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Biostimulants

– effects on root development in barley (*Hordeum vulgare)*

Biostimulanter – effekter på rotutvecklingen hos korn (Hordeum vulgare)

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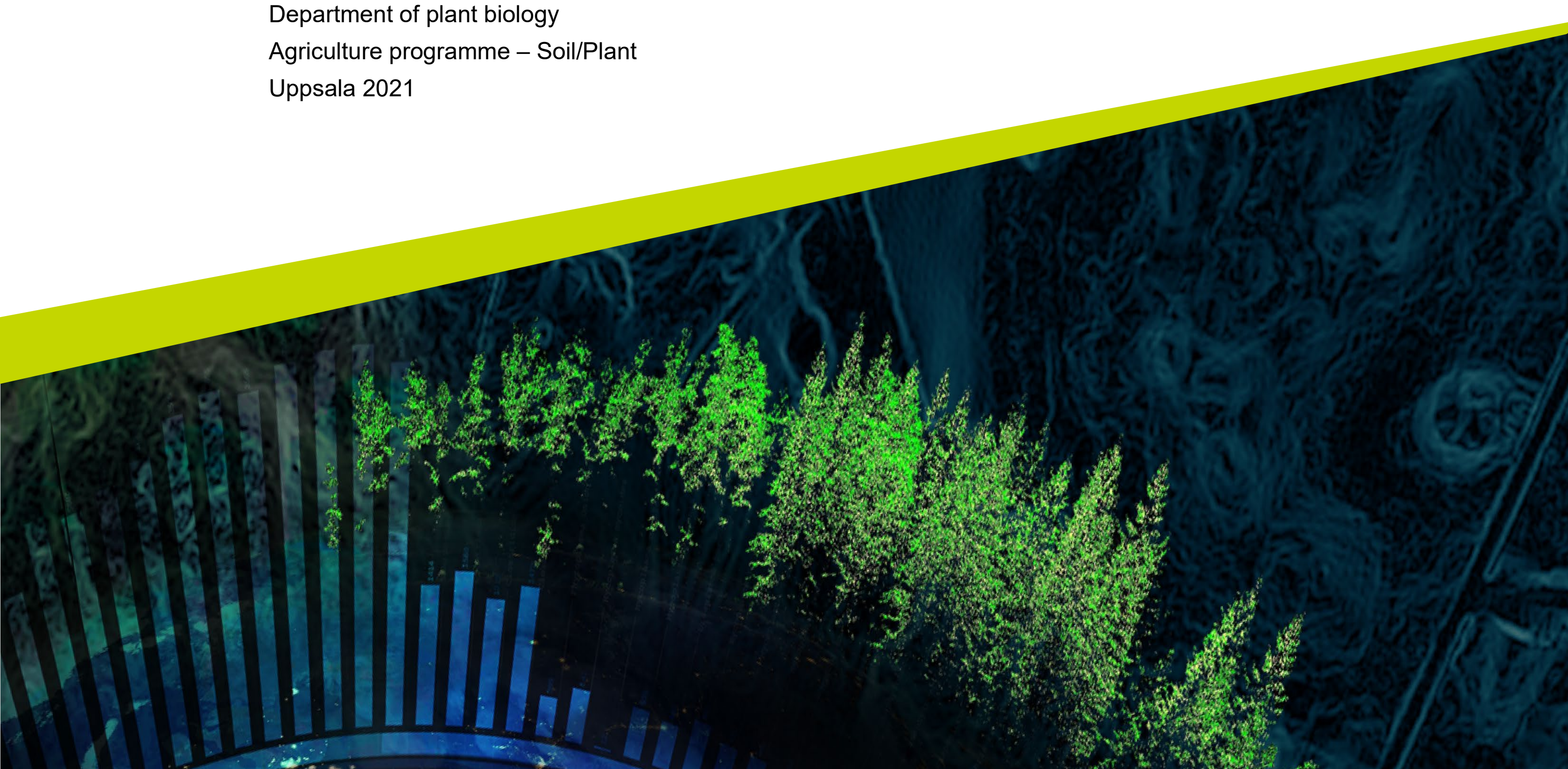

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Abstract

The use of biostimulants has increased greatly in the last 20 years. In year 2012 over 6 million hectares in Europe was treated with biostimulants and the market is expected to almost double in the coming 5 years compared to present levels. Biostimulants are used in a wide range of crops and new biostimulant products are continuously appearing on the market. The effect of the biostimulants is often debated but sales agencies promote their products intensely as yield increasing and profitable. This thesis aimed to study the effect of four different biostimulants on the root development of barley (*Hordeum vulgare*) and to investigate if root development has any correlation with grain yield. The four products tested were Physiolith, Demetias V, Stimplex and Quantis. Stimplex and Demetias V are based on seaweed extracts while Quantis is produced by fermentation of sugar cane and Physiolith is based on aminopurins. To study the root development, root samples were collected in barley field trials at two locations (Örberga and Borgeby, Sweden) that had been treated with the biostimulants. The samples were flushed clean from soil and analysed for length, fresh weight and dry weight. Grain yield data was later acquired when the trial plots were harvested. The results showed very small differences between the biostimulants and the control. The only significant results found were for root length where Stimplex and Quantis treated plants showed approximately 7 % longer roots than the control. Noticeable was that there were no significant differences in grain yield between the tested biostimulants and the control, and that the increased root length from Stimplex and Quantis did not seem to affect the grain yield. The results of this study are well in line with investigations showing that biostimulants tested under field conditions may not reach expectations of improved crop properties raised from tests conducted in controlled environments.

Keywords: Biostimulants, root development, barley, root length, root fresh weight, root dry weight, grain yield

Popular Science Summary

Biostimulants are products that are applied to crops to increase plant growth and yield. These products do not primarily consist of nutrients but rather compounds such as amino acids, hormones or microbes that affect the growth and yield of the crops. Some of these compounds enhance the uptake of nutrients such as nitrogen (N), potassium (K), phosphorus (P) and several other compounds. The biostimulants are often used as a complement to fertilizers. Even if the biostimulant enhances the crop's ability to use the naturally existing nutrients, this is seldom sufficient to achieve a good yield. With the increased demand for food to feed a growing global population with a limited amount of arable land, the use of biostimulants has increased a lot during the last decades. The global use of biostimulants increases with 11 % every year. With the increased market for biostimulants follows a wide spectrum of new biostimulants produced by companies who see a potential new profitable market. The effect of the biostimulants is sometimes questionable and seems to differ from crop to crop and between locations. It is important to evaluate the effects of biostimulants for several reasons. Firstly, it is important for the farmers to estimate if the products they are planning to use on their crop has any economical benefit. If the cost of the product is larger than the value of the potential increase in yield the farmer most likely will lose money. Secondly, it is a waste of resources if biostimulants are used on crops where they have no benefits when they instead could be used on crops where they actually would make a difference.

The aim with this thesis was to evaluate if four different biostimulants affect the root development of barley and if there exists is any correlation between root development and grain yield. The tested biostimulants were Physiolith, Demetias V, Stimplex and Quantis. Barley is a cereal and the fourth most important crop globally when it comes to produced volume. It can be used as food for human consumption, but the largest volumes go as animal feed or as malt for brewing. In this study barley plants with roots were collected in two field trials treated with biostimulants. The soil was washed away with water and the roots removed from the stem. The roots were later measured for length, root fresh weight and root dry weight. The field where the roots came from were also harvested, and the grain yield measured.

The results of this study showed that the four tested biostimulants had very little, or no, effect on the root development. Only Stimplex and Quantis showed significantly longer roots than the control, ca 7 %. There were no significant effects on the grain yield for any of the biostimulants. The results from this study shows that the tested biostimulants did not display any substantial benefits on the barley under the conditions they were tested and that farmers might not gain economically by using them on barley. Given that, it is however possible that the result would differ if the growing conditions would be different for the barley or if another cultivar was used. More experiments and research are needed in order to get a better understanding of factors that affect in-field performance of biostimulants on crop plants.

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1. Introduction

1.1. Biostimulants

During the last decades the use of biostimulants in crop production has increased substantially. Only in Europe over 6 million hectares were treated with biostimulants in 2012 (Calvo et al. 2014). In 2021, the market value for biostimulants in Europe was $906 million and is growing with 11 % per year. In 2026 the market value is expected to reach $1500 million (Market Data Forecast 2021). There are several definitions of biostimulants. The European Biostimulants Industry Council (EBIC) has defined biostimulants as "*containing substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality*" (EBIC, 2012). The equivalent organisation in America, the Biostimulant Coalition defines biostimulants as "*Substances, including microorganisms, that are applied to plant, seed, soil other growing media that may enhance the plants' ability to assimilate applied nutrients or provide benefits to plant development. Biostimulants are not plant nutrients and therefore may not make any nutrient claims or guarantees*" (Biostimulant Coalition 2021). Since EBICs definition does not exclude nutrients as the Biostimulant Coalitions definition does, they have added "*Biostimulants operate through different mechanisms than fertilisers, regardless of the presence of nutrients in the products*" to the definition, since many biostimulant products also contains nutrients to different degrees (Calvo et al. 2014). In 2019 the EU resolved a new regulation that biostimulants will become a product category on its own by the 16th of July 2022. Then it will also be possible for biostimulant products to get CE-marking. A CE marking shows that the product follows all the regulations set by the EU, but it will not be a requirement for selling biostimulants in EU (European Parliament 2019). The most common biostimulant categories are microbial inoculants, humic acids, fulvic acids, protein hydrolysates and amino acids, and seaweed extracts (Calvo et al. 2014)

1.1.1. Microbial inoculants

Microbial inoculants are living microorganisms such as fungi and bacteria. *Pseudomonas spp, Streptomyces spp, Trichoderma spp* and *Bacillus spp* are some of the most used microbial inoculants (Alori et al. 2017). These can be applied both to the soil and directly to plant surfaces. Microbial inoculants can influence the growth of the plant in several ways. By providing provide better nutrition efficiency and uptake, as well by stimulating formation of larger roots with more extensive root systems. Products that contain microbial components can also be classified as biopesticides or biofertilizers. A well-studied type of microbial inoculants is plant growth-promoting rhizobacteria (PGPR), that thrive in the rhizosphere around the roots. PGPR is regarded as an ecosystem service with great potential for crop production. When applying microbial products it is very important to consider the species and variety of the plant to become inoculated. One reason for this is that different species and varieties produce different root exudates, which will affect the microbial inoculant as well as the soil microbiota. A big challenge with microbial inoculants is to keep the cells alive in the formulation until the application. Further, the microbes must be able to cope with fertilizers and chemicals in conventional agriculture as well as to show high rhizosphere competence not to be outcompeted by the natural soil microbiota present (Calvo et al. 2014).

