Arginine as a Biostimulant for Enhancing Growth and Establishment of Spinach Transplants

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Arginins roll som biostimulant för att minska transplantationschock och främja tillväxt hos spenatplantor

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Abstract

Transplantation shock can severely reduce plant yield by damaging roots and impairing water and nutrient uptake, making plants more vulnerable to stress and infections. This study investigates whether arginine, in the form of arGrow Granules, can act as a biostimulant to reduce transplantation shock and promote spinach seedling growth.

Spinach seedlings were transplanted into humus-rich muddy moraine soil, with *arGrow Granules* placed under the roots of the biostimulant group. Stress symptoms (bolting, leaf discoloration, wilting, and death), leaf size, leaf count, plant height, and biomass (leaf and root) were measured.

The biostimulant group that received arginine at transplantation exhibited more stress symptoms and had smaller leaves, height, and biomass compared to the control group. Therefore, this study does not support arginine as a biostimulant to reduce transplantation shock in spinach. Future studies could explore different soils, dosages, and application methods for non-forestry applications.

Keywords: Arginine, biostimulant, transplantation, spinach

Sammanfattning

Transplantationschock kan minska skörden genom att skada rötterna, försämra näringsupptag och öka mottaglighet för stress och infektioner. Det har bevisats i flera andra studier att biostimulanter kan lindra transplantationschock och öka tillväxten vid transplantation. Denna studie undersöker om en biostimulant bestående av arginin i form av *arGrow Granulat* kan främja tillväxt och rotutveckling hos spenatplantor.

Krukor fylldes med humusrik lerig moränjord och krukorna tillhörande behandlingsgruppen fick en dos arGrow Granulat i substratet. Därefter transplanterades spenatplantor till alla krukor. Under studien mättes stressymtom (blomning, missfärgning, vissnande), bladstorlek, bladantal, höjd och biomassa.

Resultaten visade att argininbehandlade plantor uppvisade fler stressymtom samt mindre blad, höjd och biomassa jämfört med kontrollgruppen. Studien stödjer därför inte användning av arginin som biostimulant för att minska transplantationschock hos spenat. Vidare studier med annan jord, dosering och appliceringsmetod kan ge mer insikt.

Nyckelord: Arginin, biostimulant, transplantation, spenat

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Abbreviations

AACP	Amino-acid containing product
arGrow	arGrow Granulat
Ca	Calcium
Cm	Centimeters
EC	Electrical conductivity
g	Grams
HCP	Hormone containing product
HS	Humic substances
K	Potassium
mS/cm	Millisiemens per centimer
N	Nitrogen
NPK	Nitrogen-phosphorus-potassium
P	Phosphorus
PAR	Photosynthetically active radiation
RSA	Root system architecture
SLU	Swedish University of Agricultural Sciences

1. Introduction

Transplantation is an essential activity in the production of many plants (Schoeneweiss 1975). Transplantation often causes significant root damage which impacts the acquisition of nutrients and water (Berkowitz 1987; Bloom & Sukrapanna 1990). This leads to a higher risk of plant disease and a weaker resistance to abiotic stress (Berkowitz 1987; Bloom & Sukrapanna 1990). The root system architecture (RSA) can easily be impacted negatively by soil composition, water, and nutrients (Schoeneweiss 1975). Nitrogen and phosphorus are essential for RSA regulation and root growth (ibid). When roots are disturbed or even gently physically manipulated, essential nutrient uptake may diminish (Bloom & Sukrapanna 1990). As a result, the absorption of key nutrients such as K, P, and Ca can be greatly reduced (Bloom & Sukrapanna 1990). Transplantation shock is the term used to summarize symptoms experienced after transplantation (Schoeneweiss 1975). These symptoms include growth retardation, leaf wilt, developmental delay, altered metabolic processes, and in worst case death (Dong *et al.* 2020). The risk of plant death is high during transplantation (Zeljkovic *et al.* 2010). As a result, transplantation shock can have a devasting impact on agricultural yield for producers. Root growth must be resumed quickly after transplantation for the plant to survive and to reduce the impact on yield (Dong *et al.* 2020).

Many amino acid-based biostimulants have been shown to be effective in minimizing the negative effects of transplantation shock as well as abiotic stressors such as drought caused by climate change (Zeljkovic *et al.* 2010; Tkalec *et al.* 2012; du Jardin 2015; Matysiak *et al.* 2020; Cui *et al.* 2022; Häggström 2023). Biostimulants are a growing product sector on the global agricultural market. These products come in multiple forms with different functions and different types of ingredients as their base (Kauffman *et al.* 2007; Markets 2022). According to Markets and Markets, the market value for biostimulants globally in 2022 was valued at USD 3.5 billion and is estimated to increase to USD 6.2 billion in 2027 (2022). This means that the compounded annual growth rate is 10-12% (Markets 2022). One of the reasons for the projected increase in value is that agricultural producers are under pressure due to increased food demand to make production more sustainable, effective, and efficient which means they need to be able to produce crops with a higher yield (Colla *et al.* 2014; Markets 2022). According to the European Biostimulant Industry Council biostimulants can help to increase yield with a minimum of 5-10% (Council 2021).

arGrow Granulat is an established product and approved biostimulant within the forestry industry (Arevo 2023b). It is typically used when transplanting tree seedlings (Arevo 2023a). According to the company Arevo, their product,

arGrow, increases the survival rate of forestry plants by encouraging plants to grow fine roots and establish a strong root system (Arevo 2023b). The strong root system enables the plant to increase their uptake of nutrients and water which as a result leads to enhanced growth, stress tolerance and increased biomass (ibid). The product is based on an organic N source, the amino acid arginine, from crystalized arginine-phosphate (Arevo 2023c). One advantage of using organic sources of N is that plants are able to directly utilize and absorb this form of N. In contrast, using an inorganic N source requires the use of transporters to absorb N (Chen *et al.* 2022). Arevo claims that using granulates based on crystalized arginine-phosphate allows for a slow release of N and P with almost no leakage to the environment (Arevo 2023c). arGrow is used by placing a small amount of granulates underneath the plant when planting the seedling (ibid).

Plant growth is dependent on the availability of nutrients (Winter *et al.* 2015). A shortage of N, an essential macronutrient, is often a limiting factor to plant growth and can as a result have a huge impact on agricultural productivity (ibid). However, it is essential not to overuse nitrogen fertilizers due to the negative impact on the environment such as soil acidification and water eutrophication (Winter *et al.* 2015; Chen *et al.* 2022). Biostimulants that improve the accumulation and assimilation of N can thus optimize N usage to increase plant growth and minimize the negative effects on the environment (Hedwall *et al.* 2018; Chen *et al.* 2022).

There are multiple studies showing the effects of using arGrow when transplanting conifer saplings (Öhlund & Näsholm 2001; Hedwall *et al.* 2018; Häggström 2023). However, there have been few scientific studies showing the effects of using the product outside of forestry. This study aims to show whether biostimulants based on arginine, such as arGrow, can be used to minimize the effect of transplantation by enhancing plant growth and root establishment.

This study uses spinach seedlings as the focus culture. Spinach is a popular leafy green vegetable that is grown all over the world (Bhattarai & Shi 2021). It is ideal for this study as it is a quick culture that needs only a short period of time to reach maturity (Joshi *et al.* 2022). Spinach is typically cultivated during cooler weather as it sensitive to warmer temperatures and longer photoperiods (Chun *et al.* 2000; Li *et al.* 2022). Additionally, it is common practice for fresh market spinach growers to transplant spinach (Leskovar & Stein 2000). This is because transplanting spinach allows for a homogenous spinach yield that can be harvested earlier than spinach sown directly in the field (Yoshida *et al.* ; Drost 2020).

The aim of the current study is to answer the following research question; 'Can arginine be used as a biostimulant to enhance plant growth and root establishment when transplanting spinach seedlings?' In doing so, background information on biostimulants, arginine, and spinach will be presented. Thereafter, an experiment was conducted comparing spinach seedlings that have been transplanted with the biostimulant, arGrow, with spinach seedlings that have been transplanted without the use of a biostimulant. The data gathered at the end of the experiment will be statistically analyzed to determine

whether the arginine-based biostimulant has a statistically significant effect on the transplanted seedlings.

1.1 Background

1.1.1 Biostimulants

Zhang and Schmidt defined biostimulants as "materials, other than nutrients, fertilizers, that promote plant growth when applied in small quantities" and are also known as "metabolic enhancers" (Zhang & Schmidt 1997 se Kauffman et al. 2007). They differ from nutrients, fertilizers and soil amendments which also aim to enhance growth but require much larger quantities. Biostimulants do not include pesticides (ibid).

