., southern highbush, rabbiteye, plant biomass and size, leaf pigment content, biometric measures, biostimulants, microbial abundance, ammonia-oxidizing archaea, ammonia-oxidizing bacteria, soil respiration, phosphatase, urease, FDA activity, enzymatic activity qPCR, soil bacteria

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CHAPTER 1

INTRODUCTION

Blueberry production has been experiencing a rapid growth in Georgia (USA). It has been estimated that from 2000 to 2018, the harvested area in the state rose from 4,600 acres to 13,300 acres, putting Georgia in fifth place in the nation (https://tinyurl.com/t2rny5n). The majority of blueberry growing area is located in the south Georgia, in the region called the Lower Costal Plane, characterized by sandy soils and high water table, an ideal environment for its growth. The main blueberry types grown in Georgia are the southern highbush and rabbiteye. Those two types are the best adapted to the Georgia climate.

The increase in production of blueberries has come along with a change in agricultural practices. In fact, farmers are moving towards sustainable practices, using products claimed to be environmentally friendly. Among these products are biostimulants, which are collections of products that may contain microbial inoculants and/or organic constituents. They are commonly advertised as having ingredients that promote plant and soil health, but such claims are largely not proven with research.

Biostimulants may contain measurable but biologically irrelevant concentrations of known active ingredients but their concentrations may not be effective when those products are applied at the recommended rates (Yakhin et al., 2017). The success of microbe-containing biostimulants is also largely dependent on storage conditions and a good understanding of the soil environment to which they are applied. Lack of such knowledge leads to poor survival of the microorganisms in soil. As a result, the complex types of molecules and microorganisms in biostimulant products and their effect on the plant growth and productivity need to be further studied. Biostimulants are also advertised as having a positive impact on soil biological health, which is capacity of soil microorganisms to provide beneficial services such nutrient cycling, soil organic matter decomposition and disease suppression to plants (Parađiković et al., 2018, Rouphael et al., 2020, Trevisan et al., 2019). However, there has not been adequate research to support this claim.

The objective of this study was, therefore, to examine the impact of select biostimulants on blueberry growth and soil biological health. The information used to design this research was obtained directly from Georgia blueberry growers who were surveyed to better understand their expertise and experience in using biostimulants. Based on their response from the survey, a greenhouse study was designed and performed to test the impact of eight commonly used biostimulants on blueberry growth with four cultivars in Georgia. More specifically, the objectives of the study were to:

a) determine the impact of biostimulants on blueberry growth in greenhouse

b) determine the impact of biostimulants on soil biological health

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CHAPTER 2

LITERATURE REVIEW

Growth of blueberry production in the USA

Blueberry is one of the most promising fruit crops worldwide. Studies have shown its health benefits in preventing or reducing diseases such as dementia (Krikorian et al., 2010) and myocardial damage caused by infarct (Ahmet et al., 2009). The awareness campaign for healthy diet increased the US per capita blueberry consumption by 599% between 1994 and 2014. This is an incredible rise that has not been registered for other fruits and vegetables in the same time range (https://tinyurl.com/yb5lhj9e). To meet this growing demand, the harvest area for blueberries rose from 54,829 to 109,270 ha worldwide between 2000 and 2018. In the same years, the area harvested in the USA grew by more than two-fold, rising from 16,520 ha to 36,098 ha, resulting in the USA to be the biggest blueberry producer in the world (FAOSTAT, https://tinyurl.com/y8efbtro). In the USA, the blueberry cultivation is spread across 38 states (FDA, https://tinyurl.com/y4kmcqdg), thanks to the variability in soil and climatic conditions that allow the growth of different blueberry types. According to the 2018 data, the major blueberry producers in the US were Michigan, Washington, Oregon and Georgia (https://tinyurl.com/t2rny5n).

Currently, Georgia is one of the most promising blueberry growers in the country. Georgia is better known as the Peach State, but since 2004 the blueberry production has surpassed that of peach (Plattner, 2008). It has been estimated that from 2000 to the 2018, the area harvested rose from 1,861 ha to 5,382 ha (https://tinyurl.com/sqarjwx, https://tinyurl.com/t2rny5n), resulting in

Georgia to be the most important producer in the Southeast. Despite this success, Georgia's blueberry yields are below the United States average. Since 2004, yields in Georgia have been constant or slightly decreasing, while across the other states yield has been continuously increasing. In 2016, the average yield was 6,340 lbs per acre in the USA against 4,140 lbs per acre in Georgia (https://tinyurl.com/un6xhx2). For this reason, Georgia blueberry yield must increase to be economically competitive in the U.S. market (Fonsah et al., 2016).

Nowadays, growers are moving towards sustainable management practices, driven by market trends. Consumers are shifting their attention to the organic market as it is perceived to be healthy and environmental-friendly (Plattner et al., 2008). Not surprisingly, acres destined to organic production are growing in the USA, as in the rest of the world (Krewer, 2006). In 2016 the value of organic blueberry production in Georgia amounted to $4,282,987 produced over 506 acres. For comparison, in 2014 the value was $2,002,414 over just 263 acres. Organic producers face more challenges compared to conventional ones. They usually rely on ecological practices to solve the common production issues, or they have fewer products that can be used in organic production. Those products must be approved by OMRI (Organic Material Research Institute), an organization that verifies all the products can be used as input in organic agriculture (https://tinyurl.com/y7sxapbf). There are some products available to be against the most common blueberry problems such as mummy berry and the maggot fly (Krewer and Walker, 2006), but growers are always looking for new innovative products suitable for organic production.

Blueberry varieties grown in the Southeast

According to Itle (personal communication, 4/12/2021), 40% of Georgia blueberry production comes from rabbiteye (*Vaccinium ashei*) and the rest from southern highbush (*V.*

corymbosum hybrids). Ninety percent of the production is located in few counties in South Georgia: Appling, Bacon, Clinch, Pierce and Ware (Scherm et al., 2001).

Rabbiteye (RE) are native of south Georgia and have been considered the most important blueberry type in the state. They can grow in soils with low organic matter (1-2%) and pH between 4.5 and 5.3 (Cline, 2007). Rabbiteye are typically considered to be a low-input blueberry type and can be highly productive for a long period of time (NeSmith, 2006). Their main horticultural problems are poor fruit set (NeSmith, 2006) and flower damage at blooming (NeSmith, 2006, Krewer at al., 2006, Scherm et al., 2008). These problems are overcome by applying gibberellic acid during flowering and improving pollination with honeybees. Another major problem is the mummy berry disease, which is caused by the fungus *Monilia vaccinii-corymbosi* (Reade) Honey (Scherm et al., 2004) that leads to the mummification of the fruits. Growers usually apply three different fungicides in two-three applications to overcome this problem. Eighty percent of growers treat the fields in this way to achieve a good control of the disease (Scherm et al., 2001).

In general, RE are less demanding in terms of management practices and pest problems compared to southern highbush (SHB) (Scherm et al., 2003). In fact, SHB grow only in soil with high organic matter (more than 3%) and pH 4-5. It is not recommended to cultivate this blueberry type on infertile soils, as they can suffer of low vigor (NeSmith, 2006). The major horticultural problems are Phytophothora root rot and stem blight diseases. The root rot is caused by the fungus *Phytophthora cinnamomi* and is particularly destructive when the plants are grown in poorly drained soils (Smith, 2006). This disease can be controlled with fungicide application and improving soil drainage (Scherm et al., 2001, Yeo et al., 2016, Smith, 2006). Stem blight is caused by *Botryosphaeria dothidea* that can infect the plants through cuts (Smith, 2006). Fungicides are not particularly effective against this disease. The removal of infected canes is the main treatment.

Typically, SHB are cultivated in two areas located in southeast Georgia: the Lower Coastal Plain and Coastal Flatwoods (Scherm et al., 2001). They represent 60% of the total blueberry acreage (Itle, personal communication 4/12/2021).

These two blueberry types are the best adapted to the Georgia climate, which is characterized by warm temperatures. In fact, climate is the most important limiting factor for blueberries growth, as these plants need a period of "dormancy" to set the fruits. In general SHB require less than 400 chilling hrs (Krewer and NeSmith, 2006), while RE need 600 hrs of dormancy (Retamales et al., 2012). To make a comparison, the northern highbush cultivars, which are well adapted to the North America, need 800-1000 hrs of chilling, and they are resistant to temperatures below -20°C (Retamales et al., 2012). The average ripening window in south Georgia for RE goes from June to July (Krewer et al., 2006), while for SHB is April-May (Krewer et al., 2006).

Biostimulant: definition and description

Plant biostimulants include a wide group of products that are described as being suitable in agriculture to improve crop productivity. In the past years they have attracted the attention of the scientific community for their claim to be a sustainable-oriented approach. They are often marketed as being suitable for meeting the current agricultural challenges: the production of food in a sustainable way for a growing population, decreasing the space allocated to agriculture (Povero et al., 2016).

Despite their recent popularity, the history of biostimulants is not new. According to scientific literatures, professor V.P Filatov was working with “biogenetic stimulant” as early as 1933. He claimed that every living tissue in contact with a non-lethal stress can produce molecules stimulating the metabolism of those organisms (Yakhin et al., 2017). In the following years, many scientists have researched and written about biostimulants albeit under different names. In 2007 the term “biostimulant” was used for the first time by Kauffman (du Jardin, 2015). More recently, biostimulant was defined as, “*any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content*” (du Jardin, 2015). They were also delineated according to their chemistry and impact on physiological activity of plants. The main ingredients of biostimulants, according to du Jardin (2015), are: humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extract and botanicals, chitosan and other biopolymers, inorganic compounds, beneficial fungi and bacteria.

Humic substances are natural components of the soil. They are the final products of microbial degradation of animal, plants and organic residues (Calvo et al., 2014). They are categorized into humin, humic acids and fulvic acids according to their weight and solubility. Humic substances play various key roles in soil fertility and, therefore, on plant physiology. Their function is strictly related to plant roots and microbes as they can influence the structure of colloids, enhancing nutrient uptake from the soil. Their molecular structure is composed of a wide number of functional groups that can increase cation exchange capacity and ion chelation (Calvo et al., 2014). They can also stimulate proton pumping on the plasma membrane, and improve cellular respiration (du Jardin, 2015). Studies have shown that they can increase plant growth, resulting in better yield both quantitatively and qualitatively. Calvo et al. (2014) summarized the benefits of humic substances on 16 plants of fruit and agronomic crops. In most cases, improved root system is their most citated effect on plants.

Protein hydrolysates and other N-containing compounds have shown to have an important role in the enhancement of plant growth and tolerance to biotic and abiotic stresses (Calvo et al., 2014). Protein hydrolysates are composed of proteins and amino acids of organic origin, and other single amino acids (the most important is glycine betaine). This category plays an important role in plant and animal waste recycling as most of those proteins come from industrial processing of wastes (Calvo et al., 2014). The other category is composed of 20 individual amino acids. They also play an important role in providing protection from stresses as a result of salinity, drought, heat and heavy metals.

Seaweed extracts have been used in agriculture from ancient time. They contain many molecules, including polysaccharides that are involved in gel formation. Gels contribute to water storage (du Jardin, 2015). Their impact on crops is wide-ranging and includes the stimulation of root growth, increased yield and better resistance to biotic and abiotic stresses (Calvo et al., 2014). Other beneficial effects include improvements in microbial activity and diversity, enhanced seed germination and plant development. Seaweed extracts also contain many hormones, the most reported ones are cytokinin and auxins. The hormones can enhance vegetative growth and improve resistance to stresses (Calvo et al., 2014).

Chitosan is a polysaccharide derived from chitin. It has been used for years in agriculture to improve resistance to drought, salinity and extreme temperatures. It plays an important role especially in stomata regulation during stress. In fact, it can induce stomata closure due to environmental stresses (du Jardin, 2015. Caradona et al., 2018)

Inorganic compounds include a small group of chemical elements called beneficial elements. These are aluminum, cobalt, silicon, sodium and selenium (du Jardin, 2015). Those elements are not fundamental for all plant species but can be helpful in case of stress with some plants. They can enhance plant growth and protect from abiotic stresses. For example, silica can fortify cell walls, selenium is helpful during the contact with a pathogen. Sodium is related to osmoregulation. Even if their mode of action has not been determined, the inorganic salts of Al, Co, Na, Se and Si has been applied to protect from fungi (du Jardin, 2015).

Beneficial fungi are known for years to establish beneficial interactions with plants. Those interaction are called mutualistic symbiosis, where both microorganisms involved in the interaction get benefits. There are many mechanisms of interaction between plants and fungi. The *Arbuscule-Forming Mycorrhiza* (AMF) is one of the most important in agriculture. The mechanism foresees the penetration of the fungal hypha into the root of the plant, creating the arbuscules. In this way, the root system of the plant is expanded thanks to the contact with that of the fungus. The main effects of this interaction are enhanced water and nutrient uptake, increased resistance to biotic and abiotic stresses, and increased yield (Calvo et al., 2014).

Beneficial bacteria are gaining increasing attention recently. They are often categorized into *plant growth-promoting rhizobacteria* (PGPR) and *plant growth-promoting bacteria* (PGPB) (Calvo et al., 2014). They can establish symbiotic relationships with the plant in the rhizosphere. Their action on the plant is variable because it is influenced by soil type, plant species and other environmental factors (du Jardin, 2015, Calvo et al., 2014). In general, the main effect can be seen on plant nutrition (e.g N_2 fixation) plant health and development since some PGPR can enhance the production of plant hormones.

Currently, the regulation on the use of plants biostimulants is unclear. The categories mentioned above are the widely recognized by the scientific community, but to date there is not a legislation on biostimulants' definition and classification anywhere. In Europe and America level, there is no common legislation. Rather, biostimulants are legislated at state level and they usually fall into regulations for "beneficial substances" (Caradonia et al., 2019. du Jardin, 2015). The debate is still open among the scientific community, especially on their classification and efficacy.

Some consider it appropriate to classify them according to their effect on plants (du Jardin, 2015, Bulgari et al., 2014), others in regards to their modes of actions (Basak, 2008).

It is important to underline that for most biostimulants it is difficult to detect mode of action. They can contain many molecules that influence plant physiology but are found in a very low concentration in the products. For this reason, they may not always be effective (Yakhin et al., 2017). According to Yakhin et al. (2017), one of the main challenges of biostimulants is the inability to describe their mode of action or mechanism of action. Mode of action is defined as *"specific effect on a discrete biochemical or regulatory process"*. Many biostimulants do not have a biochemical target and few products are known to have a mode of action with a specific biochemical target site (Yakhin et al., 2017).

Popularity of biostimulants

Given their recent popularity, there are limited data regarding the economic impact of biostimulants. The European Biostimulants Industry Council (EBIC) estimated an annual increase of 10% for the European biostimulant market (EBIC, 2013), reaching € 800 million in 2018 (Traon et al., 2013). In USA, the value was $ 313.0 million in 2014 (Yakhin et al., 2017). A research of Povero et al. (2016) estimated that the value of biostimulants market was $ 1,402.15 million in 2014.

The main drivers that are leading to the growth of this sector are the new agricultural policies, which are pushing towards sustainable agriculture (du Jardin, 2015). The EBIC has documented some effects of the integration of biostimulants in agricultural practices. The use of pesticides is reduced from 10 to 15%. The fertilized use efficiency improved by 5 to 25% and yield increased by 5-10% (EBIC, 2013). Biostimulants also play an important role in recycling waste.

Many of the molecules contained in biostimulants are derived from food, animal waste and aquaculture as well as compost and waste from sewage treatment (Xu et al., 2018).

Previous studies on efficacy of biostimulants on crops

The effect of biostimulants has been studied on several food and non-food crop crops. As already mentioned, the effects of biostimulants on plants are varied and related to alleviating biotic and abiotic stresses, improving plant growth and biological soil quality. Research on corn indicated that biostimulants can play an important role in the stimulation of metabolic and physiological changes (Rouphael et al., 2020). The application of protein hydrolysate, alone or in combination with AMF, increased shoot production by 16.6%. The combination of AMF and *Trichoderma koningii* increased the dry root mass by 48%. Another biostimulant containing protein hydrolysate was also reported to affect the growth of roots and stems on corn in aquaculture, the beneficial effect being greater in the presence of multiple stresses (Trevisan et al., 2019). In addition, it can regulate the transcription of genes for the transport of nitrates and metabolism of reactive oxygen species (Trevisan et al., 2019). Similarly, Ertani et al. (2009) reported that two protein hydrolysates improved nitrate reductase activity, highlighting the effect biostimulants on nitrogen metabolism.

Research on wheat grown in greenhouse indicated that a combination of N fertilization and PGPR application increased the root biomass by 45%, and the stem-elongation and production of ears by 23% in some growth stages (Nguyen et al., 2018). In another study, the combination of herbicide and a biostimulant mixture composed of seaweed extract and nitrophenols enhanced the gluten amount in grains, even if there was no control for weeds (Matysiak et al., 2018). Popko et al. (2018) reported that yield of winter wheat increased by up to 11% with the application of amino acids-based biostimulant. In addition, the biostimulant application enhanced the qualitative characteristics of the grain. Parađiković et al. (2018), in their summary of the effect of various biostimulants on some horticultural plants, concluded that many biostimulants improved biotic and abiotic stress tolerance, nutrient uptake and promoted plant growth and seed establishment. These benefits can reduce fertilization and enhanced production.

There are also some recent studies on blueberry and biostimulants. Schoebitz et al. (2019) assessed the effect of humic substances, microbial inoculants and their combinations on multiple aspects of the fruit. In particular, the effect was evaluated by looking at multiple vegetative parameters, including cane diameter, plant height, cane and root dry weight, and leaf nutrient concentration. The microbial inoculants increased dry weight of roots and canes by 70% and 35% respectively, while the combined use of microbes and humic substances increased roots and canes dry weight by 33% and 20% respectively. In general, they reported an increase in fruit yield, especially with the mixed treatment. Their results were not in agreement with another study by Loyola et al (2009) in which the effect of an algal-based biostimulant was evaluated on plant growth at different stages, with no treatment effect on total fruit weight.

Soil health: definition and characteristics

The concept of soil health is gaining importance as it is key to a sustainable agriculture (Larkin, 2015). The soil, thanks to its various functions, plays a fundamental role in the concept of sustainability. Considering the soil as a mere substrate of plant growth is reductive. The soil must be studied as a complex ecosystem in which every component, plants, microbes and animals, plays a fundamental role in maintaining the equilibrium. In fact, the USDA defines soil health as *the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans* (USDA, https://tinyurl.com/ycbbmt24). If just one component of the system is missing, it inevitably leads to an imbalance and soil health can be damaged. Poor soil health can lead to low productivity due to pest and weeds problems. Poor soil structure results in less water drainage, nutrient loss, and soil erosion (Larkin, 2015).

The concept of healthy soil is also closely linked to that of resilience. A resilient soil can recover and adapt to changes, maintaining a stable and healthy soil (Lehman et al., 2015). It is essential to keep the soil healthy as it has a primary role in many functions. The most important for humans is the efficient production of food and fiber. There are others ecosystem services that a healthy soil can provide: carbon sequestration, nutrient cycle, soil detoxification and water regulation are the main (Moebius-Clune, 2017). A soil with a good structure can store water, making it available to plants that will need less irrigation. A big advantage considering that agriculture consumes 70% of the global fresh water reserves (FAO, 2017).

It has been shown that healthy soils also play an important role in disease suppression against soil-borne pathogens. This is because pathogens fail to compete for resources against the resident microbial communities (Raaijmakers et al., 2016, Expósito et al., 2017). These are complex interactions in which different taxa play fundamental roles in soil disease suppressiveness (Expósito et al., 2017). Soil disease suppressiveness can be improved through sustainable farming practices, including conservation tillage and crop rotation (Chandrashekara et al., 2012, Schlatter et al., 2017). These practices prevent pathogens from entering the soil and allow the formation of a strengthened and fortified bacterial community. Not surprisingly, monocultures are on average more susceptible to pathogens with some exceptions like cereals (Hoestra, 1975). The upside is a lesser pesticide application, and therefore savings for the growers and a great environmental benefit.

According to Larkin (2015), the characteristics of a healthy soil are many. The most important is a high content of soil organic matter (SOM). The SOM is not only a source of energy for microorganisms, which will therefore be more abundant and active, but it affects the chemical and physical characteristics of the soil with a direct effect on plant growth.

Other important features are the presence of an adequate nutrient content, the ability to stimulate radical growth thanks to an enhanced soil structure, the presence of a diverse bacterial community, low incidence of pathogens and good water retention (Moebius-Clune et al., 2016, Larkin, 2015). These characteristics contributed to the formation of a stress-resistant and resilient soil for the ability to recover from any disturbance.

How to achieve soil health

Every farmer deal with unique problems related to their field. This is because there are many types of soils, with different features that make each soil unique. The following is a general overview of the management options available to improve soil health.

As already mentioned, increasing the amount of SOM is crucial to achieve soil health. This because SOM influences multiple mechanisms of lithosphere and atmosphere that have been described in detail in many reviews (Bot et al., 2005, Johnston et al., 2009, Larkin, 2015).

Crop rotation and cover crops provide many soil benefits. Are both excellent techniques to improve the structure, favoring the developments of healthy root system. In general, they favor water management and reduce erosion. In particular, cover crops play a key role in contrasting nitrate leaching, as they improve the efficiency of nitrogenous use and improve water drainage (Novara et al., 2013, Staver et al., 1998, Yeo et al., 2014).

Conservation tillage is another practice to improve soil health. In contrast to traditional tillage, conservation tillage allows the creation of stable aggregates and improves the SOM content, with important effects on soil health. Traditional tillage, in fact, destroys the soil aggregates and the interactions created between microorganisms. The excessive aeration of the soil involves the respiration of the organic substance which is then lost.

Finally, the application of organic amendments should be encouraged rather than chemical fertilizers. It is known that the application of chemical fertilizers decreases the bacterial biomass in the soil. This can be caused by the increase in osmotic potential due to the application of nitrogen fertilizers (Treseder, 2008). In addition, thanks to the abundant availability of nutrients, the plant no longer needs to expend energy to support the interaction with mycorrhizal fungi (Treseder, 2004). All these mechanisms lead to the loss of microorganisms, with consequences on the formation of humic substances.

Microorganisms play a fundamental role in the mineralization of organic P (Bargaz et al., 2018). It is estimated that the organic P represents between 30 and 65% of the total P content in soil (Shen et al., 2011). Phosphorous can be made available by microorganisms through mineralization processes, and AMF contributes up to 80% of P uptake (Bargaz et al., 2018). These have also been studied on blueberry (Montalba et al., 2010). A study comparing plants fertilized with ammonium sulfate or with organic fertilizer indicated that the chemical fertilizer reduces microbial activity. On the other side, blueberries treated with organic fertilizer had a higher concentration of antioxidants in their leaves. Not surprisingly, the incidence of *Fusarium* wilt in organically fertilized plants was lower than those under the conventional practice. The presence of more microorganisms in soil may be linked to increased plant resistance and health.

As already mentioned, biostimulants have the ability to influence soil health. Some biostimulants contain PGPR that directly interact with plant roots. Other substances, including humic substance, can significantly impact microbial activity. In a study by Schoebitz et al. (2019), the activity of several enzymes, including phosphatase and urease, was analyzed. They also assessed fluorescein diacetate (FDA) activity and soil respiration following the application of humic substance and/or microbial inoculum. Phosphatase increased by 43% in the mixed treatment, and urease activity was 10 times larger in the mixed treatment than the control. Similarly, the biostimulant treatments increased FDA activity by up to 140% as compared to the control.

Parameters for measuring soil health

Soil health can be evaluated by assaying for different parameters. Some soil health indicators such as organic matter color, level of erosion, crop appearance and soil compaction can be directly assessed in the field (Moebius-Clune et al., 2017). Other indicators are directly related to microbial activity. Microbes mediate the biogeochemical cycle of nutrients. Microorganisms, through production of enzymes, catalyze reactions in the soil that transform organic substances to nutrients that are available for plant use (Vinhal-Freitas et al., 2010). It is therefore important to evaluate indicators that quantify microbial activity of the soil. One way of evaluating microbial activity is by assaying for the activity of enzymes they produce. The enzyme urease, for instance, is a good example as it is microbial produced and is responsible for the hydrolysis of urea to ammonia in soils (Kandeler, 2007). Assaying for urease activity is often used a proxy for measuring soil microbial activity in relation to nitrogen cycling. Another example is the enzyme phosphatase that catalyzes the production of orthophosphates from organic forms of phosphorous

(Plante, 2007; Dick et al., 1994). The two enzymes can bind to colloids and soil organic matter after cell death and remain active and perform their catalytic functions even in the absence of active microbes (Burns ert al., 2013; Vinhal-Freitas et al., 2010). In general, enzymes are very sensitive to changes and are therefore excellent indicators of the impacts of management practices on microbial activity and soil health (Baldrian, 2009, Vinhal-Freitas et al., 2010).

The common indicator of generic microbial activity is soil respiration. Soil respiration quantifies the amount of CO_2 produced from the soil organic matter decomposition through microbial activity (Wildung et al., 1975). Since soil organic matter is derived from plants, animals and microorganisms, its turnover can give an idea of trophic interactions in ecosystems, and therefore of its health (Barrios, 2007). The generic microbial activity can also be assessed through fluorescein diacetate assay (Green et al., 2005). This assay allows the quantification of the microbial biomass C indirectly due to the presence of fluorescein which is produced by the activities of many soil enzymes including protease, lipase and esterase. Therefore, production of fluorescein can be a good indicator of soil microbial activity.

Quantifying the abundance of some key microorganisms can also be a good indicator of soil biological health. Quantitative polymerase chain reaction is often employed to quantify the abundance of key groups of microorganisms that mediate certain aspects of a nutrient cycle. One such example are ammonia-oxidizing bacteria and archaea. These organisms mediate the first and rate limiting step of nitrification that produces nitrate which can easily be lost from the soil via leaching and/or denitrification (Hatzenpichler, 2012, Norton et al., 2011). Therefore, their activity has important agronomic and environmental implications (Norton et al., 2011, Qiao et al., 2014).

It should be noted that a combination of indicators needs to be used to accurately capture any changes in soil biological health in response to inputs or management practices (Baldrain, 2009).

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CHAPTER 3

EVALUATING THE EFFECT OF BIOSTIMULANTS ON BLUEBERRY GROWTH[1]

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ABSTRACT

The drive to adopt sustainable practices in agriculture has led to the use of alternative products that either supplement and/or replace commonly used inputs. Among these products are biostimulants. However, there is a lack of research when it comes to their efficacy, especially with blueberries. For this purpose, a 9-month greenhouse study was conducted to assess the effect of eight commonly used biostimulants on plant growth with four blueberry cultivars (Brightwell, Farthing, Legacy and Premier). Monthly measurements were taken on parameters that are indicators of plant growth (height, width, volume, cane number, cane diameter, leaf area index), plant biomass (dry weight) and plant leaf pigment (chlorophyll, anthocyanin, flavonols and NBI index) multiple times during the study period. None of the plant growth and biomass parameters were significantly affected by treatment. Similarly, no significant difference was detected between the Control and the biostimulants for the leaf pigment indicators, with few exceptions. The biostimulant Terra Grow resulted in significant higher chlorophyll content than Control in Brightwell in February. Chlorophyll content was significantly higher in the Control than the biostimulant Fertiactyl GZ in Legacy in May. Similarly, there were significant differences in anthocyanin and flavonols contents between the Control and the biostimulants Terra Grow and Fertiactyl GZ in Legacy and Brightwell at two timepoints. However, the effects were not consistent over time or cultivar type. While biostimulants are often described as capable of providing benefits to plants in improving growth or alleviating biotic and abiotic stresses, such benefits were not evident with these products in a manner that is consistent in this study. Overall, the effects of the biostimulants differed by blueberry cultivar and time. To confirm the findings of the greenhouse study and gain a better insight on the impact of biostimulants on blueberry growth, the study needs to be repeated under field conditions.

INTRODUCTION

In the past decades, the blueberry industry has experienced a rapid growth worldwide (FAOSTAT, https://tinyurl.com/y8efbtro). The demand for blueberry increased exponentially, along with the acreage planted (Brazelton, 2013). Georgia is one of the most important blueberry producers. According to Itle (personal communication, 4/12/2021), 40% of Georgia blueberry production comes from rabbiteye (*Vaccinium ashei*) and the rest from southern highbush (*V. corymbosum* hybrids). Ninety percent of the production is located in few counties in South Georgia: Appling, Bacon, Clinch, Pierce and Ware (Scherm et al., 2001). SHB are cultivated in two areas located in southeast Georgia: the Lower Coastal Plain and Coastal Flatwoods (Scherm et al., 2001). They represent 60% of the total blueberry acreage (Itle, personal communication, 4/12/2021).

Rabbiteye are native of south Georgia and has been considered the most important blueberry type in the state.

The expansion in blueberry production has also led to interest in using products that are marketed as being sustainable and environmentally friendly as compared to conventional inputs. One such group of products are collectively called biostimulants. Biostimulants are defined as "*any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content*" (du Jardin, 2015). They are marketed to growers as being more sustainable and suitable for organic agriculture than traditional pesticides and chemical fertilizers. The market for plant biostimulant was valued at $313.0 million in 2014 (Yakhin et al., 2017). The prediction is that their market value will increase to $4.1 to 4.9 billion in 2025 (Povero et al. 2016, Kumar et al., 2020).