1.1.2. Humic acids

Humic acids are substances that are formed due to microbial decomposition of plant and animal matter (Canellas et al. 2015). Humic acids generally have high molecular mass and the structure varies depending on the speed of the transformation and the structure of the original material (Calvo et al. 2014). Humic acids mainly consists of hydrophobic compounds that are stabilized at neutral pH by hydrophobic dispersive forces. If the humic acid is exposed to acidic media it starts to precipitate (Canellas et al. 2015). Many of the most common crops have displayed some kind of increased growth when humic acids have been applied under controlled conditions (i.e. growth chamber or hydroponic conditions). There are also some studies that show increased yield under field conditions. Among some crops such as wheat and maize (*Zea mays*) the development of the root system has increased, due to larger lateral roots and/or a more developed root system at the early seedling stages. The ability of humic acids to chelate ions increases plants ability to take up nutrients. Plant physiology and metabolism can also be affected by humic acids. Some studies have showed that humic acids can affect the H^+-ATPase activity. For example humic acids can trigger the H^+-ATPase to create acidic conditions in the root apoplast, which results in loosening of cell walls and allow root cells to elongate (Calvo et al. 2014).

1.1.3. Fulvic acids

Fulvic acids are also humic substances as the humic acids but many commercial biostimulant manufacturers mark their products as one of either. Compared to the humic acids, fulvic acids have lower molecular weights and can withstand acidic conditions (Calvo et al. 2014). In contrast to humic acids, the fulvic acids are able to pass micropores of biological membrane systems due to their smaller sizes. This ability gives fulvic acids the capability to move nutrients and specially micronutrients into the plant cells (Bocanegra et al. 2006). Rauthan & Schnitzers (1981) study on cucumber grown in solution showed increased uptake of N, P, K, Ca, Mg, Cu, Fe and Mn when fulvic acids were added to the solution. Several studies reports that fulvic acids can have effect on the root system of crops. Some examples are increased numbers of root initials on the hypocotyl of common bean, longer roots in maize and longer lateral roots in tomato (Calvo et al. 2014).

1.1.4. Protein hydrolysates and amino acids

Protein hydrolysates and amino acids are protein-based molecules that can be used as biostimulants (Ertani et al. 2009). Protein hydrolysates are produced from hydrolysis (chemical, enzymatic or thermal) of different kind of organic matters, both plant and animal-based. The result of the hydrolysis is a mixture of peptides and amino acids (Cavani et al. 2006). Some biostimulants contain amino acids only, such as alanine, glycine, proline, glutamate and glutamine (Calvo et al. 2014). Exogenous amino acids that are added to plants can serve as signalling molecules or as protection against abiotic stresses (Sharma & Dietz 2006). A study conducted by Fan et al. (2006) showed that glutamine affects the uptake of nitrogen by barley roots. Glutamine can act as a signalling molecule that regulates the nitrate uptake systems. If the size of the glutamine (and some other amino acids) pool is larger than normal it might signal to the plant that the nitrogen status is too high and that the nitrate uptake and assimilation needs to be altered (Fan et al. 2006). Glycine and proline are two examples of amino acids that can function as osmolytes and protect plant cells from high temperatures and high salt concentrations (Calvo et al. 2014).

1.1.5. Seaweed extracts

Man has used seaweed as a soil conditioner improvement for at least a millennium, both by first composting seaweed or applying it directly to the soil (Calvo et al. 2014). Before the mechanization of the agriculture it was mainly fields close to the costal lines that were "fertilized" with seaweed due to the labour intensive work connected with transport of seaweed (Craigie 2011). In the 1950s the first liquid seaweed extracts for agricultural use were produced and became available on the market (Calvo et al. 2014). In 2012 the market value of biostimulants based on seaweed extracts was valued to $412 million, and by 2019 it has increased to $944 million (Stirk et al. 2020). Seaweed extracts can both act as chelators and biostimulants depending on their properties. Chelators improve soil structure, which is beneficial for root growth, and also enhance the uptake of mineral ions for the plants. The current research suggests that the positive effects of biostimulants that originate from seaweed extracts is attributed to two factors. Firstly, it is the presence of growth hormones and low molecular weight compounds. Secondly, special seaweed polysaccharides and polyphenols make the plants more resistant to stress and serve as allelochemicals (Calvo et al. 2014). Presently it is mainly brown seaweeds that are used in biostimulants. Common species are *Sargassum* spp*.,* *Laminaria* spp,. *Ecklonia maxima* and *Ascophyllum nodosum,* some non-brown seaweed species who also are used in biostimulants are *Fucus serrathus* and *Enteromorpha intestinalis.* The colour of the extracts has a wide span from dark brown to almost colourless, other properties such as viscosities, particle matter content and odors also vary between extracts (Craigie 2011).

The dominant procedure to produce seaweed extracts is trough alkali extraction. The seaweed is first milled or disrupted by high pressure to create a mildly acidic suspension. To the suspension is then added acids, alkalis or water to create the extracts. In some cases, high temperatures are used to speed up the production of extracts. Another procedure is "cold cell burst", where high pressure cracks the cell membranes and the cytosolic components are released. It is then possible to collect these cytosolic components through different methods and make extracts from these. When extraction is final, the extract is dried to a powder or is prepared as a solution with pH 7-10 to create the biostimulant products. To some products the manufacturers add micronutrients and/or fertilizers to make a more diverse product and to prevent trace ions from the seaweed extract to precipitate and lower the degree of efficiency. There are several different types of bioactive molecules in seaweed extracts. Common ones are plant hormones, brassinosteriods, glycine betaines and polyamines (Stirk et al. 2020).

Plant hormones

Plant hormones are among the most common bioactive molecules present in seaweed extracts. Examples of such are cytokinins, abscisic acid (ABA), auxins, gibberellic acids (GA) and ethylene. These hormones modulate environmental responses and physiological growth. Of the plant hormones, cytokinins and auxins are more frequently found in seaweed extracts. From an agricultural point of view the cytokinins are most important because they increase both productivity and stress tolerance. Plant hormones can interact with each other and can through positive and negative feedback loops send both hormonal and non-hormonal signals. For example the interaction between cytokinin and auxin control lateral root initiation and shoot branching (Stirk et al. 2020). It's important to consider that not all of the plant hormones identified in seaweed extracts are biologically active because they can be present as inactive storage forms or biosynthetic precursors (Stirk et al. 2020). However, these forms may become activated through plant or rhizosphere metabolism

Brassinosteriods

Brassinosteroids (BRs) are steroidal hormones connected to the plants responses to abiotic stresses (Nephali et al. 2020), biotic stress (Lucini et al. 2018) and function as plant growth promoters (Stirk et al. 2020). There are special receptors and BRs responsive genes that complete the BRs pathway. Since BRs serve as growth promoters these are connected to a wide range of biochemical and physical responses such as seed germination, cell division, photosynthesis, senescence and many others. Stress factors that BRs respond to are e.g. salinity, draught and pathogens. BRs also affect the structure of roots by controlling the epidermal cells in roots. Because of BRs ability to control epidermal cell development BRs regulate the differentiation into root hair cells. BRs can be taken up by the roots and then translocated within the plant. However, there are some studies that showed that BRs are quite immobile when taken up (Lucini et al. 2018). BRs also affects many of the other common plant hormones, e.g. by stimulating the production of ethylene and cytokinin (Stirk et al. 2020).

Glycine betaines

Glycine betaine (GB)(*N,N,N*-trimethylglycine) is an osmolyte consisting of a soluble quaternary ammonium compound (Stirk et al. 2020), which serves as an osmoprotectant. Osmoprotectans help plant cells to stabilize membranes and maintain the osmotic balance (Nephali et al. 2020). The synthesis of GB takes place in the chloroplast and the precursor molecule is choline via several intermediates. Naturally, the largest concentration of GB is found in the chloroplast where it protects the thylakoid membrane and hence the photosynthetic machinery (Ashraf & Foolad 2007). GB has the ability to accumulate in plants when they are exposed to abiotic stresses. Examples of abiotic stresses are freezing, draught, salinity and oxidative stress (Nephali et al. 2020). Increased GB accumulation in plants exposed to stress is common in crops such as wheat (*Triticum aestivum)* and barley. In many crops the production of GB is smaller than what is sufficient to prevent negative consequences of dehydration and exogenous applications can thus be beneficial (Ashraf & Foolad 2007).

Polyamines

Polyamines are nitrogen-containing compounds with low molecular mass and that contain more than one amine group. Some common polyamines in plant cells are spermine, spermidine and putrescine. Polyamines participate in several different processes in the plant such as defence against pathogens and abiotic stresses, senescence and plant development (Alzahrani & Rady 2019). Polyamines also have the ability to interoperate with plant hormones and other metabolites and hence also work in signalling. In some studies with nitrogen deficient conditions, biostimulants containing polyamines have improved the yield. It has been suggested that these kind of biostimulants could be used to decrease the need of chemical fertilizers (Stirk et al. 2020).

