Prevention and control of water emission in greenhouses

WP 3.4 Effect of humates on plants grown under high sodium levels

Barbara Eveleens, Chris Blok

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Referaat
De effectiviteit van humaat bij het verminderen van groeiremmende effecten van natrium werd getest. Een niveau van 10 mmol natrium per liter was effectief in het remmen van de vers gewicht groei van Chinese kool met 10%. De toevoeging van humaat aan de 10 mmol / l natriumvoedingsoplossing in de concentraties van 2,5 12.5, 25, 50 en 250 mg / l gaf een verbetering in kleur, maar in het eerste experiment werd geen toename in vers gewicht gemeten. In het tweede experiment werden minder zaden per pot geplant. De planten in dit experiment vertoonden ook lichtere bladeren wanneer natrium aanwezig was in de voedingsoplossing. Een laag humaatgehalte verbeterde de kleur niet, maar toen meer dan 20 mg / l humaat werd toegevoegd, verbeterde de kleur van de bladeren. Bovendien toonde dit tweede experiment een hoger vers gewicht met de toevoeging van meer dan 20 mg/l humaat aan de 10 mmol/l natrium oplossing.

Abstract
A level of 10 mmol sodium per litre was used in these experiments to test the effectiveness of humate in the prevention the inhibitory effects of sodium. This level of sodium was effective in inhibiting the fresh weight growth of Chinese cabbage by 10%. The addition of humate to the 10 mmol/l sodium nutrient solution at the concentrations 2.5, 12.5, 25, 50 and 250 mg/l gave an improvement in colour but in the first experiment no fresh weight increase was measured. In the second experiment less seeds were planted per pot. The plants in this experiment also showed lighter leaves when sodium was present in the nutrient solution. A low level of humate did not improve the colour but when more than 20 mg/l humate was added the colour of the leaves improved. Additionally this second experiment showed a higher fresh weight with addition of more than 20 mg/l humate to the 10 mmol/l sodium nutrient solution.

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Address

Wageningen University & Research
Business unit Greenhouse Horticulture
Violierenweg 1, 2665 MV Bleiswijk
P.O. Box 20, 2665 ZG Bleiswijk
The Netherlands
T +31 (0) 317 - 48 56 06
F +31 (0) 10 - 522 51 93
glastuinbouw@wur.nl
www.wur.eu/greenhousehorticulture

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Summary

The greenhouse horticulture sector in the Netherlands has agreed with the government to work towards a (more or less) zero emission for nutrients and plant protection products in 2027. To achieve this the following points must be understood (1) the effectiveness of purification in non-regular spray streams, and (2) accumulation of sodium and other unwanted bottlenecks with prolonged recycling of the nutrient solution. This project aims to investigate whether the sodium tolerance of a crop can be affected using humates. Partner in this project HuminTech, supplied the humates and these were tested on a Chinese cabbage in perlite in pots.

A level of 10 mmol sodium per litre was used in these experiments to test the effectiveness of humate in the prevention the inhibitory effects of sodium. This level of sodium was effective in inhibiting the fresh weight growth of Chinese cabbage by 10%. In these preliminary experiments the EC and the nitrate concentration were similar in all treatments so only the negative effects of sodium could be measured. In the first experiment no fresh weight increase was measured on addition of humate although the colour of the plants was clearly improved. This was due to competition between the plants in the pot and therefore in the second experiment less seeds were planted per pot.

The colour of the plants was measured with a SPAD meter (directly related to chlorophyll content). The SPAD index measurements of colour did show differences between the treatments, the control without sodium was much greener than the other treatments and the treatments with humate were, in general, greener than the treatment without added humate. This was more apparent with a humate concentration of 25 mg/l or more. These results were confirmed in a repeat experiment.

Addition of humate at levels of 25 mg/l or more reduced the inhibitory effects of 10 mmol/l sodium in the nutrient solution. The fresh weight of plants treated with a 10 mmol/l sodium solution plus 25 or 250 mg/l humate was significantly higher than the fresh weight of plants treated with a 10 mmol/l sodium solution plus 2,5 or no humate. The dry weight is significantly lower in the treatment with sodium and 2.5 mg/l humate and low in the treatment with sodium without or with intermediate humate level. The highest humate level with sodium gives a significantly higher dry weight than the lower humate level with sodium.

It seems that the plants treated with the 10 mmol/l solution all accumulate sodium in the plant. The two controls without sodium have much lower sodium concentrations in the leaves. The sodium levels found in the dried plant analyses corresponded to the treatment. The dried plant material of the plants treated with sodium and humate had a lower sodium concentration in the plant than the control with sodium but without humate.