Despite their growing importance, the mode of action of active molecules in plant biostimulants is not well defined (Yakhin et al., 2017, Brown at al., 2015). They can contain many molecules that can influence plant physiology, but the molecules might be found in a very low concentration in the products. For this reason, they may not always be effective (Yakhin et al., 2017). According to Yakhin et al. (2017), one of the main challenges of biostimulants is the inability to describe their mode of action or mechanism of action. Mode of action is defined as *"specific effect on a discrete biochemical or regulatory process"*. Many biostimulants do not have a biochemical target, and few products are known to have a mode of action with a specific biochemical target site (Yakhin et al., 2017). Also, they are usually composed of a wide range of natural ingredients and their composition could vary (Calvo et al., 2014, Brown at al., 2015).

There are studies that reported positive crop response to biostimulants (Nguyen et al., 2018, Parađiković et al., 2018, Popko et al. 2018). Research on corn indicated that biostimulants can play an important role in the stimulation of metabolic and physiological changes (Rouphael et al., 2020). The application of protein hydrolysate, alone or in combination with arbuscular mycorrhizal fungi (AMF), increased shoot production by 16.6%. The combination of AMF and *Trichoderma koningii* increased the dry root mass by 48%. Another biostimulant containing protein hydrolysate was also reported to affect the growth of roots and stems on corn in aquaculture, the beneficial effect being greater in the presence of multiple stresses (Trevisan et al., 2019). Schoebitz et al. (2019) assessed the effect of humic substances, microbial inoculants and their combinations on multiple aspects of the fruit. In particular, the effect was evaluated by looking at multiple vegetative parameters, including cane diameter, plant height, cane and root dry weight, and leaf nutrient concentration. The microbial inoculants increased dry weight of roots and canes by 70% and 35% respectively, while the combined use of microbes and humic substances increased roots and canes dry weight by 33% and 20% respectively. However, little research has been done on blueberries. To fill this knowledge gap, a greenhouse experiment was conducted to evaluate the effect of eight biostimulants on blueberry growth with four cultivars - Premier and Brightwell, Legacy and Farthing belonging to RE and SHB types, respectively. The objectives of this study were to evaluate the impact of eight biostimulants on blueberry growth, and if the impact varies by blueberry type and cultivar. This study can provide useful information to blueberry growers about the effectiveness of plant biostimulants.

MATERIALS AND METHODS

Blueberry cultivars

Four cultivars (Brightwell, Premier, Legacy and Farthing) belonging to two different blueberries types (RE and SHB) were studied during this experiment. The two RE, Brightwell and Premier, were obtained from the Alma Nursery and Blueberry Farm in Alma, GA. The two SHB, Legacy and Farthing, were obtained from the Cornelius Farm in Manor, GA. These choices represent the commonly used blueberry cultivars in GA since more than 40% of the state's blueberry acreage is planted to RE (*Vaccinium ashei*) cultivars, with the remaining to SHB (*V. corymbosum hybrids*) cultivars (Itle, personal communication, 4/1/2021). The plants were transported to the University of Georgia, Griffin campus on November 10, 2019. The four cultivars were similar in terms of plant age (17-18 months old), but the SHB experienced pruning during the summer. As a result, they were shorter in size. All of them were contained in a one-gallon pot originally.

Plant preparation

Initially, the plants underwent a period of chilling in a Conviron CG72 walk-in growth chamber (Conviron, Manitoba, Canada) at the Dempsey Farm of University of Georgia, Griffin campus. The plants were chilled to speed up the dormancy period so that they could be actively growing from the same growth stage once out of the chamber. The chamber was set at 4°C with no light. The blueberry plants spent about 600 hrs in the growth chambers, from October 28th to December 18th. The plants were irrigated four times during the chilling period (November 3rd, 13th, 21st and December 1st, 2019). Pictures of the plant were taken constantly to capture their change during the dormancy. On December 18th, 2019, the plants were moved to the greenhouse of the University of Georgia, Griffin campus. Despite the dormancy period, some plants maintained their leaves. These plants were defoliated before the start of the experiment. On December 19th, 2019, the blueberry plants were transplanted into three-gallon pots to provide more space for the plant roots to grow.

The growth medium contained 75% pine bark and 25% sand (Foothills Compost, Royston, GA, US). The same day the plants were pruned to homogenize the heights of all the cultivars. The plants of Farthing cultivar were used as references as they were the shortest. After pruning, the plants were assigned to the bench spaces in the greenhouse house in a randomized complete block design as described below. The plants were irrigated daily. To speed up the growth, growth lights were placed above the plants. The lights were programmed to come on 1 hour before sunset and to turn off 1 hour before sunrise. The light timer was set to be adjusted every day and adapt to the duration of the day. The lights were turned off on April 7th.

The pots were fertilized once on April 7th, 2020. One tablespoon of slow release osmocote (15-9-12) was applied per pot.

We observed a problem with aphid infestation in the greenhouse on February 13th, 2020. The plants were then treated with Endeavor (Syngenta, Basel, Switzerland) as per the application rate on the label (¼ tsp per gallon of water). On April 28th there was a problem with caterpillars. To address the problem, the blueberry plants were also sprayed with Xentari (Abbott Labs, Chicago, IL, US) at an application rate of 2 tsp per gallon of water. To avoid the introduction of *Bacillus turingensis* from the product, soil was covered with plastic bags before the treatment. Since just the vegetative growth was the subject of the study, flower buds were periodically removed on Monday and Wednesday.

Treatments

The treatments included 8 biostimulants and a control (water), resulting in 9 treatments. The biostimulants were chosen based on blueberry growers' responses to a questionnaire about the types of products they commonly use. The questionnaire was distributed to growers during the Georgia Blueberry Grower Association Meeting in Alma, GA in summer 2019. The questionnaire was also distributed to growers through an email listserv through the UGA Extension Service with the help of Ms. Renee Allen who is the Area Blueberry Extension Agent. Based on the results of the survey, eight biostimulants were chosen for the greenhouse study. The list of the biostimulants along with their information is shown in Table 3.1. The products were applied according to instructions on the labels. If there were any doubts about the instructions in the labels, we reached out to company representatives for more information or clarification.

Experimental design

The experimental design used was a randomized complete block design. There were 9 treatments, 4 cultivars, and 5 replications per treatment. In total, it was a factorial study that involved 180 blueberry plants (9 * 4 * 5 = 180). The design schematic is shown in Table 3.2. The plants were put in five benches (blocks) in the greenhouse. Each bench had a dimension of 1.52 m width and 3.01 m length. Each bench had 36 plants organized in 9 rows, with each row having 4 plants, following treatment and cultivar randomization process that was generated in Excel. The pots were evenly spread on each bench.

The treatment schedule included application of some products biweekly and others monthly. Suntton Micron Nutrients, Quantum Organic Total and Radiate were applied biweekly. Fertiactyl GZ, Huma Gro Zap, Inocucor, Soil Set and Terra Grow were applied monthly (Table 3.3). Radiate was applied just four times. This was based on the recommendation of the company representative for the product that contains plant hormones.

The treatment solutions were prepared in 30 L volume where the biostimulants were mixed with water as per the application rates that were adjusted based on the dimensions of the pot, with some contingency in case of loss due to spilling during the treatment application. Buckets and measuring cups were used to apply the treatments. One liter of solution per pot was applied to ensure a homogeneous application throughout the pot. A sample of the solution was also taken to test for pH. This was done to monitor the pH of the treatment solutions to avoid any significant shift in pH of the growth medium that is needed to be acidic for blueberry plants.

Measurement of plant growth parameters

To evaluate the response of the plants to the biostimulants, the following parameters were measured monthly: plant height, width, volume, cane number and diameter and leaf pigments content. The plant mass was taken at the beginning and end of the experiment. Leaf area was taken at the end of the experiment. The measurements schedule is shown in Table 3.4.

Plant height, width and volume

Plant height was taken with a measuring tape perpendicularly from the soil surface up to the topmost leaf. Plant width was measured two times per plant, with one perpendicular to the other. The two width measurements were then averaged. Plant volume was calculated using the cylinder formula: $V=\pi*r^2*h$ (Hoover et al., 2014). The measurements were performed on all the plants.

Cane number and diameter

For cane number and diameter, the primary branches were counted. Primary branches originate below the soil surface. The diameter measurement was performed in the same place to have a better evaluation of cane development. The diameter measurement was performed with an electronic caliper (Model CD-6’’BS, Mitutoyo Corporation, Kanagawa, Japan).

Pigment analysis

The pigment analysis was performed with the Dualex ForceA (Dualex, Force A, Université Paris Sud, France). This is a leaf clip optical instrument that allows estimation of not only the chlorophyll content, but also flavonols and anthocyanins relative absorbance in leaves and nitrogen balance index (NBI®). The relative absorbance allows the estimation of flavonols and anthocyanin content, while the NBI index combines chlorophyll and flavonols and is related to N to C ratio (https://tinyurl.com/uawhvx6). The measurements were taken for three different leaves per plant, both on superior and inferior sides of the leaf, on all the plants in the experimental design. The calibration of the instrument was performed at the beginning and every 18 plant-interval. With each calibration, the sensor was cleaned with wipes moistened with demineralized water to prevent dust or other residues that might affect the accuracy of the equipment.

Plant weight

Plant weight measurement was performed on five plants per variety at the beginning of the study. This was done by separating the top and bottom parts of each plant, washing the roots, weighing the stems and roots, and recording the fresh weights using a digital balance (Model V11P6, Ohaus Corporation, Parsippany, NJ, US). After 72 hours in drying oven (Model 13-261-27A, the Grieve Corporation, Round Lake, IL, US) at 149°F, dry weights were recorded. Since the plants were dormant at the beginning of the study, we recorded the weights just for canes and roots. Weight was taken also at the end of the experiment. At this time, all the plants in the experimental design were included in the analysis. The measurements were performed the same way as before, the only difference was the plants were defoliated before drying them. Two different balances were used to record the fresh weight (Model V11P6, Ohaus Corporation, Parsippany, NJ, US. Model PM11-N, Mettler Toledo, Greifensee, Switzerland), and just one was used to record the dry weight (Model PM11-N, Mettler Toledo, Greifensee, Switzerland). The samples were dried in two drying ovens (Model 13-261-27A, the Grieve Corporation, Round Lake, IL, US and Model POM-324CX, Blue M Electric co., Blue Island, IL, US). One replication at the time was put in the oven to dry.

Leaf area

Leaf area measurement was performed on 30 leaves per plant at the end of the experiment (NeSmith, 1991) using the Licor 3100 area meter (LI-COR Biosciences, Lincoln, NE, US). The leaves were sampled randomly on the plants. The instrument was calibrated after every 30 leaves.

The total area resulting from the measurements of 30 leaves has been divided by 30 to have the average leaf area. Leaf area measurement was performed on replications 1, 2 and 3.

Statistical analysis

The data for plant height, average width, plant volume, cane number and diameter, average leaf area, chlorophyll, anthocyanin, flavonols content and NBI index, plant dry mass were averaged by treatment and measurement date. The data were analyzed with two ways ANOVA to determine the statistical significance of treatment (T), cultivar (CV) and cultivar-treatment interaction (CV*T) on plant growth parameters by time and type (RE vs SHB). Cultivar, T and CV*T were considered as categorical variables and time was a continuous variable and the replication was considered as random effect. We also analyzed data with one-way ANOVA to look at the impact of T by CV and time. Tukey's honest significant difference (HSD) test was used for the post hoc analyses for mean separation to identify significant difference among the T by CV.

RESULTS AND DISCUSSION

Plant height, width, volume, cane number and diameter, leaf area and dry biomass

From the mixed model analysis, treatment did not significantly influence plant height, average width, plant volume, cane number and diameter, average leaf area and dry biomass in either blueberry types (Tables 3.5, 3.6). Cultivar-treatment interaction was not significant either for these growth parameters in any of the months (Table 3.5, 3.6). In many of the timepoints, there were significant differences among the cultivars. Since our focus was on the impact of the treatments or their interactions with the varieties, we will not discuss just the impact of the varieties here. The data for these parameters that are not shown here are found in appendix A (Tables A.1-5, Figures A.1-4).

Chlorophyll content

Treatment, CV and CV*T had significant impact on chlorophyll content in both varieties albeit at different times (Table 3.7). The treatment application significant influenced adaxial chlorophyll content in February, March, April and June in RE, and in April, May and July in SHB (Table 3.7). The interaction effects were significant in January, February, March and April in RE, and in April, May and June in SHB (Table 3.7). Results that show mean separation for the CV-T interaction effects are shown in appendix A (Table A.7-10).

Analysis of variance by variety and month indicated that treatment had significant impact on adaxial chlorophyll content in Brightwell in January, February and April (Tab 3.8). In January, Brightwell treated with Quantum O.T had higher chlorophyll content than Fertiactyl GZ and Huma Gro. In February, Terra Grow showed significantly higher chlorophyll content than Radiate and Control. In April, Inocucor, Huma Gro and Fertiactyl GZ had significantly higher chlorophyll content than Radiate. In Farthing, treatment effect was significant only in June when Terra Grow and Huma Gro applications resulted in higher chlorophyll content than Soil Set. For the Legacy cultivar, treatment had a significant impact in April, May and June. In the three months, Radiate resulted in higher chlorophyll content than most of the other biostimulants but not the Control. In April, Radiate resulted in higher chlorophyll content than the rest except for the Control and Micron Nutrients, while in May Radiate had a higher chlorophyll content than the rest except for Fertiactyl GZ and Terra Grow in May. Fertiactyl GZ was the only treatment that resulted in significantly lower chlorophyl content than the Control in May. In June, the only significant differences were between Radiate, Terra Grow and Fertiactyl GZ in descending order.

In Premier, T application had a significant impact on adaxial chlorophyll content only in February and March. Radiate resulted in significantly higher chlorophyll content than Soil Set in February while the other treatments did not show any significant differences among them. In March, however, Radiate resulted in significantly higher chlorophyl content than Soil Set and Fertiactyl GZ. Additionally, Soil Set was significantly lower than Huma Gro.

In all the months, no significant difference was detected between the biostimulants and the Control, with the exception of February with Brightwell (Terra Grow > Control) and May with Legacy (Fertiactyl GZ < Control). In Brightwell, Terra Grow had a chlorophyll content 32% higher that Control. In Legacy, Fertiactyl GZ induced a chlorophyll content 40% lower that the Control. This is an indication that for most of the growth period the biostimulants did not impact the chlorophyl content in any meaningful way. Fertiactyl GZ contains seaweed, humic acids and fulvic acids, and Terra Grow contains a microbial inoculant and humic acids. Many studies reported that seaweed application can improve chlorophyll content in different crops (Khan et al., 2009; Thirumaran et al., 2009). Humic acid application mitigated the effect of salinity stress on bean, with an increase of chlorophyll concentration (Meganid et a., 2015). Fulvic acid can enhance chlorophyll content in potato (Wadas et a., 2020). Baqual et a. (2005) reported an effect of microbial inoculant on chlorophyll content because of an increased photosynthesis. Our Legacy results are in contrast with these studies. It looks like Fertiactyl GZ depressed chlorophyll biosynthesis compared to the non-treated plant.

While analyzing the March-April results, the light stress problem must be considered. Due to a power outage, the timer of the growth light in the greenhouse stopped working and the hours of artificial light overlapped with those of the natural light, causing a light stress (photoinhibition) that resulted in the leaves turning red and brown in color (Routray et al., 2011). The problem was noticed at the beginning of April, but it is possible that it started at the end of March. Most of the plants completely recovered by mid-May, with some exceptions in Legacy and Farthing. The reddish color was due to the high content of anthocyanin in response to the stress. Aarti et al. (2006) in their study on light stress on cucumber concluded that high light frequency can inhibit chlorophyll formation, with serious consequences on plant health, potentially compounding the treatment effects. However, the incident might also give the opportunity to examine the data to see if any of the biostimulants helped the plants recover from the light stress. In April, Inocucor, Fertiactyl GZ and Huma Gro had the significant higher chlorophyll content than Radiate in Brightwell. It is possible that the active ingredients contained in those products contributed to light stress problem recovery (Bulgari et al., 2019). On the other hand, Radiate was the best performer with Legacy and Premier during the light stress months, and Fertiactyl GZ the worst in April for Legacy as compared to other biostimulants, not the Control. Radiate contains indole-3-butyric acid (IBA) and cytokinins and both can potentially increase chlorophyll concentration (Dobránszki et al., 2014, Gilani et al., 2019). However, it is important to note that none of the biostimulants were better in helping the plant respond to the light stress as compared to the Control. Fertiactyl GZ even resulted in a significant lower chlorophyll content compared to the Control in May for Legacy. It is plausible to conclude that for most of the months the biostimulants did not enhanced chlorophyll biosynthesis, and in some cases it have been depressed by the treatments.

The effects of the biostimulants were not consistent over time in the different cultivars. The differences among the biostimulants were early in the growth months in Brightwell and Premier while they were in the later growth periods for Farthing and Legacy.

A trend in the biostimulant ranking can be suggested for Legacy and Premier. In Legacy, Radiate was the best performing product over three months, while Terra Grow and Fertiactyl GZ were the worst. In Premier, Radiate was the best over two months, while Soil Set the least effective. For Brightwell, the treatments did not show any discernable patterns. Fertiactyl GZ and Huma Gro, the least performing in January, were the best in April, but Radiate was the worst performing in both February and April. In Farthing the only significant difference was detected in June. It is important to note that this pattern is not reflective of the effect of the biostimulant in comparison to the Control but mainly among each other.

The abaxial chlorophyll content followed similar trend to the adaxial, with the only exception in July in which significant treatment was detected in Brightwell. In this month, Terra Grow and Quantum O.T had significant higher chlorophyll than Fertiactyl GZ. Significant difference was also detected in January for Legacy, where Soil Set resulted in higher chlorophyll content than Radiate. There was no significant difference between the treatments and Control in any of these cases. The result Table for this parameter is shown in the appendix A (Table A.6).

Anthocyanin content

Treatment, CV and CV*T had significant impact on anthocyanin content in both varieties albeit at different times (Table 3.7). The treatment application significant influenced adaxial anthocyanin content in March, in RE, and in April, May, June and July in SHB (Table 3.7). The interaction effects were significant in January, February, April and May in RE, and in April, May and June in SHB (Table 3.7). Results that show mean separation for the CV-T interaction effects are shown in appendix A (Table A.12-15)

Analysis of variance by variety and month indicated that T had significant impact on adaxial anthocyanin content in Brightwell in January, February, March and April (Table 3.9). In January and February, Huma Gro application induced a significantly higher anthocyanin content than Quantum O.T. In March, Fertiactyl GZ led to a significantly higher anthocyanin content than Terra Grow. In April, Radiate resulted in higher chlorophyll content than Terra Grow, Huma Gro, Fertiactyl GZ and Inocucor. In Farthing, T effect was significant in May, June and July. In May, Radiate application resulted in higher anthocyanin concentration than Micron Nutrients. In June and July, Soil Set resulted in significantly higher anthocyanin content than Terra Grow in June, and Micron Nutrients in July.

In Legacy, significant difference was detected in January, April, May and June. In January, Radiate application significantly increased anthocyanin content as compared to Fertiactyl GZ. In April and May, Fertiactyl GZ and Quantum O.T. led to a higher anthocyanin content than Radiate. In May and June, Terra Grow resulted in increased anthocyanin content as compared to Radiate in May, and Control, Soil Set and Radiate in June. In Premier, T application did not impact anthocyanin content.

In all the months, no significant difference was detected between the biostimulants and the Control with the exception of June in Legacy (Terra Grow > Control) in which Terra Grow resulted in 60% higher anthocyanin than the Control. This suggests that the plants that received the biostimulant were more stressed than the Control. This is contrary to one's expectation based on the product description. Terra Grow is described as having microbial inoculants and humic acids that would help reduce stress in plants. It is plausible that the microorganisms in the products were not able to compete with soil microorganisms, or they did not survive during product storage to be effective (Kaminsky et al., 2019). It is also possible that they might have failed to interact with the plant or failed to survive the acidic soil where blueberry grows (Kaminsky et al., 2019). Moreover, the addition of extra humic acids to soil that already has enough might not lead to any significant impact. All these might explain the lack of positive impact of Terra Grow or the other biostimulants, but does not still explain why Terra Grow led to more anthocyanin content that the Control, which is an indication of plant stress. There is no literature on this topic to my knowledge. The results from abaxial anthocyanin content indicated that for most of the growth period the biostimulants did not impact anthocyanin content in any significant way, even in response to the March-April light stress problem. In fact, plants accumulate anthocyanin during stresses to prevent damages to the photosynthetic system (Trojak et al., 2017). Anthocyanin content increases when the plants are under stress condition and decreases when they are recovering (Chalker-Scott, 2002).

When comparing the different biostimulants, in March with Brightwell, Fertiactyl GZ had a significant higher anthocyanin content than Terra Grow. In April, Radiate had a significant higher anthocyanin content than Terra Grow, Fertiactyl GZ, Huma Gro and Inocucor. It is plausible to conclude that the active molecules contained in Fertiactyl GZ (seaweed, humic acids and fulvic) and Radiate (IBA and cytokinins) did not contribute to alleviate the light stress problem as compared to other biostimulants. On the other side, Fertiactyl GZ was effective in lowering anthocyanin content in April. This result shows again that the effect of the same biostimulant was not consistent over the months. In Legacy in April, Fertiactyl GZ and Quantum O.T had a significant higher anthocyanin concentration than Radiate. This is another evidence that the same biostimulant interact differently with different cultivars.

The effects of the biostimulants were not consistent over time in the different cultivars. A trend in the biostimulant ranking can be suggested for Farthing and Legacy. In Farthing, Soil Set had the overall highest anthocyanin content, and Micron Nutrients had the overall lowest. In

Legacy, Quantum O.T., Fertiactyl GZ and Terra Grow had the highest anthocyanin content, while Radiate had the overall lowest. For Brightwell, it is again hard to rank the biostimulants based on their impact. Huma Gro had the highest anthocyanin content in January and February, and the lowest in April. In March, Fertiactyl GZ had the highest content, but the lowest in April. Terra Grow had the lowest concentration in both March and April, the time the light stress occurred.

The abaxial chlorophyll content followed similar trend as the adaxial, with the only exception in January in which no significant difference was detected among the treatments in Brightwell, and July in which significant difference was detected between the Control and Terra Grow. Significant difference was detected for Legacy in February with the Control having a higher anthocyanin content compared to Soil Set, but not in January. The result Table for this parameter is shown in the appendix A (Table A.11).

Flavonols content

Treatment, CV and CV*T had significant impact on flavonols content in both varieties albeit at different times (Table 3.7). The treatment application significant influenced adaxial flavonols content in March, in RE, and in May, June and July in SHB (Table 3.7). The interaction effects were significant in January, February and July in RE, and in January and April in SHB (Table 3.7). Results that show mean separation for the CV-T interaction effects are shown in appendix A (Table A.17-20).

Analysis of variance by variety and month indicated that T had significant impact on adaxial flavonols content in Brightwell in February and July (Table 3.10). In February, Soil Set application induced a significantly higher flavonols content than Terra Grow. In July, Soil Set and Control led to significant higher flavonols content than Fertiactyl GZ. In Legacy, significant difference was detected in February and May. In February, Quantum O.T., Radiate, Huma Gro, Inocucor and Fertiactyl GZ significantly increased flavonols content than Soil Set. In May, Fertiactyl GZ led to a higher flavonols content than Radiate. No significant was detect among the biostimulants in Farthing and Premier.

In all the months, no significant difference was detected between the biostimulants and the Control, with the exception of July with Brightwell (Control > Fertiactyl GZ). In particular, Fertiactyl GZ had a flavonols content about 14% lower than the Control. The results are an indication that for most of the growth period, the biostimulants did not impact the flavonols content in any significant way. Flavonols are a subcategory of flavonols and are known to be stress protectors (Brodowska, 2017, Samanta et al., 2011). In particular, they are produced in case of high irradiance (Treutter, 2006). No significant difference was detected between the treatments in March-April, the months of the light stress problem. The only interesting result came from Brightwell in July was that the Control and Soil Set resulted in higher flavonols concentration than Fertiactyl GZ. This suggests that Brightwell without treatment application was significantly more stressed than the ones treated with Fertiactyl GZ. This can be due to several factors, including the high light intensity in July. As already mentioned, Fertiactyl GZ contains seaweed, humic acids and fulvic acids. Those molecules have a positive effect on light stress protection, and they could have helped the plant during the stress period. This was not true for Legacy, in which Fertiactyl GZ application led to the overall highest flavonols content in February and May, Soil Set resulted in the overall highest flavonols concentration for Brightwell in February and July. This result shows again that the effect of the same biostimulant varies from cultivar to cultivar.

The effects of the biostimulants were not consistent over time in the different cultivars. A trend in the biostimulant ranking can be suggested for Brightwell and Legacy. In Brightwell, Soil

Set application led to the highest flavonols content. In Legacy, Fertiactyl GZ had the overall higher flavonols concentration.

The results for abaxial chlorophyll content showed similar trends as the adaxial, with the only exception in Brightwell in which no significant difference was detected among the treatments. In Legacy, significant difference has been detected in April where Inocucor had a significant higher flavonols content compared to Radiate, and June in which Fertiactyl GZ had a significant higher flavonols content compared to Radiate. The result Table for this parameter is shown in the appendix A (Table A.16).

Chlorophyll/flavonols ratio (NBI index)

Treatment, CV and CV*T had significant impact on NBI index in both varieties albeit at different times (Table 3.7). The treatment application significant influenced adaxial NBI index in March and June in RE, and in April, May, June and July in SHB (Table 3.7). The interaction effects were significant in January, February, March and April in RE, and in April, May and June in SHB (Table 3.7). Results that show mean separation for the CV-T interaction effects are shown in appendix A (Table A.17-20).

Analysis of variance by variety and month indicated that T had significant impact on adaxial NBI index in Brightwell in February, March and April (Table 3.11). In February, Terra Grow induced a significantly higher NBI index compared to Huma Gro, Radiate, Control and Soil Set. In March, Terra Grow had a significantly higher NBI index than Soil Set. In April, Micron Nutrients, Inocucor, Fertiactyl GZ and Huma Gro applications resulted in a higher NBI index compared to Radiate. In Farthing, Huma Gro had a significantly higher NBI index compared to Control and Soil Set in June.

In Legacy, significant differences were detected in April, May and June. In these months, Radiate application led to the higher NBI index. In April, Soil Set, Inocucor, Quantum O.T, Huma Gro and Fertiactyl GZ had the lowest NBI index. In May, Inocucor, Huma Gro and Fertiactyl GZ had the lowest NBI index. In June, Terra Grow resulted in the lowest NBI index. Over the 3 months, Radiate had the highest NBI index.

In Premier, significant differences were assessed in March, where Radiate had a significant higher NBI index compared to Fertiactyl GZ and Soil Set.

In all the months, no significant difference was detected between the biostimulants and the Control, with the exception of Brightwell in February (Terra Grow > Control), and Farthing in June (Huma Gro > Control). In particular, in Brightwell in February, Terra Grow had an NBI index 54.5% higher than Control. In Farthing in June, Huma Gro had an NBI index 41% higher than Control.

The NBI index expressed as chlorophyll/flavonols ratio is related to the N status in the leaves since it is related to nitrogen and carbon allocation (Diago et al., 2016). The two values depend on many environmental factors like solar radiation and physiological plant status. Chlorophyll and flavonols increase during vegetative growth, and generally chlorophyll decreases during the reproductive stage while flavonols remain constant (Cerovic et al., 2015). During senescence, chlorophyll content decreases while flavonols increase (Mettila et al., 2018). Mahdavian et al. (2007) reported that increasing UV radiation can lead to a reduction of chlorophyll content, and an increase in the flavonols one. It is reported that biostimulants can help in case of abiotic stresses, and could improve light management (Yakhin et al., 2017). This is the case of Brightwell in February, and Farthing in June. As already mentioned, Terra Grow contains a microbial inoculant that can increase the chlorophyll content. Huma Gro contains a mixture of organic derived components. It is plausible to conclude that the active molecules in those products induced a better allocation of N in the leaves compared to the Control.

The effect of the biostimulants were not consistent over time, but a trend can be defined for Brightwell and Legacy. In Brightwell, Terra Grow had the best in February and March, while Soil Set the worst. Radiate has been the worst performing in February and April. In Legacy, Radiate was consistently the best performing in April, May and June. Inocucor, Huma Gro and Fertiactyl GZ were the worst in April and May. Farthing showed significant difference only in June, with Huma Gro having a higher NBI index compared to the Control. In Premier, significant difference has been detected only in March, Radiate had a higher NBI index compared to Fertiactyl GZ and Soil Set.

The abaxial chlorophyll content showed a similar trend to the adaxial one, with the only exception in Brightwell in which significant difference was detected also in January between Quantum O.T, and Control and Soil Set, but not in February and March. In Legacy, significant difference was detected also in April, with Radiate having higher NBI index compared to Fertiactyl GZ, Inocucor, Quantum O.T., Huma Gro and Terra Grow. In Premier, no significant difference was detected between treatments. The result Table for this parameter is shown in the appendix A (Table A.21).

SUMMARY AND CONCLUSIONS

The effect of the biostimulants on blueberry growth varied among the parameters measured. We can conclude that the effect of the biostimulants on chlorophyll content is variable among blueberry cv and timepoint. For all the cultivars, no significant difference was detected between the Control and the biostimulants over the months, with the exception of Brightwell in

February. Terra Grow resulted in significant higher chlorophyll content than Control in February. Another exception was Legacy in May, with the Control having a higher chlorophyll content than Fertiactyl GZ. In Legacy, Radiate appears to be the best performing biostimulant as compared to the other biostimulants, while Fertiactyl GZ and Terra Grow the worst. In Premier, Radiate application led to the highest overall chlorophyll concentration, while Soil Set resulted in the lowest.

For anthocyanin content, Legacy treated with Terra Grow in June showed a significant higher anthocyanin content than the Control, which is a sign of stress in plants receiving Terra Grow. In Legacy, Radiate was consistently the biostimulant with the lowest anthocyanin content.