1.2. Barley

1.2.1. Barley

Barely (*Hordeum vulgare*) is an annual cereal that originates from western Asia. Barley was domesticated from *Hordeum spontaneum C* 7-8000 years BC (Jones et al. 2011). In dry and often poor areas such as North Africa, Central Asia and the horn of Africa, barley is the major staple food because of its ability to grow in harsh environments and with limited resources. Barley is grown over a very wide geographical and environmental range, from 46°S in Chile to 70°N in Norway. Globally the consumption of food barley has decreased during recent decades, mainly due to increased wheat consumption (Grando & Macpherson 2005). Barely is the fourth most important crop in the world when considering the volume produced. Only corn, wheat and rice are cultivated in larger volumes. Europe is the leading producer of barley (Tricase et al. 2018). In Europe barely is primarily used as animal feed or as malt for brewing (Jones et al. 2011). One advantage of barley is that it can grow under a wide range of environmental conditions and with smaller management efforts compared with the larger crops (i.e. corn and wheat) and still produce significant yields (Tricase et al. 2018).

In 2019 barley was cultivated on 299 900 hectares in Sweden, which is approximately 10 % of the total farmland (Jordbruksverket 2020). Globally barley is grown annually on 48 million hectares (Gebeyehu et al. 2021). The mean yield in Sweden 2019 was 5 148 kg ha^{-1} and the total yield in Sweden was 1 406 100 tonnes (Jordbruksverket 2020). The global production volume 2019 was around 156 million tonnes (Statista 2020). The mean yield varies a lot depending on the local conditions, for example the mean yield in North Africa is only 1 tonne ha^{-1} (Grando & Macpherson 2005).

Barley can be cultivated in several different types of cropping systems. When it comes to seedbed preparations all three "major" techniques are commonly used, conventional tillage (mouldboard plow), reduced tillage (disc cultivator) and no tillage (direct sowing) (Soane & Ball 1998). Several studies have compared conventional tillage with no tillage in barley cultivation, but the results are inconsistent. Many of the studies showed that no tillage gave a few percent lower yield than conventional tillage. The inconsistent results are suggested to depend on the soil type, crop rotation, climatic conditions and application of fertilizers (Malecka et al. 2012). Fertilization is one of the most important cultivation measures when growing barley and achieving high yields. The purpose with the fertilizers is to compensate with the nutrients that the soil cannot deliver naturally to obtain optimal crop yields. Different soils deliver different nutrients and amounts of nutrients. Accordingly, choice of fertilizers and levels used vary on different soils and crop varieties. Nitrogen (N) and Phosphorus (P) are the most common nutrients in fertilizers used in barley cultivation. Fertilizers containing other essential nutrients are used locally where the soil cannot supply these nutrients in adequate amounts (Gebeyehu et al. 2021). In Sweden the Board of Agriculture recommend use of fertilizers in the range 50-145 kg N ha^{-1} depending on where in the country the barley is grown and what the desired yield is (Jordbruksverket 2021).

1.2.2. Root development of barley

During germination 5-7 seminal rootlets emerge from the coleorhiza and continues into the soil. These rootlets form branches and develop a system of roots that can extend deep downwards in the soil. After a while the adventitious root system develops from the base of the crown as atypical whorls. Initially the adventitious (also called nodal) roots grow horizontally and is usually thicker than the seminal roots and do not branch to the same extent (Briggs 1978). Different barley cultivars develop various types of root systems. Two common types are "mesophytic" and "xerophytic" root architectures. Mesophytic varieties form a shallow root system that grows mainly horizontally. These varieties are common in areas with adequate soil moisture. Xerophytic varieties form a lot of roots that stretches more vertically into the soil, which is advantageous in dry and arid regions (Briggs 1978). Plants have developed various sensors that can direct root elongation into more favourable directions in the soil to support adequate supply of soil resources.

In some extreme conditions such as extreme draught the barley plant does not develop any adventitious roots, and only relay on the seminal roots to acquire nutrients and water. Under "normal" conditions the seminal roots stop to function properly sometime during the development of the plant. Some roots even start to die while the plant is still growing (Briggs 1978). The total root mass might even start to decrease when the ear emerges from the boot. This decrease is suggested to mainly be due to the death of roots and that recourses (i.e nutrients and various substances) is relocated from the roots to the ear (Weaver 1926). Resource allocation in plants is highly dynamic and will differ during development and adaptation to various growth conditions.

Barley root elongation is due to cell divisions in the apex located at the root tip. The continuous differentiation of new cells powers the apex onwards into the soil. To protect the apex from physical damage when it pushes through the soil, there is a root cap; also known as the calyptra, which forms a slimy structure at the very top of the root. When the root moves on due to the continuous cell divisions the cells that are "left behind" keep growing in size and with time differentiate into various cell layers and functions. When the root ages, sclerenchyma cells are formed in the outer cortex. This results in a corky layer that increases the mechanical strength of the root (Briggs 1978).

Barley root development is dependent on soil moisture and soil structure. The roots will not grow under the wilting point nor will they grow when the soil is water saturated. Obviously the amount of available nutrients also affects the root growth. As for the whole plant the roots reach the largest mass when the nutrient levels are adequate and the levels of growth-inhibiting substances are low (Briggs 1978). The physical structure of the soil affects the root development to a large extent. If the soil is too dense the roots will not be able to grow in that direction and their elongation will be arrested. The root tips will then probe other directions if a more favourable soil texture exists elsewhere. Barley roots prefer a porous soil for maximum development and growth. When the circumstances are optimal the barley roots can reach a depth of 1.8-2.1 m (Briggs 1978).

Fertilization can affect the roots in several ways. High concentrations of nitrate enhance branching of barley roots while the length will be reduced. Phosphate and potassium have the opposite effect on length; these elements will increase the root length (Weaver 1926). If there is a lack of potassium and phosphate the number of branches on each root and their lengths will decrease. These effects mainly apply to the adventitious roots and not so much to the seminal roots. A strong potassium deficiency can force a root to not develop any secondary tillers (branch of the first branch) (Hackett 1968).

The barley cultivars in Sweden have in the last 100 years undergone extensive phenotypic changes. The breeding programs have caused a decline of root weight of barley seedlings with 33.9 % and the root length with 10 % (Bertholdsson & Kolodinska Brantestam 2009). However, the modern cultivars seem to develop more finer roots than the older ones. Some studies in the most recent years have shown that this historical decrease in root growth parameters may have come to an end or even an increase. Mainly because of some new high-yielding cultivars that also show positive correlations between root parameters and grain yield (Bertholdsson & Kolodinska Brantestam 2009). In more general terms the modern barley breeding has resulted in a loss of genetic variation for stress tolerance. Modern cultivars are optimized for stress free environments with plenty of nutrients and moisture in contrast of the old landraces who were adapted to more poor conditions (Pswarayi et al. 2008). The breeding of modern cultivars has led to changes in allele frequencies in the close proximity of quantitative trait loci (QLT) attributed to high yield and growth under favourable conditions. The amount of alleles around certain QLTs varies even within the modern cultivars and can explain why they respond differently to various conditions and treatments (Pswarayi et al. 2008)

It is difficult to study roots under field conditions. Some examples of ways to study roots are to grow plants in water, take soil cores, to dig plants up and wash the soil away from the roots or apply tomographical analysis to soil grown plants. The growth pattern of roots varies a lot due to local growing conditions that can be very different even between neighbouring plants. Plants that are grown isolated develop a substantial larger root system than plants that are sown in rows. The largest contributing factor to this variation is inter-plant competition (Briggs 1978). This can be a combination of competition for nutrients, microbiota effects as well as allelopathic effects.

1.2.3. Root parameters connection to the grain yield

The root systems size and architecture is central for the crops ability to acquire nutrients and water, and hence the productivity of the crop (Robinson et al. 2018). Chloupek et al (2010) conducted field trials on barley cultivars at different locations over several years, where the root system size was measured using electrical capacitance. The study showed that during the driest conditions there was a significant correlation between the size of the root system and the grain yield levels. Chloupek et al (2010) concluded that if the root system size increased from a low to an average level there was a significant yield increase. However, if the root system size increased from an already average level no significant yield increase was acquired.

Bertholdsson & Kolodinska Brantestam (2009) investigated seedling and root growth of barley cultivars cultivated in Sweden and Denmark in hydroponics and then compared the root data with the yield data from the official cultivar trials in the countries. The results showed that at low nitrogen levels combined with low aeration, both the total and lateral root length showed a significant positive correlation with grain yield. At high nitrogen levels the results were the opposite with a negative correlation. All the results from fully aerated hydroponics showed low correlations (no significance) with grain yield (Bertholdsson & Kolodinska Brantestam 2009). The seminal root traits may affect the grain yield levels but the relationship is highly context dependent. Factors that could affect the outcome include rainfall, sowing depth, pre-sowing moisture and soil moisture during the growing period (Robinson et al. 2018).

1.3. The tested biostimulants

1.3.1. Physiolith

Physiolith is a biostimulant sold by Timac Agro. It is in granulated form and is often applied at or before sowing of the crop. Physiolith contains aminopurine that, according to Timac Agro, is “a biological substance that in a naturally way stimulates the plant increased growth”. It also contains calcium and magnesium in the form of carbonate. In Physiolith's product data sheet, Timac Agro also declares that “Physiolith will activate the calcium uptake which will benefit the root development” (Timac Agro 2021a).

1.3.2. Demetias V

Demetias V is a biostimulant sold by Timac Agro. It is a product based on seaweed extract and is applied in granulated form at the time of or before sowing of the crop. Timac Agro claims that the biostimulant has an effect as “bio-messenger” and that the zinc (Zn) in the product works as a regulator of the plant’s metabolism. In addition to the zinc and “bio-messenger”, Demetias V also contains P_2O_5, K_2O and SO_3 (Timac Agro 2021b).

1.3.3. Stimplex

Stimplex is a biostimulant sold by Lantmännen (in Sweden). It is a liquid seaweed extract that is derived from *A. nodosum* which is a brown algae common in the North Atlantic. Stimplex contains micronutrients, amino acids and the chelating agents mannitol, fucoidins and alginic acids. These are supposed to increase the nutrient availability and uptake. The biostimulant is also supposed to promote plants production of auxins and cytokinins and because of that develop a larger root system. *A. nodosum* is supposed to increase the amount of enzymes that degrade the toxins that the plant produces when it is exposed to stresses. Finally, Stimplex is suggested to stimulate protein synthesis, which will support the natural defences and create a more vigorous crop (Ilex EnvrioSciences 2021).