According to du Jardin, the main uses of biostimulants comprise of "growth promotion, modulation of development and of quality traits, increased tolerance to environmental stress" (2015). Biostimulants can protect against heavy metals, enhance plant establishment, maximize root development, and can improve the uptake and usage of N (du Jardin 2015). Stress tolerance can be improved by adding active compounds such as amino acids, polysaccharides, glycosides via the application of biostimulants (Zeljkovic *et al.* 2010). Additionally, biostimulants can be used to positively influence cotyledon formation and seed germination (Fries 1951; Zeljkovic *et al.* 2010).

Biostimulants are typically divided into three main categories based on their formation and ingredients: humic substances (HS), hormone containing products (HCP), and amino acid containing products (AACP) (du Jardin 2015). Biostimulants can also derive their function on utilizing bacteria such as plant growth-promoting rhizobacteria (PGPR), fungi such as mycorrhiza, or other microorganisms (ibid).

HS products are based on soil organic matter and are categorized into humins, humic acids, and fulvic acids (ibid). The product needs to positively maximize the complex interaction between roots, microbes, and organic matter to achieve consistent results such as increased growth or yield. As a result, the effect of HS varies (ibid).

du Jardin (2015) explains in his review that the category HCP includes many different types of products ranging from products that are based on seaweed extracts, sterols and active hormones that increase growth such as auxins and cytokinins. Seaweed has soil and nutrient enhancing properties but can also include specific macro and micronutrients as well as N-containing compounds to promote plant growth (ibid). The HCPs can use hormonal and antioxidant effects to improve seed germination, enhance plant establishment and to decrease the impact of environmental stresses (ibid).

According to du Jardin (2015), AACPs are used to help plants take up N and assimilate it "by regulation of enzymes involved in N assimilation and of their structural genes, and by acting on the signaling pathway of N acquisition in roots" (2015). The regulation of enzymes also improves the interaction between carbon and N (ibid). Additionally, some AACPs utilize certain amino acids to enhance the uptake and mobility of micronutrients (ibid). AACPs can

help minimize the impact of environmental stress by utilizing antioxidant activity to take advantage of free radicals of certain nitrogenous compounds (ibid). The amino-acids and peptides found in AACPs are from protein hydrolysis of plant and animal wastes from the agroindustrial industry (Jonsson 2021). Protein hydrolysates can have an indirect positive effect on plant nutrition and thus growth by enhancing microbial activity, soil respiration and fertility (du Jardin 2015). This helps make nutrients more available to the plants and help the roots acquire them (ibid). Additionally, biostimulants with certain amino acids such as betain, can be used to maintain the correct osmotic pressure in plant cells (Jonsson 2021). These products can then be used to protect against drought and saltstress caused by climate change by upholding the right level of osmotic pressure (Jonsson 2021; Cui *et al.* 2022). AACPs based on plant-derived protein hydrolysate have shown to have auxin-like effects (du Jardin 2015). A study reported an increase of 272 % in the coleoptile elongation rate of corn, increased shoot length of dwarf pea plants, and a significantly increased shoot, root dry weight, root length and root area of tomato plants after applying a plant-derived protein hydrolysate (Colla *et al.* 2014). The study also reported auxin and gibberellin-like effects, a higher uptake of N and higher yield in corn, tomato and dwarf pea plants (ibid). Leaf N content increased by 21.5 % as a result of enhanced N uptake and absorption (ibid). The biostimulant, arGrow, utilized in the experiment of this study is based on amino-acids and can thereby be classified as an AACP.

1.1.2 Arginine

The amino-acid, arginine, has the highest nitrogen and carbon ratio of all the proteinogenic amino-acids (Winter et al. 2015). This enables arginine to be "a major storage and transport form for organic N in plants in addition to its role as an amino acid for protein synthesis, a precursor for polyamines and nitric oxide (NO) and an essential metabolite for many cellular and developmental processes" (Winter et al. 2015). Arginine is a dominant storage form of N for many plant species, especially boreal species, but it can also be used to enhance the accumulation of N (Nordin & Näsholm 1997). However, for N transport to occur, plants that use arginine as their main storage form for N must metabolize arginine to glutamine first (Nordin & Näsholm 1997).

Many studies have shown that arginine can be used as a biostimulant to enhance root:shoot ratios and increase mycorrhizal activity at roots for species such as Scots pine (*Pinus sylvestris* L.*),* Norway spruce (*Picea abies* (L.) Karst), and silver birch (*Betula pendula* Roth) (Hedwall *et al.* 2018; Häggström 2023). Studies have also shown that roots have a higher uptake of arginine than ammonium or nitrate while demonstrating a lower leakage rate of N (Öhlund & Näsholm 2001; Hedwall *et al.* 2018). Arginine binds well to soil particles due to its cation properties whereas nitrate is negatively charged and moves freely in the soil allowing leaching (Padilla *et al.* 2018; Häggström 2023).

Arginine plays an important role in the stress tolerance of many plants (Hamid *et al.* 2019). It has been proven that applying arginine-based biostimulants can reduce the damage caused by many abiotic stressors such as thermal stress (cold and hot temperatures), drought, virus infections and pest

damage by increasing yield (Zeljkovic *et al.* 2010; Matysiak *et al.* 2020; Häggström 2023). Using arginine as a foliar spray has been effective in reducing the damage of water stress while also increasing plant yield for tomato plants (Hamid *et al.* 2019). Another common stress factor is N deficiency in soil (Chen *et al.* 2022). Applying exogenous arginine to environments that are N deficient has shown to increase plants' acquisition of N, P, and K as well as the transport rate of these nutrients (ibid). Temperature stress is a common abiotic stress factor that affects many plants (Matysiak *et al.* 2020). Applying arginine via spray to maize plants caused roots and shoots to grow significantly despite being constantly exposed to the stress of highly fluctuating temperatures (ibid).

For plants to synthesize arginine, synthesis via ornithine is required (Winter *et al.* 2015). Ornithine is first integrated from glutamate and then arginine is integrated from ornithine (ibid). The synthesis of arginine from ornithine utilizes multiple enzymes (ibid). Arginine accumulates together with ornithine, citrulline, and proline, which leads to increased tolerance to abiotic stressors such as drought and salinity (ibid).

Furthermore, it has been shown that applying arginine can enhance plant growth due to its ability to increase the uptake of macro and micronutrients, increase photosynthetic capacity and improve root vitality (Chen *et al.* 2022). Enhancing growth and development of roots, stems, and leaves helps plants adapt to the stress of transplantation (Zeljkovic *et al.* 2010). A well-established root system is essential to take up nutrients and water and thus for plant growth (Dong *et al.* 2020). Significant root growth is in turn required to surviving abiotic stress and to resume plant growth after transplantation (ibid). Studies on biostimulants based on arginine have shown that arginine stimulates root development, increases root mass, increases stem height, and increases overall mass of leaf material of transplanted rose, tomato, strawberry and scarlet sage plants (Zeljkovic *et al.* 2010; Tkalec *et al.* 2012; Dong *et al.* 2020).

Additionally, applying arginine has been shown to positively impact cotyledon formation and increase growth and development of lateral roots (Fries 1951).

Additional studies on maize, soya bean, celery, parsley, lettuce, and leek have proven that using arginine has positive effects on seed germination and the vitality of older seeds (Yildirim *et al.* 2002; Vinkovic *et al.* 2007; Zeljkovic *et al.* 2010).

1.1.3 Spinach

Spinach (Spinacia oleracea L.) is a popular leafy vegetable that is known for its nutritious qualities such as being an excellent source of vitamin A, iron, vitamin C, folate, calcium, and antioxidants (Leskovar & Stein 2000; Bhattarai & Shi 2021). Spinach grows in leafy rosettes and the leaves are harvested for consumption (Padilla et al. 2018; Ribera et al. 2020). It is popular worldwide and consumed in a variety of forms and cuisines. The increased demand for healthy food and increased awareness of the nutritious benefits of spinach has led to an increase in demand for spinach during the last decades (Bhattarai & Shi 2021). In 2021, the global production of spinach was 30.1 million tons (ibid).

Spinach can be grown year-round; however, it prefers mild to cool temperature (ibid). It is an annual crop of the Amaranthaceae family that is diploid and dioecious (ibid). Female plants typically flower later and produce larger leaves than males (Welbaum & International 2015)The growth cycle of spinach is short (Joshi *et al.* 2022). Spinach is sensitive to temperature and day length also known as photoperiod sensitivity (Chun *et al.* 2000). It has a shallow root system and does not utilize nitrogen efficiently (Joshi *et al.* 2022). Stress factors such as nutrient stress and low levels of N, P and K can cause growth reduction or reduced dry weight (Ryder 1979). However, fertilizing with too much nitrogen can lead to a toxic build-up of nitrate in the leaves, especially in low light conditions which leads to a less preferable taste and in worst cases the amount can be lethal or poisonous to humans (Bradley *et al.* 1975; Ryder 1979).