For flavonols content, the only cultivar in which significant difference between the biostimulants and the Control was observed was Brightwell in July. In this month, the Control had a significant higher flavonols content than Fertiactyl GZ. Since flavonols are produced during stress, this suggests that Fertiactyl GZ might have helped in alleviating the stress as compared to the Control. When comparing the biostimulants, the overall trend, although not always statistically significant, was that Soil Set application resulted in the highest flavonols content in Brightwell. In Legacy, Fertiactyl GZ had the overall highest. This suggests that the two biostimulants did not help the plants with stress alleviation.

In the case of NBI index, significant difference against the Control was detected with Brightwell in February, and Farthing in June. In Brightwell, Terra Grow showed significantly higher NBI index than Control. In Farthing, Huma Gro induced a higher NBI index than the Control. Since the NBI index is a nitrogen status indicator, it is plausible to conclude that these two biostimulants induced a better N allocation in the leaves. In Legacy, Radiate was the biostimulant with the overall highest NBI index.

This study provides insights on the effect of biostimulants on blueberry vegetative growth in greenhouse environment. Overall, the products had differing effect according to blueberry cv and time. Another fact to highlight is the lack of storage information on products' labels. Since many biostimulants contain microorganisms, lack of those information can lead to low survival rate of microbes, and poor effect on plants (Wang et al., 2017). Another problem that we faced is the lack of information about application rates. Some of the products tested had limited information on the label, and it was difficult to find more instructions online and from contacts of the product retailers. Improvement in these areas can help the products performance and commercialization, increasing farmers' faith on biostimulants.

To confirm the findings of the greenhouse study and gain a better insight on the impact of biostimulants on blueberry growth, the study needs to be repeated under field conditions. This is important to better understand the interaction of the products with the various environmental factors.

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Table 3.1: Product name, active ingredients, manufacturer information of biostimulants in the study

Product name	*Company name*	*Active ingredients*	*Application rate*	*Application frequency*	**OMRI listed*
Fertiactyl GZ	Alltech	Urea nitrogen 13%, K_2O 5%, Humic acids 10%, Fulvic acids 5%, Seaweed 20%	1.42 L/A	Monthly	no
Huma Gro ZAP	Huma Gro	N 8%, S 1%, Fe 0.1%, Mn 0.05%, Zn 0.05%	10 L/ha	Monthly	yes
Inocucor	Inocucor Technologies	Bacillus subtilis Saccharomyces cerevisiae	45 mL/gal	Monthly	yes
Micron Nutrients	Suntton International Inc.	N 2%, K 0.2%, Fe 0.15%, Mn 0.1%, Mo 0.001%, Zn 0.1%	1.8 fl. Oz/A	Bi-weekly	no
Quantum Organic Total	Ecological Laboratories	Rhodopseudomonas palustris, Bacillus subtilis B. lichniformis, B. pumilus B. amyloliquefaciens	64 Oz/A** 32 Oz/A***	Bi-weekly	yes
Radiate	Loveland Products	Iba 0.85% Cytokinin 0.15%	2 fl. Oz/A	Bi-weekly	yes
Soil Set	Alltech Crop Science	Cu 2.0%, Fe 1.6%, Mn 0.8%, Zn 3.2%	2 L/ha** 1 L/ha ***	Monthly	yes
Terra Grow	BioSafe Systems	Microbial inoculant 0.30%: Bacillus lichniformis B. subtilis, B. pumilus, B. amyloliquefaciens B. megaterium, Trichoderma hazianum Humic acids 35%, Microbial food 28%	0.68 kg/A	Monthly	yes

*OMRI = Organic Material Review Institute. Certifies that the product can be used for growing USDA-certified organic produce

**first application ***following applications

Table 3.2: Experimental design for the greenhouse study

🟨 Soil set	🟦 Huma gro	🟨 Quantum O. T	🟧 Inocucor	🟩 Radiate	🟨 Terra grow	🟩 Fertiactyl GZ	🟦 Micron nutrients	🟦 Soil set
🟦 Fertiactyl GZ	🟨 Control	🟩 Huma gro	🟦 Control	🟨 Huma gro	🟩 Soil set	🟦 Quantum O. T	🟩 Control	🟧 Terra grow
🟩 Micron nutrients	🟧 Radiate	🟨 Inocucor	🟧 Soil set	🟦 Radiate	🟧 Control	🟨 Radiate	🟦 Terra grow	🟩 Inocucor
🟧 Quantum O. T	🟩 Terra grow	🟧 Fertiactyle GZ	🟩 Quantum O. T	🟨 Fertiactyl GZ	🟦 Inocucor	🟧 Micron nutrients	🟨 Micron nutrients	🟧 Huma gro
🟧 Huma gro	🟨 Quantum O. T	🟨 Micron nutrients	🟦 Radiate	🟩 Inocucor	🟦 Control	🟧 Fertiactyl GZ	🟩 Control	🟨 Inocucor
🟦 Inocucor	🟦 Micron nutrients	🟩 Fertiactyl GZ	🟩 Soil set	🟦 Soil set	🟧 Quantum O. T	🟩 Quantum O. T	🟨 Soil set	🟧 Micron nutrients
🟩 Huma gro	🟧 Terra grow	🟨 Radiate	🟦 Quantum O. T	🟨 Terra grow	🟩 Radiate	🟨 Control	🟦 Huma gro	🟩 Terra grow
🟨 Fertiactyl GZ	🟦 Terra grow	🟧 Inocucor	🟨 Huma gro	🟧 Control	🟦 Fertiactyl GZ	🟧 Radiate	🟩 Micron nutrients	🟧 Soil set
🟩 Fertiactyl GZ	🟦 Terra grow	🟨 Inocucor	🟩 Control	🟨 Micron nutrients	🟦 Inocucor	🟩 Terra grow	🟩 Fertiactyl GZ	🟦 Micron nutrients
🟧 Control	🟩 Huma gro	🟧 Micron nutrients	🟧 Inocucor	🟩 Soil set	🟧 Fertiactyl GZ	🟨 Soil set	🟧 Terra grow	🟨 Quantum O. T
🟨 Terra grow	🟧 Huma gro	🟦 Control	🟨 Huma gro	🟦 Quantum O. T	🟨 Radiate	🟧 Quantum O. T	🟦 Soil set	🟩 Micron nutrients
🟩 Inocucor	🟨 Fertiactyl GZ	🟧 Soil set	🟦 Radiate	🟩 Quantum O. T	🟦 Huma gro	🟩 Radiate	🟨 Control	🟧 Radiate
🟦 Terra grow	🟧 Radiate	🟩 Terra grow	🟧 Quantum O. T	🟧 Huma gro	🟦 Quantum O. T	🟩 Soil set	🟧 Fertiactyl GZ	🟦 Radiate
🟩 Inocucor	🟨 Radiate	🟦 Soil set	🟩 Micron nutrients	🟨 Huma gro	🟩 Fertiactyl GZ	🟨 Inocucor	🟨 Micron nutrients	🟩 Control
🟧 Micron nutrients	🟩 Quantum O. T	🟨 Control	🟦 Huma gro	🟧 Terra grow	🟦 Micron nutirients	🟦 Control	🟧 Inocucor	🟧 Soil set
🟨 Fertiactyl GZ	🟩 Radiate	🟧 Control	🟨 Soil set	🟦 Fertiactyl GZ	🟩 Huma gro	🟨 Quantum O. T	🟦 Inocucor	🟨 Terra grow
🟩 Soil set	🟧 Radiate	🟩 Radiate	🟦 Quantum O. T	🟩 Fertiactyl GZ	🟨 Inocucor	🟧 Quantum O. T	🟩 Huma gro	🟨 Control
🟨 Quantum O. T	🟩 Micron nutrients	🟦 Inocucor	🟨 Micron nutrients	🟦 Control	🟧 Terra grow	🟦 Soil set	🟧 Control	🟩 Inocucor
🟧 Inocucor	🟦 Fertiactyl GZ	🟨 Huma gro	🟦 Radiate	🟨 Terra grow	🟦 Micron nutrients	🟨 Fertiactyl GZ	🟩 Terra grow	🟨 Soil set
🟦 Terra grow	🟧 Huma gro	🟩 Control	🟧 Fertiactyl GZ	🟩 Quantum O. T	🟧 Soil set	🟦 Huma gro	🟨 Radiate	🟧 Micron nutirients

🟨 Premier
🟧 Farthing
🟩 Legacy
🟦 Brightwell

Table 3.3: Product name and application schedule

Product name	Application time						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Sunnton Micron nutrient	16th, 30	13th, 26th	12th, 26th	9th, 23rd	7th, 21st	4rt, 18th	2nd, 16th
Quantum Organic Total	16th, 30	13th, 26th	12th, 26th	9th, 23rd	7th, 21st	4rt, 18th	2nd, 16th
Radiate	16th, 30	13th, 26th					
Fertiactyl GZ	16th	15th	16th	15th	15th	15th	15th
Huma Gro Zap	16th	15th	16th	15th	15th	15th	15th
Inocucor	16th	15th	16th	15th	15th	15th	15th
Soil Set	16th	15th	16th	15th	15th	15th	15th
Terra Grow	16th	15th	16th	15th	15th	15th	15th

Table 3.4: Canopy growth measurements schedule

Task	Schedule								
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Height and width	19th	28th	29th	28th	25th	23rd	20,21st	18th, 19th	
Cane number and diameter		21st		2nd, 30	29th	25th	22nd	20	
Pigment analysis			4th	31st	28th	26th	23rd	21st	
Photosynthetic rate					27th	31st			
Plant mass-fresh	20								3rd,4th,5th,6th,7th
Plant mass-dry	23rd								6th,8th,9th
Flower removal		30	12th, 17th, 26th	2nd,4th, 11th, 18th, 25th, 31st			3rd, 17th, 22nd		

Table 3.5: ANOVA table for plant size measurement parameters

	Main effect *p*-value (α=0.05)					
Response parameters	Rabbiteye			Southern Highbush		
	Cultivar	Treatment	CV-T	Cultivar	Treatment	CV-T
January						
Height	<0.0001	0.6549	0.7910	<0.0001	0.8630	0.8080
Width	0.0287	0.6861	0.9620	<0.0001	0.4885	0.2208
Volume	0.0009	0.6348	0.9388	<0.0001	0.5596	0.5303
Total cane number	0.0293	0.6413	0.7586	0.0185	0.8345	0.0754
Total cane diameter	0.0054	0.6184	0.6920	0.2391	0.6453	0.4859
February						
Height	<0.0001	0.1611	0.0667	<0.0001	0.8196	0.9069
Width	0.0009	0.9008	0.6286	<0.0001	0.3847	0.7350
Volume	<0.0001	0.8788	0.7882	<0.0001	0.5979	0.8985
Total cane number	0.0487	0.4391	0.6341	0.0185	0.8345	0.0754
Total cane diameter	0.0096	0.8220	0.3461	0.1920	0.6337	0.5685
March						
Height	<0.0001	0.4035	0.0847	<0.0001	0.8441	0.8877
Width	0.0030	0.8734	0.7255	<0.0001	0.6899	0.6654
Volume	<0.0001	0.6028	0.5030	<0.0001	0.6189	0.6542
Total cane number	0.0487	0.4391	0.6341	0.0185	0.8345	0.0754
Total cane diameter	0.0060	0.5645	0.3674	0.1580	0.5873	0.5201
April						
Height	<0.0001	0.4947	0.0811	<0.0001	0.7766	0.9447
Width	0.0018	0.7673	0.9316	<0.0001	0.3442	0.3741
Volume	<0.0001	0.6820	0.6228	0.0035	0.3975	0.3814
Total cane number	0.0293	0.4613	0.7586	0.0421	0.8863	0.1223
Total cane diameter	0.0374	0.7225	0.5831	0.2899	0.7486	0.7679
May						
Height	<0.0001	0.4947	0.0630	<0.0001	0.9247	0.8384
Width	0.3745	0.5062	0.8668	<0.0001	0.8230	0.8206
Volume	0.0001	0.0892	0.5137	<0.0001	0.9417	0.9601
Total cane number	0.0232	0.7046	0.3774	0.1371	0.5975	0.1298
Total cane diameter	0.1300	0.9557	0.3947	0.9182	0.5082	0.7767
June						
Height	0.4912	0.9378	0.8957	<0.0001	0.6140	0.1777
Width	0.0005	0.3518	0.3267	<0.0001	0.0803	0.7364
Volume	0.0215	0.2521	0.2924	<0.0001	0.2009	0.5133
Total cane number	0.0030	0.4766	0.2663	0.1469	0.6011	0.2230
Total cane diameter	0.8351	0.6407	0.1848	0.3840	0.4802	0.5797

Response parameters	Main effect *p*-value (α=0.05)					
	Rabbiteye			Southern Highbush		
	Cultivar	Treatment	CV-T	Cultivar	Treatment	CV-T
July						
Height	0.0015	0.8150	0.5928	<0.0001	0.6361	0.2585
Width	0.4155	0.2802	0.4599	<0.0001	0.2569	0.6207
Volume	0.0147	0.4169	0.7629	<0.0001	0.5033	0.5914
Total cane number	0.0030	0.4766	0.2663	0.1619	0.6500	0.3320
Total cane diameter	0.8444	0.8800	0.2191	0.1952	0.4954	0.5800
August						
Average leaf area	0.0776	0.7865	0.5006	<0.0001	0.6645	0.5578

Table 3.6: ANOVA table for plant mass measurement parameters

Response parameters	Rabbiteye			Southern Highbush		
	Main effect p-value (α=0.05)					
	Cultivar	Treatment	CV-T	Cultivar	Treatment	CV-T
August						
Leaves	0.0355	0.7594	0.7358	<0.0001	0.6595	0.7951
Stems	<0.0001	0.9845	0.3061	<0.0001	0.7405	0.7953
Roots	0.4100	0.8551	0.6611	<0.0001	0.4314	0.4426

Table 3.7: ANOVA table for pigment measurement parameters

	Main effect *p*-value (α=0.05)					
Response parameters	Rabbiteye			Southern Highbush		
	Cultivar	Treatment	CV-T	Cultivar	Treatment	CV-T
January						
Chlorophyll sup	0.0089	0.1865	0.0013	<0.0001	0.1989	0.5248
Anthocyanin sup	0.0058	0.0899	0.0340	<0.0001	0.0842	0.0686
Flavonols sup	0.1802	0.0898	0.0433	0.2783	0.4325	0.0139
NBI index sup	0.0374	0.4346	0.0297	<0.0001	0.7798	0.2976
Chlorophyll inf	0.025	0.1109	0.0281	<0.0001	0.0480	0.5277
Anthocyanin inf	<0.0001	0.0750	0.0970	<0.0001	0.0605	0.2333
Flavonols inf	<0.0001	0.2383	0.0867	<0.0001	0.9623	0.2832
NBI index inf	0.0014	0.2445	0.0441	<0.0001	0.6473	0.3149
February						
Chlorophyll sup	0.4123	0.0175	0.0035	<0.0001	0.1329	0.9199
Anthocyanin sup	0.0215	0.2658	0.0373	<0.0001	0.1473	0.1934
Flavonols sup	0.1062	0.8044	0.0159	<0.0001	0.4264	0.1128
NBI index sup	0.8604	0.0743	0.0010	<0.0001	0.1425	0.7995
Chlorophyll inf	0.3771	0.0411	0.0032	<0.0001	0.2183	0.7749
Anthocyanin inf	0.0979	0.0892	0.0202	<0.0001	0.0291	0.1783
Flavonols inf	<0.0001	0.3926	0.5963	<0.0001	0.3079	0.3902
NBI index inf	0.0167	0.1820	0.0312	<0.0001	0.2124	0.8832
March						
Chlorophyll sup	0.0431	0.0011	0.0106	<0.0001	0.5433	0.4703
Anthocyanin sup	0.0842	0.0046	0.1026	<0.0001	0.2102	0.1888
Flavonols sup	0.1556	0.0256	0.3475	<0.0001	0.4956	0.1728
NBI index sup	0.0194	0.001	0.0356	<0.0001	0.7163	0.8773
Chlorophyll inf	0.0071	0.0037	0.0151	<0.0001	0.5948	0.3225
Anthocyanin inf	0.0026	0.0008	0.1485	<0.0001	0.1442	0.1640
Flavonols inf	<0.0001	0.4668	0.6348	<0.0001	0.3189	0.1256
NBI index inf	<0.0001	0.0617	0.2741	<0.0001	0.6709	0.4920
April						
Chlorophyll sup	0.0004	0.0459	0.0063	0.0009	0.0068	0.0005
Anthocyanin sup	0.0002	0.0940	0.0316	<0.0001	0.0011	0.0062
Flavonols sup	0.0088	0.4768	0.8933	<0.0001	0.4764	0.0077
NBI index sup	0.0019	0.0503	0.0007	<0.001	0.0089	0.0023
Chlorophyll inf	<0.0001	0.0519	0.0296	0.0002	0.0142	<0.0001
Anthocyanin inf	<0.0001	0.3087	0.0296	<0.0001	0.0001	0.0010
Flavonols inf	<0.0001	0.4336	0.6001	<0.0001	0.0021	0.2514
NBI index inf	<0.0001	0.0433	0.1509	<0.0001	0.0401	0.0010

	Main effect *p*-value (α=0.05)					
Response parameters	Rabbiteye			Southern Highbush		
	Cultivar	Treatment	CV-T	Cultivar	Treatment	CV-T
May						
Chlorophyll sup	0.1577	0.8340	0.3291	0.2276	0.0450	0.0001
Anthocyanin sup	<0.0001	0.7873	0.0423	<0.0001	0.0012	0.0001
Flavonols sup	0.0133	0.9246	0.3315	0.8789	0.0193	0.1271
NBI index sup	0.0105	0.8766	0.0607	0.7046	0.0249	0.0003
Chlorophyll inf	0.2309	0.7434	0.2195	0.1510	0.0532	0.0001
Anthocyanin inf	<0.0001	0.7340	0.1455	<0.0001	0.0049	0.0003
Flavonols inf	<0.0001	0.3614	0.0561	<0.0001	0.0066	0.0056
NBI index inf	<0.0001	0.9187	0.0124	0.0026	0.0196	0.0001
June						
Chlorophyll sup	0.4253	0.0470	0.9579	<0.0001	0.1372	0.0007
Anthocyanin sup	<0.0001	0.1843	0.1048	<0.0001	0.0178	0.0001
Flavonols sup	0.0254	0.3977	0.2909	0.0006	0.0492	0.8579
NBI index sup	0.0191	0.0079	0.7173	<0.0001	0.0241	0.0121
Chlorophyll inf	0.5669	0.0434	0.9695	<0.0001	0.0703	0.0013
Anthocyanin inf	<0.0001	0.0671	0.4055	0.0007	0.0496	0.0005
Flavonols inf	<0.0001	0.2646	0.0526	<0.0001	0.0179	01584
NBI index inf	<0.0001	0.0884	0.4057	<0.0001	0.0081	0.0405
July						
Chlorophyll sup	0.0240	0.2718	0.0761	<0.0001	0.0090	0.7592
Anthocyanin sup	<0.0001	0.5702	0.6340	<0.0001	0.0006	0.1482
Flavonols sup	0.1131	0.0618	0.0333	<0.0001	0.0169	0.9379
NBI index sup	0.0092	0.1232	0.4658	<0.0001	0.0007	0.8584
Chlorophyll inf	0.0210	0.1483	0.0092	<0.0001	0.0049	0.6415
Anthocyanin inf	0.0132	0.2128	0.3698	0.0032	0.0006	0.4693
Flavonols inf	0.0030	0.1652	0.0407	<0.0001	0.3216	0.0353
NBI index inf	<0.0001	0.4311	0.1527	<0.0001	0.0342	0.3173

Table 3.8: Mean adaxial chlorophyl content (µg/cm²) in response to eight biostimulants in two blueberry varieties of RE and SHB types. Means with same letter suffixes are not significantly different from each other. Comparison is valid only by cultivar and month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Quantum O.T	19.5 a	19.6 ab	15.6 a	18.1 ab	30.4 a	32.6 a	33.8 a
	Micron Nutrients	19.1 ab	17.8 ab	15.8 a	20.5 ab	31.3 a	32.6 a	32.2 a
	Terra Grow	18.9 ab	20.5 a	16.8 a	20.2 ab	28.0 a	32.1 a	33.6 a
	Radiate	18.2 ab	15.6 b	13.9 a	15.5 b	30.9 a	29.1 a	32.2 a
	Soil Set	17.8 ab	15.7 ab	12.9 a	20.1 ab	26.8 a	29.0 a	30.6 a
	Inocucor	17.4 ab	17.4 ab	14.8 a	23.0 a	32.2 a	28.1 a	32.9 a
	Control	16.5 ab	15.5 b	13.7 a	19.3 ab	29.5 a	29.4 a	30.5 a
	Fertiactyl GZ	16.4 b	16.7 ab	13.7 a	21.2 a	30.4 a	31.7 a	28.7 a
	Huma Gro	16.1 b	16.5 ab	13.2 a	22.5 a	29.6 a	30.0 a	31.2 a
Farthing	Terra Grow	22.0 a	20.8 a	19.5 a	17.2 a	23.3 a	30.6 a	27.5 a
	Huma Gro	22.6 a	22.9 a	19.7 a	18.4 a	24.4 a	30.3 a	32.3 a
	Quantum O.T	21.9 a	22.6 a	20.4 a	16.0 a	22.2 a	27.2 ab	26.8 a
	Fertiactyl GZ	23.4 a	22.6 a	19.5 a	16.9 a	22.4 a	27.1 ab	28.5 a
	Micron n.	21.9 a	22.4 a	18.7 a	19.4 a	23.8 a	27.1 ab	34.6 a
	Radiate	21.6 a	22.8 a	18.7 a	15.5 a	19.4 a	26.2 ab	27.4 a
	Inocucor	22.9 a	23.5 a	21.2 a	17.9 a	23.3 a	25.6 ab	29.3 a
	Control	23.1 a	22.6 a	20.2 a	16.8 a	21.9 a	24.0 ab	29.8 a
	Soil Set	23.1 a	24.7 a	19.9 a	17.0 a	18.9 a	21.7 b	26.8 a
Legacy	Radiate	16.3 a	15.0 a	11.7 a	21.3 a	28.5 a	27.9 a	26.7 a
	Micron n.	19.4 a	14.8 a	12.8 a	17.7 ab	24.3 ab	26.2 ab	26.4 a
	Control	17.8 a	14.9 a	10.9 a	16.5 ab	23.6 ab	22.1 ab	25.7 a
	Soil Set	19.3 a	17.0 a	12.2 a	14.7 b	22.1 abc	23.3 ab	22.7 a
	Terra Grow	19.3 a	14.9 a	11.8 a	14.5 b	17.6 bc	17.3 b	20.7 a
	Quantum O.T	18.9 a	15.6 a	12.8 a	14.0 b	19.9 abc	21.2 ab	21.3 a
	Inocucor	18.0 a	15.1 a	12.2 a	14.0 b	20.4 abc	23.4 ab	24.7 a
	Huma Gro	17.1 a	14.8 a	10.8 a	13.4 b	19.6 abc	22.0 ab	25.6 a
	Fertiactyl GZ	19.5 a	16.0 a	12.1 a	11.5 b	14.1 c	17.7 b	21.4 a
Premier	Radiate	19.7 a	19.8 a	16.0 a	18.8 a	27.9 a	29.9 a	30.6 a
	Terra Grow	18.9 a	17.2 ab	14.6 abc	18.6 a	31.7 a	31.5 a	31.5 a
	Control	19.2 a	17.1 ab	13.1 abc	18.3 a	28.7 a	29.0 a	29.1 a
	Fertiactyl GZ	19.2 a	17.0 ab	12.5 bc	18.5 a	29.4 a	30.1 a	32.4 a
	Huma Gro	19.9 a	16.7 ab	15.1 ab	19.6 a	29.4 a	31.2 a	29.9 a
	Micron n.	17.9 a	16.7 ab	13.0 abc	16.5 a	27.8 a	31.2 a	28.8 a
	Inocucor	17.4 a	16.6 ab	14.8 abc	17.1 a	26.2 a	28.5 a	28.6 a
	Quantum O.T	18.4 a	16.4 ab	13.1 abc	18.6 a	30.0 a	31.7 a	31.6 a
	Soil Set	17.3 a	14.5 b	11.7 c	17.2 a	27.2 a	27.5 a	31.4 a

Table 3.9: Mean adaxial anthocyanin content (relative absorbance) in response to eight biostimulants in two blueberry varieties RE and SHB types. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a cultivar and month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Huma Gro	0.15 a	0.24 a	0.33 ab	0.17 b	0.11 a	0.11 a	0.12 a
	Control	0.15 ab	0.22 ab	0.31 ab	0.21 ab	0.12 a	0.10 a	0.13 a
	Inocucor	0.15 ab	0.18 ab	0.29 ab	0.16 b	0.10 a	0.10 a	0.11 a
	Fertiactyl GZ	0.15 ab	0.19 ab	0.44 a	0.17 b	0.11 a	0.09 a	0.11 a
	Micron Nutrients	0.13 ab	0.18 ab	0.24 ab	0.19 ab	0.12 a	0.10 a	0.11 a
	Soil Set	0.13 ab	0.23 ab	0.37 ab	0.19 ab	0.12 a	0.11 a	0.12 a
	Radiate	0.13 ab	0.20 ab	0.35 ab	0.26 a	0.12 a	0.11 a	0.11 a
	Terra Grow	0.13 ab	0.18 ab	0.22 b	0.18 b	0.13 a	0.10 a	0.11 a
	Quantum O.T	0.13 b	0.16 b	0.23 ab	0.19 ab	0.12 a	0.11 a	0.10 a
Farthing	Radiate	0.13 a	0.18 a	0.21 a	0.26 a	0.23 a	0.16 ab	0.16 ab
	Soil Set	0.12 a	0.16 a	0.23 a	0.27 a	0.23 ab	0.22 a	0.19 a
	Control	0.14 a	0.18 a	0.24 a	0.27 a	0.20 ab	0.20 ab	0.16 ab
	Fertiactyl GZ	0.13 a	0.18 a	0.21 a	0.28 a	0.19 ab	0.15 ab	0.15 ab
	Inocucor	0.13 a	0.13 a	0.18 a	0.24 a	0.17 ab	0.16 ab	0.15 ab
	Terra Grow	0.13 a	0.19 a	0.18 a	0.25 a	0.17 ab	0.13 b	0.17 ab
	Quantum O.T	0.13 a	0.17 a	0.21 a	0.31 a	0.16 ab	0.13 ab	0.18 ab
	Huma Gro	0.12 a	0.16 a	0.21 a	0.22 a	0.16 ab	0.14 ab	0.14 ab
	Micron Nutrients	0.14 a	0.17 a	0.20 a	0.19 a	0.15 b	0.15 ab	0.11 b
Legacy	Radiate	0.17 a	0.45 a	0.80 a	0.61 b	0.13 b	0.12 b	0.15 a
	Huma Gro	0.16 ab	0.51 a	1.00 a	0.95 ab	0.43 ab	0.27 ab	0.18 a
	Control	0.16 ab	0.58 a	1.05 a	0.81 ab	0.27 ab	0.18 b	0.16 a
	Quantum O.T	0.15 ab	0.52 a	0.82 a	1.07 a	0.66 a	0.37 ab	0.27 a
	Terra Grow	0.15 ab	0.47 a	0.92 a	1.05 ab	0.75 a	0.45 a	0.28 a
	Soil Set	0.14 ab	0.31 a	0.81 a	0.84 ab	0.40 ab	0.16 b	0.21 a
	Micron Nutrients	0.14 ab	0.40 a	0.86 a	0.86 ab	0.25 ab	0.22 ab	0.15 a
	Inocucor	0.14 ab	0.50 a	0.99 a	0.94 ab	0.45 ab	0.23 ab	0.22 a
	Fertiactyl GZ	0.13 b	0.45 a	0.91 a	1.21 a	0.70 a	0.31 ab	0.22 a
Premier	Micron Nutrients	0.14 a	0.20 a	0.37 a	0.26 a	0.14 a	0.11 a	0.13 a
	Soil Set	0.14 a	0.23 a	0.37 a	0.21 a	0.13 a	0.12 a	0.12 a
	Terra Grow	0.13 a	0.22 a	0.30 a	0.29 a	0.12 a	0.10 a	0.13 a
	Inocucor	0.13 a	0.26 a	0.28 a	0.22 a	0.14 a	0.14 a	0.14 a
	Fertiactyl GZ	0.13 a	0.24 a	0.38 a	0.20 a	0.13 a	0.13 a	0.13 a
	Control	0.13 a	0.22 a	0.37 a	0.26 a	0.14 a	0.13 a	0.14 a
	Huma Gro	0.13 a	0.21 a	0.37 a	0.22 a	0.14 a	0.12 a	0.13 a
	Radiate	0.13 a	0.18 a	0.27 a	0.20 a	0.14 a	0.12 a	0.13 a
	Quantum O.T	0.13 a	0.23 a	0.35 a	0.21 a	0.12 a	0.11 a	0.12 a