1.3.4. Quantis

Quantis is a biostimulant sold by Syngenta. Table 1 displays the content of Quantis. It is a liquid product and is a decay product from fermentation of sugar cane (*Saccharum officinarum*). The biostimulant is supposed to protect the plants from different kinds of stresses and increase its defences, especially against draught, low temperatures and heat stress. Quantis consists of amino acids, peptides and nutrients (Table 1). A large part of the amino acid content is the tripeptide glutathione consisting of glycine, glutamate and cysteine and has a major role as redox regulator in cells (Syngenta 2021).

Table 1. Table showing the content in Quantis

Content	Minimum	Average	Maximum
Dry substance (%)	50	52	55
C organic (%)	12.8	16	19.3
K soluble (%)	8.1	16	19.3
Amino acids (%)	1.8	2	2.2
Ca (%)	0.8	0.9	1.7
N total (%)	0.1	1	1.2
N organic (%)	0.6	0.8	1
Zn (mg/kg)			400
Ni (mg/kg)			10
Cu (mg/kg)			6
Pb (mg/kg)			4
As (mg/kg)			1
CrBI (mg/kg)			1
Se (mg/kg)			1
Cd (mg/kg)			0.5
HG (mg/kg)			0.05

1.3.5. Financial aspect

The cost of Physiolith (as of September 30 2021) is 4.39 SEK/kg and the applied amount in the trials is 300 kg ha^{-1}, which correspond to a cost of 1317 SEK ha^{-1}. The final price of ecological malting barley in 2020 from Svenska Foder that is one of the largest buyers in Sweden is 2.55 SEK/kg (Svenska Foder 2021). To cover the cost of Physiolith the grain yield needs to increase with ca 500 kg ha^{-1}. The cost of Demetias V was 7.64 SEK/kg (as of September 30 2021) and 200 kg ha^{-1} was applied in the trials which corresponds to a cost of 1528 SEK ha^{-1} and the grain yield needs to increase with ca 600 kg ha^{-1} to cover the cost of Demetias V. Stimplex and Quantis are not commercially available yet (2021) so the price of the products are not yet set.

1.4. Aims

In recent years the number of biostimulants on the Swedish market has increased. The knowledge about when and how they should be used is inadequate, and there is a lack of studies in field-trials under Swedish conditions. The aims of this project were to:

- evaluate if a few chosen biostimulants had any effect on the root system of barley under field conditions,
- investigate if the effect of the biostimulants varies between locations,
- analyse the relationship between the root system, yield and fertilization.

2. Method

2.1. Overall

Two root studies in two organic field trials with biostimulants were conducted in June 2021. The trials were situated in Örberga (Östergötland) (58°26´3.7´´N, 14°48´33.0´´E) and Borgeby (Skåne) (55°45´2.2´´N, 13°3´2.5´´E), data about the locations is presented in Table 2. The crop in the trials was barley of the variety Planet (Svenska Foder). Four different biostimulants in total were examined in the two root studies, but all biostimulants were not included in both studies. The biostimulants were Physiolith, Demetias V, Stimplex and Quantis. Physiolith (300 kg ha^{-1}) and Demetias V (200 kg ha^{-1}) are granulated and applied at sowing. Stimplex is fluid and applied to the barley at development stages BBCH 21 and BBCH 32 (2*2 l ha^{-1}) (Figure 1). Quantis is also a fluid and applied to the barley in BBCH 32 (1.5 l ha^{-1}). These trials were also two-factorial with two different N-levels, 40 kg ha^{-1} and 80 kg ha^{-1}. An ecological fertilizer who is produced from meat meal and vinasse was used to achieve the N-levels. The fertilizer is an NPKS product with the composition 8-3-5-3, it also contains some micronutrients. Each site had three replications of each treatment in randomized block design and all replications of the plots included in the study were sampled. The first root study was conducted two weeks after the BBCH 21 treatment of Stimplex, the second study two weeks after the BBCH 32 treatment of Stimplex and Quantis. The samples from both Örberga and Borgeby was transported to Hushållningsällskapet Östergötlands research station Klostergården located at Vreta Kloster.

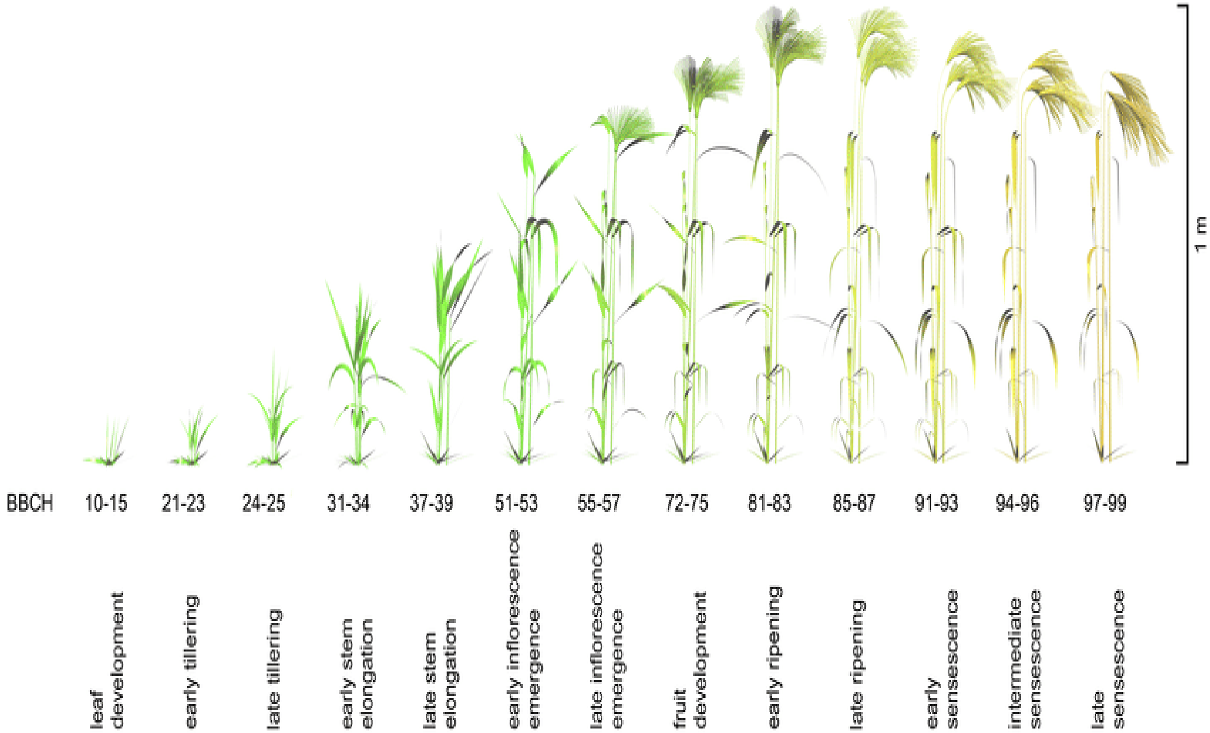

Figure 1. Displays development stages (BBCH) in barley (Kuester & Spengler 2018).

Table 2. Table showing location data from the trial locations.
** Data collected between 30/4-28/6*
*** Data collected between 8/4-21/6*

Data	Skåne	Östergötland
Day degrees	548*	434**
Precipitation	57*	81**
pH	8	7,7
P-Al	IVB	III
K-Al	III	III
K/Mg-Al	1,4	1
Mull (%)	1,2	3
Clay (%)	25	36
Silt (%)	17	27
Sand (%)	57	34
Sowing date	30/4	08/4
Root study 1	18/6	15/6
Root study 2	28/6	21/6

In the first root study three biostimulants (Physiolith, Demetias V and Stimplex) and a control were examined. The barley was in BBCH 49 in both trials when the first root study was conducted. In the second root study, two biostimulants (Stimplex and Quantis) and a control were examined. The barley was in BBCH 59 in both trials when the second root study was conducted.

The trial in Skåne suffered quite hard from draught from the very beginning and was irrigated several times during the growing period. In Östergötland the trial was irrigated once, but it was not until after root study 2. Overall the trial in Östergötland did not suffer from the lack of water to the same extent that the Borgeby trial did, but it got a slow start due to the quite cold temperatures in April.

2.2. Data collection

Four samples that contain ca six plants in one clod each were dug up from every plot. The samples were taken at the exact same spot (four spots) in every plot, approximately 1.5 m "into" the plot from each corner and in the outer row. Every clod with plants were dug up from a depth of 21 cm. A mark on the shovel was used to measure the depth so each clod got the same height. The clods were then put two and two in plastic bags (Figure 2) for transport to the research station for further sample treatment.

Figure 2. Two clods ready to be put into a bag for transport.

When the samples arrived at Klostergården they were taken out of the plastic bags and put into a water bath during approximately 12 h to clear the roots from soil and plant debris in order to simplify the following wash. To clear the roots the material was flushed with a fine water muzzle on net trays (Figure 3). For each clod sample with six plants, the two plants on each fore-edge were discarded. These two plants served as protection for the other plants in the clod during the dug work, transport and handling. This resulted in 16 plants from each plot, 48 plants from each treatment/location/root study so in total including both root studies 1344 plants were examined. The roots were separated from the crown, the crown roots were also cut of and was included in the measurements.

Figure 3. Washing of roots on net trays with a water muzzle.

After the roots were cut off their length and fresh weight were measured individually before all the roots from the same plot were put into a perforated plastic bag for drying. The roots were first dried for three days in drawer dryers to be able to be stored. Later the samples were dried to “dry weight” in a hot air drier (65-75°C) for two days. After the drying the dry weight of the samples were measured.

The grain yield data were provided through ordinary harvest of the field-trial, the harvest was carried out by the field staff at Klostergården.