Spinach plants have a high nitrogen requirement and typically require more nitrogen than legumes or root vegetables (Nonnecke 1989). Leafy vegetables such as spinach have a higher nitrogen requirement when grown in the fall or winter due to low temperatures (ibid). Nonnecke recommended adding 168kg/ha N, P_2O_5 112kg/ha, and K_2O 168kg/ha via a general fertilizer when growing spinach in soil of unknown qualities (1989). Additional studies have shown that using higher amounts of nitrogen fertilizers such as 200kg/ha and up to 450kg/ha, leads to higher spinach yields (Goh & Vityakon 1983; Williams *et al.* 2003). However, this level of fertilization can lead to nitrogen leaching (Williams *et al.* 2003).

The highest yield of spinach is produced when grown in optimal temperature conditions for the specific variety (Matysiak *et al.* 2020). Spinach can handle lower temperatures (Li *et al.* 2022). However, spinach is sensitive to high temperatures (ibid). Spinach can handle temperatures around 0 degrees Celsius but do not grow well at temperatures above 23 degrees Celsius (Drost 2020).

Bolting is a significant issue in spinach production due to photoperiod sensitivity (Chun *et al.* 2000). Bolting is when the plant suddenly grows significantly in height and the reproduction phase for spinach is initiated (Ribera *et al.* 2020). The risk of bolting and flowering increases with increased temperature and day length which is why spinach is typically grown in the fall or spring (Bradley *et al.* 1975; Chun *et al.* 2000). Early bolting causes the leaves to have a bitter and undesirable taste and often leads economic loss from not being able to sell the spinach (Chun *et al.* 2000; Bhattarai & Shi 2021).

Fresh market spinach is typically transplanted (Leskovar & Stein 2000). However, it is also common during winter and early spring in northern Europe to produce spinach in commercial greenhouses (Welbaum & International 2015). It is common to sow seeds of leafy greens in peat blocks or trays with planting soil prior to transplanting to the field (Yoshida *et al.*). Plants sown in peat blocks often have well developed root systems (ibid). Transplanting leafy greens is advantageous because it can enhance initial growth (ibid). Additionally, it is easier to ensure homogeneity amongst plants which is important in commercial production (ibid). It is also advantageous as farmers can remove the weeds from the field prior to transplantation and thus disturb the plants less once planted (Ögren & Jonsson 2021). Another advantage to transplanting spinach is that it allows for an earlier harvest (Yoshida *et al.*). Spinach should be transplanted when the plants have 4 to 6 mature leaves and an established root system (Drost 2020).

It was suspected that transplanting caused bolting in spinach plants, however studies have shown that this is not the case (Yoshida *et al.*). Bolting rate is higher for spinach grown in water-deficient conditions (ibid). This limits leaf expansion and can delay harvest and reduce yield if bolting occurs before the plants have grown to a marketable size (ibid). Transplanting spinach can cause water stress because the roots may have smaller dimensions than the root distributions of plants sown directly in the field (ibid). Transplanting can also cause the leaves of spinach plants to unfold at a smaller size which can lead to bolting (ibid).

2. Aims

The aim of the project is to see whether arginine, using the product arGrow, can be used as a biostimulant to reduce the effect of transplantation shock on spinach seedlings by enhancing plant growth and root establishment. Additionally, this project aims to show whether arGrow has the potential to be used successfully as a biostimulant outside of the forestry industry by testing its usage on a common agricultural culture.

2.1 Research Question

Can arginine be used as a biostimulant to enhance plant growth and root establishment when transplanting spinach seedlings?

2.2 Hypothesis

Null hypothesis: $H0:\mu A=\mu B$
There will not be a statistically significant difference in the mean leaf size, number of leaves, height and biomass between the treatment groups.
   Alternate hypothesis: $H1:\mu B>\mu A$
The group of plants treated with arGrow will have a larger mean leaf size, number of leaves, height and biomass than the control group that was not treated with arGrow.

2.3 Limitations

The experiment is limited in time due to the length of the course. The experiment will run for three weeks from the transplantation date. Ideally, the experiment would run for four weeks to fully examine the effects of transplantation and the recovery afterwards. The spinach plants would be at full maturity by that time.
   The treatment groups and number of seedlings used are limited to two groups with 16 seedlings in each. This will give sufficient data to determine whether any potential differences in results are significant. Additionally, had time and space in the growth chamber allowed more treatment groups with varying dosage amounts of arGrow would be studied.

Typically, transplantation of spinach would be to a field or soil plot outside in early spring. However, given that this experiment is during the winter months in Sweden, this is not possible. The growth chamber settings allow the experiment to mimic spring conditions in a controlled environment; however, the experiment would be more realistic had it taken place outside. All factors that would typically affect transplants outside in the spring are not able to be studied such as drought, heavy rainfall, and sudden temperature changes such as drops to below freezing temperatures. The scope of the experiment's environmental parameters is limited to the soil, temperature, humidity, and light. The individual effects of these different parameters will not be studied but will be grouped into the effect of the transplantation.

The amount of arGrow to be used is based on direct recommendations from Arevo. However, there is limited published research material on using this specific product outside of the forestry industry or for spinach. Therefore, it is uncertain whether the dosage amount or application method used is correct.

3. Material and Method

3.1 Plant Materials

The experiment uses organic seeds of the spinach variety 'Giant Winter' distributed by Florea. 'Giant Winter' is ideal for fall, winter or early spring cultivation as it favors cold temperatures (Spinach 'Giant Winter' 2023). 'Giant Winter' will bolt during summer months or periods of high temperatures and is not considered bolt resistance (ibid). Spinach seeds prefer cool soil (Nonnecke 1989). This variety has large, dark green leaves (Spinach 'Giant Winter' 2023). Optimal plant spacing is 30cm with a row spacing of 40cm (Florea 2023). It prefers a loose, loamy, well-draining soil but can be grown in most soils (Florea 2023). Spinach is sensitive to acidic soil and the ideal pH for soil should be between 6-7 (Ryder 1979; Spinach - Giant Winter 2017). The plant typically grows to a height of 10 to 19 cm (Florea 2023). This variety takes approximately 45-60 days to reach maturity (Spinach - Giant Winter 2017).

3.2 Experiment

3.2.1 Treatment groups

The study compares two treatment groups, the control group and the biostimulant group. Both groups consist of 16 spinach seedlings of the variety 'Giant Winter'.

Control group (A): 16 spinach seedlings that have been transplanted approximately two weeks after sprouting. The seedlings have 3-5 true leaves each. The seedlings did not receive the biostimulant treatment. The control group is also referred to as group A.

Biostimulant group (B): 16 spinach seedlings that have been transplanted approximately two weeks after sprouting. The seedlings have 3-5 true leaves each. The seedlings received the biostimulant treatment of 4.5 grams of arGrow at transplantation. The biostimulant group is also referred to as group B.

3.2.2 Experimental set-up

The growth chamber is divided into 4 block sections. Each block contains 4 trays with two plants of the same treatment group on each tray. Each block has a total of 8 plants within it of which 4 plants from treatment A and 4 plants of treatment B. The placement of pots is according to a randomized block design. An online randomizing program was utilized to guarantee a random placement. In total the experiment has 16 replications of each treatment group, totaling 32 plants. Utilizing a random block design ensures that the placement of the plants is not an impacting factor on the experiment and that the placement is unbiased towards the treatiment group (Forkman 2012).
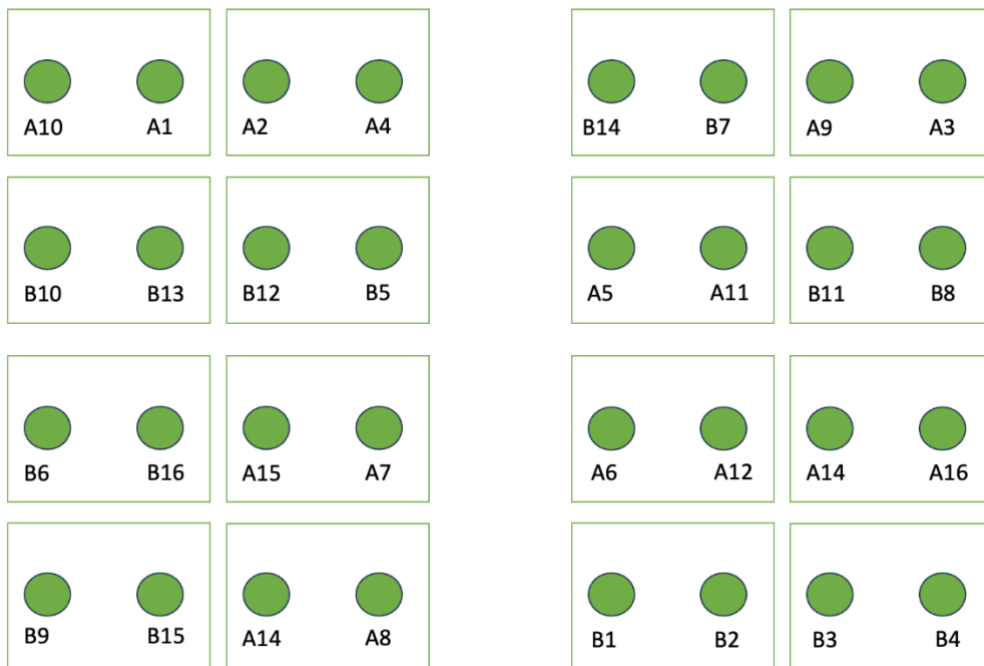
Figure 1. Image of the experiments block design and plant placement

The image above displays the random block design and the randomized plant placement. There are 4 groups of 4 trays and each block contains 8 individuals. Each block of plants contains two rows. The plants are spaced in the blocks according to the seed distributors recommendations at 30cm with a row spacing of 40cm (Florea 2023).