1 Introduction

1.1 Background

As from 2018 protected horticulture has an obligation to purify water containing plant protection products. In addition, the greenhouse horticulture sector has agreed with the government to work towards a (more or less) zero emission for nutrients and plant protection products in 2027. Both are necessary to dramatically improve water quality in horticultural areas. Horticultural entrepreneurs are seriously working on this problem in an endeavour to maintain social support and license to operate, but run against various knowledge questions. These relate to (1) the effectiveness of purification in non-regular spray streams, and (2) accumulation of sodium and other unwanted bottlenecks with prolonged recycling of the nutrient solution. With respect to this last point this project aims to investigate whether the sodium tolerance of a crop can be affected using humates. Our industrial partner for this work package is supplier of humates, Humintech. Partner HuminTech supplies customized humates, some of which contain K+ for agricultural applications instead of sodium (Na+).

1.2 Aim

Investigate the effect of humates on plant tolerance at an acceptable but high level of Na.

1.3 Approach

1. A sodium test to find a realistic sodium concentration at which the plants show (slight) damage. The Na level to be used for the crops in subsequent experiments will first be established following the CEN 16086-1 protocol (2011) using a series 0, 5, 10, 20 mmol/l Na for Chinese cabbage, barley and lettuce. This can be conducted simultaneously for all crops.

2. The test plant is Chinese cabbage. One Na level is chosen. A control with no Na to test the effect of the Na on growth is also included. A proposed range of humate is 0, 1, 5, 10, 20 and 100 mg/l humate. These 5 treatments and two controls give 7 2a. Chinese Cabbage.

The dry matter of the plants must be analysed to measure the levels of Na in the plants. This requires a minimum of 5 g dry matter per sample. This requires 50 g fresh material (20 plants per pot and 3 pots). Plants will be grown on inert perlite as in the CEN 16086 protocol (2011). The nutrient solutions are analysed prior to the experiment to check pH, EC which should be similar. The elements in the solution are also analysed and a zero measurement of the perlite will also be done. At the end of the experiment the following analyses will be done:

a. Na and elements measured in perlite in pot
b. Na and elements measured in dried plant parts

1.4 Personnel involved

The project leader of Work Package 3.4 Effect of humates on plants grown under high sodium levels was Wim Voogt and the work on humates was led by Chris Blok. Barbara Eveleens conducted the experiments and was assisted by Johan Steenhuizen. The substrate analysis (1:1,5 method) and the dried crop analysis was carried out by Groen Agro Control in Delfgauw, the Netherlands.

2 Material and method

2.1 Experiment 1

A sodium test to find a realistic sodium concentration at which the plants show (slight) damage. The Na level to be used for the crops in subsequent experiments will first be established following the CEN 16086 protocol (2011). A series 0, 5, 10, 20 mmol/l Na was proposed for Chinese cabbage, barley and lettuce. The EC and nitrate levels in the control and four treatments should be similar so that only the effects of sodium are investigated. The high Na of 20 mmol/l was not used as it was not possible to make this solution without raising the EC. A series of 0, 4, 8 14 mmol/l Na was used for Chinese cabbage, barley and lettuce. This was conducted simultaneously for all crops.

A nutrient solution was made to ensure a similar nitrate concentration while maintaining similar EC levels for the 3 sodium levels. This was done so as not to influence the growth by EC or nitrate but to just concentrate on the humates in experiments 2 and 3. The respective nutrient solutions were added to the plant saucers and after 14 days (barley and lettuce) and 16 days (Chinese cabbage) each plant was measured. There were 20 plants per pot with 3 pots per treatment (n = 60) (*Figure 1* **Test set up in experiment 1**).

Figure 1 Test set up in experiment 1.

2.2 Experiment 2

One sodium concentration was chosen after analysing the fresh weight in experiment 1. This nutrient solution was made following the same method as in the first experiment and 2.5, 12.5, 25 or 250 mg/l humate was added to this 10 mmol/l sodium solution. Two controls were included, one with 10 mmol/l sodium and no humate and one with 0 mmol/l sodium to check if there was in fact inhibition of growth by the 10 mmol/l Na solution (**Error! Reference source not found.**. The plants were sown in week 31 and harvested 4 weeks later. There were 20 plants per pot with 3 pots per treatment (n = 60) **Error! Reference source not found.** shows the treatments. In this experiment the colour of the plants was measured with a SPAD meter (directly related to chlorophyll content).

Table 1 Treatments in experiment 2.