2.3. Statistical analysis

The data from the root studies (root length, root fresh weight and root dry weight) and the grain-yield data were analysed with the statistical software JMP Pro (version 16.0.0). All the data were transformed in JMP into mean values before the analysis. A full factorial variance analysis (fixed model) was conducted for each data set (root length, root fresh weight, root dry weight and yield). The parameters included in the full factorial variance analysis was biostimulant, N-level and location. If the variance was not homogenous, the dataset was transformed into logarithmic values to receive a homogeneous variance. For parameters that displayed significant results/differences, a test with Tukeys method was carried out to distinguish treatments that were significantly segregated.

The root data was also correlated with yield, N-uptake and protein values. This correlation was done in JMP Pro (version 16.0.0) with a multivariate method that explores correlations between multiple numeric variables. The result from the multivariate method is a correlation number between 1 and -1, where positive numbers indicate a positive correlation and negative numbers indicate a negative correlation. No statistical P-values can be acquired from the correlation, hence caution needs to be considered for any conclusions drawn from these results.

3. Results

Only a few of the many variance analyses conducted on the root data showed significant results (Table 3). In root study 1 there was no significantly different result for any of the tested parameters. However, in root study 2 some significant results were found, especially for root length. There were effects from "Biostimulant", "Location*Biostimulant" and "N*Biostimulant". The dry weight in root study 2 showed significant effects from "N".

Table 3. Analysis of root growth in barley after treatment with biostimulants and different N-levels. The table shows what root parameters that showed statistically significant differences from the control for at least one treatment. - = no significant difference. S = significant difference.

Root study 1	**N**	**Location*N**	**Biostimulant**	**Location*Biostimulant**	**N*Biostimulant**	**Location*N*Biostimulant**
Length	-	-	-	-	-	-
Fresh weight	-	-	-	-	-	-
Dry weight	-	-	-	-	-	-
Root study 2						
Length	-	-	S	S	S	-
Fresh weight	-	-	-	-	-	-
Dry weight	S	-	-	-	-	-

3.1. Root length

In root study 1 there was no significant difference in results for root length between the treatments with biostimulants and the control, but it is possible to see some tendencies (Figure 4). In Skåne the results varied between the two N-levels. At 40 kg N, Physiolit and Demetias V got the longest roots with around 16.1 cm, but at 80 kg N their root length decreased and they both got shorter roots than the control and Stimplex. Especially Stimplex root length seemed to increase (ca 1 cm) with the higher N-level. The results for Östergötland followed the same intergroup ranking at both N-levels. Physiolit got the longest roots and Stimplex the shortest ones, but there were no statistically significant differences.

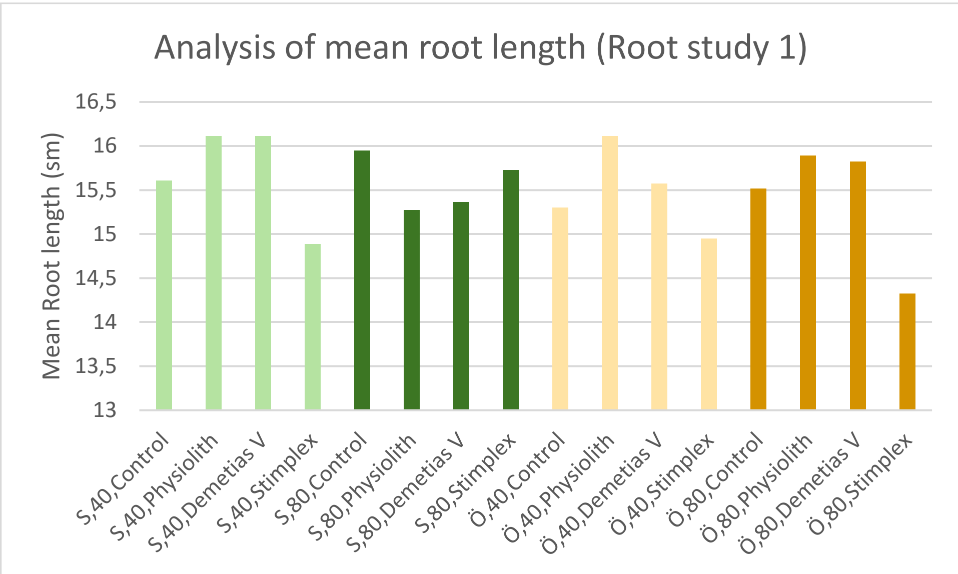

Figure 4. Mean root length from root study 1. No significant differences. S = Skåne, Ö = Östergötland, 40 = 40 kg N ha^{-1} and 80 = 80 kg N ha^{-1}.

In root study 2 the overall effect of the biostimulants Quantis and Stimplex resulted in significantly longer roots than the control (Figure 5). The overall effect includes both location and N-levels. Both Quantis and Stimplex got ca 1 cm/ 7 % longer roots. There was no significant difference between the overall effect of Quantis and Stimplex.

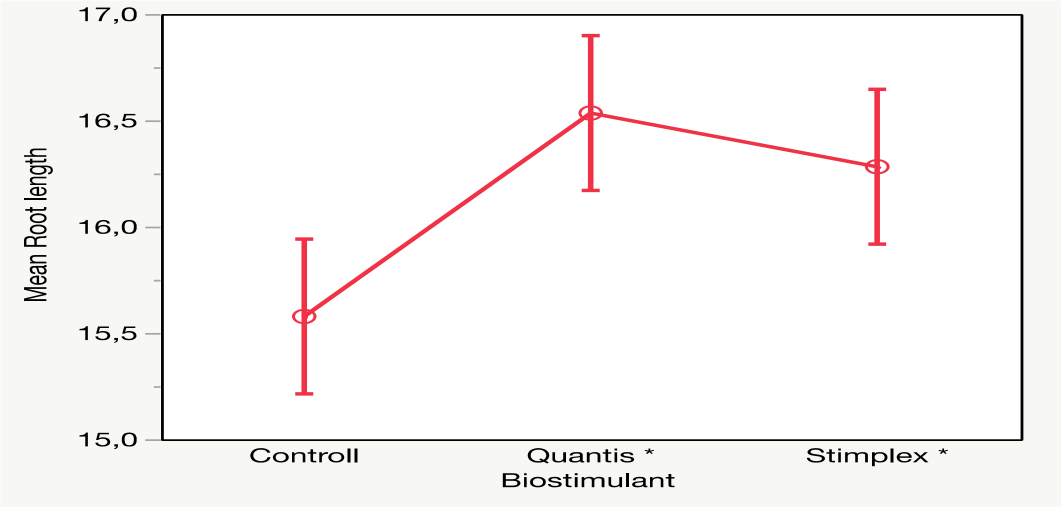

*Figure 5. Graph showing the overall (both location and N levels included) mean root length (cm) in root study 2. Both Quantis and Stimplex have significantly longer roots. * = significantly different results compared to the control.*

At 40 kg N ha^{-1} (both locations included) in root study 2 there was significant differences (Figure 6). Both Stimplex and Quantis got significantly longer roots than the control. The difference had roughly the same magnitude as for the overall effect. There was no significant difference between the lengths of Quantis and Stimplex.

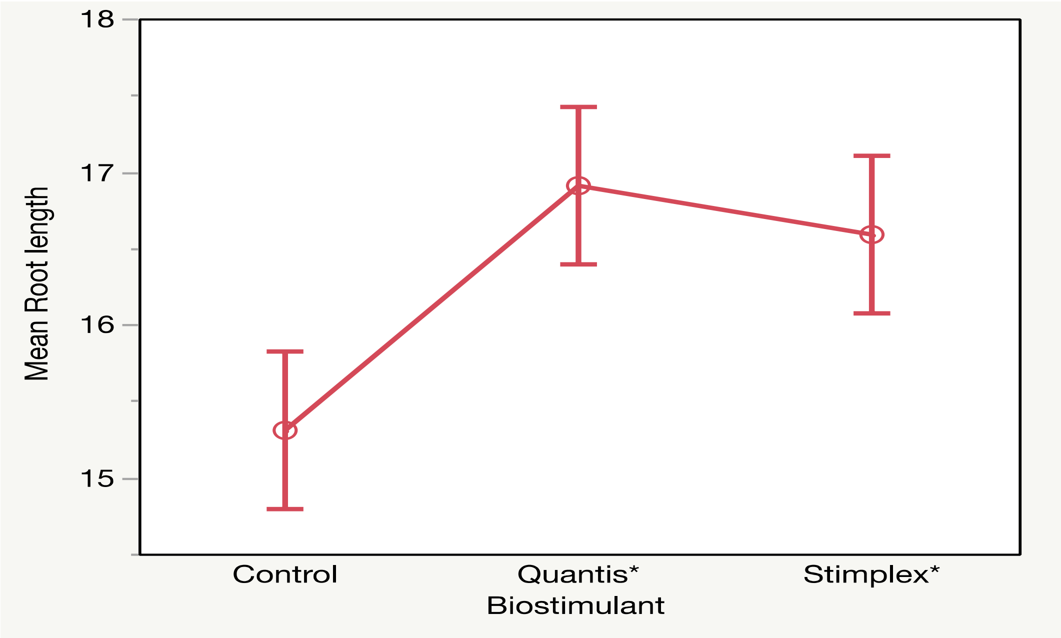

*Figure 6. Graph showing the mean root lengths (cm) at 40 kg N ha^{-1} (both locations included) in root study 2. Both Quantis and Stimplex has significantly longer roots than the control. * = significantly different results compared to the control.*

There was no significant difference between the biostimulants and the control in Östergötland in root study 2 when analysing the root length at both N-levels together, however there was significant results in Skåne (Figure 3). The P-value in the comparison between Quantis and the control in Skåne is just below 0.05. In this situation one must be careful to thrust the effect. The study indicates that there is a difference, but the significance is weak and a new larger study is needed to verify the result. However, the graph for Skåne in Figure 7 follows the same pattern as Figure 5 & 6 which strengthen its credibility.