    The study uses 32 flowerpots with a diameter of 17 cm, depth of 10 cm and a volume of 2 liters. The flowerpots stand on tray carts. Appropriate measuring utensils such as scales, teaspoons, and ml measuring cups are used to measure dosages of biostiumlant and fertilizer. Scales and drying cabinets are used to to measure weight data. A ruler is used to measure plant height and leaf size. Safety equipment such as nitrile gloves, filtering half mask and protective clothing is worn as a safety measure when handling all materials.

3.2.3 Cultivation chamber and conditions

The study takes place in a growth chamber in the SLU Biotron. The growth chamber is an artificial light chamber that provides a controlled environment and allows for accurate climatization. The area of the room is 11,5 m2 (SLU 2020). Temperature, relative humidity, light intensity, light hours and carbon dioxide levels can be systemized and installed (ibid). This allows the study environment to mimic outdoor temperature and light levels typical in the month of April in Alnarp, Sweden. Carbon dioxide levels were not adjusted.

  Yellow glue traps hang in each block to indicate the presence and number of potential pests. Koppert Swirski Ulti-Mite pouches also hang in each block for control of potential thrips or lice.

   Light intensity is set to 203 μmol m^{-2} s^{-1} for 14 hours a day. The day starts at 04:00. The light intensity is set towards a lower level of the recommended PAR for spinach according to Cornell University (Brechner & de Villiers 2013). However, this is to reflect the light levels that the plants would have received had they been transplanted outdoors.

Daytime temperature is set to 15 degrees Celsius. Nighttime temperature is set to 5 degrees Celsius. These settings are close to the average temperatures during April in Alnarp, Sweden (Vackertväder.se 2023). Relative humidity is set to 70% 24 hours a day.

3.2.4 Fertilizer

All 32 pots are filled with soil and mineral fertilizer (Hasselfors Universell Växtnäring) with NPK value of 19-4-20 mixed into it (Granngården 2023). The fertilizer also contains magnesium, sulfur and micronutrients (ibid). The pots contain approximately 2 liters of soil each. 4.2 g of fertilizer is added to each pot and thoroughly mixed into the soil. The total amount of N added to each pot is 798 mg N. The total amount of P added to each pot is 168 mg P. The total amount of potassium added to each pot is 84 mg K.

   Fertilizer = 2.1 g/L

19 % N x 4.2 g = 0.798 g N.

   Nitrogen = 0.399g/L = 399 mg/L = 399 kg/ha

4 % P x 4.2 g = 0.168 g P

   Phosphorus = 0.084 g/L = 8.4mg/L = 8.4 kg/ha

20 % K x 4.2 g = 0.84 g K

   Potassium = 0.42 g/L = 42 mg/L = 42 kg/ha

4. Method

4.1 Transplantation

The day before transplantation, soil was dug up from Grobruket AB's market garden in Alnarp, Scania province in Sweden. The soil type is humus-rich muddy moraine (SGU 1994). The soil was sifted to remove large particles and worms and to break up large aggregates to change the soil into a finer format ready for planting.

A soil sample was sent to a soil analysis lab, AB Lennart Månsson International (LMI), for Spurway analysis prior to transplantation.

All pots are prepared with soil and fertilizer. The pots are marked with treatment group and plant number, A1-A16 or B1-B16. A space is dug into the soil for the plant to be placed.

In pots marked 'B' arGrow granules are deposited into the space without blending into the soil. Arevo recommended that the study use a dosage of 4,5 grams arGrow per plant in the treatment group (Höög 2023). The granules are directly under the plant roots as recommended by Arevo (Arevo 2023c).

Thereafter, seedlings are gently removed from the seed tray, keeping the roots as best intact as possible, and placed into all the pots. The arGrow granules are thus located directly under the roots of the plants. Additional soil was added if needed.

The pots are then moved to the growth chamber and placed according to the random block design. All pots are watered once with Koppert Entonem on the day of transplantation to reduce the risk of pests.

During the experiment, the plants are watered every other day and the amount varied based on soil dryness.

4.2 Data Collection

Nutrient conditions in the soil were analyzed at the start of the experiment. The study has 4 measurement occasions when data for different parameters are collected.

The plant height in centimeters is measured on a weekly basis from the growth point to the top of the highest leaves extended upwards.

Average leaf length per plant is measured on a weekly basis from the leaf tip to the end of the leaf blade. The leaf stem is not included in length. The leaf

selected for measurement is a middle-aged leaf (approximately the third leaf from the top of the plant).

Additional observations are taken on a weekly basis and recorded. These observations are indications of stress such as whether the leaves are discolored or indicate a nutritional imbalance, whether the plant has flowered or not, whether growth seems to be stunted, whether the plant has wilted or died. The electrical conductivity (EC) will be measured in millisiemens per centimenter (mS/cm) of the leachate of each group on the 3rd measurement occasion.

At the end of the experiment the final plant height and leaf length are measured again as well as the total number of leaves per planted.
The fresh weights of the above ground material and root material are collected at the end of the experiment. The plants are moved from the Biotron to a tabletop workspace and are placed into groups according to treatment. All A plants stand together, and all B plants stand together. Photos are taken of the two treatment groups. The plants are carefully removed from the pots, being careful to keep all the roots as intact as possible. The soil is delicately removed by hand. Thereafter, the roots are rinsed in a bucket of water to further remove soil to the point where the roots are as bare as possible. The plants are placed on a paper labelled with the plant number. The plant is cut underneath the growth point to separate the leaf material from the root material.

The leaf material is weighed on a scale, the weight is recorded, the plant material is placed in the aluminum foil envelope marked with the plant number and AG. The root material is weighed on a scale, the weight is recorded, the root material is placed in the aluminum foil envelope marked with the plant number and R. The 64 aluminum foil envelopes are placed into a drying cabinet with the heat set to 105 degrees Celsius.

The dry weight is collected after the material has spent five days in the drying cabinet. The 64 envelopes are removed from the drying cabinet. A piece of paper is folded into an open box and used as a plate on top of the scale. The scale is tared with the paper box on it. The dry weight of the leaf material is taken individually by placing the plant material in the paper box and thereafter placed on the scale. The weight is recorded in grams.

All data variables collected for each group of plants will be compared via statistical analysis and tests to determine whether differences in the investigated parameters between the groups are significant. The mean and standard error will be examined for each parameter. Thereafter, the data will be run through a Shapiro-Wilks test for normality in the statistical software, R Studio. A one-sided two-sample t-test for determining significance in the difference of the mean will be used if the data is normally distributed (Swinscow 1997). A non-parametric test called the Kruskal-Wallis test can be used for samples that are not normally distributed (Xia 2020). The p-value will be used to indicate whether the null or alternate hypothesis can be accepted (Swinscow 1997). If the p-value is less than the significance value of 5% the null hypothesis can be rejected and assume that the difference between the mean values is statistically significant and not a result of chance (Ibid).

5. Results

5.1 Soil nutrient content

The values in the table below represent the analysis results (LMI 2023). The column marked "Present in soil (kg/ha)" in the table displays LMI's recommended values for spinach based on previous studies of the culture that the company has performed (ibid). The values that are colored green indicate satisfactory levels of the subject (ibid). The values that are colored yellow indicate that the levels of that subject are low (ibid). The values that are colored red indicate extremely low levels of that subject (ibid).

Table 1. Spurway analysis results & recommended values for spinach

Subject	Value:	Present in soil (kg/ha):
pH	7.3	
Electrical conductivity (mS/cm)	0.46	
Nitrogen (mg/l)	5.0	10
Phosphur (mg/l)	42.0	
Potassium (mg/l)	91.0	140
Magnesium (mg/l)	72.0	50
Sulfur (mg/l)	4.0	5
Calcium (mg/l)	1,000.0	
Manganese (mg/l)	1.3	
Boron (mg/l)	1.0	2
Copper (mg/l)	3.0	
Iron (mg/l)	1.3	
Zinc (mg/l)	6.2	
Molybdenum (mg/l)	0.10	
Sodium (mg/l)	28	
Aluminum (mg/l)	1.9	

The analysis indicates that the soil contains an insufficient amount of nitrogen, enough phosphorus, and a generally low amount of potassium. Additionally, multiple micronutrients are low. The analysis provides insight as to the amount of fertilizer needed for the study.