Table 3.10: Mean adaxial flavonols content (relative absorbance) in response to eight biostimulants in two blueberry varieties RE and SHB types. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a cultivar and month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Soil Set	1.60 a	2.13 a	2.23 a	2.10 a	1.90 a	2.00 a	2.13 a
	Huma Gro	1.80 a	2.10 ab	2.18 a	2.15 a	1.93 a	1.96 a	2.01 ab
	Control	1.79 a	2.10 ab	2.19 a	2.15 a	2.02 a	2.01 a	2.11 a
	Radiate	1.70 a	2.09 ab	2.21 a	2.16 a	2.02 a	1.84 a	1.91 ab
	Micron Nutrients	1.85 a	2.02 ab	2.18 a	2.11 a	2.03 a	1.94 a	2.03 ab
	Inocucor	1.78 a	2.01 ab	2.17 a	2.15 a	1.89 a	1.88 a	1.92 ab
	Quantum O.T	1.66 a	1.98 ab	2.11 a	2.13 a	2.05 a	1.93 a	1.93 ab
	Fertiactyl GZ	1.78 a	1.97 ab	2.18 a	2.10 a	1.91 a	1.75 a	1.82 b
	Terra Grow	1.66 a	1.89 b	2.10 a	2.14 a	2.02 a	1.91 a	1.95 ab
Farthing	Control	1.75 a	1.96 a	2.14 a	2.08 a	2.11 a	2.05 a	1.99 a
	Micron Nutrients	1.75 a	1.97 a	2.08 a	2.05 a	1.93 a	1.95 a	1.90 a
	Fertiactyl GZ	1.73 a	1.95 a	2.11 a	2.07 a	2.07 a	2.01 a	1.99 a
	Inocucor	1.72 a	1.85 a	2.06 a	2.04 a	2.02 a	1.99 a	1.89 a
	Soil Set	1.71 a	1.96 a	2.12 a	1.96 a	2.07 a	2.03 a	2.10 a
	Radiate	1.71 a	1.94 a	2.02 a	2.01 a	2.08 a	1.98 a	1.97 a
	Terra Grow	1.65 a	1.97 a	2.06 a	1.98 a	2.01 a	1.98 a	2.06 a
	Huma Gro	1.54 a	1.98 a	2.16 a	2.07 a	1.96 a	1.88 a	1.88 a
	Quantum O.T	1.53 a	1.93 a	2.11 a	2.05 a	2.03 a	1.99 a	2.00 a
Legacy	Quantum O.T	1.82 a	2.22 a	2.24 a	2.09 a	1.97 ab	2.02 a	2.15 a
	Radiate	1.66 a	2.19 a	2.24 a	2.17 a	1.86 b	1.98 a	2.12 a
	Huma Gro	1.68 a	2.19 a	2.22 a	2.09 a	2.08 ab	1.99 a	2.05 a
	Inocucor	1.68 a	2.18 a	2.26 a	2.07 a	2.12 ab	2.06 a	2.07 a
	Fertiactyl GZ	1.68 a	2.17 a	2.24 a	2.09 a	2.14 a	2.10 a	2.16 a
	Control	1.75 a	2.17 ab	2.26 a	2.13 a	2.07 ab	2.14 a	2.10 a
	Micron Nutrients	1.71 a	2.16 ab	2.26 a	2.11 a	1.94 ab	2.07 a	2.07 a
	Terra Grow	1.74 a	2.15 ab	2.23 a	2.10 a	1.98 ab	2.12 a	2.17 a
	Soil Set	1.65 a	2.03 b	2.19 a	2.18 a	2.10 ab	2.15 a	2.14 a
Premier	Terra Grow	1.80 a	2.05 a	2.14 a	2.09 a	1.99 a	1.96 a	2.07 a
	Radiate	1.79 a	1.97 a	2.19 a	2.06 a	2.08 a	1.99 a	2.06 a
	Fertiactyl GZ	1.75 a	2.05 a	2.01 a	2.10 a	2.07 a	2.00 a	2.06 a
	Micron Nutrients	1.74 a	1.98 a	2.20 a	2.09 a	2.01 a	1.90 a	1.99 a
	Huma Gro	1.74 a	2.03 a	2.21 a	2.11 a	2.07 a	2.04 a	1.89 a
	Soil Set	1.68 a	1.92 a	2.17 a	2.04 a	2.04 a	1.98 a	2.09 a
	Control	1.62 a	1.95 a	2.20 a	2.13 a	2.05 a	2.03 a	2.02 a
	Quantum O.T	1.61 a	2.01 a	2.20 a	2.08 a	2.00 a	1.90 a	1.94 a
	Inocucor	1.53 a	1.96 a	2.16 a	2.13 a	2.07 a	2.03 a	2.06 a

Table 3.11: Mean adaxial NBI index in response to eight biostimulants in two blueberry varieties RE and SHB types. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a cultivar and month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Terra Grow	11.6 a	11.6 a	8.3 a	9.4 ab	13.9 a	17.4 a	17.6 a
	Quantum O.T	12.3 a	10.0 ab	7.6 ab	8.5 ab	15.3 a	17.1 a	18.0 a
	Micron Nutrients	10.7 a	8.6 ab	7.3 ab	9.7 a	15.5 a	17.1 a	15.9 a
	Inocucor	10.1 a	8.9 ab	7.0 ab	10.7 a	17.2 a	15.2 a	17.6 a
	Fertiactyl GZ	9.4 a	8.8 ab	6.4 ab	10.1 a	16.2 a	19.0 a	17.0 a
	Huma Gro	9.4 a	8.0 b	6.2 ab	10.7 a	15.6 a	15.7 a	15.9 a
	Radiate	11.0 a	7.5 b	6.3 ab	7.2 b	15.7 a	16.4 a	17.5 a
	Control	9.4 a	7.5 b	6.3 ab	9.0 ab	14.8 a	14.8 a	14.8 a
	Soil Set	11.5 a	7.5 b	5.8 b	9.6 ab	14.0 a	14.7 a	14.4 a
Farthing	Huma Gro	15.1 a	11.7 a	9.1 a	8.9 a	13.1 a	16.5 a	17.8 a
	Terra Grow	13.8 a	10.8 a	9.6 a	8.8 a	11.7 a	15.6 ab	13.4 a
	Micron nutrients	12.7 a	11.6 a	9.3 a	9.5 a	12.5 a	14.2 ab	18.5 a
	Quantum O.T	15.2 a	11.8 a	9.7 a	7.8 a	11.1 a	13.9 ab	13.8 a
	Radiate	13.0 a	12.2 a	9.6 a	7.7 a	9.5 a	13.6 ab	14.2 a
	Fertiactyl GZ	13.6 a	11.7 a	9.3 a	8.2 a	11.0 a	13.6 ab	14.5 a
	Inocucor	13.6 a	12.9 a	10.4 a	8.8 a	11.6 a	13.2 ab	15.9 a
	Control	13.6 a	11.7 a	9.5 a	8.1 a	10.3 a	11.7 b	15.3 a
	Soil Set	13.8 a	12.9 a	9.4 a	8.8 a	9.3 a	10.9 b	13.0 a
Legacy	Radiate	10.1 a	6.9 a	5.2 a	9.8 a	15.8 a	14.5 a	12.6 a
	Micron nutrients	11.7 a	6.9 a	5.7 a	8.4 ab	13.3 ab	12.9 ab	13.3 a
	Control	10.2 a	6.9 a	4.8 a	7.7 ab	11.6 abc	10.4 ab	12.5 a
	Terra Grow	11.3 a	7.0 a	5.3 a	7.2 ab	9.8 abc	8.2 b	9.6 a
	Soil Set	11.9 a	8.7 a	5.6 a	6.8 b	10.6 abc	10.8 ab	10.7 a
	Inocucor	11.0 a	6.9 a	5.4 a	6.7 b	9.7 bc	11.5 ab	12.2 a
	Quantum O.T	10.4 a	7.0 a	5.7 a	6.6 b	11.2 abc	10.9 ab	10.1 a
	Huma Gro	1.5 a	6.8 a	4.9 a	6.4 b	9.6 bc	11.6 ab	13.0 a
	Fertiactyl GZ	11.9 a	7.4 a	5.4 a	5.5 b	6.6 c	8.9 ab	10.0 a
Premier	Radiate	11.2 a	10.2 a	7.3 a	9.1 a	13.5 a	15.1 a	15.0 a
	Inocucor	11.6 a	8.8 a	6.9 ab	8.0 a	12.6 a	14.0 a	13.9 a
	Huma Gro	11.9 a	8.3 a	6.8 ab	9.3 a	14.3 a	15.4 a	16.3 a
	Terra Grow	11.1 a	8.5 a	6.8 ab	8.8 a	16.5 a	16.2 a	15.3 a
	Quantum O.T	11.6 a	8.2 a	6.0 ab	9.0 a	15.1 a	17.3 a	16.9 a
	Control	12.0 a	9.1 a	5.9 ab	8.7 a	14.0 a	14.3 a	14.6 a
	Micron Nutrients	10.5 a	8.5 a	5.9 ab	7.9 a	14.0 a	16.3 a	14.7 a
	Fertiactyl GZ	11.3 a	8.4 a	5.6 b	8.8 a	14.2 a	15.1 a	15.7 a
	Soil Set	10.5 a	8.2 a	5.4 b	8.4 a	13.4 a	13.8 a	15.0 a

CHAPTER 4

EVALUATING THE EFFECT OF BIOSTIMULANTS ON SOIL BIOLOGICAL HEALTH[2]

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[2] Drocco C., Habteselassie M.Y., Itle R., and Meggio F. To be submitted to *Journal of Environmental Quality*

ABSTRACT

The use of biostimulants in crop production is growing as they are marketed to be sustainable alternatives and/or supplements to conventional inputs. However, there has been little study to confirm these claims. A greenhouse study was carried out to evaluate the impact of eight biostimulants on soil biological health with four blueberry cultivars that are commonly grown in GA. Soil biological health was evaluated by measuring parameters that are indicators of microbial activity and abundance. The activity parameters included soil respiration, urease, phosphatase and fluorescein diacetate hydrolysis (FDA) activities. Microbial abundance was evaluated with quantitative polymerase chain reaction to quantify ammonia-oxidizing bacteria (AOB) and archaea (AOB). No significant treatment effect was detected on soil respiration, urease and phosphatase activities while the biostimulant Fertiactyl GZ resulted in higher FDA activity than Micron Nutrients in the Legacy cultivar, but was not significantly different against the Control. With AOA and AOB abundance, the biostimulants Inocucor, Huma Gro, Terra Grow and Micron Nutrients resulted in significantly higher abundance than Control in Farthing and Premier cultivars. This suggests this biostimulants might have stimulated growth of these microorganisms, potentially impacting the fate of nitrogen in the system as well. However, the results were not consistent across cultivars and biostimulants, and it was not easy to link the change to ingredients in the product. To confirm the findings of the greenhouse study and gain a better insight on the impact of biostimulants on blueberry growth, the study needs to be repeated under field conditions.

INTRODUCTION

Sustainability is becoming a primary objective in agricultural production (Latruffe et al., 2016). Considering an increasing world population, and an increasing need for food and fibers, growers must produce more food in a small agricultural land (Supuwiningsih et al., 2017). A key challenge in sustainable agriculture is the optimization of resource use efficiency, especially with fertilizers. It has been estimated that nitrogen fertilization has an overall efficiency of 50%. The nutrient use efficiency decreases to 10% and 40% with phosphorus and potassium respectively (Baligar et al., 2001). Biostimulants are often marketed as sustainable alternative to conventional inputs or as being capable of improving the efficiency of conventional inputs when used together. Yakhin et al. (2017) define biostimulant as "*formulated product of biological origin that improves plant productivity as a consequence of the novel or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds*". Although this is not a universally accepted definition of biostimulants.

One mechanism by which biostimulants are stipulated to improve efficient use of nutrients by crops is through their positive impact on soil microbial communities that provide a number of beneficial services (Chen et al., 2003, Tejada et al., 2011). Soil microorganisms are directly involved in organic matter decomposition, nutrient cycling and plant nutrient absorption (Aislabie at al., 2013, Miransari, 2013, Schimel, 2013). Healthy soil promotes plant nutrient uptake, disease suppression, and water retention through their impact on organic matter (Aislabie at al., 2013, Singh, 2015, Moebius-Clune, 2017). The ability of soil microorganisms to provide these beneficial services is described as soil biological health. Parameters of microbial activity and abundance are commonly used as indicators of soil biological health.

Microbial activity can be characterized by measuring for soil respiration and enzyme activities. Soil respiration quantifies the amount of CO_2 evolved from the decomposition of SOM (Wildung et al., 1975). Since soil organic matter is composed of dead plants, animals and microorganisms, its turnover can give an idea of trophic interactions in ecosystems, and therefore of its soil health (Barrios, 2007). The generic microbial activity can also be measured with fluorescein diacetate assay (Green et al., 2005). This assay quantifies microbial biomass C due to the presence of fluorescein, a molecule produced by the activities of protease, lipase and esterase. Enzymes are very sensitive to environmental changes. As such, they can be good indicators of the impacts of management practices on soil microorganisms (Baldrian, 2009, Vinhal-Freitas et al., 2010). The enzyme urease, for instance, is produced by microbes for urea hydrolysis to ammonia in soils and is directly related to nitrogen cycling (Kandeler, 2007). The enzyme phosphatase catalyzes the production of orthophophosphates from organic forms of phosphorous (Plante, 2007; Dick et al., 1994).

Quantifying the abundance of some key microorganisms is also a good way to assess soil biological health. Quantitative polymerase chain reaction is commonly employed to quantify the abundance of key groups of microorganisms that mediate nutrient cycling. For instance, ammonia-oxidizing bacteria and archaea mediate the first and rate limiting step of nitrification that produces nitrate which can easily be lost from the soil via leaching and/or denitrification (Hatzenpichler, 2012, Norton et al., 2011). Therefore, their activity has important agronomic and environmental implications (Norton et al., 2011, Qiao et al., 2014). They are also sensitive to agricultural management practices. As such, they are good indicators of soil biological health.

There are limited studies that evaluated the impact of biostimulants on blueberry soil biological health. Schoebitz et al. (2019) analyzed the activity enzymes and soil respiration in response to the application of biostimulants containing humic substance and/or microbial inoculum on blueberry. Phosphatase activity increased by 43% in the mixed treatment, and urease activity was 10-fold higher in the mixed treatment than the control. Similarly, the biostimulant treatments increased FDA activity by up to 140% as compared to the control.

The objective of this study was to evaluate the impact of eight biostimulants on soil biological health in greenhouse study with four blueberry cultivars.

MATERIALS AND METHODS

Soil sampling

At the end of the study (between August 4 and 7, 2020), the blueberry plants described in Chapter 3 were removed from their 3-gal pots, and the rhizosphere was sampled by dislodging the soil that was attached to the roots. Once the soil samples were obtained from the rhizosphere, they were thoroughly mixed in a Ziplock bag and similar amount of soil samples were kept after having it passed through 2 mm sieve to remove any plant material or bark and were subsequently stored at 4°C until analysis. A portion of each sample was also transferred to another Ziplock bag and stored in the freezer for DNA analysis. Before each analysis, soil samples of the same replication were taken out from the fridge, mixed thoroughly and made to equilibrate to room temperature.

Soil moisture

Fresh and dry weights of the soil samples were determined to calculate soil moisture. To do this, approximately 10 g of fresh soil was weighed (Model Adventurer Pro AV2102C, Ohaus Corp., Pine Brook, NJ) into an aluminum tin and dried in an oven at 100°C for 24 hours. The soil was then cooled down in a desiccator for 30 min and was weighed to obtain the oven-dry weight after calculating for the soil moisture content as follows:

Gravimetric water content (g) = (Fresh Weight-Dry Weight)/(Dry Weight-Tin Weight)*100

Soil texture and pH

The soil texture of the growth medium was determined from four sub samples. Briefly, 100 g of sieved dry soil were mixed in a blender with 25 mL of sodium metaphosphate solution (10 g/L) and 100 mL of water. The suspension was quantitatively poured in a hydrometer jar and diluted to 1 L. After covering with parafilm, the suspension was inverted 10 times and rested on a counter. A hydrometer was carefully placed in the jar, and the first reading (clay+silt) after 40 secs was taken along with the temperature. After 6 h, the second hydrometer (clay) and temperature reading were taken. After calculating for the weights of the sand, silt and clay fractions, texture was determined with the help of a textural triangle after adjusting the hydrometer readings for the temperature. The texture was sandy, with an average of 89.1% sand, 4.02% clay, 6.8% silt.

Soil pH was also determined to evaluate if there was any change due to treatment application and plant growth. Soil suspensions were prepared from samples a ratio of 1:5 (10 g of soil and 50 mL of DI water). The pH of the suspension was determined with a pH meter (Thermo Scientific, Orion 2-Star, Beverly, MA, US). The pH of the soil before treatment application started was 5.76. For Control, the average pH was 4.98, for Soil Set 4.79, for Radiate 4.53, for Terra Grow 4.29, for Inocucor 4.46, for Quantum O.T 4.35, for Micron Nutrients 4.60, for Fertiactyl GZ 4.73 and Huma Gro 4.30.

Soil respiration

This assay was done on soil samples from four of the five replications as described in Zibilske (1994) protocol. Briefly, twenty grams of soil from each sample was weighted and placed in mason jar. A glass vial containing 10 ml of 0.08 N $Ba(OH)_2$ was placed in each mason jar, which was then sealed. Two control jars with no soil were also included as blank controls along with all the samples. All the containers were then put in an incubator (Model 1545, Sheldon Manufacturing Inc., Cornelius, OR, US) set at 26°C for 24-hr incubation. After one day, the jars were removed from the incubator and left open for 1 minute to re-equilibrate with the atmosphere. The $Ba(OH)_2$ from the glass vial was poured in a 125 ml Erlenmeyer flask. Two drops of phenolphthalein were added into the flask, which was swirled. The alkaline traps were titrated with 0.08 N HCl and the amount of HCl used was recorded. Soil respiration (mg CO_2 $*g^{-1}$ $soil*d^{-1}$) was calculated as shown below:

$$\text{mg CO2}*g^{-1}\ \text{soil}*d^{-1} = [0.08\ \text{N}\ Ba(OH)_2*10\ \text{mL}\ Ba(OH)_2]-[0.08\ \text{N HCl}*(\text{HCl Control-HCl Treatment})\ \text{mL}]*[22/(\text{dry soil weight (g)}]$$

Urease assay

For this assay, the protocol described by Mobley and Hausinger (1989) was followed. Briefly, for each sample, two biplates were labeled with the variety name, repetition and treatment. One of the biplates was used as control, and the other one as a treatment. One gram of soil was placed in one side of the biplate, followed by addition of 3 mL of 0.5-M Tris malate buffer with 0.1% sodium azide. Three milliliters of 2%-Boric acid indicator solution were pipetted into the other side of the petri plate. One milliliter of 6 M Urea was also added to the soil in the treatment plate. At this point the lid was placed on the petri dishes for incubation for 1 hour at room temperature.

After incubation, 50 mL of 10 mM $AgSO_4$ were added to the soil to terminate the reaction. Subsequently, 1 mL of 3-M K_2CO_3 was then added to the soil to allow the release of NH_3 to the other side of the petri plate where the boric acid trap was found. The petri plates were then put in Ziplock bags for incubation for 24 hours at room temperature. After the incubation, boric acid-indicator solutions of both control and treatment plates were titrated with 0.02 N HCl solution. The amount of HCl used has been recorded, and the equation below was used to express the urease activity in NH_3*g^{-1} soil$*h^{-1}$ in dry soil weight basis:

$$\mu\text{mol NH}_3\text{ evolved}*g^{-1}\cdot h^{-1} = (\text{mL HCL})*(0.02\text{ mol/L})*(1\text{ L/1000 mL})*(106\mu\text{mol/mol})/\text{dry soil weight (g)})$$

Phosphatase assay

The protocol described by Tabatabai (1994) was followed for phosphatase assay. Briefly, two 15 mL brown centrifuge tube, used to minimize light exposure, were used to contain 1g of soil per sample, one for the treatment and the other for the control. Four milliliters of 0.5-M Tris malate buffer (pH 7.0) were added into the treatment and control tubes. To initiate the reaction, 1 mL of 100 mM para-nitrophenyl phosphate (pNPP) was added in the treatment tube. All the centrifuge tubes were incubated on a rotary shaker for 1 hour at 175 rpm. At the end of the incubation period, 1 mL of pNPP was added to the control tubes. To terminate the reaction, 1 mL of 0.5-M $CaCl_2$ and then 4 mL of 0.5-M NaOH were added to both control and treatment tubes. The containers were then swirled gently, and the content was poured into a clean 16-mL centrifuge tube and then centrifuged at 10,000 rpm at 4°C for 5 minutes. A subsample of the supernatant from each centrifuge tube was transferred to a 96-well plate and diluted 10 times with 0.5-M Tris malate buffer (pH 7.0) as needed. Colorimetric reading of the plate was undertaken at 400 nm with a plate reader (Epoch Microplate Spectrophotometer, BioTech Instruments, Inc. Winooski, VT, US). Along with the samples, the standard solutions of *p*-nitrophenol ranging in concentration between 0 to 10 µM were used to generate the standard curve to convert the spectrophotometric reading to concentration unit. Phosphatase activity was expressed in μmol PNP*g^{-1} soil*h^{-1} as follows:

$$\mu\text{mol pNP evolved}*g^{-1}*h^{-1} = ((\mu\text{mol pNP})/L)*(10\text{mL}/(\text{dry soil weight (g)}))* (1\text{ L}/1000\text{ mL})*\text{dilution factor}$$

Fluorescein Diacetate Hydrolysis Activity (FDA)

The protocol described by Green et al (2006) was followed for phosphatase assay. Briefly, 1 g of soil has been put into two 125 mL Erlenmeyer flasks, one representing treatment and the other the control. One extra flask with no soil (blank) was also run. Fifty mL of THAM buffer (0.1 M, pH 7.6) was added to each flask. Half milliliter of acetone was added just to the controls, and 0.5 mL of FDA was added to the treatment and blank. Each flask was capped with a stopper and put in the incubator at 37°C for incubation for 3h.

After the incubation period the samples were removed from the incubator, and 2 mL of acetone was quicky added to the flask. The content was poured into 50 mL flask, and 0.5mL of FDA was added even to the controls. The contents were then centrifuged for at 4°C for 5 minutes at 4000 rpm. Standard solutions of fluorescein ranging in concentration between 0 and 10 mg/L were prepared. The samples were diluted with THAM buffer (pH 7.6) as needed and transferred into a 96-well plate along with the standards for spectrophotometric reading with a plate reader (Epoch microplate spectrophotometer, BioTech Instruments, Inc. Winooski, VT, US) at a wwavelengthof 490 nm. The values for the sample controls were subtracted from that of the samples', and FDA hydrolysis activity was calculated using the formula:

mg fluorescein*g^{-1} dry soil*day^{-1} = (mg fluorescein/L)*((50 ml THAM)/(adjusted soil weight(g)))*(1 L)/(1000 mL))*(1/3)*dilution factor*24h

AOA and AOB abundance

The quantitative polymerase chain reaction (qPCR) was performed to quantify abundance of ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB). Table 4.1 shows detailed information about primers and cycling conditions. The DNA was extracted from 0.25 g of rhizosphere soil samples using DNeasy PowerSoil DNA extraction kit (QIAGEN, Germantown, MD, US). The qPCR reaction volume was 20µL and was composed by 10 µL PowerUp SYBR Green Master Mix (ThermoFisher Scientific), 6.4 µL of nuclease-free PCR water, 2 µL of DNA template, 0.8 µL of forward primer and 0.8 µL of reverse primer. The final primer concentrations were 200 nM. Standard ranging from 30 to $3*10^5$ copies of DNA/2µL were prepared and run along with 3 negative controls and the samples in duplicates (Mundepi et al., 2017). To analyze the results from the qPCR, StepOne Software (Applied Biosystems) was used. The mean reaction efficiencie for AOA was 49.64, and the mean R^2 value was 0.92, while for AOB they were 0.94, and 117.07 respectively. The abundance data for each sample was calculated (copy*g^{-1} soil) as indicated below:

$$\text{Copy}*\text{g}^{-1}\ \text{soil} = (\text{x copies})/(2\ \mu\text{L})*((100\ \mu\text{L})/(0.25\ \text{g}))$$

Statistical analysis

The data for soil respiration, urease, phosphatase, FDA and microbial abundance were averaged by treatment. The data were analyzed with two ways ANOVA to determine the statistical significance of treatment (T), cultivar (CV) and cultivar-treatment interaction (CV*T) on plant growth parameters by type (SHB vs RE). Cultivar, T and CV*T were considered as categorical variables and replication was considered as random effect. We also analyzed data with one-way ANOVA to look at the impact of T by CV. Tukey's honest significant difference (HSD) test was used for the post hoc analyses for mean separation to identify significant difference among the T by CV.

RESULTS AND DISCUSSION

Soil respiration, urease and phosphatase activities

From the mixed model analysis, the treatment application did not significantly influence soil respiration, urease and phosphatase activity in either blueberry types (Table 4.2). The cultivar-treatment interaction was not significant either. However, soil respiration and phosphatase were significantly influenced by cultivar in the SHB and both blueberry types respectively (Table 4.2). One-way ANOVA by cultivar did not indicate any treatment effect either. Summaries of soil respiration, urease and phosphatase data for RE and SHB varieties are shown in Figures 4.1-4.3. In Brightwell, mean soil respiration was 1.80 mg CO_2 g^{-1} soil d^{-1}, in Farthing 2.18 mg CO_2 g^{-1} soil d^{-1}, in Legacy 1.99 mg CO_2 g^{-1} soil d^{-1} and in Premier from 1.83 mg CO_2 g^{-1} soil d^{-1}. The mean urease activity in Brightwell was 9.50 µmol NH_3 g^{-1} soil h^{-1}, in Farthing 18.99 µmol NH_3 g^{-1} soil

h^{-1}, in Legacy 17.97 µmol NH_3 g^{-1} soil $h^{-1}$and in Premier 9.98 µmol NH_3 g^{-1} soil h^{-1}. The mean phosphatase activity in Brightwell was 0.66 µmol pNP g^{-1} soil h^{-1}, 1.66 µmol pNP g^{-1} soil h^{-1} in Farthing, 0.98 µmol pNP g^{-1} soil h^{-1} in Legacy and 0.92 µmol pNP g^{-1} soil h^{-1} in Premier.

Our results, in which no significant impact was seen from the biostimulants, are in contrast with the ones from Schoebitz et al. (2019). In their blueberry field study, two biostimulants that had microbial consortium and a combination of microbial consortium and humic acids improved soil biological health as indicated by measurements of soil respiration rate, urease and phosphatase activities. The differences in the results with our studies could be due to differences in the biostimulants that they used and the study design and factors. The fact that no significant differences was observed in our study can be due to several factors. It is plausible that the soil already had enough organic matter and/or other ingredients in the biostimulants that are supposed to bring about any change and that adding a little more might not make a significant difference. Another reason could be related the survival rate of the microorganisms in the biostimulants during storage and application. The success of microbe-containing biostimulants is largely dependent on storage conditions and a good understanding of the soil environment to which they are applied (Abbott et al., 2018, Halpern et al., 2015). Lack of such knowledge could lead to poor survival of the microorganisms contained in some of the biostimulants in soil.

Fluorescein Diacetate Hydrolysis Activity (FDA)

From the mixed model analysis, the treatment application did not significantly influence the FDA activity in either blueberry types (Table 4.2). The cultivar-treatment interaction was not significant either. However, the FDA activity was significantly influenced by the cultivar in the SHB type (Table 4.2). Summaries of FDA data for RE and SHB varieties are shown in Figure 4.4.

Mean FDA activity was 2.47 mg fluorescein g^{-1} soil d^{-1} in Brightwell, 3.90 mg fluorescein g^{-1} soil d^{-1} in Farthing, 3.07 mg fluorescein g^{-1} soil d^{-1} in Legacy and 2.60 mg fluorescein g^{-1} soil d^{-1} in Premier.

The results from one-way ANOVA, however, showed significant treatment effect in the Legacy cultivar between Fertiactyl GZ and Micron Nutrients, but not against the Control. The FDA assay is useful to evaluate the overall microbial activity (Green et al., 2006). The results for this parameter parallel those of soil respiration and phosphatase data, with the exception of Legacy. In Legacy, Fertiactyl GZ resulted in a significant higher FDA activity than Micron Nutrients. Fertiactyl GZ contains seaweed, humic acids and fulvic acids. No study could be found on the effect of those molecules on FDA activity, but seaweed extracts can enhance soil enzymatic activity, leading to a higher nutrient availability in the soil (EL Boukhari et al., 2020). Humic acids are known to have a positive effect on microorganisms as it can increase catalase, dehydrogenase and phosphatase activities in sugarcane (Sellamuthu et al., 2003). In a 3 years trail on peanut, humic acid applications increased the activity of urease, sucrase and phosphatase compared to the control (Li et al., 2019).

Our results partially reflect the ones from Schoebitz et al. (2019). In their field experiment, they observed an increase in FDA activity with the Legacy blueberry cultivar treated with a microbial consortium, and a combination of microbial consortium and humic substances. The differences in the results could be due to the nature of the biostimulants that they used, and study factors.

AOA and AOB abundance

From the mixed model analysis, the treatment application did not significantly influence AOB abundance (Table 4.2). However, the cultivar-treatment interaction showed significant difference in both RE and SHB. AOB abundance was significantly influenced by the cultivar in RE (Table 4.2). Summaries of AOA and AOB abundance are shown in Figure 4.5-4.6. Mean AOA abundance was 5.20 copy g^{-1} soil in Brightwell, 6.13 copy g^{-1} soil in Farthing, 5.72 copy g^{-1} soil in Legacy and 5.61 copy g^{-1} soil in Premier. Mean AOB abundance was 5.97 copy g^{-1} soil in Brightwell, 7.00 copy g^{-1} soil in Farthing, 6.75 copy g^{-1} soil in Legacy and 6.30 copy g^{-1} soil in Premier.