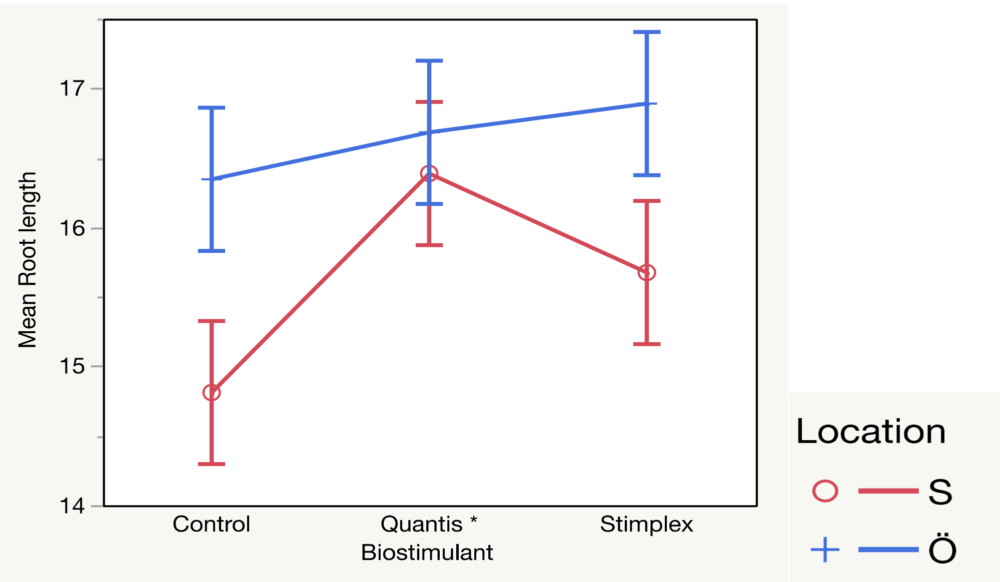

*Figure 7. Graph showing the mean root lengths (cm) (both N levels included) for both locations, Skåne and Östergötland. In Skåne was Quantis significantly longer than the control. S = Skåne, Ö = Östergötland. * = significantly different results compared to the control.*

3.2. Root fresh weight

The root fresh weight showed no statistically significant differences in the results from root study 1 (Figure 8). The values for the different biostimulants in Skåne displayed very small variations and differed very little from the control, at both N-levels. In Östergötland, there was a little larger variance, especially at 40 kg N ha^{-1} where the control plants had the highest root fresh weight but still showed no significant differences. Similar to root study 1, there was no significant results for root fresh weight in root study 2 (Figure 9). In Skåne the control even got the highest root fresh weight at both N-levels. In Östergötland the variance between the treatments was a little larger at 80 kg N ha^{-1}, but still no significant difference was found. The control and Stimplex treatments who was sampled in both root studies seem to have decreased their root fresh weight from the first to the second root study at both locations and N-levels.

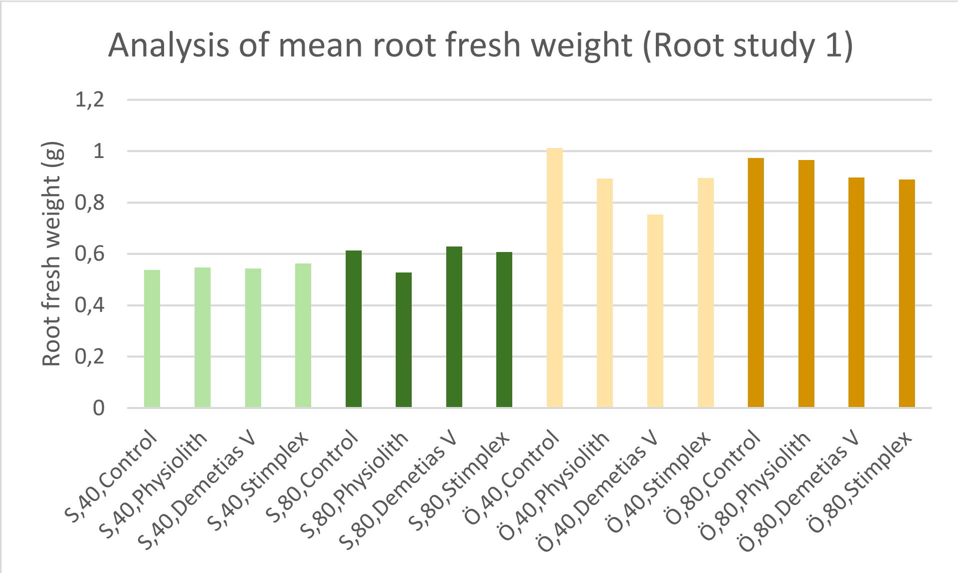

Figure 8. Mean root fresh weight from root study 1. No significant differences within the locations, significant differences between the locations. S = Skåne, Ö = Östergötland, 40 = 40 kg N ha^{-1} *and 80 = 80 kg N ha*$^{-1}$.

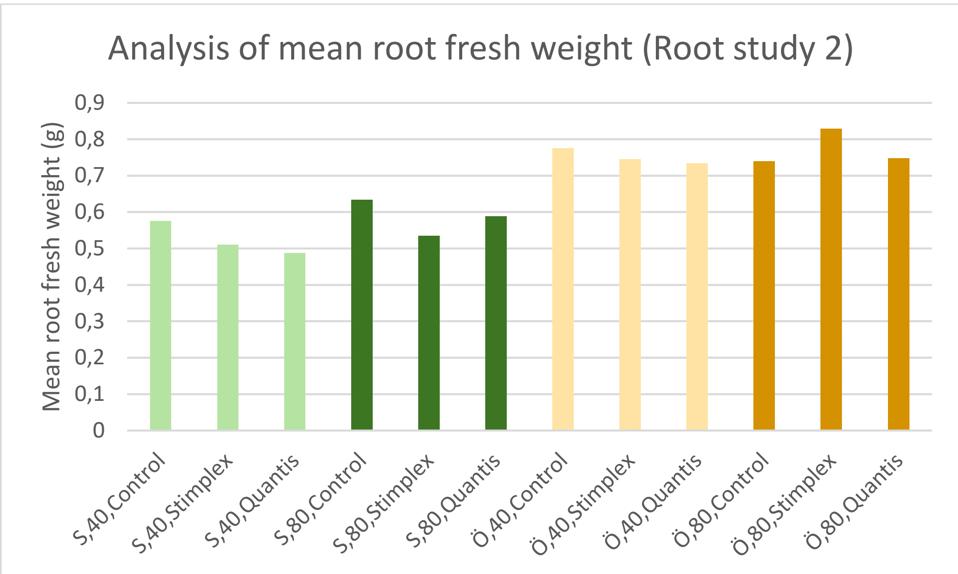

Figure 9. Mean root fresh weight from root study 2. No significant differences within the locations, significant differences between the locations. S = Skåne, Ö = Östergötland, 40 = 40 kg N ha^{-1} *and 80 = 80 kg N ha*$^{-1.}$

3.3. Root dry weight

There were no statistically significant differences in root dry weight between the biostimulants and the control in root study 1 (Figure 10). In Skåne the root dry weight hardly showed any differences among the treatments. In Östergötland, the control got the highest root dry weight at both N-levels. As in root study 1, there was no significant differences in root dry weight in root study 2 (Figure 11). Noticeable is that the difference between the locations is smaller in root study 2 than in 1. As for the root fresh weight, the root dry weight for the control and Stimplex seems to decrease from the first to the second root study.

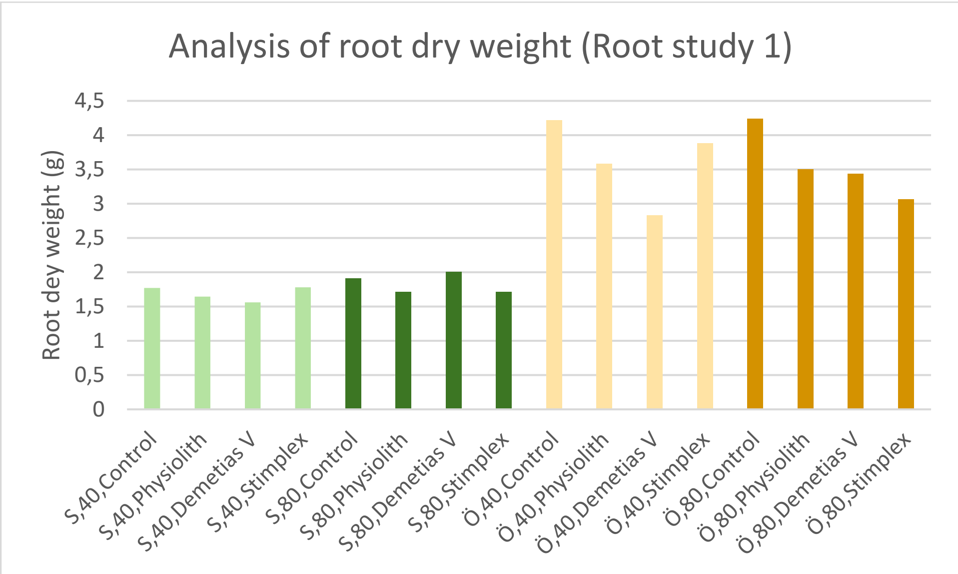

Figure 10. Mean root dry weight of 16 (aggregated) barley plants from root study 1. No significant differences within the locations, significant differences between the locations. S = Skåne, Ö = Östergötland, 40 = 40 kg N ha^{-1} and 80 = 80 kg N ha^{-1}.