5.2 Growth parameters

The full data sets collected during the study including the data for each plant individual can be seen in Appendix 1.

5.2.1 Measured leaf size

Average leaf size was measured in centimeters throughout the length of the study. This provides insight as to how the plants have developed over time.

Table 2. Average Leaf Size (cm) collected over four measurement occasions. The standard error (SE) is presented to the right. The table shows that the average leaf size for group A is larger at every measurement occasion. The standard error for the mean at each measurement occasion is similar for both groups.

Occasion	Mean A	SE A	Mean B	SE B
1	2.86	±0.18	2.83	±0.15
2	3.38	±0.23	3.20	±0.23
3	5.97	±0.51	2.71	±0.43
4	8.03	±0.62	2.69	±0.57

The average leaf size increased over time for the plants in group A whereas the average leaf size over time decreased for the plants in group B. The average leaf size for group A was consistently larger than group B's throughout the study.

The Shapiro-Wilks tests showed that the data for average leaf size collected throughout the experiment was normally distributed during the first 3 measurement occasions ($p > 0.05$). However, on the final measurement occasion the data no longer is normally distributed for group A ($p = 0.018$) or for group B ($p = 0.028$). The non-parametric Kruskal-Wallis test results showed that there was no significant difference between the average leaf size of first two measurements ($p > 0.05$). The difference between the average leaf size became significant for the last two measurements. The p-value for occasion 3 is 0.0003 and 0.000022) for occasion 4. Thus, the null hypothesis can be rejected as Group A had significantly larger leaves than group B at the end of the experiment.

5.2.2 Total number of leaves

The total number of leaves were counted for each plant as a comparative measure of growth.

Table 3. The total number of leaves was measured on the last measurement occasion. Group A had on average a larger of total number of leaves per plant with a small standard error (SE) as compared with group B.

Occasion	Mean A	SE A	Mean B	SE A
4	18.81	±1.74	13.00	±2.16

The average number of leaves shown by the mean value for plant group A was higher than the average number of leaves for plant group B was 13.00 at the

end of the study. This indicates that on average, plants in group A have more leaves than plants in plant group B. The plants in group A have a lower standard error which suggests that the number of leaves in group A is less spread out around the mean compared to group B, where there is more variability in the number of leaves.

The Shapiro-Wilks test for normality showed that the data for group A has a normal distribution ($p = 0.23$) and group B has a borderline normal distribution ($p \approx 0.05$). The p-values generated by the non-parametric Kruskal-Wallis test ($p = 0.027$) as well as for a one-sided ($p = 0.022$) were below the level of significance ($p = 0.05$). This indicates that the difference in total number of leaves is significantly different between the groups and that the null hypothesis can hereby be rejected.

5.2.3 Plant height

The plants' heights at the end of the study show whether the biostimulant impacted the plant's growth.

Table 4: The average plant height was measured in centimeters on four occasions during the study period. The average plant height was consistently higher for group A as compared with group B throughout the study. The standard error (SE) is within similar levels for each measurement occasion for both groups.

Occasion	Mean A	SE A	Mean B	SE B
1	8.61	±0.32	8.59	±0.29
2	9.76	±0.44	9.51	±0.54
3	12.93	±0.81	7.54	±1.07
4	15.71	±1.03	7.74	±1.38

The average plant height for group A increased over time and was higher than those in group B throughout the entirety of the study. The average height of plants in group B decreased from the second measuring occasion. The mean indicates that the average height of plants in group A was higher than the average height of plants in group B. The average height of plants in group A was more than twice the average height of plants in group B. Group B has a higher standard error than group A for majority of the occasions, indicating greater variability in plant heights in group B.

The Shapiro-Wilk test for normality showed that the data for group A on occasion 2 is not normally distributed ($p = 0.012$). The data on occasion 3 for group B is not normally distributed ($p = 0.034$). All other groups and occasions are approximately normally distributed. The Kruskal-Wallis test showed that there are significant differences between the heights of plants in group A versus plants in group B for occasions 3 ($p = 0.00049$) and 4 ($p = 0.00015$) but not for occasions 1 ($p = 0.75$) and 2 ($p = 0.623$). For the 4th occasion, the Kruskal-Wallis chi-squared value is 14.38, the degrees of freedom is equal to 1, and the p-value is 0.00015. The high chi-squared test statistic indicates that the groups distributions differ significantly. A p-value of 0.00015 is extremely small, indicating that it's highly unlikely the differences observed between the groups happened by chance. For occasions 3 and 4, the one-sided t-test also indicates a significant difference between groups ($p = 0.0002$ and $p =$

0.000038) but similarly to the Kruskal-Wallis test it did not show significant differences for occasions 1 and 2 (p > 0.05). Both the Kruskal-Wallis test and the t-test suggest that the null hypothesis should be rejected.

5.2.4 Biomass – leaf material

The leaf material was measured in grams to compare the biomass of the plants. The weight of the leaf material was measured as fresh weight as well as dried.

Table 5. The biomass for leaf material was measured as a fresh weight and dry weight in grams. The mean was larger for group A as compared with group B for both fresh and dry weight. The standard error (SE) was larger for group A than group B for both fresh and dry weights.

Group	Mean (fresh weight g)	SE (fresh weight g)	Mean (dry weight g)	SE (dry weight g)
A	13.44	±2.16	1.21	±0.19
B	2.35	±0.62	0.28	±0.06

The average fresh weight shown by the mean for Group A is 13.44 g, which is significantly higher than Group B's average of 2.35 g. The dry weight follows the same trend as the fresh weight and group A had a higher average dry weight than group B. Group A has a larger standard error compared to Group B for both fresh weight and dry weight. This indicates that the weights in Group A are more spread out, showing greater variability. Group A tends to have heavier weights than Group B.

The Shapiro-Wilk test for normality showed that group A had normal data distribution for both fresh weight (p = 0.31) and dry weight (p = 0.15). On the other hand, group B had a non-normal distribution for both fresh (p = 0.0038) and dry weight (p = 0.035). The Kruskal-Wallis test gave very low p-values for both fresh (p = 0.000064) and dry weights (p = 0.00024). The p-value for the fresh and dry weights are lower than the level of significance (p < 0.05) which indicates that there are significant differences in the fresh and dry weights of group A and B that are unlikely a result of chance.

5.2.5 Biomass – Root material

The root material was also measured in grams as an additional comparative measure of biomass. The root material was measured as fresh weight and dry weight.

Table 6. The biomass for the root material was measured as a fresh weight and dry weight in grams. The mean was larger for group A as compared with group B for both fresh and dry weight. The standard error (SE) was larger for group A than group B for both fresh and dry weights.

Group	Mean (fresh weight g)	SE (fresh weight g)	Mean (dry weight g)	SE (dry weight g)
A	3.72	±0.60	0.59	±0.31
B	1.19	±0.25	0.25	±0.11

The average weight of plants in group A (3.72 g) is much higher than that of plants in group B (1.19 g). Plants in group A have a higher standard error compared to the plants in group B. This suggests that group A's weights are more spread out and variable compared to group B.

The Shapiro-Wilks test for normality showed that the data for group A ($p = 0.00000088$) and group B ($p = 00000065$) is not normally distributed. The Kruskal-Wallis test showed that there is a significant difference between the fresh weights of the groups ($p = 0.00084$). However, there is no significant difference between the dry weights of group A and group B ($p = 0.35$). The null hypothesis can be rejected for the fresh weight but not for the dry weight.

5.2.6 Additional Observations

Additional observations were studied continuously. Symptoms of transplantation shock and stress such as wilting, leaf discoloration, flowering and death were noted each week.

Table 7: The number of plants in group A and B that displayed symptoms of wilting, leaf discoloration, flowering or death throughout the study. The data was collected at 4 measurement occasions.

Symptom	Occasion	Group A	Group B
Wilting	1	1	6
	2	0	7
	3	0	6
	4	0	8
Leaf Discoloration	1	4	3
	2	6	13
	3	8	10
	4	7	9
Flowering	1	2	5
	2	2	6
	3	2	6
	4	3	6
Dead	1	0	0
	2	0	0
	3	0	3
	4	0	4

The increase in plants exhibiting symptoms of wilting or dying increased over time for plants in group B. This coincides with the average leaf size and average plant height decreasing over time for plants in group B. By the end of the study group A exhibited zero plants wilting or dying whereas group B had exhibited 8 plants with symptoms of wilting and 4 plants that had died. Group B generally had more individuals exhibiting this symptom as compared to group A apart from the first measurement occasion.