The results from the one-way ANOVA by cultivar indicated a significant treatment effect in Premier for AOA abundance. Terra Grow resulted in a significant higher AOA abundance than the Control and Inocucor, while Terra Grow had a 13.5% higher AOA abundance than the Control. Terra Grow contains a microbial inoculant and humic acids. The microbial inoculant might have contributed to the AOA DNA pool. About the humic acids, no research could be found on the effect of humic acids fertilization on AOA, but it is known that humic acids fertilization can enhance microbial diversity and enzymatic activity. As such, they might have affected microbial abundance (Li et al., 2019). Our result is in contrast with the results of Wang et al. (2020). In their field study on the effect of the incorporation of microbial inoculant in straw mulch, they could not detect any significant difference in AOA abundance between the different treatments and the control (no straw).

The results from the one-way ANOVA also highlighted a significant difference in Farthing and Premier for AOB abundance. In Farthing, Inocucor resulted in a significant higher AOB abundance than Control and Soil Set. In particular, Inocucor had a 18.7% higher AOB abundance than the Control. In Premier, Micron Nutrients, Terra Grow and Huma Gro resulted in significantly higher AOB abundance than the Control. Micron Nutrients and Huma Gro had a 21.5% and 21% higher AOB abundance compared to the Control, respectively. Inocucor contains *B. subtilis* and other microorganisms. Visconti et al., (2020) in their study on biostimulants and compost applied to grass, concluded that the most effective biostimulant on soil contained a mixture of microorganisms. This reflects our result in the Premium cultivar in which the highest AOB abundance was detected for Inocucor, a biostimulant that contains a mixture of microorganisms.

Huma Gro and Micron Nutrients contain a mixture of organic derived components. It is plausible that those molecules stimulated AOB growth as compared to the Control and other biostimulants. I was not able to find studies on the effect of organic and protein based biostimulants, but it is known that the application of organic fertilizers (manure or biofertilizers) enhances AOB soil population (Tao et al., 2017, Wang et al., 2013). The results from our study show that the effect of the same biostimulant is not consistent over different cultivars.

SUMMARY AND CONCLUSIONS

Overall, the biostimulants did not seem to have any significant impact on most of the soil biological health (soil respiration and enzyme activities). Differences were mainly between the cultivars and the interaction between treatment and cultivar. In fact, changes in microbial community are strongly related to soil characteristics. To have a long term effect on microbial community, the biostimulants have to modify soil properties mainly through root exudates (Hellequin et al., 2020). Since no significant differences was detected among the products, it is possible that the biostimulants tested did not impact soil properties, and hence the indicators.

However, this explanation did not apply to AOA and AOB abundance in which significant difference was observed between some of the biostimulants and the Control in Farthing and Premier cultivars. In these two cultivars, biostimulants Inocucor, Huma Gro, Terra Grow and Micron Nutrients significantly increased the abundance of AOA and AOB as compared to the Control. The fact that the significant difference was detected between some of the biostimulants and the Control is an indication that some of the products were effective in stimulating AOA and AOB growth. This implies that some of the biostimulants had affected the N soil cycle and can potentially enhance microbial transformation of ammonia to nitrate, with the resulting consequences on nitrogen availability and movement in the environment (Hatzenpichler, 2012, Norton et al., 2011, Qiao et al., 2014).

To have a better understanding those results, more frequents soil enzymatic evaluations should be performed through the growth period. In this way we could evaluate if the biostimulants had a short-term effect on the microbial community, and if there was a change in the enzymatic activity and microbial abundance over time. The differences among the blueberry cultivars are obvious as different plants produce different root exudates that support the microbial community in a specific way (Calvo et al., 2014).

A challenge worth highlighting here is the lack of storage information on products’ labels. Since many biostimulants contain microorganisms, lack of such information can cause uncertainty about viability of the microbial inoculants, and hence their effectiveness (Wang et al., 2017). Additionally, it was challenging not to be able to get some basic information such as application rates on the product labels. Some of the products tested did not have much information on the label and was difficult to find additional information online or from listed contacts. Finally, to have a better evaluation of the impact of the biostimulants on soil biological health, they should be tested in field conditions. Also, a long-term study and the evaluation of additional soil parameters will provide more information regarding the impact of those products on soil biological health.

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Table 4.1: Primer sequences and thermal cycling conditions used for qPCR analysis

Target group	Gene	Amplicon length (bp)	Primers		Thermocycling conditions	Ref.
			Name	Sequence		
AOA	Archaeal amo*A*	635	Arch*amoA*F	5’-TTATGGTCTGGCTTAGACG-3’	95ºC for 10 min; 40 cycles (95ºC for 1 min, 56ºC for 1 min, and 72ºC for 3 min)	(Francis et al., 2005; Wynngaard et al., 2016)
			Arch*amoA*R	5’-GCGGCCATCCATCTGTATGT-3’		
AOB	Bacterial amo*A*	491	amoA-1F	5’-GGGGTTTCTACTGGTGGT-3’	95ºC for 10 min; 40 cycles (95ºC for 1 min, 57ºC for 1 min, and ºC for 3 min)	(Rotthauwe et al., 1997; Wynngaard et al., 2016)
			amoA-2R	5’-CCCCTCGGGAAAGCCTTCTTC -3		

Table 4.2: Mixed model analysis of enzyme activities, soil respiration, fluorescein diacetate hydrolysis and microbial abundance in RE and SHB types treated with eight biostimulants

Response variables	Main effect *p*-value (α=0.05)					
	Rabbiteye			Southern Highbush		
	Cultivar	Treatment	CV*T	Cultivar	Treatment	CV*T
Soil respiration (mg CO_2 g^{-1} soil d^{-1})	0.0704	0.5790	0.5002	<0.0001	0.2232	0.1583
Urease activity (µmol NH_3 g^{-1} soil h^{-1})	0.6958	0.2595	0.3882	0.7287	0.4250	0.6103
Phosphatase activity (µmol pNP g^{-1} soil h^{-1})	0.0088	0.7793	0.8288	0.0018	0.4382	0.1187
FDA activity (mg fluorescein g^{-1} soil d^{-1})	0.2333	0.0907	0.1942	0.0020	0.2651	0.2107
AOA abundance (copy g^{-1} soil)	<0.0001	0.0932	0.0578	0.4879	0.1964	0.0331
AOB abundance (copy g^{-1} soil)	0.0007	0.0612	0.0001	0.0587	0.7194	0.0002

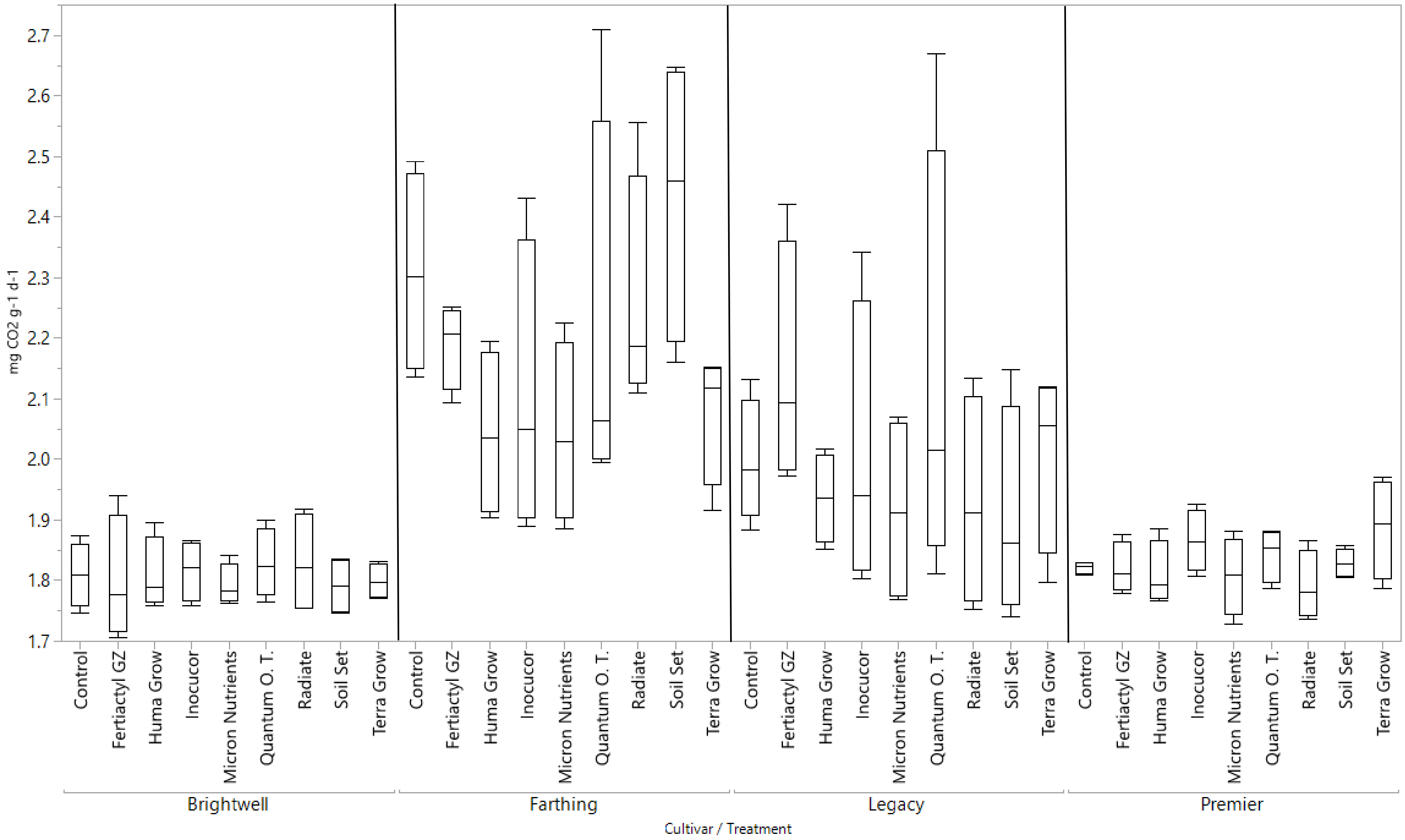

Figure 4.1: Soil respiration (mg CO_2 g^{-1} d^{-1}) in response to application of eight biostimulants in RE and SHB types. None of the biostimulants significantly affected soil respiration in any of the cultivars

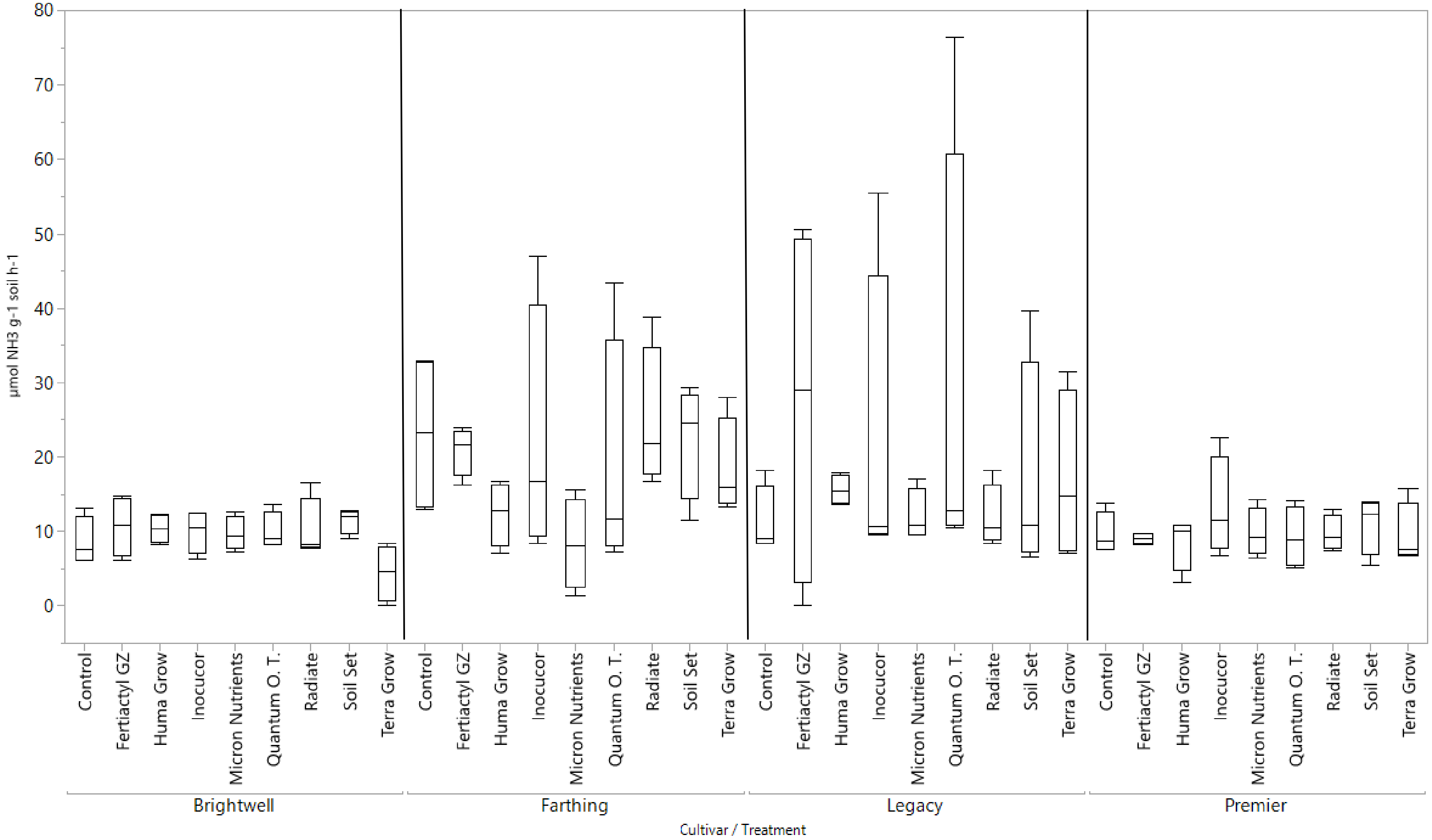

Figure 4.2: Urease activity (µmol NH_3 g^{-1} soil h^{-1}) in response to application of eight biostimulants in RE and SHB types. None of the biostimulants significantly affected urease activity in any of the cultivars

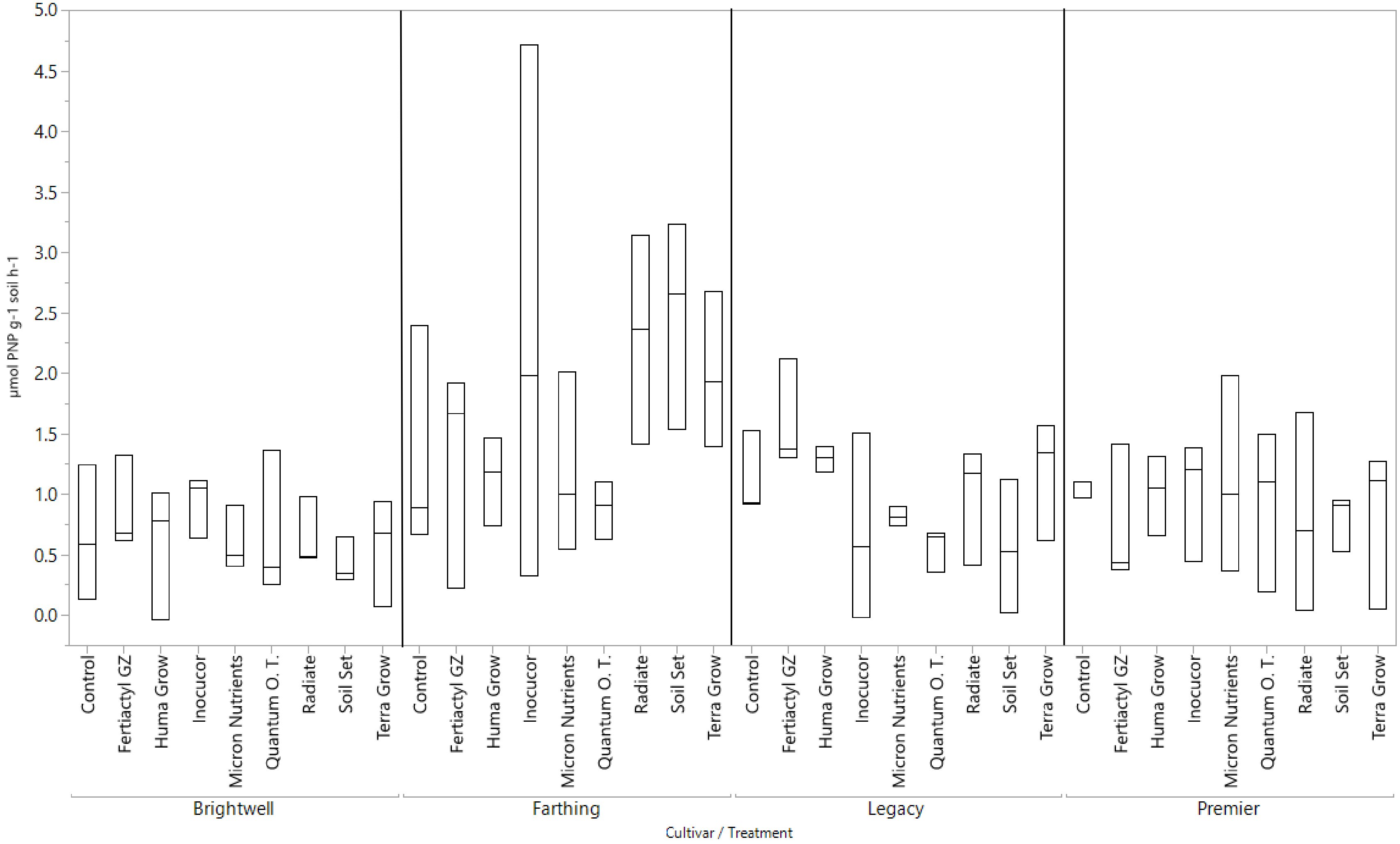

Figure 4.3: Phosphatase activity (µmol pNP g^{-1} soil h^{-1}) in response to application of eight biostimulants in RE and SHB types. None of the biostimulants significantly affected phosphatase activity in any of the cultivars

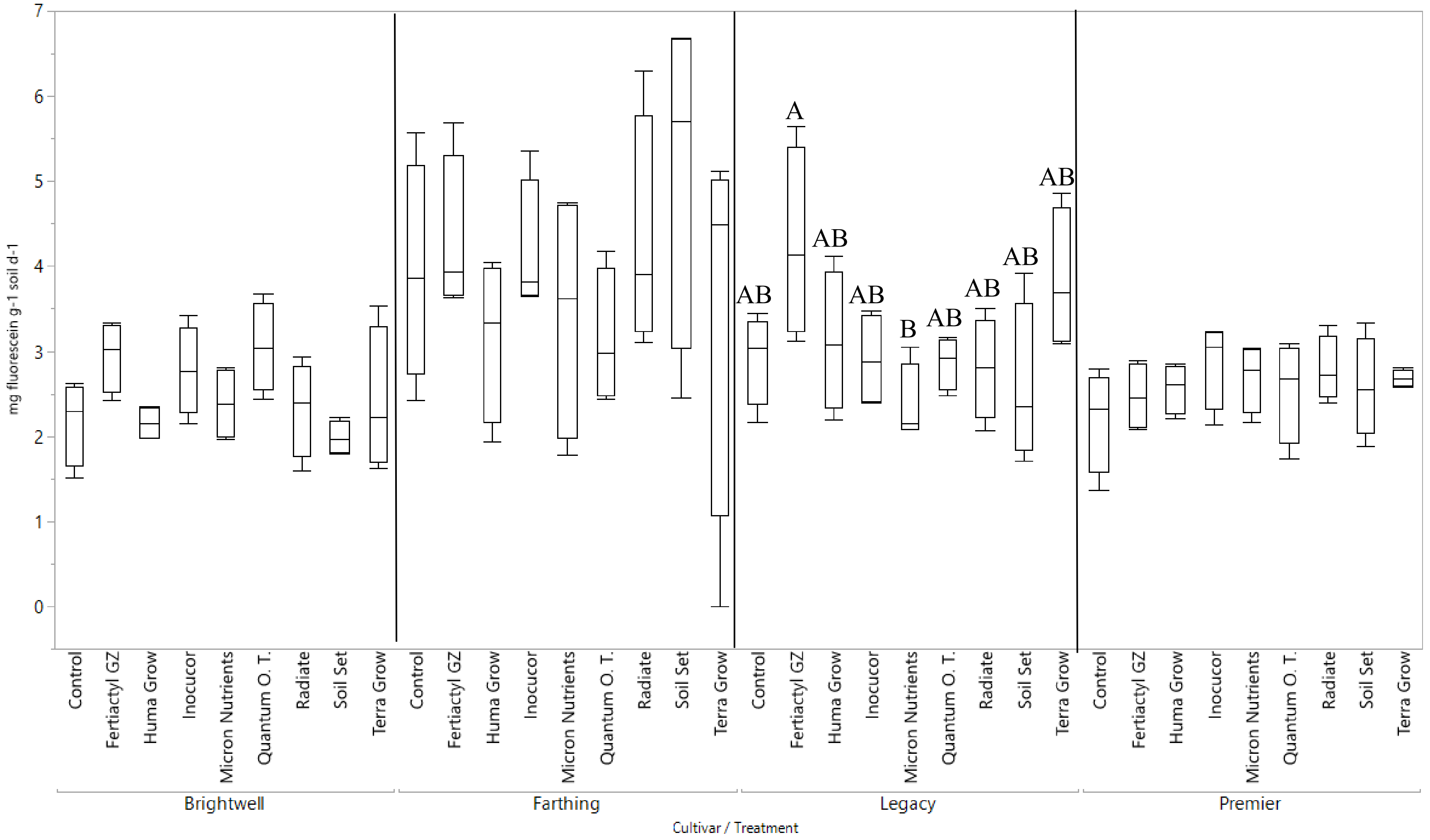

Figure 4.4: FDA activity (mg fluorescein g^{-1} soil d^{-1}) in response to application of eight biostimulants in RE and SHB types. Treatment effect by cultivar showed there was significant difference only in Legacy cultivar.

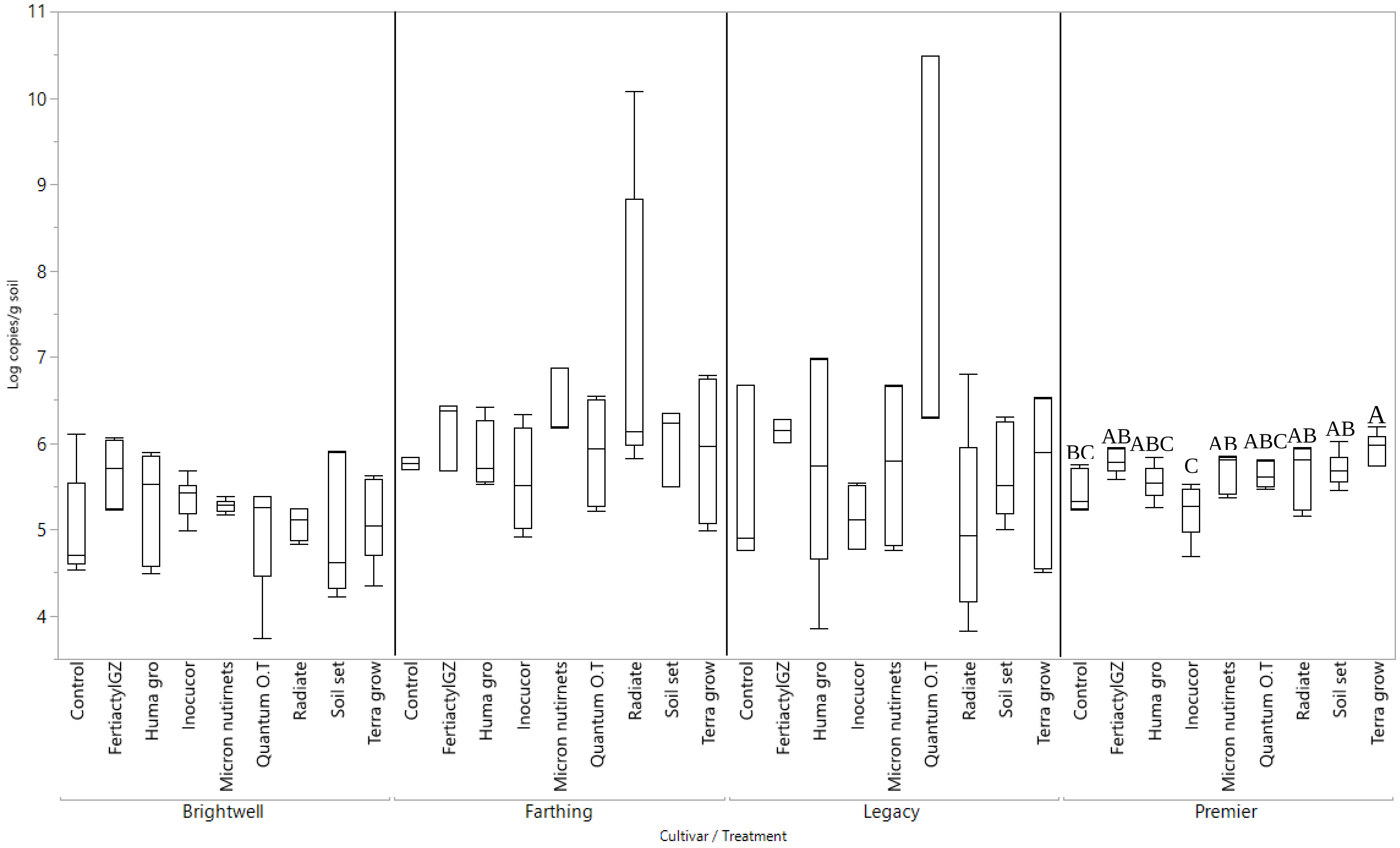

Figure 4.5: AOA abundance (copy g^{-1} soil) in response to application of eight biostimulants in RE and SHB types. Treatment effect by cultivar showed there was significant difference only in Premier

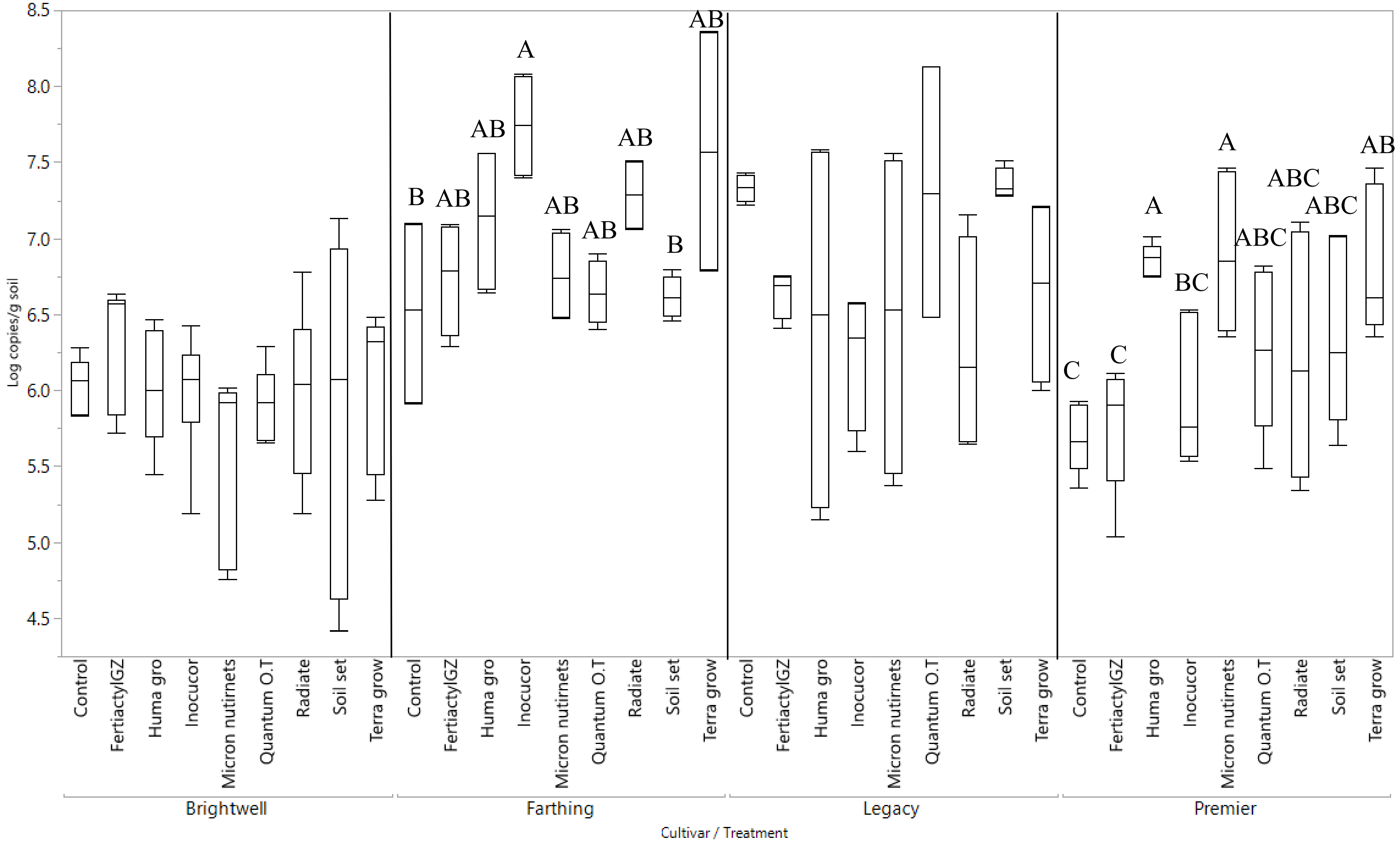

Figure 4.6: AOB abundance (copy g^{-1} soil) in response to application of eight biostimulants in RE and SHB types. Treatment effect by cultivar showed there was significant difference only in Farthing and Premier

CHAPTER 5

SUMMARY AND CONCLUSIONS

In this study a nine-month greenhouse study was conducted to assess the effect of eight commonly used biostimulants (Fertiactyl GZ, Huma Gro, Inocucor, Micron Nutrients, Quantum O.T., Radiate, Soil Set and Terra Grow) on blueberry growth with four cultivars (Brightwell, Premier, Legacy and Farthing) and on soil biological health. Monthly measurements were taken on parameters that are indicators of plant growth (height, width, volume, cane number, cane diameter, leaf area index), plant biomass (dry weight) and plant leaf pigment (chlorophyll, anthocyanin, flavonols and NBI index) multiple times during the study period. At the end of the study, the rhizospheres of the blueberry plants were sampled and analyzed for indicators of soil biological health that reflected microbial activity (soil respiration, urease, phosphatase and fluorescein diacetate hydrolysis activities) and microbial abundance (abundance of ammonia-oxidizing bacteria and archaea, AOB and AOA).