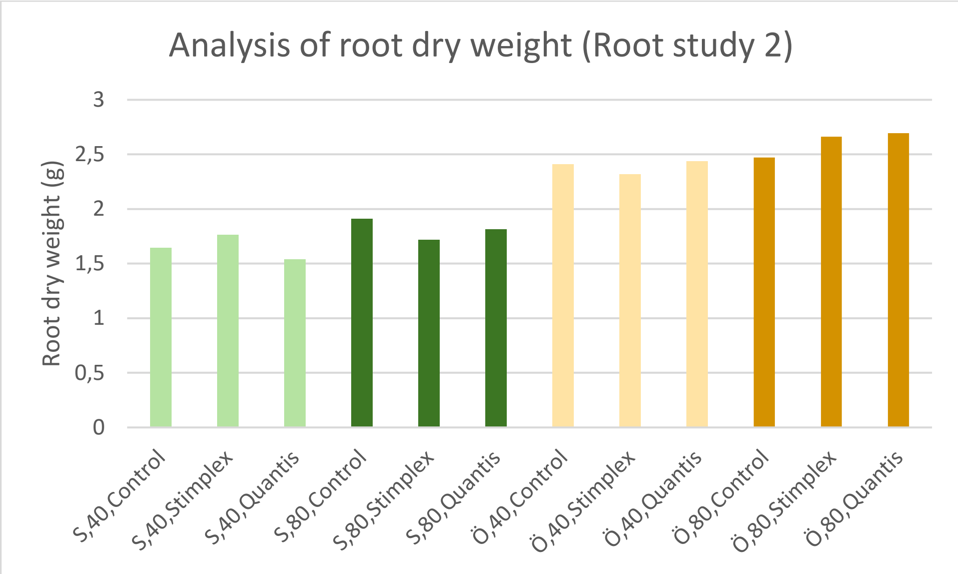

Figure 11. Mean root dry weight of 16 (aggregated) barley plants from root study. No significant differences between the biostimulants. S = Skåne, Ö = Östergötland, 40 = 40 kg N ha^{-1} and 80 = 80 kg N ha^{-1} .

3.4. Grain yield

There were no statistically significant differences in yield between the biostimulant treatments included in this experiment (Figure 12 and 13). All treatments at both locations gave a higher yield at 80 kg N ha^{-1} compared to 40 kg N ha^{-1}. The intergroup yield level between the treatments varies at the two locations and N-levels. Quantis receives the lowest yield at both locations at 40 kg N ha^{-1} but is also the biostimulant that responded best to the increased N-level. In Skåne, Quantis received the highest yield at 80 kg N ha^{-1}. Noticeable is that Demetias V and Stimplex received some of the lowest yields in Skåne but the highest in Östergötland. The control and Physiolit differs little from each other at both locations and N-levels.

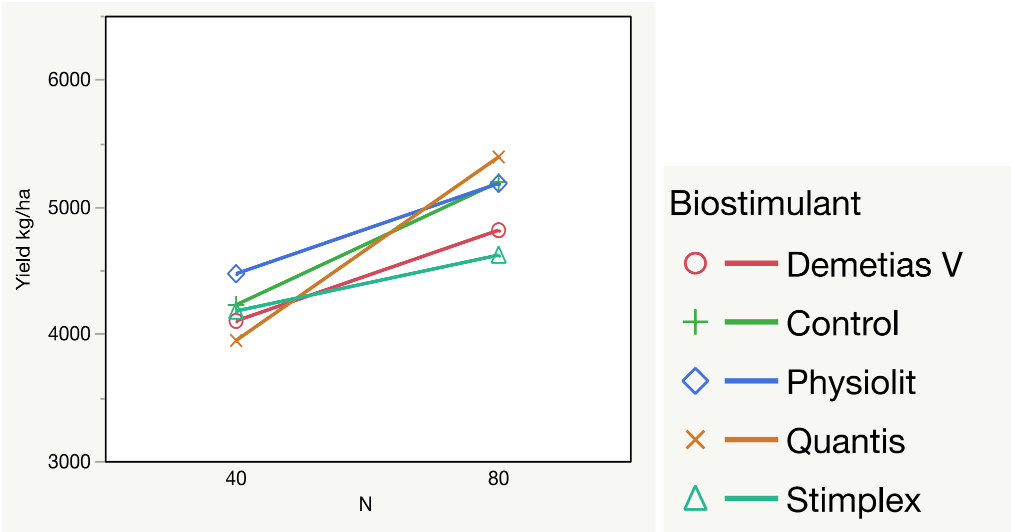

Figure 12. Graph showing the yield from the trial in Skåne. No significant results.

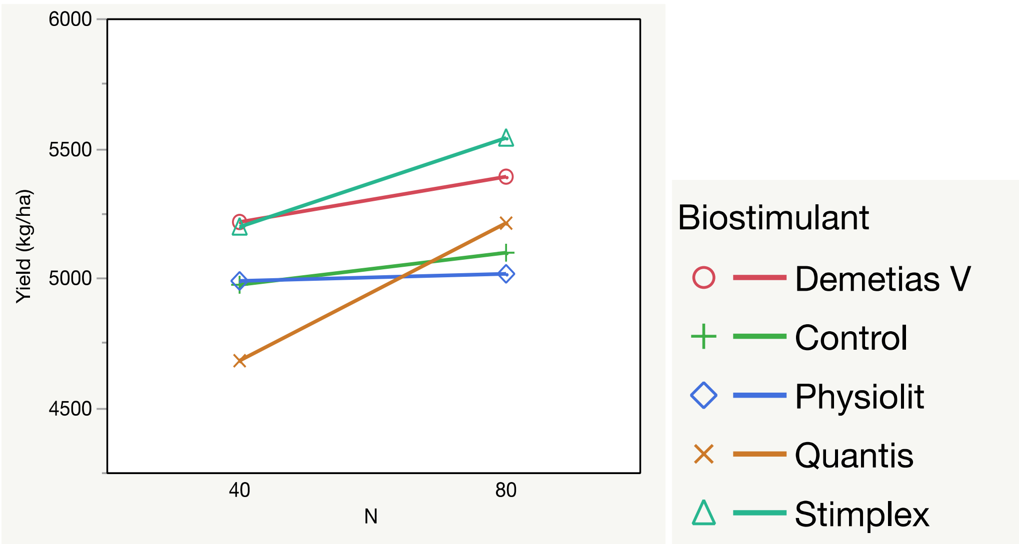

Figure 13. Graph showing the yield from the trial in Östergötland. No significant results.

3.5. Correlations between root data, yield, N-uptake and protein

The values received from the correlation between the root data from the root studies and the yield showed a large variation (Table 4). Due to the small number of observations in each correlation analysis and lack of P-values, cautiousness must be considered when analysing the results. A problem with the correlation data is that the result for the controls varies to a large degree. Some of them show a positive correlation for 40 kg N $ha^{-1}$and a negative for 80 kg N ha^{-1} and vice versa, also some correlations values are quite small. If the control is hard to interpret, the effect of the biostimulants becomes very hard to legitimize. One thing that is noticeable is that in Östergötland quite many of the treatments showed a strong negative correlation between root data and yield. That is possible to interpret as "plots with shorter and less dense roots receives higher yields".

Table 4. Table showing the result of the correlation test (multivariate method) between root data acquired from the root studies and yield data. Each plots (3 for each treatment and location) root data is correlated to its yield. Positive numbers indicate a positive correlation, negative numbers indicate a negative correlation. The scale is from 1 to -1.

Skåne Root study 1				
Biostimulant	N - level	Root length - Yield	Root fresh weight - yield	Root dry weight - yield
Demetias V	40	0.8785	-0.9996	-0.7898
Demetias V	80	-0.8253	-0.8897	-0.9314
Control	40	0.0616	0.9236	0.7523
Control	80	0.4282	0.8701	0.9933
Physiolit	40	0.4267	-0.9048	-0.8298
Physiolit	80	-0.9957	-0.2169	-0.9149
Stimplex	40	0.0704	0.6302	0.9474
Stimplex	80	-0.65	-0.9358	-0.7871
Skåne Root study 2				
Control	40	0.802	-0.4966	0.1189
Control	80	0.989	0.9984	0.8045
Stimplex	40	0.9713	0.4482	0.9443
Stimplex	80	0.3595	0.211	-0.7011
Quantis	40	-0.9991	-0.7496	-0.6298
Quantis	80	0.4084	0.0842	0.289
Östergötland Root study 1				
Demetias V	40	-0.9812	-0.552	0.0837
Demetias V	80	0.0365	-0.3878	-0.1751

Control	40	-0.9952	-0.9803	-0.943
Control	80	0.757	-0.9627	-0.9763
Physiolit	40	0.5871	-0.7551	-0.6719
Physiolit	80	0.9843	0.8262	0.5149
Stimplex	40	-0.9609	0.3765	0.3899
Stimplex	80	0.778	0.7842	-0.9829
Östergötland Root study 2				
Control	40	-0.6607	-0.3003	0.2943
Control	80	-0.5372	-0.9278	-0.9937
Stimplex	40	-0.8998	-0.965	-0.9712
Stimplex	80	-0.8269	0.8835	0.7395
Quantis	40	-0.8238	0.7273	-0.1667
Quantis	80	-0.0667	0.463	0.957

To investigate if N-uptake and protein-levels affected by root properties correlation analysis were conducted with the root data received from the root studies and the N-uptake and protein-level data from the analysis of the harvested grains. The results are presented in Table 5. The overall low correlation values received from this study indicates that there is little or no correlation between the root parameters and the N-uptake and protein-levels.