Group B had more than double the number of individuals that flowered prior to maturity. as compared to group A, apart from the last measurement date. Flowering occurring at the last measurement date may be a result of the plant reaching maturity or be a symptom of bolting. Group B had 6 plant individuals that flowered from the 2nd measurement occasion and onwards (37.5 % of group B). Whereas from the 2nd measurement occasion group A only had 2 individuals with flowers (12.5 %) and on the 4th measurement occasion had 3 individuals with flowers (18.6 %).
None of the plant individuals in group A died whereas by the end of the study group B had 4 dead plant individuals.

To investigate whether the observed stress symptoms could be a result of overfertilization, the water on the trays of each plant group was sampled and the electrical conductivity (EC) was measured on the 3rd measurement occasion. Group A had an EC level at 2.5 mS/cm whereas group B had an EC level at 2.4 mS/cm.

Additionally, it was observed at the end of the study that for group B, the arGrow granulates had not broken down sufficiently and formed a gray mass underneath the roots of all the plants in the B pots. The roots did not grow through this. The root length was limited to where the roots touched the granulates. In a few cases the roots grew to the side of the arGrow but in most cases the root growth seemed to be limited.

6. Discussion

The study aimed to see whether arginine, using the product arGrow, can be used as a biostimulant to reduce the effect of transplantation shock on spinach seedlings by enhancing plant growth and root establishment. At the end of the study, data for the plants' leaf size, number of leaves, height and biomass were collected for each group. Applying arginine to plants in other studies have shown an increased stress tolerance (Winter et al. 2015; Hamid et al. 2019; Matysiak et al. 2020). The hypothesis was that spinach plants treated with the arginine biostimulant, arGrow, (group B) at transplantation will have a larger average leaf size, a larger number of leaves, be taller than the control plants (group A) and will show larger biomass (fresh and dry weights) of leaf and root plant material than the plants that were not given the treatment. Furthermore, symptoms such as flowering, leaf discoloration, wilting, and plant death were monitored throughout the study to assess potential stress or shock resulting from transplantation.

Previous studies have shown that arginine can help enhance root development thus helping plants increase their uptake of vital macro and micronutrients (Chen *et al.* 2022). A healthy root system and higher uptake of nutrients can lead to enhanced plant growth (ibid). Furthermore, a well-developed root system assists plants in surviving abiotic stress such as transplantation (Dong *et al.* 2020).

The results showed that when comparing groups, A and B there was a significant difference in the mean leaf size number of leaves, height, fresh weight and dry weight of the leaf material and fresh weight of the roots. However, the dry weight of the roots did not have a significant difference. The results of the dry weight of the roots can be impacted and skewed by an inconsistent level of moisture removed from the material and that the root material was so little for some individuals that the root mass amounted to 0 g. Overall, majority of the results and coinciding statistical tests indicate that the null hypothesis stating that there is no significant difference between the means, should be rejected. The alternate hypothesis stated that the means should be significantly larger for group B. The alternate hypothesis, cannot be accepted as the mean for all data parameters was higher for group A. To conclude, the data collected at the end of the study does not support the theory that the biostimulant treatment using arginine, arGrow, enhances plant and root growth.

Furthermore, the data does not indicate that arginine can reduce the impact of transplantation shock. The plant group treated with the arginine biostimulant exhibited more individuals that bolted (flowered) as compared to the control group that did not receive the treatment. Bolting can be a reaction to abiotic

stress (Guoliang *et al.* 2020). Leaf discoloration can occur when the plant either is unable to absorb available nutrients or when there is a lack of available nutrients (Evert & Eichhorn 2013). The biostimulant group also had more individuals that exhibited symptoms of wilting and leaf discoloration than the comparative group of plants. Finally, group B which was treated with arginine had 4 individuals that died by the end of the study as opposed to 0 dead individuals in group A.

Transplantation shock causes symptoms of growth retardation, leaf wilt, developmental delay, and death (Dong *et al.* 2020). Transplantation significantly disturbs roots which can impact the absorption of nutrients (Berkowitz 1987; Bloom & Sukrapanna 1990). Transplantation shock needs to be minimized and roots must resume growth quickly after transplantation in order for the plant to survive, obtain nutrients and grow (Dong *et al.* 2020). Group B exhibited more individuals that suffered from wilt, leaf discoloration, death and flowering as compared to group A. This means that the plants that were treated with arginine, did not show fewer or milder symptoms of transplantation shock.

Bolt, wilt, leaf discoloration and death are symptoms that a plant is experiencing stress (Dong *et al.* 2020; Guoliang *et al.* 2020). However, both plant groups have seedlings originating from the same population and were transplanted on the same date and under the same conditions other than the biostimulant treatment given to group B. The biostimulant did not reduce the symptoms of transplantation shock as group B exhibit more individuals with these symptoms compared to group A.

A common cause of stress for plants is high salinity levels (Machado & Serralheiro 2017). Overfertilization is one of the leading causes of soil salinity (ibid). Excess salinity in the soil can lead to significant decrease in yield of many vegetable crops due to low salinity tolerance (ibid). High salinity causes an increase in osmotic pressure in the soil which leads to a reduced water uptake by plants (ibid). Symptoms of salt stress are yellowing leaves, wilting, reduced or stunted plant growth, imbalanced uptake of nutrition, inhibition of photosynthesis and decreased leaf growth (ibid). Spinach has a low tolerance of soil salinity and can handle only up to 2,0 ($dS \cdot m{-}1$) (ibid). The concentration of dissolved salts can be measured by measuring EC (ibid). High nitrogen salt in the soil caused by excess fertilizers causes dehydration in the plant (Bibi & Ilyas 2020). Excessive fertilizing causes a decrease in water uptake which can lead to root burn, also known as, fertilizer burn (ibid).

Many of the spinach plants in group B exhibit symptoms that align with symptoms of salt stress described by Machado and Serralheiro (2017). Already after one week from transplantation, plants in group B exhibit wilting, stunted growth, yellowing leaves and bolting. The plants received of 399 mg/L N (399 kg N/ha) via fertilizer when transplanting. The arGrow dosage of 4.5 g per pot added an additional of 540 mg of N (0.12 N x 4.5 g granulate = 0.54 g = 540 mg N/L). The total amount of N when combining the amount from arGrow with the amount from the fertilizer, administered to group B is above the optimal levels of N for spinach (Goh & Vityakon 1983; Nonnecke 1989; Williams *et al.* 2003). ArGrow is marketed as a biostimulant, not as a fertilizer (Arevo 2024). However, combining arGrow with a fertilizer that also contains

N might cause overdose or salt stress like the symptoms seen in treatment group B of this study. To investigate whether the high amount of N was causing the stress symptoms the water on the trays of each plant group were sampled and the electrical conductivity was measured on the 3rd measurement occasion.

Group A had an EC level at 2.5 mS/cm whereas group B had an EC level at 2.4 mS/cm. It is possible that overfertilization is a cause of the symptoms as both EC levels are above the tolerance level of salinity for spinach (Machado & Serralheiro 2017). However, the result does not clarify as to why group B shows more symptoms of stress as compared to group A. It is unlikely that the difference in mean is a result of salt stress for group B, given that group A has approximately the same EC level.

The handling and drying process of the roots may have influenced the biomass measurements. Additionally, it is possible that not all soil was completely removed from the roots prior to weighing the plant material, as thoroughly cleaning the roots without damaging the fine structures proved challenging. This was particularly evident in group B plants, where the roots were extremely delicate and prone to breaking during soil removal. As a result, more soil may have been left on B plants as it was difficult to remove the soil while preserving the root material. Furthermore, the root material for 4 plant individuals in group A and 1 individual in group B did not dry properly. Another factor that may have influenced the results is that the arGrow granules did not fully break down in the soil, instead forming a clay-like mass beneath the roots. This could have hindered nutrient uptake and may be attributed to the clay content of the soil.

Taking soil directly from a market garden provides the study with soil that is representative of soil that spinach and many other agricultural crops are typically transplanted into. Humus-rich muddy moraine soil is common in many agricultural areas of Sweden (Ekström 1927). A muddy moraine soil contains clay (Wallander *et al.* 2016). Clay soil can become very moist and compact upon heavy rain (ibid). It had rained recently prior to extracting the soil which made the soil dense, compact and saturated with water (Ekström 1927). The soil pores are very small which increases the capillary force (Wallander *et al.* 2016). The capillary force enables the soil to hold large amounts of water but the water is not always available to the plants (ibid). This type of soil is typically calcium-rich and often contains a high amount of phosphorus (Ekström 1927). The results might have differed if a conventional potting soil from a garden center had been used instead.

Additional analyses, such as plant sap analysis, could have provided deeper insights into whether the observed stress symptoms were linked to specific nutrient levels.