No treatment	Treatment	Sodium	Humate
		mmol /l	mg/l
1	Control 1	10	0
2	Treatment 2	10	2.5
3	Treatment 3	10	12.5
4	Treatment 4	10	25
5	Treatment 5	10	50
6	Treatment 6	10	250

7	Control 2	0	0

2.3 Experiment 3

In previous experiments an effect of humate was seen on fresh weight after 2 weeks but at final harvest 2 weeks later this was not found. Therefore in this experiment less plants per pot were used to reduce the effect of plants on each other and the plants were grown for a longer period. The growing conditions and nutrient solutions were similar to the previous test* (*Table 2*) . The amount of plant material in this previous experiment was not sufficient for an analysis of the elements in the plant material. In this experiment this analysis will be carried out on plant material and on the perlite. For the initial levels in the perlite see Annex 1.

Three concentrations of humate (2.5, 24 and 250 mg/L nutrient solution) were used to test the effect of humate on plants grown in perlite under high sodium concentrations (10 mmol/l). In this experiment 3 control treatments were used:

- One with salt and without humate to check for if sodium inhibition does occur.
- One without salt and without humate to act as standard for no salt inhibition.
- One without salt and with a high level of humate to check if humate alone has an effect on growth. The EC and nitrate level of the nutrient solutions was similar

The other 3 treatments were treated with a nutrient solution with 10 mmol/l sodium and 2.5, 25 and 250 mg humate per litre. For all treatments see *Table 2* and for the nutrient solutions *Table 3*.

Table 2 Treatments in experiment 3.

	treatment	sodium	Humate
		mmol/l	mg/l
1	no humate	10	0
2	low humate	10	2.5
3	middle humate	10	25
4	high humate	10	250
5	control no salt no humate	0	0
6	control no salt high humate	0	250

The plants were sown in week 42 and harvested in week 48.

Figure 2 The experimental set-up for experiment 3.

Table 3 Example of 3 nutrient solutions, one with 10 mmol sodium and no humate (treatment 1) one without sodium and humate (5), and one with 10 mmol/l sodium and 250 mg/l humate (6).

		1 10 mmol sodium 0 mg/l humate	5 Control O mmol/l sodium 0 mg/l humate	6 10 mmol/l sodium 250 mg/l humate
pHRapport		5.9	5.9	6.4
EC	mS/cm	2.1	2.1	2.1
NH4	mmol/l	< 0.1	1	< 0.1
K	mmol/l	4.7	7.9	4.8
Na	mmol/l	11.3	0.3	10.3
Ca	mmol/l	0.6	3.5	0.7
Mg	mmol/l	1.4	1.4	1.5
Si	mmol/l	< 0.1	< 0.1	0.3
NO3	mmol/l	15.3	14.4	15
Cl	mmol/l	0.4	0.3	0.3
SO4	mmol/l	1.4	1.4	1.6
HCO3	mmol/l	0.3	0.3	0.6
P	mmol/l	1.5	1.3	1.8
Fe	µmol/l	8.4	9.4	64.3
Mn	µmol/l	8.9	9.3	11.7
Zn	µmol/l	5.5	7.9	7.3
B	µmol/l	49	48	56
Cu	µmol/l	0.9	0.8	1.3
Mo	µmol/l	0.44	0.41	0.6

3 Results

3.1 Experiment 1

The solutions based on a standard solution as stated in CEN 16086-1 (2011) were made with increasing sodium concentrations of 4, 8 and 14 mmol/l. These solutions were analysed and *Table 4* shows that the solutions all had an EC of 2 and a nitrate concentration of 14 mmol/l.

Table 4 Analysis of the nutrient solutions used for experiment 1 to test for sodium damage.

		control	4 mmol/l Na	8 mmol/l Na	14 mmol/l Na
pH		4.8	5.3	5	5.8
EC	mS/cm	2.0	2.0	1.9	1.9
NH_4	mmol/l	0.6	0.6	0.5	< 0.1
K	mmol/l	7.4	5.6	3.9	1.4
Na	mmol/l	**0.3**	**3.8**	**7.5**	**13.5**
Ca	mmol/l	3.6	2.7	1.9	0.7
Mg	mmol/l	1.4	1.4	0.9	1.2
NO_3	mmol/l	14.2	14.1	14.5	14.5
Cl	mmol/l	0.2	0.1	0.1	0.1
HCO_3	mmol/l	0.1	0.1	0.1	0.1
P	mmol/l	1.2	1.1	1.2	1.4

The plants were harvested according to EN 16086, (2011). For the Chinese cabbage the fifth true leaf was visible in 50% of the plants. For the barley when the second leaf is larger than the first leaf in 50% of the plants *Figure 4* en *Figure 5*.

The graph in *Figure 3* shows the % inhibition in fresh weight compared to the control and the photos illustrate the growth of the lettuce, barley and Chinese cabbage at final harvest under the increasing sodium concentrations. A sodium concentration of 10 mmol/l was chosen for the following experiments on Chinese cabbage.