Table 5. Table showing results of the correlation test (multivariate method) between root data acquired from the root studies, the N-uptake and protein levels in the grains. Positive numbers indicate a positive correlation, negative numbers indicate a negative correlation. The scale is from 1 to -1

Correlation parameters	**Skåne Root study 1**	**Skåne Root study 2**	**Östergötland Root study 1**	**Östergötland Root study 2**
Root length - N-uptake	-0.1015	0.0306	0.0682	-0.3008
Root length - Protein	0.0609	0.0379	0.2229	-0.0982
Root fresh weight - N-uptake	-0.1904	0.3105	-0.3866	0.3246
Root fresh weight - Protein	0.1442	0.115	-0.3126	0.2876
Root dry weight – N-uptake	-0.0506	0.3032	-0.4644	0.1312
Root dry weight - Protein	0.1097	0.0149	-0.3608	-0.0004

4. Discussion

The biostimulants generally had small effects on the root development and yield in this study. Physiolith and Demetias V did not show any significant effects on any of the growth parameters measured. Stimplex and Quantis showed some significant effects on root length. The roots were around 7 % longer after Stimplex and Quantis treatment compared with the control. One may speculate what benefits the barley plant will acquire due to this relatively small increase in root length, and since the roots in the trials was quite short overall the significance for the plant may be minimal. There was no correlation in this study that the increased root length for Stimplex and Quantis and affected the grain yield. If the root system size of the barley plants in the trials is assumed to be of medium size, the results with increased root length and no effect on yield correlates with the findings of Chloupek et al. (2010). If the root system size, which is affected partly by the root length, increases from an already average level to a higher level the grain yield will not increase. On the other hand, Chloupek et al. (2010) found a positive correlation between root system size and grain yield under dry and water stressed conditions. The trial in Skåne experienced water stress, and Quantis treated plants had significantly longer roots than the control, but the yield of Quantis did not differ to any large extent from the control. It is hard to estimate how severe the water stress of the trial in Skåne was before it was irrigated so the plants might not have reached the degree of stress to achieve the correlation that Chloupek et al. (2010) obtained. Bertholdsson & Kolodinska Brantestam (2009) only found a positive correlation between the root system size and grain yield when the plants were grown under conditions with low oxygen and N supply. Since the biostimulants were tested in trials with estimated good aeration and "sufficient" N supply the probability that the biostimulants would have any impact on the grain yield because of an increased root system is small if the findings of Bertholdsson & Kolodinska Brantestam (2009) are general.

The somewhat dry conditions at both locations (more enhanced in Skåne) for the trials could explain the significantly longer roots in the Stimplex and Quantis treated plants. Both these products contain amino acids and application of some amino acids to plants under stressed conditions can increase water and K uptake (Haghighi et al. 2020). Quantis contains glycine which is one of the amino acids with this property. The amino acid content of Stimplex is not specified by the sales agent, however. Quantis also contains K in soluble form which is known to have a positive effect on root growth (Weaver 1926). On the other hand, Demetias V also contains some K but this product did not increase the root length, which lower the probability that it was the K content in Quantis alone that caused the observed increase in root length. An additional reason why the K content in Quantis did not affect the root length is that the amount of accessible K in the soil at the locations of the trials was around 15 mg 100 g^{-1} soil (K-AL class III), which cover a large part of the needs of barley (depending on the yield level). Furthermore, the "N-fertilizer" also contains quite a lot of K. The treatments where 40 kg N ha^{-1} was applied also received 25 kg K ha^{-1}. Hence, it is more likely that it is the amino acid content of the biostimulants that causes the increased root length rather than the K present even if it is not possible to prove that with the data from this study.

Steveni et al. (1992) conducted experiments on barley with a biostimulant based on *A. nodosum*, the same raw material used in Stimplex. Their study showed that the root growth increased significantly when the seaweed extract was applied. It is not proven which of the substances in the seaweed concentrate that were responsible for this increased growth but, Steveni et al. (1992) suggest that the most likely group is cytokinins. Cytokinins are known to have the ability to enhance root growth when applied in low concentrations (Featonby-Smith & van Staden 1984). There are also studies were application of pure cytokinins to growing plants results in similar effects as the ones seen in studies testing seaweed extracts (Steveni et al. 1992). Since the seaweed extract in Stimplex originates from *A. nodosum* it is possible to suggest that the increased root length of Stimplex in root study 2 is due to increased cytokinin levels in the roots. Physiolith might also contain some cytokinins because some aminopurines are synthetic variants of cytokinins. 6-Benzylaminopurine is an example of such a cytokinin mimic (Verma et al. 2020), but unfortunately the manufacturer has not specified which aminopurines Physiolith contains. Physiolith did not show any increase in root length as Stimplex did and it is only possible to hypothesize the reasons for this difference. One possible explanation is that the cytokinins in Physiolith (if any) were not available when the barley needed these, maybe because of the product's granulated form result in a too slow release. Stimplex on the other hand was applied as a fluid in BBCH 21 and 32 and was available instantly for the plant. Szczepanek et al. (2018) reported increased root growth in field trials with barley when applying a similar biostimulant as Stimplex at almost the same development stages. Szczepanek et al. (2018b) conducted a similar field trial in spring wheat (*T. aestivum L.*) and found significantly larger root weight and grain yield when a seaweed based biostimulant was applied at two development stages, Drygas et al. (2021) used extract from the same algae (*A. nodosum*) and reported significantly higher grain yield in oats (*Avena sativa*). This indicates that some biostimulants might have similar effects in other cereals but trials with the same biostimulant in every cereal is needed to confirm this.

There was a loss of root fresh and dry weight for the control and Stimplex from the first to the second root study. The physiological differences between the development stages of barley can be a reason for this difference. At root study 1 the plants were in BBCH 49, which means that the bristle just has emerged. At root study 2, the ear was fully emerged and developed but had not started to bloom (BBCH 59). Barley relocates nutrients from the roots to the ear when it starts to grow and some roots actually will die during the development of the plant (Weaver 1926). This relocation of nutrients and loss of root tissue could explain the loss of fresh and dry weight seen in this study.

The correlation values in Table 4 show very mixed results but there are more strong negative correlations than there are strong positive correlations. One may hypothesize based on this that the grain yield decreases with larger root systems. This could be connected with what Bertholdsson & Kolodinska Brantestam (2009) writes, that the breeding programmes of barley cultivars in Sweden during the last 100 years have resulted in a decrease in root system size while the yields have increased. On the other hand, Robinson et al. (2018) concludes that some of the new barley cultivars in the last decade show positive correlations between root system size and grain yield. It is important to consider that the correlation between root system size and grain yield could be a cultivar specific trait that varies between cultivars. To be able to draw a firmer conclusion on this topic, several different cultivars need to be tested with biostimulants to study if generic or cultivar specific effects occur. This study also scrutinized if there were any general correlations between root parameters, N-uptake and protein values in the grain (Table 5). The correlation analysis showed very small values indicating no correlation between N-uptake and protein values in the grain with the root parameters. Szczepanek et al. (2018) measured N-uptake in trials with seaweed based biostimulants but found no correlations between the N-uptake and grain yield. Due to the setup of the trials in this study no p-values could be acquired during the correlation analysis. Accordingly a random factor cannot be disregarded but the conformity in the results strengthens their credibility, the majority of the correlations values being in the range between 0.3 and – 0.3.

To compare and analyse results from different soils and climatic conditions is difficult because there are many factors that interact at the same time (Bertholdsson & Kolodinska Brantestam 2009). For example, the soils in the present trials are quite similar in pH, K-AL and P-AL content but differs some when it comes to clay and mull content. The trial in Skåne also got a lot more day degrees and less precipitation than the Östergötland trial. Although all these factors interact on the trial, there were barely any significant variation of the effects of the biostimulants between the two locations. The overall low numbers of significant results in the study reduces the possibility that there would be any variation in biostimulant effect between the two sites. Another factor that could affect the results between the two trial locations is batch variation of the biostimulants. Especially biostimulants based on seaweed extracts seem to differ in content between batches. This is mainly suggested to be due to that seaweed species are natural products so the content can vary depending on location and season of harvest, which will affect the biostimulant product (Lötze & Hoffman 2016). Since the majority of biostimulants are based on natural products from other types of plants one may speculate that batch variation might not be uncommon. If the biostimulants used in the field trials at the two locations in this study belong to the same batch is not known.

The roots were dug up from a depth of 21 cm and some roots might be longer than that. If that would be a major fraction the results for the root length and biomass study would not reflect the true situation. Briggs (1978) writes that barley roots can reach a depth of 1.8 – 2 m under optimal conditions. Usually the conditions in field are far from optimal, there could be compaction, water stress, nutrient deficiency, inter plant competition etc. that affect root development. The result indicates that the mean root lengths in this study are quite a bit shorter than 21 cm. Only a few of the 1,344 plants in this study had a root length longer than 20 cm indicating that the roots were cut off by the shovel when the samples were taken. These few plants were spread randomly between plots and locations and there was no hint of clustering. If a larger proportion of the plants would have had a root length over and around 20 cm the method with taking samples from a depth of 21 cm would need to be revised.

Taking large amount of root samples containing whole root systems is very labour intensive and time consuming, the handling and transport is also a bit problematic because of the sheer weight of the samples but there are not so many other options if one wants to record more exact root length and weight. If a more general root system study would be done in these trials, the method of Chloupek et al. (2010) with electrical capacitance as a value on root system size would be much more time effective and less labour intensive. The disadvantage with this method is that one does not know what parts of the root system that the biostimulants potentially would affect.

There are some studies that showed increased yields for cereals treated with seaweed extracts when grown under controlled environments while field trials found no effect on the yield (Möller & Smith 1999). A common observation is that seaweed extracts has small effect on plants under normal conditions but can make a difference under stressed conditions (Möller & Smith 1999). Generally when reviewing the literature there are quite many studies that displayed effects on root development when biostimulants were applied under controlled conditions such as growth chambers and hydroponically grown plants, but when the products were tested in field studies the effects ceased to occur. Szcepanek (2017) and Steveni et al. (1992) are examples of studies that examined similar biostimulants that follow this pattern. The data from this study indicates that it is hard to derive what causes potential effects of biostimulants in field trials but nevertheless they give important knowledge of their in-field effects.

5. Conclusion

The tested biostimulants seems to have little or no effect on the root development and grain yield of barley. Quantis and Stimplex had some effect on root length but it is questionable if the small increase observed is of any significant advantage to the crop. The treatments with Physiolith and Quantis showed no significant effects. The cost of some of the biostimulants are quite high and the yield increase must be substantial to cover this expense, which was not the case in this study. There is a large variation in effects of biostimulants when reviewing the literature, even within the same crop that has been treated with similar biostimulants. Since the results differ widely hence it is important to take great caution when interpreting their results because the conditions can have large impact on the effect of the biostimulants. This study shows that it is important to continue to conduct independent field trials with standardised conditions when new biostimulants are introduced to the market, so farmers can make informed decisions about their cultivation strategies.

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