The product, arGrow, was developed to be used in the forestry industry and multiple studies have shown its success in this area (Öhlund & Näsholm 2001; Häggström 2023). As mentioned earlier, the plants treated with the biostimulant in this study had a smaller average biomass, leaf size, number of leaves and plant height while also exhibiting multiple stress symptoms. The yield must be significantly increased by using arginine to be relevant to market gardeners. According to the European Biostimulant Council, biostimulants can

increased the yield with a minimum of 5-10% (Council 2021). The study did not show that using arginine increased yield as the plants that were treated with arginine at transplantation had on average a smaller biomass than the non-treated plants. However, running new experiments using the product with other plant cultures that have a more robust root system than spinach, soil types, testing different dosage amounts and application methods may lead to different results. Thus, studying arginine as a biostimulant further may be recommended to answer whether it can be a relevant product outside of the forestry industry and or relevant for market gardeners.

7. Conclusion

This study examined whether arginine in the form of the product, arGrow, can be used as a biostimulant to enhance plant and root growth when transplanting spinach plants. Other studies have shown that arginine can impact cotyledon formation, increase the development of roots, and enhance growth of boreal, rose, strawberry, tomato and scarlet sage species. This is based on the proposition that arginine can increase the uptake of macro and micronutrients, increase photosynthetic capacity and improve root vitality. These factors further enhance the plants' ability to adapt to the stress of transplantation, as healthy plants with robust root systems are more resilient. It was hypothesized that plants that received arginine in the form of arGrow, would have on average a larger leaf size, number of leaves, plant height, fresh weight and dry weight of leaf and root material than spinach plants that were not treated with arginine. The results showed that there was a significant difference between the mean values of most of the studied variables between the two groups. Thus, the null hypothesis is rejected. However, plants that did not receive the arginine treatment at transplantation had on average larger leaf sizes, more leaves, taller plants with larger biomass compared to the treated group of plants. Both groups of plants exhibited symptoms of stress, however, the symptoms were more frequent and dire for individuals that received arginine. Transplantation shock syndromes were not reduced for the arginine-treated plant group in comparison to the group that did not receive the treatment. Contrastingly, the group that received the arginine biosimulant had more plant individuals that died, exhibited symptoms of wilt and leaf discoloration and bolting. Therefore, the alternate hypothesis could not be accepted either.

The study also considers whether an arginine biostimulant can be a relevant product outside of the forestry industry and for market gardeners. The yield should be significantly increased for the product to biostimulant to be considered relevant outside of the forestry industry for market gardeners. The treatment did not lead to an increase in yield and therefore does not provide support for the relevance of the product outside of forestry. Studying arginine as a biostimulant further may be recommended to determine whether it can be a relevant product outside of the forestry industry and or relevant for market gardeners. It is possible that the product arGrow, could perform differently with different plant species or with different agricultural conditions such as soil type. Additional studies could review the dosage amount and application methods of arginine. Furthermore, this study could be replicated with a conventional potting soil to determine whether the results of using arginine at transplantation are impacted by soil types.

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Acknowledgements

I would like to express my deepest gratitude to my supervisor, Samar Khalil at SLU, and my mentor, Niklas Hjelm at Grobruket AB. Your invaluable support, insightful guidance, and unwavering encouragement have been truly inspiring and instrumental throughout my thesis journey.

A heartfelt thank you to my husband, Joakim Faxå, for your love, steadfast support and hours of proofreading, which have been immensely helpful. Lastly, to my wonderful children, Klara and Liam, thank you for the boundless love and joy you bring into my life every single day. Your presence has been my greatest source of strength and happiness.

Appendix 1 – Full data sets

Appendix 1 includes the full data sets collected during the experiment

Table 1. Leaf size (leaf blade without stem (cm)) of all plants.

Plant:	1	2	3	4
A1	4.1	5	8.6	10.2
A10	2.7	3.1	4	5.9
A11	3.3	3.5	6.1	8
A12	2.8	3.5	6	9
A13	2.5	3.4	5.5	9
A14	2.7	2.6	4.3	7.4
A15	2.1	2.8	3	3.5
A16	3.5	3.5	6.8	9.5
A2	2	1.8	2.2	3
A3	2.5	2.2	6	9.5
A4	2.1	3	4.1	4.7
A5	2.3	2.5	7.5	10.4
A6	2.7	4.5	8	10.3
A7	3.6	4.2	8.4	9.5
A8	4.4	5	9	10.6
A9	2.5	3.5	6	8
B1	2.5	2	2.6	2.3
B10	2.8	3.7	3.3	3
B11	1.8	2	1.9	0
B12	2.2	2.6	2.5	2.5
B13	2.1	3	3.2	3.1
B14	2.9	3.4	3.3	3.1
B15	2.6	3.5	6.5	8.3
B16	3.7	3.9	4.5	6.3
B2	2.2	1.7	0	0
B3	3	2.8	2.9	2.7
B4	3.4	3.1	2.6	2.4
B5	3.2	4	0	0
B6	3	3	0	0
B7	4.2	4	4.1	4.3
B8	2.7	5.5	2.5	2.2
B9	2.9	3	3.4	2.9
A1	4.1	5	8.6	10.2
A10	2.7	3.1	4	5.9
A11	3.3	3.5	6.1	8
A12	2.8	3.5	6	9
A13	2.5	3.4	5.5	9
A14	2.7	2.6	4.3	7.4

A15	2.1	2.8	3	3.5
A16	3.5	3.5	6.8	9.5
A2	2	1.8	2.2	3
A3	2.5	2.2	6	9.5
A4	2.1	3	4.1	4.7
A5	2.3	2.5	7.5	10.4
A6	2.7	4.5	8	10.3
A7	3.6	4.2	8.4	9.5
A8	4.4	5	9	10.6
A9	2.5	3.5	6	8
B1	2.5	2	2.6	2.3
B10	2.8	3.7	3.3	3
B11	1.8	2	1.9	0
B12	2.2	2.6	2.5	2.5
B13	2.1	3	3.2	3.1
B14	2.9	3.4	3.3	3.1
B15	2.6	3.5	6.5	8.3
B16	3.7	3.9	4.5	6.3
B2	2.2	1.7	0	0
B3	3	2.8	2.9	2.7
B4	3.4	3.1	2.6	2.4
B5	3.2	4	0	0
B6	3	3	0	0
B7	4.2	4	4.1	4.3
B8	2.7	5.5	2.5	2.2
B9	2.9	3	3.4	2.9

Table 2. The total number of leaves for each plant on 4th measurement occasion.

Plant A:	Number of Leaves:	Plant B:	Number of Leaves:
A1	26	B1	11
A10	12	B10	38
A11	10	B11	10
A12	22	B12	7
A13	16	B13	18
A14	14	B14	11
A15	24	B15	12
A16	25	B16	20
A2	14	B2	3
A3	18	B3	7
A4	13	B4	8
A5	10	B5	14
A6	29	B6	0
A7	32	B7	17
A8	22	B8	15
A9	14	B9	17

Table 3. Heights (cm) of the individual plants collected over the duration of the study.

Plant:	1	2	3	4
A1	11.3	12.5	18	21
A10	9.1	10	9.5	11.5
A11	6.9	8.3	11	14
A12	7.7	8.5	11.3	14.6
A13	8.5	9.3	13.4	18.3
A14	7.6	8.8	10.5	11.6
A15	7.4	7.5	8.8	11.1
A16	9.6	9.5	13.5	18.8
A2	9.5	10	9.4	7.9
A3	7	8.6	12.1	17.5
A4	8.8	9.1	10.1	11.6
A5	7.8	9.5	15	16.8
A6	8	9.6	15.1	19.8
A7	10.1	12.5	17.7	21.3
A8	10.5	14	19	20.3
A9	8	8.5	12.5	15.3
B1	8.3	8.5	8	6.6
B10	8.5	8.9	12.5	15
B11	6	15.5	5	0
B12	7	6.6	6.8	6.5
B13	9.1	9.5	9.2	9.8
B14	7.8	9.2	8.5	8.2
B15	9	10.5	12	15
B16	10.5	11.6	13	16.8
B2	7.8	7.2	0	0
B3	9.1	9.1	7.5	7.5
B4	8.7	8.5	8.1	7.8
B5	9	8.7	0	0
B6	8.3	7	0	0
B7	10.2	11.1	9.5	10.5
B8	9.9	11	10	9.5
B9	8.3	9.3	10.5	10.7

Table 4. Fresh weight of the plants' leaf material collected on the 4th measurement occasion.

Plant A:	Weight (g):	Plant B:	Weight (g):
A1	17.7	B1	0.9
A2	1.5	B2	0.1
A3	18.1	B3	0.8
A4	3.7	B4	0.6
A5	14.1	B5	1.5
A6	29.1	B6	0.3
A7	22.8	B7	5.1
A8	28.9	B8	1.8
A9	13.5	B9	2.4
A10	3.8	B10	4.5
A11	8.3	B11	0.4
A12	15.8	B12	0.4
A13	13.2	B13	2
A14	7.9	B14	1.7
A15	2.8	B15	8.3
A16	13.8	B16	6.8

Table 5. Fresh weight of the plants' root collected on the 4th measurement occasion.