None of the plant growth and biomass parameters were significantly affected by treatment. Similarly, no significant difference was detected between the Control and the biostimulants for the leaf pigment indicators, with few exceptions. The biostimulant Terra Grow resulted in significant higher chlorophyll content than Control in Brightwell in February. Chlorophyll content was significantly higher in the Control than the biostimulant Fertiactyl GZ in Legacy in May. Similarly, there were significant differences in anthocyanin and flavonols contents between the Control and the biostimulants Terra Grow and Fertiactyl GZ in Legacy and Brightwell at two timepoints. However, the effects were not consistent over time or cultivar type.

No significant treatment effect was detected on soil respiration, urease and phosphatase activities while the biostimulant Fertiactyl GZ resulted in higher FDA activity than Micron Nutrients in the Legacy cultivar, but was not significantly different against the Control. With AOA and AOB abundance, the biostimulants Inocucor, Huma Gro, Terra Grow and Micron Nutrients resulted in significantly higher abundance than Control in Farthing and Premier cultivars. This suggests this biostimulants might have stimulated growth of these microorganisms, potentially impacting the fate of nitrogen in the system as well. However, the results were not consistent across cultivars and biostimulants, and it was not easy to link the change to ingredients in the product.

One way by which biostimulants are often described as being capable of influencing plant growth is through their impact on soil biological health. It is stated that their positive impact on soil microbial activity and function will lead to improved nutrient availability and stress tolerance in plants. While such a direct link between plant and soil biological health parameters was not obvious (was not properly explored either) in this study, it is worth exploring with statistical analysis such as correlation or multivariate analysis. It is interesting to note that Huma Gro and Terra gro resulted in improvements in NBI index. They also appeared to have stimulated the growth of AOA and AOB in the Premier cultivar. What is common to both (NBI index and AOA and AOB) is they play some role in nitrogen cycling. Such potential links between plant and soil parameters will need to be explored further.

A challenge worth highlighting here is the lack of storage information on products' labels. Since many biostimulants contain microorganisms, lack of such information can cause uncertainty about viability of the microbial inoculants, and hence their effectiveness (Wang et al., 2017). Additionally, it was challenging not to be able to get some basic information such as application rates on the product labels. Some of the products tested did not have much information on the label and was difficult to find additional information online or from listed contacts. Finally, to have a better evaluation of the impact of the biostimulants on soil biological health, they should be tested in field conditions. Also, a long-term study and the evaluation of additional plant growth and soil parameters will provide more information regarding the impact of those products on soil biological health.

The study is an attempt in providing some insight into the effect of biostimulant on blueberry growth and soil biological health. The findings of this research can contribute to a better understanding of the efficacy of plant biostimulant on blueberries and can guide growers in the choice of the products.

APPENDIX A

Table A.1: Mean height (m) of two blueberry varieties of RE and SHB types in response to the eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within cultivar and month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Huma Gro	0.43 a	0.61 a	0.63 a	0.64 a	0.67 a	0.94 a	1.15 a
	Quantum O.T	0.43 a	0.59 a	0.67 a	0.68 a	0.69 a	0.92 a	1.04 a
	Soil Set	0.42 a	0.59 a	0.58 a	0.59 a	0.60 a	0.88 a	1.06 a
	Radiate	0.42 a	0.55 a	0.56 a	0.58 a	0.59 a	0.83 a	1.06 a
	Terra Grow	0.41 a	0.48 a	0.57 a	0.59 a	0.61 a	0.87 a	1.00 a
	Fertiactyl GZ	0.41 a	0.60 a	0.63 a	0.62 a	0.68 a	0.82 a	1.08 a
	Control	0.41 a	0.51 a	0.56 a	0.57 a	0.64 a	0.90 a	1.07 a
	Inocucor	0.40 a	0.53 a	0.59 a	0.58 a	0.63 a	0.80 a	0.98 a
	Micron Nutrients	0.40 a	0.59 a	0.63 a	0.59 a	0.65 a	0.88 a	1.05 a
Farthing	Terra Grow	0.42 a	0.47 a	0.47 a	0.47 a	0.46 a	0.49 a	0.53 a
	Quantum O.T	0.41 a	0.46 a	0.45 a	0.47 a	0.47 a	0.50 a	0.57 a
	Control	0.41 a	0.45 a	0.47 a	0.48 a	0.48 a	0.47 a	0.46 a
	Inocucor	0.40 a	0.43 a	0.44 a	0.44 a	0.45 a	0.45 a	0.47 a
	Radiate	0.39 a	0.42 a	0.44 a	0.44 a	0.45 a	0.44 a	0.46 a
	Micron Nutrients	0.39 a	0.43 a	0.42 a	0.42 a	0.45 a	0.46 a	0.48 a
	Soil Set	0.39 a	0.43 a	0.44 a	0.45 a	0.45 a	0.46 a	0.45 a
	Fertiactyl GZ	0.39 a	0.44 a	0.45 a	0.45 a	0.50 a	0.55 a	0.57 a
	Huma Gro	0.38 a	0.42 a	0.45 a	0.44 a	0.46 a	0.48 a	0.52 a
Legacy	Inocucor	0.51 a	0.57 a	0.59 a	0.58 a	0.59 a	0.69 a	0.80 a
	Soil Set	0.50 a	0.66 a	0.64 a	0.65 a	0.66 a	0.83 a	0.92 a
	Terra Grow	0.48 a	0.58 a	0.58 a	0.58 a	0.62 a	0.68 a	0.73 a
	Fertiactyl GZ	0.47 a	0.58 a	0.58 a	0.58 a	0.58 a	0.64 a	0.67 a
	Control	0.47 a	0.56 a	0.60 a	0.58 a	0.61 a	0.85 a	0.87 a
	Radiate	0.47 a	0.57 a	0.52 a	0.55 a	0.57 a	0.87 a	0.94 a
	Micron n.	0.47 a	0.58 a	0.58 a	0.56 a	0.61 a	0.89 a	1.01 a
	Quantum O.T	0.47 a	0.61 a	0.62 a	0.58 a	0.65 a	0.86 a	1.00 a
	Huma Gro	0.46 a	0.55 a	0.56 a	0.55 a	0.59 a	0.70 a	0.80 a
Premier	Huma Gro	0.50 a	0.71 ab	0.73 a	0.74 a	0.78 a	0.83 a	1.11 a
	Control	0.48 a	0.74 ab	0.79 a	0.79 a	0.81 a	0.89 a	1.20 a
	Terra Grow	0.48 a	0.68 ab	0.69 a	0.72 a	0.75 a	0.91 a	1.02 a
	Soil Set	0.47 a	0.66 ab	0.67 a	0.67 a	0.72 a	0.98 a	1.31 a
	Micron Nutrients	0.47 a	0.73 ab	0.75 a	0.74 a	0.77 a	0.89 a	1.30 a
	Radiate	0.47 a	0.76 a	0.82 a	0.84 a	0.85 a	0.92 a	1.22 a
	Inocucor	0.46 a	0.64 ab	0.69 a	0.69 a	0.69 a	0.89 a	1.34 a
	Quantum O.T	0.45 a	0.69 ab	0.72 a	0.71 a	0.74 a	0.90 a	1.20 a
	Fertiactyl GZ	0.43 a	0.62 b	0.67 a	0.66 a	0.66 a	0.85 a	1.16 a

Table A.2: Mean width (m) of two blueberry varieties of RE and SHB types in response to the eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison is valid only by cultivar and month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Control	0.48 a	0.57 a	0.54 a	0.58 a	0.58 a	0.79 a	0.96 a
	Huma Gro	0.45 a	0.51 a	0.49 a	0.54 a	0.55 a	0.75 a	0.79 a
	Terra Grow	0.43 a	0.53 a	0.55 a	0.55 a	0.58 a	0.74 a	0.76 a
	Inocucor	0.43 a	0.46 a	0.46 a	0.50 a	0.55 a	0.71 a	0.75 a
	Quantum O.T	0.42 a	0.53 a	0.54 a	0.55 a	0.56 a	0.81 a	0.98 a
	Fertiactyl GZ	0.42 a	0.47 a	0.52 a	0.49 a	0.52 a	0.70 a	0.80 a
	Radiate	0.42 a	0.49 a	0.51 a	0.51 a	0.57 a	0.71 a	0.79 a
	Micron Nutrients	0.42 a	0.49 a	0.53 a	0.53 a	0.60 a	0.84 a	0.90 a
	Soil Set	0.40 a	0.52 a	0.50 a	0.53 a	0.57 a	0.81 a	0.88 a
Farthing	Inocucor	0.32 a	0.37 a	0.38 a	0.39 a	0.38 a	0.42 a	0.45 a
	Micron Nutrients	0.31 a	0.35 a	0.34 a	0.35 a	0.38 a	0.51 a	0.52 a
	Huma Gro	0.31 a	0.32 a	0.34 a	0.35 a	0.37 a	0.45 a	0.50 a
	Soil Set	0.29 a	0.32 a	0.33 a	0.34 a	0.33 a	0.35 a	0.36 a
	Radiate	0.29 a	0.31 a	0.33 a	0.33 a	0.33 a	0.37 a	0.42 a
	Terra Grow	0.27 a	0.33 a	0.35 a	0.36 a	0.34 a	0.41 a	0.43 a
	Quantum O.T	0.26 a	0.32 a	0.34 a	0.33 a	0.34 a	0.43 a	0.48 a
	Fertiactyl GZ	0.26 a	0.30 a	0.30 a	0.31 a	0.32 a	0.34 a	0.40 a
	Control	0.25 a	0.30 a	0.32 a	0.34 a	0.33 a	0.32 a	0.33 a
Legacy	Quantum O.T	0.48 a	0.53 a	0.52 a	0.55 a	0.54 a	0.62 a	0.66 a
	Inocucor	0.46 a	0.58 a	0.57 a	0.56 a	0.59 a	0.59 a	0.65 a
	Control	0.45 a	0.55 a	0.55 a	0.58 a	0.58 a	0.59 a	0.68 a
	Fertiactyl GZ	0.44 a	0.54 a	0.57 a	0.57 a	0.56 a	0.56 a	0.59 a
	Huma Gro	0.43 a	0.51 a	0.51 a	0.91 a	0.50 a	0.59 a	0.64 a
	Soil Set	0.43 a	0.50 a	0.51 a	0.55 a	0.53 a	0.55 a	0.57 a
	Radiate	0.40 a	0.48 a	0.47 a	0.50 a	0.54 a	0.69 a	0.80 a
	Micron Nutrients	0.40 a	0.50 a	0.52 a	0.51 a	0.54 a	0.67 a	0.70 a
	Terra Grow	0.39 a	0.48 a	0.48 a	0.49 a	0.52 a	0.59 a	0.63 a
Premier	Inocucor	0.52 a	0.59 a	0.61 a	0.60 a	0.59 a	0.63 a	0.80 a
	Control	0.51 a	0.59 a	0.59 a	0.63 a	0.64 a	0.72 a	0.96 a
	Fertiactyl GZ	0.50 a	0.63 a	0.59 a	0.59 a	0.56 a	0.63 a	0.78 a
	Micron Nutrients	0.48 a	0.60 a	0.63 a	0.64 a	0.59 a	0.65 a	0.80 a
	Radiate	0.47 a	0.54 a	0.58 a	0.58 a	0.55 a	0.70 a	0.88 a
	Quantum O.T	0.46 a	0.58 a	0.55 a	0.56 a	0.58 a	0.66 a	0.85 a
	Huma Gro	0.45 a	0.55 a	0.55 a	0.59 a	0.57 a	0.69 a	0.87 a
	Terra Grow	0.44 a	0.55 a	0.53 a	0.59 a	0.56 a	0.78 a	0.98 a
	Soil Set	0.43 a	0.54 a	0.54 a	0.55 a	0.55 a	0.68 a	0.93 a

Table A.3: Average plant volume (m^3) of two blueberry varieties of RE and SHB types in response to eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison is valid by cultivar and month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Control	0.08 a	0.13 a	0.13 a	0.16 a	0.17 a	0.44 a	0.81 a
	Huma Gro	0.07 a	0.12 a	0.12 a	0.15 a	0.16 a	0.42 a	0.56 a
	Terra Grow	0.07 a	0.12 a	0.14 a	0.14 a	0.16 a	0.37 a	0.45 a
	Fertiactyl GZ	0.06 a	0.11 a	0.15 a	0.13 a	0.15 a	0.33 a	0.54 a
	Quantum O.T	0.06 a	0.14 a	0.16 a	0.17 a	0.17 a	0.48 a	0.77 a
	Radiate	0.06 a	0.11 a	0.12 a	0.12 a	0.15 a	0.34 a	0.51 a
	Inocucor	0.06 a	0.09 a	0.10 a	0.12 a	0.15 a	0.33 a	0.43 a
	Soil Set	0.06 a	0.14 a	0.11 a	0.13 a	0.16 a	0.44 a	0.64 a
	Micron Nutrients	0.06 a	0.11 a	0.14 a	0.13 a	0.18 a	0.48 a	0.68 a
Farthing	Inocucor	0.03 a	0.05 a	0.05 a	0.05 a	0.05 a	0.07 a	0.08 a
	Micron Nutrients	0.03 a	0.04 a	0.04 a	0.04 a	0.05 a	0.11 a	0.12 a
	Huma Gro	0.03 a	0.03 a	0.04 a	0.04 a	0.05 a	0.08 a	0.11 a
	Soil Set	0.03 a	0.03 a	0.04 a	0.04 a	0.04 a	0.04 a	0.04 a
	Radiate	0.03 a	0.03 a	0.04 a	0.04 a	0.04 a	0.05 a	0.07 a
	Terra Grow	0.02 a	0.04 a	0.05 a	0.05 a	0.04 a	0.07 a	0.09 a
	Quantum O.T	0.02 a	0.05 a	0.05 a	0.06 a	0.05 a	0.09 a	0.13 a
	Fertiactyl GZ	0.02 a	0.03 a	0.03 a	0.03 a	0.04 a	0.05 a	0.07 a
	Control	0.02 a	0.03 a	0.04 a	0.05 a	0.04 a	0.04 a	0.04 a
Legacy	Quantum O.T	0.09 a	0.14 a	0.13 a	0.14 a	0.15 a	0.26 a	0.38 a
	Inocucor	0.08 a	0.15 a	0.15 a	0.15 a	0.16 a	0.19 a	0.26 a
	Soil Set	0.08 a	0.13 a	0.13 a	0.16 a	0.15 a	0.20 a	0.24 a
	Control	0.07 a	0.13 a	0.14 a	0.15 a	0.17 a	0.24 a	0.39 a
	Fertiactyl GZ	0.07 a	0.13 a	0.15 a	0.15 a	0.14 a	0.15 a	0.18 a
	Huma Gro	0.07 a	0.12 a	0.12 a	0.56 a	0.13 a	0.20 a	0.26 a
	Radiate	0.06 a	0.11 a	0.10 a	0.11 a	0.14 a	0.35 a	0.53 a
	Micron Nutrients	0.06 a	0.11 a	0.13 a	0.12 a	0.15 a	0.35 a	0.43 a
	Terra Grow	0.06 a	0.11 a	0.10 a	0.11 a	0.14 a	0.22 a	0.30 a
Premier	Control	0.11 a	0.21 a	0.23 a	0.24 a	0.26 a	0.37 a	0.90 a
	Inocucor	0.10 a	0.18 a	0.21 a	0.20 a	0.19 a	0.28 a	0.69 a
	Micron Nutrients	0.08 a	0.21 a	0.23 a	0.24 a	0.21 a	0.30 a	0.69 a
	Fertiactyl GZ	0.08 a	0.19 a	0.19 a	0.18 a	0.16 a	0.28 a	0.61 a
	Radiate	0.08 a	0.17 a	0.22 a	0.22 a	0.20 a	0.37 a	0.78 a
	Huma Gro	0.08 a	0.17 a	0.18 a	0.21 a	0.20 a	0.32 a	0.66 a
	Quantum O.T	0.07 a	0.18 a	0.18 a	0.18 a	0.20 a	0.33 a	0.72 a
	Terra Grow	0.07 a	0.17 a	0.15 a	0.20 a	0.18 a	0.47 a	0.85 a
	Soil Set	0.07 a	0.15 a	0.16 a	0.16 a	0.17 a	0.37 a	0.94 a

Table A.4: Mean total cane number of two blueberry varieties of RE and SHB types in response to eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison is valid only by cultivar and month.

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Huma Gro	1.40 a	1.40 a	1.40 a	1.40 a	1.60 a	2.00 a	2.00 a
	Fertiactyl GZ	1.20 a	1.00 a	1.00 a	1.00 a	1.80 a	1.80 a	1.80 a
	Inocucor	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a
	Micron Nutrients	1.20 a	1.20 a	1.20 a	1.20 a	1.60 a	1.60 a	1.60 a
	Radiate	1.20 a	1.20 a	1.20 a	1.20 a	1.40 a	1.80 a	1.80 a
	Terra Grow	1.20 a	1.20 a	1.20 a	1.20 a	1.60 a	1.60 a	1.60 a
	Control	1.00 a	1.00 a	1.00 a	1.00 a	1.20 a	1.40 a	1.40 a
	Quantum O.T	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	Soil Set	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
Farthing	Soil Set	1.80 a	1.80 a	1.80 a	1.80 a	1.80 a	2.00 a	1.80 a
	Control	1.60 a	1.60 a	1.60 a	1.60 a	1.60 a	1.60 a	1.60 a
	Micron Nutrients	1.60 a	1.60 a	1.60 a	1.60 a	1.80 a	2.00 a	2.00 a
	Huma Gro	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a
	Radiate	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a
	Fertiactyl GZ	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a
	Inocucor	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.60 a	1.80 a
	Quantum O.T	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a
	Terra Grow	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
Legacy	Inocucor	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a
	Terra Grow	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a	1.40 a
	Fertiactyl GZ	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.40 a	1.40 a
	Quantum O.T	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a
	Soil Set	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a
	Control	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	Huma Gro	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	Micron Nutrients	1.00 a	1.00 a	1.00 a	1.00 a	1.20 a	1.20 a	1.20 a
	Radiate	1.00 a	1.00 a	1.00 a	1.20 a	1.60 a	1.80 a	1.80 a
Premier	Terra Grow	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a	1.20 a
	Control	1.00 a	1.00 a	1.00 a	1.00 a	1.20 a	1.20 a	1.20 a
	Fertiactyl GZ	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	Huma Gro	1.00 a	1.00 a	1.00 a	1.00 a	1.20 a	1.20 a	1.20 a
	Inocucor	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	Micron Nutrients	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	Quantum O.T	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	Radiate	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	Soil Set	1.00 a	1.00 a	1.00 a	1.00 a	1.40 a	1.40 a	1.40 a

Table A.5: Mean total cane dimeter (mm) of two blueberry varieties of RE and SHB types in response to eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison is valid only by cultivar and month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Huma Gro	12.62 a	13.90 a	14.57 a	16.10 a	17.33 a	22.67 a	25.91 a
	Radiate	12.50 a	11.93 a	12.74 a	12.61 a	16.91 a	21.56 a	23.37 a
	Quantum O.T	12.19 a	11.91 a	12.62 a	13.36 a	14.17 a	15.61 a	17.32 a
	Fertiactyl GZ	11.50 a	12.90 a	12.80 a	13.00 a	17.11 a	23.18 a	21.01 a
	Inocucor	11.22 a	10.39 a	11.21 a	12.36 a	13.86 a	15.60 a	18.87 a
	Terra Grow	11.07 a	11.14 a	11.77 a	12.95 a	16.36 a	18.57 a	20.42 a
	Micron Nutrients	11.80 a	10.92 a	11.31 a	12.64 a	15.53 a	18.49 a	21.07 a
	Soil Set	11.12 a	10.32 a	11.06 a	11.60 a	13.22 a	15.02 a	17.30 a
	Control	10.06 a	9.88 a	10.27 a	12.12 a	13.86 a	18.11 a	20.69 a
Farthing	Soil Set	14.86 a	15.62 a	15.77 a	15.75 a	16.40 a	16.70 a	17.14 a
	Micron Nutrients	14.14 a	14.28 a	14.56 a	14.52 a	16.35 a	18.12 a	19.07 a
	Huma Gro	13.55 a	13.72 a	13.75 a	13.24 a	14.40 a	14.29 a	15.45 a
	Inocucor	13.49 a	13.56 a	14.50 a	14.89 a	15.65 a	17.94 a	19.13 a
	Control	13.26 a	12.78 a	13.05 a	13.63 a	14.50 a	15.18 a	16.11 a
	Radiate	12.57 a	13.56 a	13.15 a	13.62 a	15.63 a	15.09 a	15.11 a
	Quantum O.T	11.53 a	12.35 a	12.17 a	12.66 a	13.18 a	13.38 a	14.40 a
	Fertiactyl GZ	11.53 a	11.93 a	12.24 a	12.18 a	12.68 a	13.37 a	14.40 a
	Terra Grow	10.43 a	10.46 a	10.50 a	10.97 a	10.92 a	11.53 a	12.22 a
Legacy	Huma Gro	14.87 a	15.17 a	14.91 a	14.23 a	15.06 a	17.07 a	18.44 a
	Quantum O.T	12.67 a	12.60 a	13.22 a	13.27 a	14.29 a	15.91 a	18.90 a
	Fertiactyl GZ	12.19 a	11.85 a	11.71 a	12.33 a	13.19 a	14.61 a	14.64 a
	Inocucor	12.00 a	12.44 a	12.10 a	12.64 a	14.59 a	15.10 a	16.85 a
	Control	11.78 a	12.09 a	12.15 a	12.34 a	14.59 a	14.95 a	16.88 a
	Terra Grow	11.67 a	12.02 a	12.08 a	12.38 a	13.72 a	14.55 a	15.13 a
	Soil Set	11.53 a	12.01 a	12.35 a	13.35 a	14.06 a	15.79 a	16.88 a
	Radiate	11.18 a	11.03 a	11.41 a	12.90 a	16.61 a	19.93 a	21.24 a
	Micron Nutrients	10.02 a	10.19 a	10.56 a	11.16 a	12.87 a	15.44 a	16.51 a
Premier	Micron Nutrients	15.61 a	14.87 a	15.18 a	15.82 a	18.49 a	21.65 a	23.53 a
	Inocucor	13.94 a	14.64 a	15.44 a	14.18 a	17.11 a	19.53 a	20.09 a
	Terra Grow	13.84 a	13.46 a	14.23 a	14.59 a	16.17 a	18.50 a	20.76 a
	Quantum O.T	13.59 a	13.33 a	13.92 a	14.37 a	15.42 a	16.68 a	19.48 a
	Radiate	12.80 a	12.42 a	12.81 a	14.09 a	15.18 a	16.41 a	17.88 a
	Huma Gro	12.42 a	11.89 a	12.75 a	13.45 a	14.98 a	16.84 a	18.75 a
	Control	12.31 a	11.67 a	12.10 a	13.24 a	18.69 a	22.41 a	22.39 a
	Fertiactyl GZ	12.19 a	12.77 a	14.01 a	14.57 a	16.60 a	18.80 a	20.49 a
	Soil Set	11.30 a	13.20 a	12.79 a	13.51 a	17.38 a	20.22 a	24.89 a

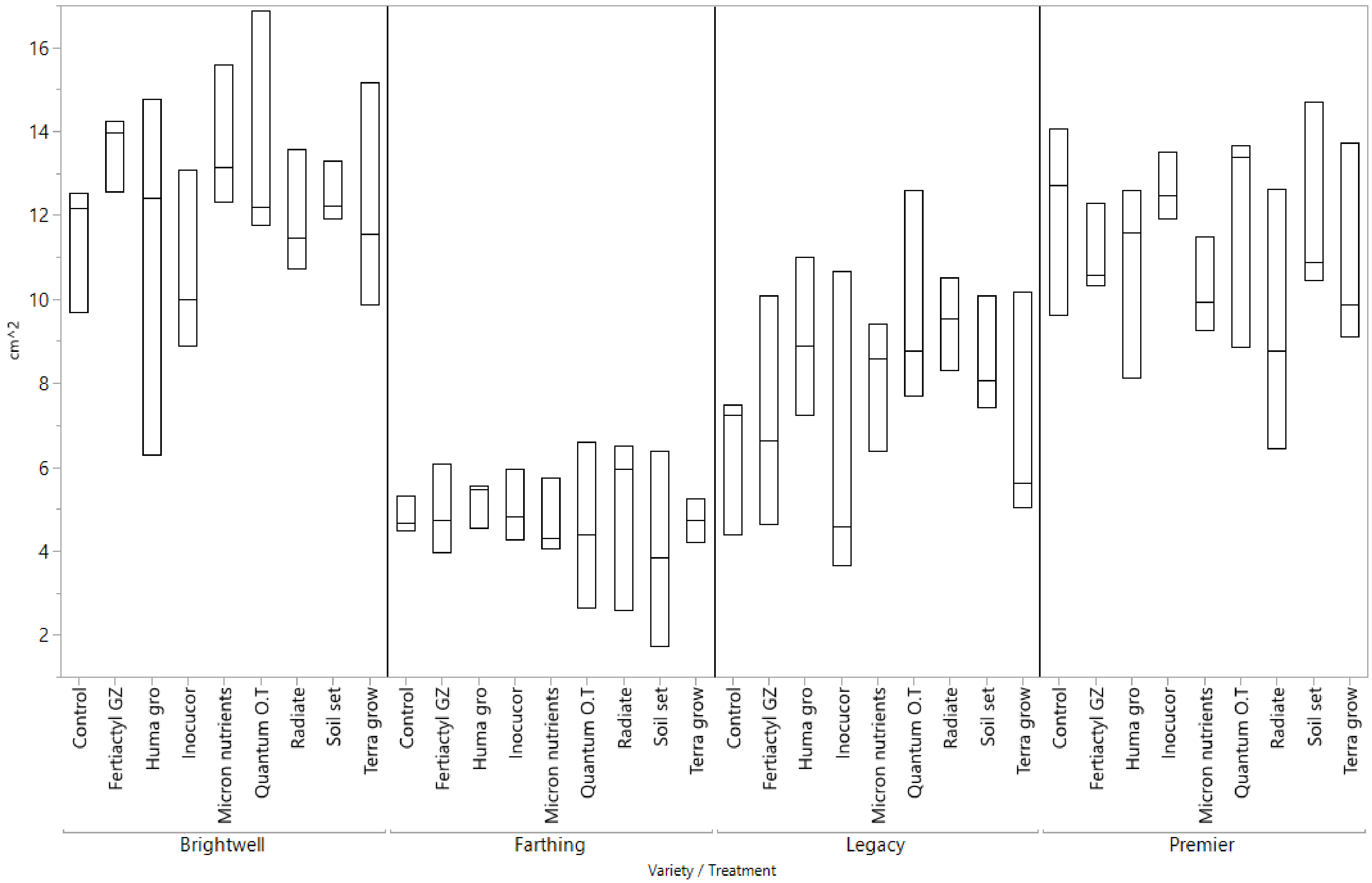

Figure A.1: Leaf area (cm^2) of blueberry plants of four cultivars in response to eight biostimulant