Plant A:	Weight (g):	Plant B:	Weight (g):
A1	4.2	B1	0.2
A2	0.3	B2	0
A3	4.3	B3	0.2
A4	1.2	B4	0.9
A5	3	B5	0.9
A6	8.6	B6	1.1
A7	5.6	B7	2.1
A8	8.8	B8	1
A9	3	B9	0.8
A10	2	B10	1
A11	3.2	B11	1.4
A12	4.8	B12	1.2
A13	3.1	B13	2.2
A14	3	B14	0.5
A15	0.7	B15	4.1

A16		3.7	B16		1.4

Table 6. Dry weight of the plants' leaf material collected after drying for a period of 5 days.

Plant A:	Weight (g):	Plant B:	Weight (g):
A1	1.6	B1	0.3
A2	0.2	B2	0.1
A3	1.3	B3	0.2
A4	0.4	B4	0.2
A5	1.2	B5	0.3
A6	2.6	B6	0.2
A7	2.2	B7	0.8
A8	2.7	B8	0.4
A9	1.1	B9	0.4
A10	0.5	B10	0.6
A11	0.9	B11	0.2
A12	1.2	B12	0.1
A13	1.1	B13	0.3
A14	0.8	B14	0.4
A15	0.3	B15	0.8
A16	1.3	B16	0.7

Table 7. Dry weight of the plants' root material collected after drying for a period of 5 days.

Plant A:	Weight (g):	Plant B:	Weight (g):	Comment:
A1	0.8	B1	0.1	A1 did not dry properly and was damp at measurement.
A2	0	B2	0	
A3	0.2	B3	0.1	
A4	0.1	B4	0.1	
A5	0.1	B5	0.1	
A6	5.1	B6	0.2	A6 did not dry properly and was very wet at measurement.
A7	0.2	B7	0.3	
A8	0.8	B8	0.1	
A9	0.2	B9	0.1	
A10	0.1	B10	0.1	
A11	0.1	B11	0.2	
A12	0.6	B12	0.3	A12 did not dry properly and was slightly damp at measurement.

A13	0.7	B13	0.2	A13 did not dry properly and was slightly damp at measurement.
A14	0.2	B14	0.1	
A15	0	B15	1.8	B15 did not dry properly and was very wet at measurement.
A16	0.2	B16	0.2	

Appendix 2 - Images

Appendix 2 displays the images of the experiment.

Image 1: All the plants in group A on the 4ᵗʰ and final measurement occasion. Group A plants did not receive the arginine treatment at transplantation. Majority of the plants have a lush green color and are relatively large. A few of the plants show clear symptoms of stunted growth and possible nutrition imbalances indicated by leaf discoloration.

Image 2: All the plants in group B on the 4ᵗʰ and final measurement occasion. Group B plants received a dose of arginine, arGrow, at transplantation. All plants show stunted growth and leaf discoloration. Many of the plants are wilting or have died.

Image 3 & 4: A side-by-side comparison of average individuals from each group on the 4ᵗʰ measurement occasion. The A plant has significantly larger leaf and root plant materials as compared with the plant in group B which was treated with arginine.

Image 5: A picture of all the plant individuals in group A that shows the roots and the leaf plant materials.
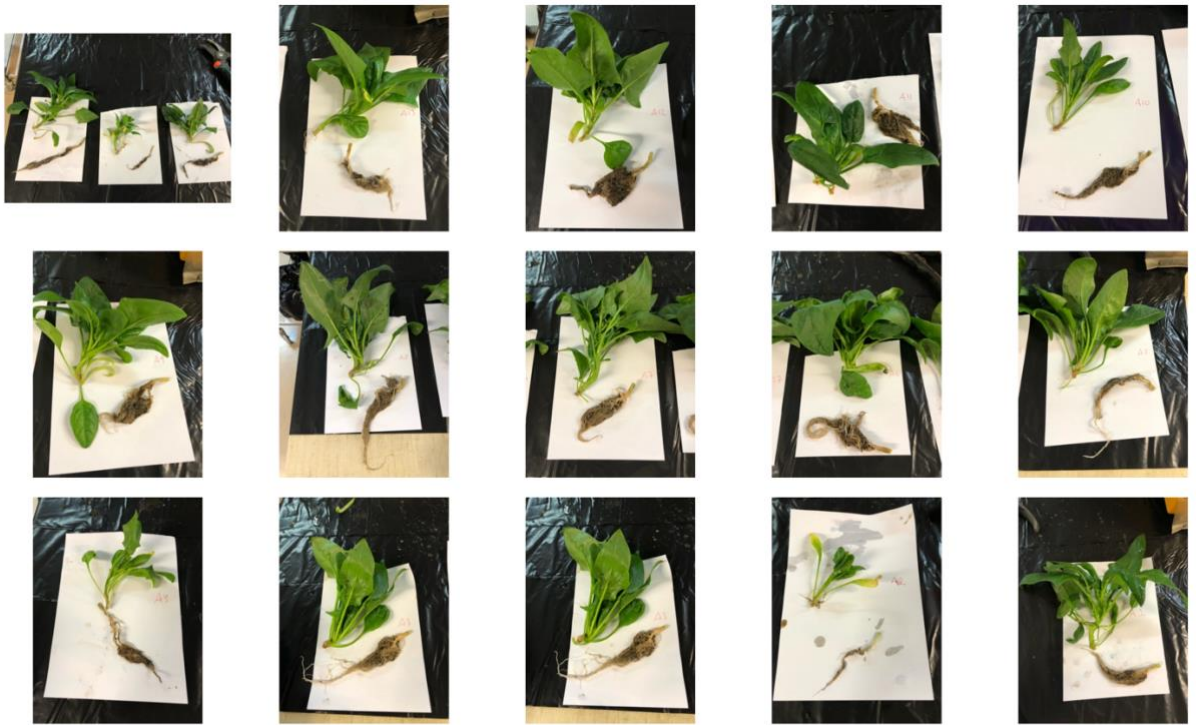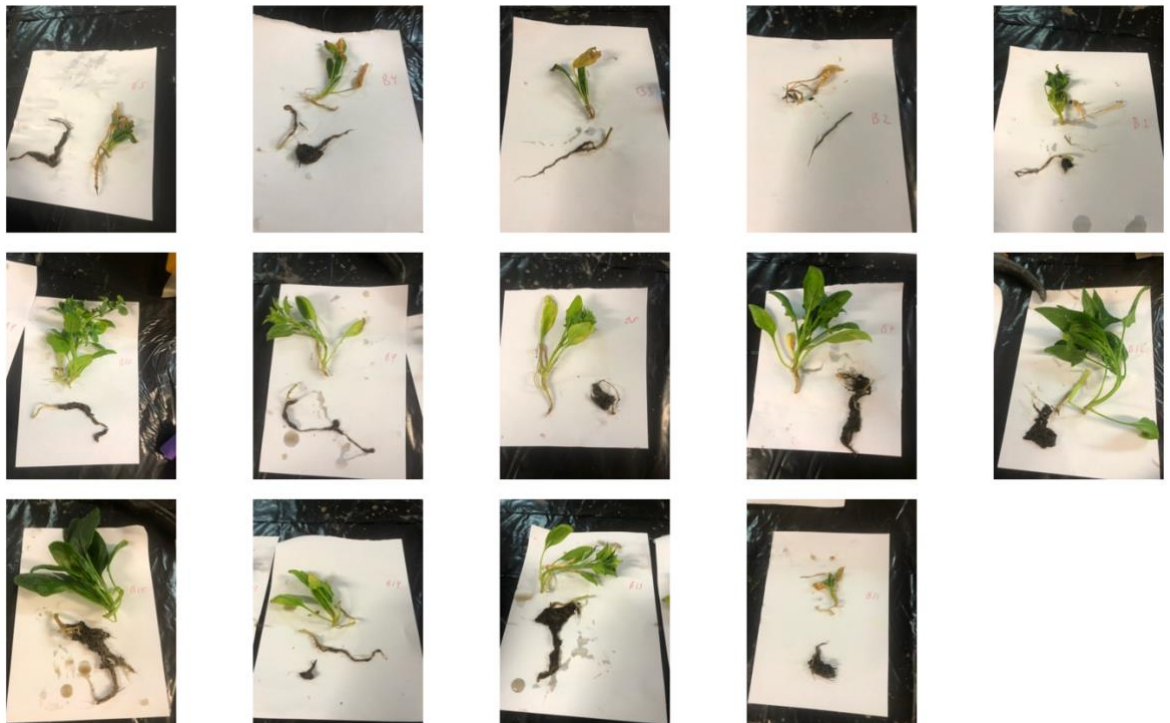

Image 6: A picture of all the plant individuals in group B that had not died during the study. The image displays the roots and leaf materials of the plants.

Image 7: The image was taken on the 4th measurement occasion and displays an example from group B that shows the clay-like mass in the soil from the arginine treatment.

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