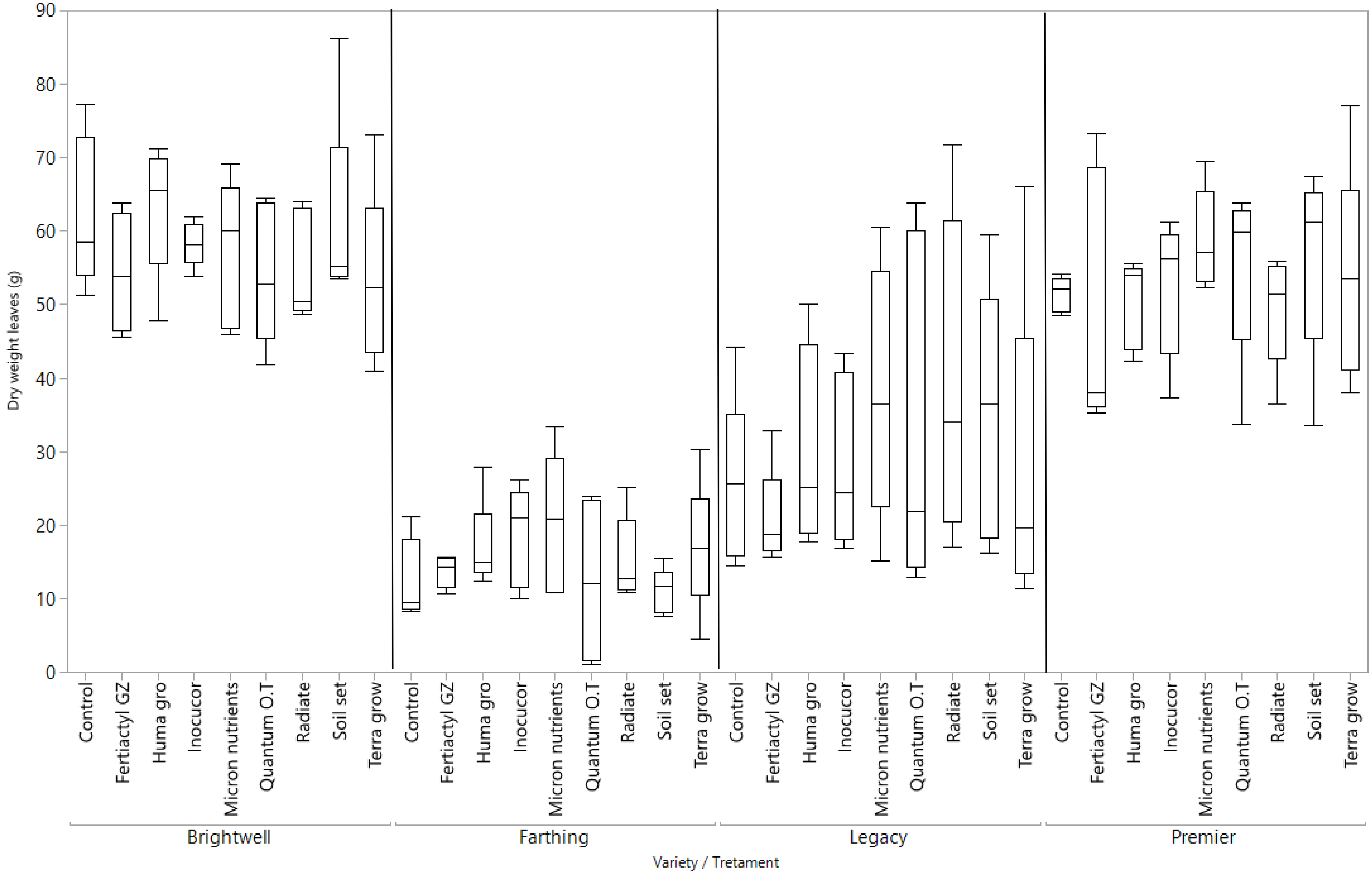

Figure A.2: Leaf dry weight (g) of blueberry plants of four cultivars in response to eight biostimulants

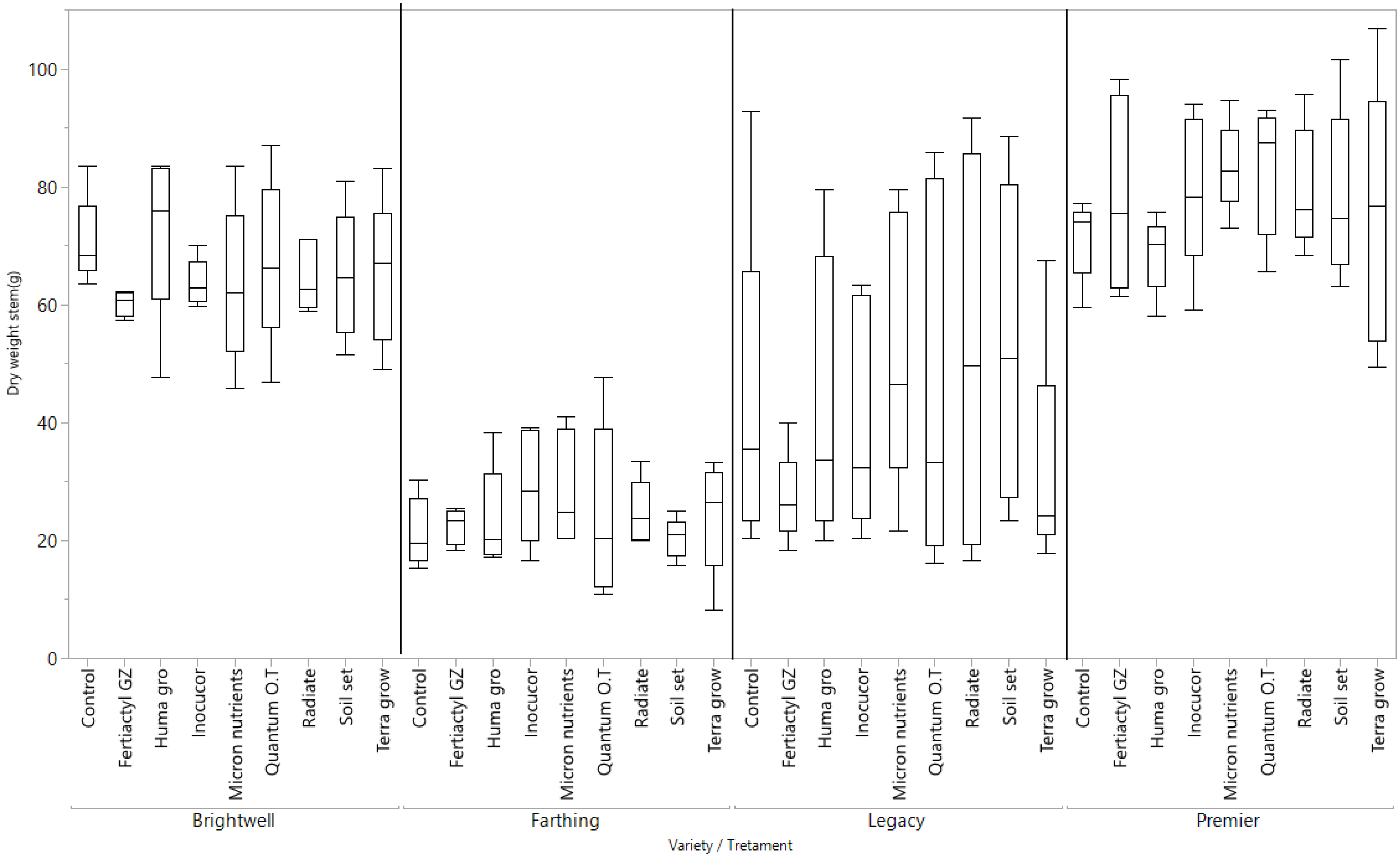

Figure A.3: Stems dry weight (g) of blueberry plants of four cultivars in response to eight biostimulants

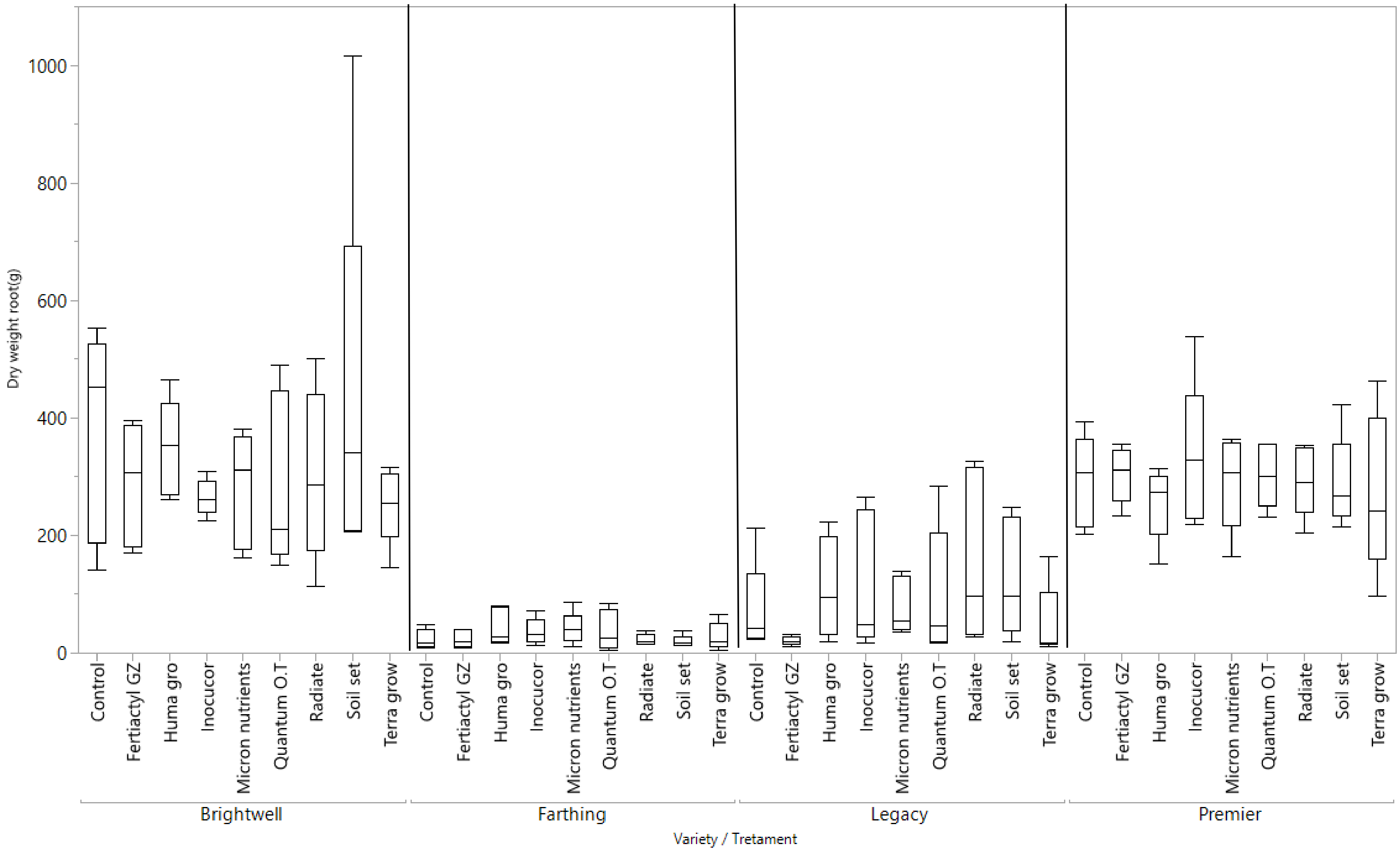

Figure A.4: Roots dry weight (g) of blueberry plants of four cultivars in response to eight biostimulants

Table A.6: Mean abaxial chlorophyl content (µg/cm^2) in two blueberry varieties of RE and SHB types in response to eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison shows treatment-cultivar interaction effect and is valid by month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Quantum O.T	18.7 a	18.9 ab	15.7 a	17.5 ab	29.9 a	31.8 a	33.3 a
	Micron Nutrients	18.3 ab	17.5 ab	15.1 a	19.7 ab	30.6 a	31.4 a	31.9 ab
	Terra Grow	17.9 ab	19.4 a	16.4 a	19.6 ab	27.4 a	31.3 a	33.4 a
	Radiate	17.4 ab	14.5 b	13.4 a	15.8 b	30.8 a	28.6 a	31.3 ab
	Soil Set	17.1 ab	14.9 ab	12.8 a	19.4 ab	25.2 a	27.8 a	29.5 ab
	Inocucor	16.1 ab	16.5 ab	14.6 a	22.8 a	31.5 a	27.8 a	31.9 ab
	Fertiactyl GZ	16.1 ab	15.5 ab	13.0 a	20.6 ab	29.6 a	30.6 a	27.4 b
	Huma Gro	16.0 ab	15.9 ab	12.9 a	22.5 a	29.0 a	29.0 a	30.4 ab
	Control	15.6 b	15.2 ab	13.6 a	18.9 ab	28.8 a	28.9 a	29.9 ab
Farthing	Terra Grow	21.5 a	20.1 a	19.5 a	17.0 a	23.3 a	30.5 a	27.0 a
	Huma Gro	21.8 a	22.6 a	19.9 a	18.1 a	24.0 a	30.1 a	32.6 a
	Quantum O.T	20.9 a	21.7 a	20.3 a	15.7 a	22.4 a	26.8 ab	25.7 a
	Micron Nutrients	20.6 a	22.1 a	18.0 a	19.2 a	23.7 a	26.8 ab	34.0 a
	Fertiactyl GZ	23.1 a	22.1 a	18.9 a	17.1 a	22.3 a	26.1 ab	28.0 a
	Radiate	21.0 a	22.4 a	18.5 a	14.6 a	18.7 a	25.7 ab	27.9 a
	Inocucor	21.8 a	22.8 a	20.8 a	17.8 a	23.4 a	25.5 ab	28.8 a
	Control	22.1 a	23.1 a	19.8 a	16.3 a	21.8 a	23.1 ab	29.1 a
	Soil Set	22.9 a	24.0 a	19.0 a	16.6 a	18.8 a	21.6 b	26.5 a
Legacy	Soil Set	19.3 a	16.5 a	12.0 a	14.2 b	20.8 abc	22.6 ab	22.3 a
	Terra Grow	19.0 ab	14.7 a	11.3 a	14.1 b	17.4 bc	17.0 b	20.8 a
	Fertiactyl GZ	18.9 ab	15.0 a	12.1 a	10.5 b	13.9 c	17.2 b	19.1 a
	Micron Nutrients	18.3 ab	14.0 a	12.3 a	16.9 ab	24.4 ab	25.7 ab	25.6 a
	Quantum O.T	17.8 ab	14.6 a	12.9 a	13.0 b	19.7 abc	20.9 ab	21.0 a
	Inocucor	17.7 ab	14.2 a	11.9 a	13.5 b	20.0 abc	23.2 ab	24.7 a
	Control	16.9 ab	14.1 a	10.5 a	16.2 ab	23.1 abc	21.2 ab	25.1 a
	Huma Gro	16.4 ab	14.6 a	10.3 a	12.6 b	19.3 abc	22.0 ab	25.1 a
	Radiate	15.8 b	14.3 a	11.7 a	21.0 a	28.2 a	27.6 a	26.4 a
Premier	Radiate	19.0 a	18.8 a	14.9 a	18.0 a	27.1 a	28.8 a	30.2 a
	Control	17.7 a	16.6 ab	12.4 ab	17.4 a	28.6 a	28.0 a	27.9 a
	Terra Grow	18.2 a	16.5 ab	13.9 ab	18.0 a	31.2 a	30.9 a	30.4 a
	Fertiactyl GZ	18.2 a	16.0 ab	12.4 ab	17.9 a	28.7 a	30.3 a	32.1 a
	Huma Gro	18.5 a	15.9 ab	14.3 ab	18.7 a	28.9 a	31.0 a	29.0 a
	Micron Nutrients	17.2 a	15.7 ab	12.7 ab	16.0 a	27.5 a	29.3 a	27.7 a
	Quantum O.T	17.5 a	15.5 ab	12.3 ab	17.9 a	29.2 a	30.9 a	30.9 a
	Inocucor	16.8 a	15.4 ab	14.3 ab	16.7 a	25.4 a	27.6 a	28.1 a
	Soil Set	16.3 a	14.3 b	11.3 b	16.2 a	26.9 a	26.7 a	30.8 a

Table A.7: The impact of treatment-variety interactions on chlorophyll content (µg/cm²) of two blueberry varieties of RE type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

CV	Treatment	Least square mean						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Premier	Huma Gro	19.96 a	16.70 abc	15.13 abc	19.61 abcd	29.56 a	31.2 a	29.9 a
	Radiate	19.68 ab	19.85 ab	15.97 ab	18.79 abcd	27.9 a	29.9 a	30.6 a
	Control	19.19 ab	17.12 abc	13.07 abc	18.28 abcd	28.7 a	29.1 a	29.1 a
	Fertiacyl GZ	19.19 ab	17.04 abc	12.48 bc	18.49 abcd	29.4 a	30.1 a	32.4 a
	Terra Grow	18.87 ab	17.18 abc	14.58 abc	18.64 abcd	31.7 a	31.5 a	31.5 a
	Quantum O.T	18.38 ab	16.42 abc	13.07 abc	18.63 abcd	30.0 a	31.7 a	31.6 a
	Micron Nutrients	17.92 ab	16.67 abc	12.97 abc	16.47 cd	27.8 a	30.2 a	28.8 a
	Inocucor	17.39 ab	16.57 abc	14.78 abc	17.09 bcd	26.2 a	28.5 a	28.6 a
	Soil Set	17.32 ab	14.62 c	11.74 c	17.25 bcd	27.4 a	27.5 a	31.4 a
Brightwell	Quantum O.T	19.50 ab	19.57 ab	15.66 abc	18.10 abcd	30.5 a	32.6 a	33.8 a
	Micron Nutrients	19.05 ab	17.82 abc	15.83 ab	20.55 abcd	31.3 a	32.6 a	32.2 a
	Terra Grow	18.86 ab	20.46 a	16.85 a	20.17 abcd	28.0 a	32.1 a	33.6 a
	Radiate	18.19 ab	15.65 bc	13.93 abc	15.54 d	31.0 a	29.2 a	32.4 a
	Soil Set	17.80 ab	15.75 abc	12.90 abc	20.12 abcd	26.8 a	29.0 a	30.6 a
	Inocucor	17.41 ab	17.42 abc	14.77 abc	22.98 a	32.2 a	28.1 a	32.9 a
	Control	16.54 ab	15.53 bc	13.74 abc	19.29 abcd	29.5 a	29.4 a	30.5 a
	Fertiactyl GZ	16.38 ab	16.75 abc	13.76 abc	21.24 abc	30.4 a	31.7 a	28.7 a
	Huma Gro	16.09 b	16.53 abc	13.25 abc	22.53 ab	29.6 a	30.0 a	31.2 a

Table A.8: The impact of eight biostimulants (treatment) on chlorophyl content (µg/cm²) two blueberry varieties of RE type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

Treatment	Least squares mean						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Terra Grow	18.9 a	18.81 a	15.71 a	19.40 ab	29.8 a	31.8 a	32.6 a
Quantum O.T	18.9 a	17.99 ab	14.36 abc	18.36 ab	30.2 a	32.2 a	32.7 a
Radiate	18.9 a	17.74 ab	14.953 ab	17.16 b	29.5 a	29.5 a	31.5 a
Micron Nutrients	18.5 a	17.24 ab	14.39 abc	18.50 ab	29.6 a	31.4 a	30.5 a
Inocucor	17.4 a	16.99 ab	14.77 abc	20.03 ab	29.2 a	28.3 a	30.7 a
Fertiactyl GZ	17.8 a	16.89 ab	13.12 bc	19.86 ab	29.9 a	30.9 a	30.6 a
Huma Gro	18.0 a	16.61 ab	14.18 abc	21.07 a	29.5 a	30.6 a	30.6 a
Control	17.9 a	16.31 ab	13.40 abc	18.78 ab	29.1 a	29.2 a	29.8 a
Soil Set	17.6 a	15.18 b	12.32 c	18.68 ab	27.0 a	28.2 a	31.0 a

Table A.9: The impact of treatment-variety interactions on chlorophyl content (µg/cm²) of two blueberry varieties of SHB type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

CV	Treatment	Least square mean						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Legacy	Radiate	16.3 d	15.0 c	11.7 b	21.34 a	28.51 a	27.87 a	26.7 abc
	Micron Nutrients	19.4 abcd	14.8 c	12.8 b	17.73 ab	24.35 ab	26.20 ab	26.4 abc
	Control	17.8 cd	14.9 c	10.9 b	16.46 abc	23.64 ab	22.13 ab	25.7 abc
	Soil Set	19.3 abcd	17.0 bc	12.2 b	14.70 bc	22.12 abc	23.34 ab	22.7 abc
	Terra Grow	19.3 abcd	14.9 c	11.8 b	14.52 bc	17.57 bc	17.33 b	20.8 c
	Quantum O.T	18.9 bcd	15.6 c	12.8 b	14.01 bc	19.90 abc	21.19 ab	21.3 c
	Inocucor	18.1 cd	15.0 c	12.2 b	14.01 bc	20.43 abc	23.39 ab	24.7 abc
	Huma Gro	17.1 d	14.8 c	10.8 b	13.42 bc	19.61 abc	21.89 ab	25.6 abc
	Fertiactyl GZ	19.5 abcd	16.0 c	12.1 b	11.49 c	14.0 5c	17.65 b	21.4 c
Farthing	Micron Nutrients	21.9 abc	22.4 a	18.7 a	19.45 ab	23.85 ab	27.11 ab	34.6 a
	Huma Gro	22.6 ab	22.9 a	19.7 a	18.41 ab	24.38 ab	30.34 a	32.3 ab
	Inocucor	22.9 ab	23.5 a	21.2 a	17.93 ab	23.30 ab	25.58 ab	29.3 abc
	Terra Grow	22.0 abc	20.8 ab	19.5 a	17.24 abc	23.27 ab	30.63 a	27.4 abc
	Soil Set	23.2 ab	24.7 a	19.9 a	17.00 abc	18.91 bc	21.71 ab	26.8 abc
	Fertiactyl GZ	23.4 a	22.6 a	19.5 a	16.94 abc	22.44 abc	27.14 ab	28.5 abc
	Control	23.1 ab	22.6 a	20.2 a	16.83 abc	21.89 abc	23.97 ab	30.1 abc
	Quantum O.T	21.9 abc	22.7 a	20.4 a	15.98 abc	22.25 abc	27.20 ab	26.8 abc
	Radiate	21.6 abc	22.8 a	18.7 a	15.51 abc	19.36 abc	26.24 ab	27.4 abc

Table A.10: The impact of eight biostimulants (treatment) on chlorophyl content (µg/cm²) of two blueberry varieties of SHB type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

Treatment	Least squares mean						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Micron Nutrients	20.7 a	18.6 ab	15.8 a	18.59 a	24.10 a	26.7 a	30.49 a
Radiate	18.9 a	18.9 ab	15.2 a	18.42 a	23.94 ab	27.1 a	27.02 ab
Control	20.4 a	18.7 ab	15.5 a	16.65 ab	22.76 ab	23.1 a	27.92 ab
Inocucor	20.5 a	19.2 ab	16.7 a	15.97 ab	21.86 ab	24.5 a	27.00 ab
Huma Gro	19.8 a	18.9 ab	15.2 a	15.91 ab	21.99 ab	26.1 a	28.94 ab
Terra Grow	20.6 a	17.9 b	15.7 a	15.88 ab	20.42 ab	24.0 a	24.10 b
Soil Set	21.2 a	20.9 a	16.0 a	15.85 ab	20.51 ab	22.5 a	24.73 ab
Quantum O.T	20.4 a	19.1 ab	16.6 a	14.99 ab	21.08 ab	24.2 a	24.07 b
Fertiactyl GZ	21.4 a	19.3 ab	15.8 a	14.22 b	18.25 b	22.4 a	24.94 ab

Table A.11: Mean abaxial anthocyanin content (relative absorbance) in two blueberry varieties of RE and SHB types in response to eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison shows treatment-cultivar interaction effect and is valid by month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Huma Gro	0.18 a	0.24 a	0.29 ab	0.20 b	0.15 a	0.14 a	0.14 ab
	Soil Set	0.17 a	0.23 a	0.31 ab	0.22 ab	0.16 a	0.15 a	0.16 ab
	Control	0.18 a	0.23 ab	0.27 ab	0.22 ab	0.16 a	0.14 a	0.16 a
	Radiate	0.17 a	0.22 ab	0.29 ab	0.26 a	0.16 a	0.15 a	0.14 ab
	Fertiactyl GZ	0.18 a	0.22 ab	0.33 a	0.20 b	0.15 a	0.13 a	0.16 ab
	Inocucor	0.18 a	0.21 ab	0.26 ab	0.19 b	0.15 a	0.14 a	0.14 ab
	Micron Nutrients	0.17 a	0.20 ab	0.23 ab	0.22 ab	0.15 a	0.14 a	0.14 ab
	Terra Grow	0.17 a	0.20 ab	0.22 b	0.21 b	0.16 a	0.14 a	0.13 b
	Quantum O.T	0.17 a	0.18 b	0.23 b	0.22 ab	0.16 a	0.14 a	0.14 ab
Farthing	Soil Set	0.16 a	0.18 a	0.24 a	0.28 a	0.26 a	0.25 a	0.23 a
	Radiate	0.17 a	0.19 a	0.22 a	0.27 a	0.26 ab	0.20 a	0.19 ab
	Control	0.17 a	0.20 a	0.23 a	0.27 a	0.24 abc	0.24 a	0.19 ab
	Fertiactyl GZ	0.16 a	0.20 a	0.22 a	0.27 a	0.22 abc	0.19 a	0.18 ab
	Inocucor	0.17 a	0.17 a	0.21 a	0.26 a	0.21 abc	0.20 a	0.21 ab
	Quantum O.T	0.16 a	0.19 a	0.22 a	0.28 a	0.20 abc	0.18 a	0.22 ab
	Terra Grow	0.17 a	0.21 a	0.21 a	0.26 a	0.20 abc	0.17 a	0.21 ab
	Huma Gro	0.16 a	0.19 a	0.22 a	0.24 a	0.19 bc	0.17 a	0.17 ab
	Micron Nutrients	0.17 a	0.19 a	0.22 a	0.23 a	0.18 c	0.18 a	0.14 b
Legacy	Control	0.18 a	0.43 a	0.65 a	0.49 abc	0.25 ab	0.21 ab	0.20 a
	Huma Gro	0.19 a	0.38 ab	0.64 a	0.64 ab	0.40 ab	0.26 ab	0.21 a
	Quantum O.T	0.18 a	0.38 ab	0.52 a	0.65 ab	0.45 ab	0.33 ab	0.28 a
	Inocucor	0.17 a	0.36 ab	0.61 a	0.58 abc	0.35 ab	0.22 ab	0.23 a
	Terra Grow	0.17 a	0.35 ab	0.60 a	0.62 ab	0.51 a	0.38 a	0.28 a
	Fertiactyl GZ	0.17 a	0.34 ab	0.56 a	0.71 a	0.51 a	0.29 ab	0.25 a
	Radiate	0.19 a	0.34 ab	0.52 a	0.36 c	0.15 b	0.16 b	0.18 a
	Micron Nutrients	0.17 a	0.32 ab	0.53 a	0.45 bc	0.26 ab	0.18 b	0.18 a
	Soil Set	0.17 a	0.25 b	0.54 a	0.57 abc	0.36 ab	0.24 ab	0.23 a
Premier	Soil Set	0.17 a	0.24 a	0.33 a	0.24 a	0.17 a	0.16 a	0.15 a
	Inocucor	0.17 a	0.25 a	0.27 a	0.25 a	0.18 a	0.16 a	0.15 a
	Micron Nutrients	0.17 a	0.21 a	0.31 a	0.26 a	0.18 a	0.15 a	0.15 a
	Control	0.17 a	0.23 a	0.32 a	0.26 a	0.18 a	0.18 a	0.17 a
	Terra Grow	0.16 a	0.23 a	0.27 a	0.25 a	0.16 a	0.15 a	0.16 a
	Fertiactyl GZ	0.16 a	0.24 a	0.32 a	0.23 a	0.17 a	0.15 a	0.16 a
	Huma Gro	0.16 a	0.22 a	0.31 a	0.25 a	0.17 a	0.16 a	0.16 a
	Quantum O.T	0.16 a	0.23 a	0.31 a	0.23 a	0.16 a	0.15 a	0.14 a
	Radiate	0.16 a	0.19 a	0.27 a	0.23 a	0.18 a	0.16 a	0.16 a

Table A.12: The impact of treatment-variety interactions on relative anthocyanin absorbance of two blueberry varieties of RE type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

CV	Treatment	Least square means						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Huma Gro	0.16 a	0.15 a	0.24 ab	0.17 b	0.11 abc	0.10 abc	0.12 a
	Control	0.15 ab	0.14 ab	0.21 ab	0.20 ab	0.12 abc	0.10 abc	0.13 a
	Inocucor	0.15ab	0.14 ab	0.18 ab	0.16 b	0.10 c	0.10 abc	0.11 a
	Fertiactyl GZ	0.5 ab	0.14 ab	0.19 ab	0.16 b	0.10 bc	0.09 c	0.11 a
	Micron Nutrients	0.13 ab	0.13 ab	0.18 ab	0.18 ab	0.11 abc	0.10 bc	0.11 a
	Soil Set	0.13 ab	0.13 ab	0.22 ab	0.19 ab	0.12 abc	0.11 abc	0.12 a
	Radiate	0.13 ab	0.13 ab	0.20 ab	0.25 ab	0.12 abc	0.10 abc	0.11 a
	Terra Grow	0.13 ab	0.13 ab	0.18 ab	0.18 b	0.13 abc	0.10 bc	0.11 a
	Quantum O.T	0.13 ab	0.13 ab	0.15 b	0.19 ab	0.12 abc	0.10 abc	0.11 a
Premier	Micron Nutrients	0.14 ab	0.13 ab	0.20 ab	0.26 ab	0.14 ab	0.11 abc	0.13 a
	Soil Set	0.14 ab	0.13 ab	0.23 ab	0.21 ab	0.13 abc	0.12 abc	0.12 a
	Terra Grow	0.13 ab	0.13 ab	0.22 ab	0.28 a	0.12 abc	0.10 abc	0.13 a
	Inocucor	0.14 ab	0.13 ab	0.26 a	0.21 ab	0.14 ab	0.14 a	0.15 a
	Fertiactyl GZ	0.13 ab	0.13 ab	0.23 ab	0.19 ab	0.13 abc	0.13 abc	0.13 a
	Control	0.13 ab	0.13 ab	0.21 ab	0.25 ab	0.14 a	0.13 ab	0.14 a
	Huma Gro	0.13 ab	0.13 ab	0.20 ab	0.21 ab	0.14 ab	0.12 abc	0.13 a
	Radiate	0.13 ab	0.13 ab	0.17 ab	0.20 ab	0.14 ab	0.11 abc	0.13 a
	Quantum O.T	0.13 b	0.12 b	0.22 ab	0.20 ab	0.12 abc	0.11 abc	0.12 a

Table A.13: The impact of eight biostimulants (treatment) on relative anthocyanin absorbance of two blueberry varieties of RE type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

Treatment	Least squares mean						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Fertiactyl GZ	0.14 a	0.22 a	0.41 a	0.18 a	0.12 a	0.11 a	0.12 a
Soil Set	0.13 a	0.23 a	0.36 ab	0.20 a	0.13 a	0.12 a	0.12 a
Huma Gro	0.14 a	0.23 a	0.35 ab	0.20 a	0.13 a	0.11 a	0.12 a
Control	0.14 a	0.22 a	0.34 ab	0.23 a	0.13 a	0.12 a	0.13 a
Radiate	0.13 a	0.19 a	0.30 ab	0.23 a	0.13 a	0.11 a	0.11 a
Micron Nutrients	0.14 a	0.19 a	0.30 ab	0.22 a	0.13 a	0.10 a	0.12 a
Quantum O.T	0.13 a	0.19 a	0.29 ab	0.20 a	0.12 a	0.11 a	0.11 a
Inocucor	0.14 a	0.22 a	0.28 ab	0.19 a	0.12 a	0.12 a	0.13 a
Terra Grow	0.13 a	0.20 a	0.25 b	0.23 a	0.13 a	0.10 a	0.12 a

Table A.14: The impact of treatment-variety interactions on relative anthocyanin absorbance of two blueberry varieties of SHB type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

CV	Treatment	Least square mean						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Legacy	Fertiactyl GZ	0.13 bcd	0.45 ab	0.91 a	1.21 a	0.70 a	0.31 abc	0.22 abc
	Quantum O.T	0.15 abcd	0.52 ab	0.82 a	1.07 ab	0.66 ab	0.37 ab	0.27 ab
	Terra Grow	0.15 abcd	0.47 ab	0.92 a	1.05 ab	0.74 a	0.45 a	0.28 a
	Huma Gro	0.17 ab	0.51 ab	1.00 a	0.95 abc	0.43 abc	0.27 abc	0.18 abc
	Inocucor	0.14 abcd	0.50 ab	0.99 a	0.94 abc	0.45 abc	0.23 bc	0.22 abc
	Micron Nutrients	0.14 abcd	0.40 abc	0.86 a	0.86 abc	0.25 c	0.16 bc	0.15 c
	Soil Set	0.14 abcd	0.31 bcd	0.81 a	0.84 bc	0.40 abc	0.22 bc	0.21 abc
	Control	0.16 abc	0.58 a	1.05 a	0.81 bc	0.26 bc	0.18 bc	0.16 abc
	Radiate	0.17 a	0.45 ab	0.80 a	0.61 cd	0.23 c	0.11 c	0.15 c
Farthing	Quantum O.T	0.13 cd	0.17 cd	0.21 b	0.31 de	0.16 c	0.13 c	0.18 abc
	Fertiactyl GZ	0.13 cd	0.18 cd	0.21 b	0.27 de	0.19 c	0.15 c	0.23 abc
	Soil Set	0.12 d	0.16 cd	0.23 b	0.26 de	0.23 c	0.21 bc	0.19 abc
	Control	0.14 abcd	0.18 cd	0.24 b	0.26 de	0.20 c	0.19 bc	0.16 abc
	Radiate	0.13 bcd	0.18 cd	0.21 b	0.26 de	0.12 c	0.15 bc	0.16 abc
	Terra Grow	0.13 bcd	0.19 cd	0.19 b	0.25 de	0.16 c	0.12 c	0.17 abc
	Inocucor	0.13 bcd	0.13 d	0.18 b	0.24 e	0.17 c	0.16 bc	0.15 bc
	Huma Gro	0.12 d	0.16 cd	0.21 b	0.21 e	0.16 c	0.13 c	0.14 c
	Micron Nutrients	0.14 abcd	0.17 cd	0.20 b	0.19 e	0.14 c	0.15 c	0.11 c

Table A.15: The impact of eight biostimulants (treatment) on relative anthocyanin absorbance of two blueberry varieties of SHB type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

Treatment	Least squares mean						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Fertiactyl GZ	0.13 a	0.32 a	0.56 a	0.74 a	0.44 ab	0.23 ab	0.18 ab
Quantum O.T	0.14 a	0.35 a	0.51 a	0.69 a	0.41 abc	0.25 ab	0.22 a
Terra Grow	0.14 a	0.33 a	0.55 a	0.65 ab	0.45 a	0.29 a	0.22 a
Inocucor	0.14 a	0.31 a	0.58 a	0.59 ab	0.31 abc	0.19 ab	0.18 ab
Huma Gro	0.14 a	0.34 a	0.60 a	0.58 ab	0.29 abc	0.20 ab	0.16 ab
Soil Set	0.13 a	0.23 a	0.52 a	0.55 ab	0.31 abc	0.22 ab	0.20 ab
Control	0.15 a	0.38 a	0.64 a	0.53 ab	0.23 abc	0.19 ab	0.16 ab
Micron Nutrients	0.14 a	0.28 a	0.53 a	0.52 ab	0.20 bc	0.15 ab	0.13 b
Radiate	0.15 a	0.31 a	0.51 a	0.43 b	0.18 c	0.13 b	0.15 ab

Table A.16: Mean abaxial flavonols content (relative absorbance) in two blueberry varieties of RE and SHB types in response to eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison shows treatment-cultivar interaction effect and is valid by month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Huma Gro	0.97 a	1.22 a	1.43 a	1.51 a	1.26 a	1.29 a	1.45 a
	Micron Nutrients	0.95 a	1.16 a	1.41 a	1.54 a	1.29 a	1.22 a	1.25 a
	Control	0.94 a	1.21 a	1.41 a	1.49 a	1.32 a	1.22 a	1.45 a
	Inocucor	0.89 a	1.20 a	1.33 a	1.46 a	1.26 a	1.16 a	1.24 a
	Fertiactyl GZ	0.89 a	1.19 a	1.39 a	1.35 a	1.21 a	1.09 a	1.17 a
	Terra Grow	0.85 a	1.21 a	1.35 a	1.49 a	1.48 a	1.35 a	1.44 a
	Radiate	0.85 a	1.24 a	1.33 a	1.45 a	1.39 a	1.10 a	1.23 a
	Quantum O.T	0.83 a	1.09 a	1.26 a	1.48 a	1.32 a	1.14 a	1.24 a
	Soil Set	0.83 a	1.20 a	1.39 a	1.39 a	1.19 a	1.26 a	1.47 a
Farthing	Soil Set	0.87 a	0.01 a	1.32 a	1.32 a	1.38 a	1.36 a	1.26 a
	Terra Grow	0.86 a	1.08 a	1.31 a	1.44 a	1.35 a	1.31 a	1.34 a
	Control	0.83 a	1.05 a	1.23 a	1.37 a	1.42 a	1.24 a	1.18 a
	Radiate	0.83 a	1.03 a	1.14 a	1.20 a	1.28 a	1.19 a	1.25 a
	Micron Nutrients	0.82 a	1.04 a	1.26 a	1.38 a	1.16 a	1.18 a	1.25 a
	Inocucor	0.82 a	1.03 a	1.14 a	1.39 a	1.29 a	1.24 a	1.20 a
	Fertiactyl GZ	0.81 a	0.99 a	1.24 a	1.32 a	1.29 a	1.18 a	1.20 a
	Quantum O.T	0.80 a	1.04 a	1.24 a	1.26 a	1.18 a	1.16 a	1.24 a
	Huma Gro	0.76 a	1.03 a	1.30 a	1.26 a	1.27 a	1.24 a	1.31 a
Legacy	Micron Nutrients	0.93 a	1.46 a	1.60 a	1.65 ab	1.44 bc	1.39 ab	1.47 a
	Inocucor	0.95 a	1.43 ab	1.73 a	1.89 a	1.80 a	1.50 ab	1.56 a
	Quantum O.T	0.99 a	1.41 ab	1.56 a	1.74 ab	1.55 abc	1.36 ab	1.46 a
	Fertiactyl GZ	0.91 a	1.40 ab	1.59 a	1.69 ab	1.73 ab	1.62 a	1.63 a
	Huma Gro	0.94 a	1.40 ab	1.62 a	1.63 ab	1.63 abc	1.41 ab	1.45 a
	Terra Grow	0.90 a	1.34 ab	1.63 a	1.69 ab	1.52 abc	1.54 ab	1.58 a
	Radiate	0.94 a	1.34 ab	1.51 a	1.55 b	1.31 c	1.21 b	1.32 a
	Control	0.94 a	1.34 ab	1.71 a	1.65 ab	1.48 abc	1.46 ab	1.41 a
	Soil Set	0.88 a	1.20 b	1.52 a	1.56 b	1.50 abc	1.43 ab	1.40 a
Premier	Terra Grow	1.09 a	1.39 a	1.56 a	1.72 a	1.55 a	1.34 a	1.54 a
	Fertiactyl GZ	1.08 a	1.41 a	1.68 a	1.62 a	1.71 a	1.63 a	1.51 a
	Huma Gro	1.08 a	1.32 a	1.56 a	1.64 a	1.61 a	1.51 a	1.26 a
	Radiate	1.07 a	1.20 a	1.51 a	1.71 a	1.54 a	1.36 a	1.38 a
	Soil Set	1.05 a	1.30 a	1.61 a	1.67 a	1.53 a	1.41 a	1.46 a
	Micron Nutrients	1.02 a	1.26 a	1.59 a	1.64 a	1.67 a	1.41 a	1.44 a
	Quantum O.T	0.98 a	1.25 a	1.61 a	1.63 a	1.51 a	1.34 a	1.47 a
	Inocucor	0.96 a	1.29 a	1.47 a	1.64 a	1.53 a	1.43 a	1.41 a
	Control	0.93 a	1.27 a	1.52 a	1.72 a	1.70 a	1.57 a	1.45 a

Table A.17: The impact of treatment-variety interactions on relative flavonols absorbance of two blueberry varieties of RE type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

CV	Treatment	Least square means						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Micron Nutrients	1.85 a	2.02 a	2.18 ab	2.11 a	2.03 a	1.94 a	2.03 ab
	Huma Gro	1.80 a	2.10 a	2.18 ab	2.15 a	1.93 a	1.96 a	2.01 ab
	Control	1.79 a	2.10 a	2.19 ab	2.15 a	2.02 a	2.01 a	2.11 ab
	Inocucor	1.78 a	2.01 a	2.17 ab	2.15 a	1.98 a	1.88 a	1.92 ab
	Fertiactyl GZ	1.78 a	1.97 a	2.18 ab	2.11 a	1.90 a	1.75 a	1.82 b
	Soil Set	1.60 a	2.13 a	2.23 a	2.10 a	1.91 a	2.00 a	2.13 a
	Radiate	1.70 a	2.09 a	2.21 ab	2.16 a	2.02 a	1.84 a	1.91 ab
	Terra Grow	1.66 a	1.89 a	2.07 b	2.14 a	2.02 a	1.91 a	1.95 ab
	Quantum O.T	1.66 a	1.98 a	2.11 ab	2.14 a	2.01 a	1.93 a	1.93 ab
Premier	Micron Nutrients	1.74 a	1.98 a	2.20 ab	2.09 a	2.01 a	1.90 a	1.99 ab
	Soil Set	1.68 a	1.91 a	2.17 ab	2.04 a	2.04 a	1.98 a	2.09 ab
	Terra Grow	1.80 a	2.05 a	2.14 ab	2.09 a	1.99 a	1.96 a	2.07 ab
	Inocucor	1.54 a	1.96 a	2.16 ab	2.13 a	2.07 a	2.03 a	2.06 ab
	Fertiactyl GZ	1.75 a	2.05 a	2.21 ab	2.10 a	2.07 a	2.01 a	2.06 ab
	Control	1.62 a	1.95 a	2.20 ab	2.13 a	2.05 a	2.03 a	2.02 ab
	Huma Gro	1.74 a	2.03 a	2.21 ab	2.11 a	2.07 a	2.04 a	1.89 ab
	Radiate	1.79 a	1.97 a	2.20 ab	2.06 a	2.08 a	1.99 a	2.05 ab
	Quantum O.T	1.61 a	2.01 a	2.20 ab	2.08 a	2.01 a	1.90 a	1.95 ab

Table A.18: The impact of eight biostimulants (treatment) on relative flavonols absorbance of two blueberry varieties of RE type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

Treatment	Least squares mean						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Micron Nutrients	1.79 a	2.00 a	2.19 ab	2.10 a	2.02 a	1.92 a	2.01 a
Huma Gro	1.77 a	2.06 a	2.19 ab	2.13 a	2.00 a	2.00 a	1.95 a
Fertiactyl GZ	1.77 a	2.01 a	2.19 ab	2.10 a	1.99 a	1.88 a	1.94 a
Radiate	1.74 a	2.03 a	2.20 a	2.11 a	2.05 a	1.92 a	1.98 a
Terra Grow	1.73 a	1.97 a	2.11 b	2.11 a	2.00 a	1.94 a	2.01 a
Control	1.71 a	2.02 a	2.19 ab	2.14 a	2.03 a	2.02 a	2.06 a
Inocucor	1.66 a	1.98 a	2.16 ab	2.14 a	1.98 a	1.95 a	1.99 a
Soil Set	1.64 a	2.02 a	2.20 ab	2.07 a	1.97 a	1.99 a	2.11 a
Quantum O.T	1.64 a	1.99 a	2.15 ab	2.11 a	2.03 a	1.92 a	1.94 a

Table A.19: The impact of treatment-variety interactions on relative flavonols absorbance of two blueberry varieties of SHB type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

CV	Treatment	Least square mean						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Legacy	Fertiactyl GZ	1.67 ab	2.18 a	2.24 a	2.09 abcd	2.14 a	2.09 ab	2.16 a
	Quantum O.T	1.83 a	2.22 a	2.24 a	2.10 abcd	1.97 ab	2.02 ab	2.15 ab
	Terra Grow	1.74 ab	2.15 abcd	2.23 ab	2.09 abcd	1.98 ab	2.12 ab	2.17 a
	Huma Gro	1.68 ab	2.19 a	2.22 ab	2.09 abcd	2.08 ab	1.99 ab	2.05 abc
	Inocucor	1.68 ab	2.18 a	2.26 a	2.07 abcd	2.12 ab	2.06 ab	2.07 abc
	Micron Nutrients	1.71 ab	2.17 abc	2.25 a	2.11 abc	1.94 ab	2.07 ab	2.07 abc
	Soil Set	1.65 ab	2.04 abcde	2.19 abc	2.17 a	2.10 ab	2.15 a	2.14 abc
	Control	1.75 ab	2.17 ab	2.26 a	2.13 ab	2.07 ab	2.14 a	2.10 abc
	Radiate	1.66 ab	2.19 a	2.24 a	2.17 a	1.86 b	1.97 ab	2.12 abc
Farthing	Quantum O.T	1.53 b	1.92 e	2.10 abcd	2.05 abcd	2.03 ab	1.99 ab	2.00 abc
	Fertiactyl GZ	1.74 ab	1.95 de	2.11 abcd	2.07 abcd	2.07 ab	2.01 ab	1.99 abc
	Soil Set	1.71 ab	1.96 de	2.13 abcd	1.96 d	2.07 ab	2.03 ab	2.10 abc
	Control	1.75 ab	1.96 de	2.14 abcd	2.08 abcd	2.12 ab	2.05 ab	1.99 abc
	Radiate	1.71 ab	1.92 e	2.02 d	2.01 bcd	2.08 ab	1.99 ab	1.97 abc
	Terra Grow	1.65 ab	1.97 cde	2.06 cd	1.98 cd	2.01 ab	1.98 ab	2.06 abc
	Inocucor	1.72 ab	1.84 e	2.06 cd	2.04 abcd	2.03 ab	1.99 ab	1.89 bc
	Huma Gro	1.54 b	1.98 bcde	2.16 abcd	2.07 abcd	1.96 ab	1.88 b	1.88 c
	Micron Nutrients	1.75 ab	1.97 cde	2.07 bcd	2.05 abcd	1.93 ab	1.95 ab	1.90 bc

Table A.20: The impact of eight biostimulants (treatment) on relative flavonols absorbance of two blueberry varieties of SHB type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

Treatment	Least squares mean						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Fertiactyl GZ	1.70 a	2.07 a	2.17 a	2.08 a	2.11 a	2.05 a	2.07 a
Quantum O.T	1.69 a	2.07 a	2.17 a	2.08 a	2.00 a	2.01 a	2.08 a
Terra Grow	1.69 a	2.06 a	2.14 a	2.03 a	1.99 a	2.05 a	2.11 a
Inocucor	1.70 a	2.01 a	2.16 a	2.06 a	2.07 a	2.02 a	1.98 a
Huma Gro	1.61 a	2.08 a	2.19 a	2.08 a	2.02 a	1.93 a	1.97 a
Soil Set	1.68 a	1.99 a	2.16 a	2.07 a	2.08 a	2.09 a	2.12 a
Control	1.75 a	2.07 a	2.20 a	2.10 a	2.10 a	2.09 a	2.05 a
Micron Nutrients	1.73 a	2.07 a	2.16 a	2.08 a	1.94 a	2.01 a	1.98 a
Radiate	1.69 a	2.06 a	2.13 a	2.09 a	1.97 a	1.98 a	2.04

Table A.21: Mean abaxial NBI index in two blueberry varieties of RE and SHB types in response to eight biostimulants. Means with same letter suffixes are not significantly different from each other. Comparison shows treatment-cultivar interaction effect and is valid by month

CV	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Quantum O.T	25.0 a	18.8 a	13.1 a	12.0 ab	23.4 a	29.5 a	28.0 a
	Soil Set	21.6 ab	12.8 a	9.5 a	14.5 ab	21.9 a	24.0 a	20.7 a
	Terra Grow	21.5 ab	17.9 a	13.0 a	13.4 ab	18.8 a	24.7 a	25.1 a
	Radiate	21.2 ab	12.1 a	10.5 a	11.0 b	22.9 a	27.7 a	27.6 a
	Micron Nutrients	21.0 ab	16.6 a	11.3 a	13.1 ab	24.7 a	28.3 a	26.1 a
	Inocucor	19.8 ab	14.8 a	11.7 a	15.9 a	25.8 a	25.9 a	28.4 a
	Fertiactyl GZ	18.7 ab	13.7 a	9.5 a	15.6 ab	26.2 a	30.0 a	27.9 a
	Control	17.4 b	13.2 a	10.4 a	13.3 ab	22.0 a	24.7 a	22.5 a
	Huma Gro	17.0 b	13.7 a	9.9 a	14.9 ab	24.5 a	23.6 a	22.4 a
Farthing	Huma Gro	29.1 a	22.6 a	16.0 a	14.8 a	20.1 a	25.4 a	26.3 a
	Quantum O.T	27.4 a	21.7 a	16.7 a	12.5 a	19.2 a	24.0 a	21.7 a
	Terra Grow	25.7 a	19.9 a	15.8 a	12.2 a	17.8 a	23.9 ab	20.2 a
	Micron Nutrients	25.5 a	22.7 a	15.1 a	14.3 a	20.8 a	22.8 ab	28.2 a
	Fertiactyl GZ	28.6 a	23.1 a	16.1 a	13.5 a	18.4 a	22.1 ab	23.9 a
	Radiate	26.0 a	23.2 a	16.9 a	12.2 a	14.9 a	22.1 ab	21.3 a
	Inocucor	27.4 a	23.3 a	18.7 a	13.0 a	19.1 a	20.6 ab	24.4 a
	Control	27.5 a	22.8 a	16.4 a	12.1 a	15.6 a	19.5 ab	25.1 a
	Soil Set	27.0 a	25.2 a	15.5 a	12.6 a	14.0 a	16.1 b	21.3 a
Legacy	Soil Set	22.3 a	14.7 a	8.1 a	10.8 ab	14.7 abc	16.3 ab	16.5 a
	Terra Grow	21.4 a	11.6 ab	7.2 a	8.5 b	14.7 abc	13.0 ab	14.5 a
	Radiate	17.5 a	11.1 ab	8.1 a	13.7 a	22.7 a	23.7 a	20.6 a
	Control	18.5 a	11.1 ab	6.3 a	9.8 ab	16.3 abc	15.2 ab	18.5 a
	Inocucor	19.2 a	10.8 ab	7.1 a	7.3 b	11.5 bc	17.1 ab	17.4 a
	Fertiactyl GZ	21.2 a	10.8 ab	7.8 a	6.3 b	8.4 c	11.8 b	12.3 a
	Huma Gro	18.1 a	10.8 ab	6.5 a	7.9 b	12.7 bc	18.0 ab	19.0 a
	Quantum O.T	18.4 a	10.5 ab	8.5 a	7.8 b	15.9 abc	18.7 ab	16.0 a
	Micron Nutrients	20.8 a	9.8 b	7.8 a	10.5 ab	18.8 ab	20.2 ab	18.2 a
Premier	Control	19.9 a	13.9 a	8.5 a	10.2 a	16.9 a	18.0 a	20.1 a
	Quantum O.T	18.8 a	13.0 a	7.8 a	10.7 a	19.6 a	25.1 a	21.7 a
	Radiate	18.5 a	16.6 a	10.5 a	10.7 a	18.0 a	21.7 a	22.3 a
	Huma Gro	18.4 a	12.6 a	9.6 a	11.9 a	18.7 a	21.2 a	24.2 a
	Inocucor	18.3 a	12.9 a	10.5 a	10.3 a	16.9 a	19.7 a	19.9 a
	Fertiactyl GZ	17.9 a	11.9 a	7.8 a	11.3 a	17.0 a	18.9 a	22.2 a
	Terra Grow	17.8 a	13.2 a	9.7 a	10.5 a	22.0 a	24.5 a	20.9 a
	Micron Nutrients	17.2 a	13.0 a	8.3 a	9.9 a	16.7 a	23.0 a	20.1 a
	Soil Set	16.1 a	11.6 a	7.2 a	9.9 a	18.5 a	19.6 a	22.1 a

Table A.22: The impact of treatment-variety interactions on NBI index in two blueberry varieties of RE type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

CV	Treatment	Least square means						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Brightwell	Terra Grow	11.6 a	11.64 a	8.3 a	9.43 abc	13.9 a	17.4 ab	17.6 a
	Quantum O.T	12.3 a	10.04 ab	7.6 ab	8.46 abc	15.3 a	17.2 ab	18.0 a
	Micron Nutrients	10.7 a	8.93 ab	7.3 ab	9.70 abc	15.5 a	17.1 ab	15.9 a
	Inocucor	10.1 a	8.90 ab	7.0 a	10.72 a	17.2 a	15.2 ab	17.6 a
	Fertiactyl GZ	9.4 a	8.78 ab	6.4 ab	10.07 ab	16.2 a	19.0 a	17.0 a
	Huma Gro	9.4 a	8.02 b	6.2 ab	10.72 a	15.6 a	15.7 ab	15.9 a
	Radiate	11.0 a	7.50 b	6.3 ab	7.18 c	15.7 a	16.4 ab	17.5 a
	Control	9.4 a	7.47 b	6.3 ab	8.99 abc	14.8 a	14.8 ab	14.8 a
	Soil Set	11.5 a	7.45 b	5.8 b	9.59 abc	14.0 a	14.7 ab	14.4 a
Premier	Radiate	11.2 a	10.19 ab	7.3 ab	9.12 abc	13.5 a	15.1 ab	15.0 a
	Control	12.0 a	9.08 ab	5.9 b	8.68 abc	14.0 a	14.3 ab	14.6 a
	Inocucor	11.6 a	8.77 ab	6.9 ab	7.98 bc	12.6 a	16.3 ab	13.9 a
	Micron Nutrients	10.5 a	8.52 ab	5.9 b	7.86 bc	14.0 a	16.3 ab	14.7 a
	Terra Grow	11.1 a	8.49 ab	6.8 ab	8.84 abc	16.5 a	16.2 ab	15.3 a
	Fertiactyl GZ	11.3 a	8.38 ab	5.6 b	8.78 abc	14.2 a	15.0 ab	15.7 a
	Huma Gro	11.09 a	8.30 b	6.8 ab	9.26 abc	14.2 a	15.4 ab	16.3 a
	Soil Set	10.5 a	8.29 ab	5.4 b	9.12 abc	13.4 a	13.9 b	15.0 a
	Quantum O.T	11.6 a	8.21 b	6.0 b	8.96 abc	15.1 a	17.3 ab	16.9 a

Table A.23: The impact of eight biostimulants (treatment) on NBI index on two blueberry varieties of RE type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

Treatment	Least squares mean						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Terra Grow	11.3 a	10.1 a	7.60 a	9.14 ab	15.2 a	16.8 a	16.4 a
Inocucor	10.9 a	8.8 ab	6.93 ab	9.35 ab	14.9 a	14.6 a	15.8 a
Radiate	11.1 a	8.8 ab	6.79 ab	8.15 b	14.6 a	15.7 a	16.2 a
Quantum O.T	12.0 a	9.1 ab	6.79 ab	8.71 ab	15.2 a	17.3 a	17.5 a
Micron Nutrients	10.6 a	8.7 ab	6.58 ab	8.78 ab	14.8 a	16.7 a	15.3 a
Huma Gro	10.6 a	8.2 ab	6.52 ab	9.99 a	14.9 a	15.5 a	16.1 a
Control	10.7 a	8.3 ab	6.12 b	8.83 ab	14.4 a	14.6 a	14.7 a
Fertiactyl GZ	10.4 a	8.6 ab	6.01 b	9.43 ab	15.2 a	17.0 a	16.4 a
Soil Set	11.0 a	7.9 b	5.61 b	9.00 ab	13.7 a	14.3 a	14.7 a

Table A.24: The impact of treatment-variety interactions NBI index on two blueberry varieties of SHB type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

CV	Treatment	Least square means						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Legacy	Radiate	10.1 b	6.9 c	5.2 b	9.85 a	15.84 a	14.48 abc	12.6 abcd
	Micron Nutrients	11.7 ab	6.9 c	5.7 b	8.39 abcd	13.28 ab	12.87 abcd	13.3 abcd
	Control	10.2 b	6.9 c	4.8 b	7.66 abcd	11.62 abc	10.42 bcd	12.5 bcd
	Terra Grow	11.3 ab	7.0 c	5.3 b	7.22 abcd	9.81 bc	8.24 d	9.6 d
	Soil Set	11.9 ab	8.7 bc	5.6 b	6.78 bcd	10.60 abc	10.84 abcd	10.7 cd
	Inocucor	10.9 b	6.9 c	5.4 b	6.72 bcd	9.74 bc	11.50 abcd	12.2 bcd
	Quantum O.T	10.4 b	7.0 c	5.7 b	6.59 cd	11.18 abc	10.97 abcd	10.1 cd
	Huma Gro	10.5 b	6.8 c	4.9 b	6.40 cd	9.58 bc	11.57 abcd	12.9 abcd
	Fertiactyl GZ	11.9 ab	7.4 c	5.4 b	5.53 d	6.63 c	8.89 cd	10.0 cd
Farthing	Micron Nutrients	12.7 ab	11.7 ab	9.2 a	9.51 ab	12.47 ab	14.22 abc	18.5 a
	Huma Gro	15.1 a	11.7 ab	9.1 a	8.89 abc	13.09 ab	16.54 a	17.8 ab
	Inocucor	13.6 ab	12.9 a	10.4 a	8.82 abc	11.58 abc	13.16 abcd	15.9 abc
	Soil Set	13.8 ab	12.9 a	9.4 a	8.79 abc	9.35 bc	10.88 abcd	12.9 abcd
	Terra Grow	13.8 ab	10.8 ab	9.6 a	8.76 abc	11.71 abc	15.62 ab	13.4 abcd
	Fertiactyl GZ	13.6 ab	11.7 ab	9.3 a	8.18 abcd	11.05 abc	13.62 abcd	14.5 abcd
	Control	13.6 ab	11.7 ab	9.5 a	8.06 abcd	10.34 abc	11.68 abcd	15.3 abcd
	Quantum O.T	15.2 a	11.8 ab	9.7 a	7.76 abcd	11.11 abc	13.90 abcd	13.8 abcd
	Radiate	13.0 ab	12.2 a	9.6 a	7.71 abcd	9.47 bc	13.64 abcd	14.2 abcd

Table A.25: The impact of eight biostimulants (treatment) on NBI index in two blueberry varieties of SHB type. Means with same letter suffixes are not significantly different from each other. Comparison is valid only within a month

Treatment	Least squares mean						
	Jan	Feb	Mar	Apr	May	Jun	Jul
Micron Nutrients	12.2 a	9.2 a	7.5 a	8.95 a	12.88 a	13.5 a	15.92 a
Radiate	11.6 a	9.5 a	7.4 a	8.78 a	12.65 a	14.0 a	13.44 abc
Terra Grow	12.5 a	8.9 a	7.5 a	7.99 ab	10.76 ab	11.9 a	11.52 c
Control	11.9 a	9.3 a	7.1 a	7.86 ab	10.98 ab	11.0 a	13.90 abc
Soil Set	12.8 a	10.8 a	7.5 a	7.79 ab	9.98 ab	10.9 a	11.82 bc
Inocucor	12.3 a	9.9 a	7.9 a	7.77 ab	10.66 ab	12.3 a	14.06 abc
Huma Gro	12.8 a	9.3 a	7.0 a	7.64 ab	11.33 ab	14.0 a	15.37 abc
Quantum O.T	12.8 a	9.4 a	7.7 a	7.17 ab	11.15 ab	12.4 a	11.96 bc
Fertiactyl GZ	12.7 a	9.6 a	7.4 a	6.85 b	8.84 b	11.2 a	12.26 abc