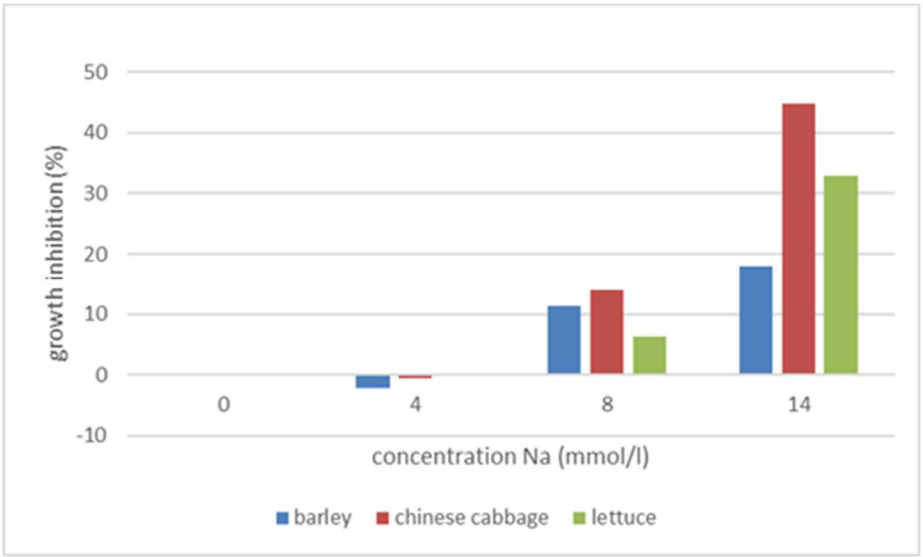

Figure 3 Left axis; growth inhibition (%) fresh weight / seedling on harvest.

Figure 4 Plants prior to havest.

Figure 5 From top to bottom; Barley, lettuce and Chinese cabbage. From left to right; control (0 mmol/l Na); 4 mmol/l Na; 8 mmol/l Na; 14 mmol/l Na.

3.2 Experiment 2

In this experiment only Chinese cabbage was used and this was sown according to the protocol with 20 seeds per pot and harvested when the fifth true leaf was visible. A sodium concentration of 10 mmol/l was chosen for this experiment and the humates were added at 2.5, 12.5, 25, 50 and 250 mg/l nutrient solution (*Table 5*). Two controls were included, one with 10 mmol/l sodium and no humate (treatment 1) and one with 0 mmol/l sodium to check if there was in fact inhibition of growth by the 10 mmol/l Na solution (treatment 7).

Table 5 Treatments in experiment 2.

No treatment	Treatment	Sodium	Humate
		mmol /l	mg/l
1	Control 1	10	0
2	Treatment 2	10	2.5
3	Treatment 3	10	12.5
4	Treatment 4	10	25
5	Treatment 5	10	50
6	Treatment 6	10	250
7	Control 2	0	0

Two weeks after sowing differences in growth of the plants were visible. Chinese cabbage plants treated with the 10 mmol /l solution (control 1, treatment 1) are inhibited when compared to the nutrient solution without sodium (control 2, treatment 7) (*Figure 6*). The treatment 6 (*Figure 7*) seemed to grow better than treatment 1 and this tendency seemed to decrease as the amount of humate decreased. The pot surface became less covered by leaves as the humate concentration

decreased. *Figure 8* shows the treatment 2 with 2.5 mg humate per litre compared to control 1. There is very little difference between the control 1 and the treatment 2.

Figure 6 Left; control 2 (treatment 7) without sodium. Right: control 1 (treatment 1) with 10 mmol/l sodium and no humate.

Figure 7 Left; treatment 6 with 10 mmol/l sodium and 250 mg/l humate. Right: control 1 (treatment 1) with 10 mmol/l sodium and no humate.

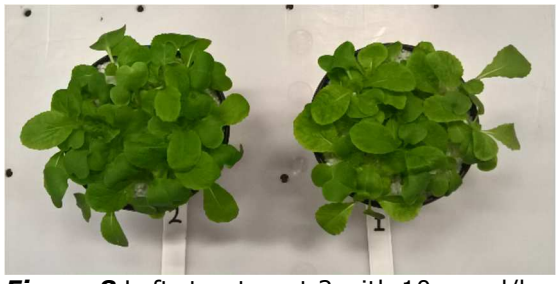

Figure 8 Left; treatment 2 with 10 mmol/l sodium and 2.5 mg/l humate. Right: control 1 (treatment 1) with 10 mmol/l sodium and no humate.

The plants were harvested after another 2 weeks but no significant difference was found in fresh weight between the humate treatments (*Figure 9*). It seemed that the original differences visible in the figures 6, 7 and 8 were reduced because the pots were too full and because of the competition the smaller plants

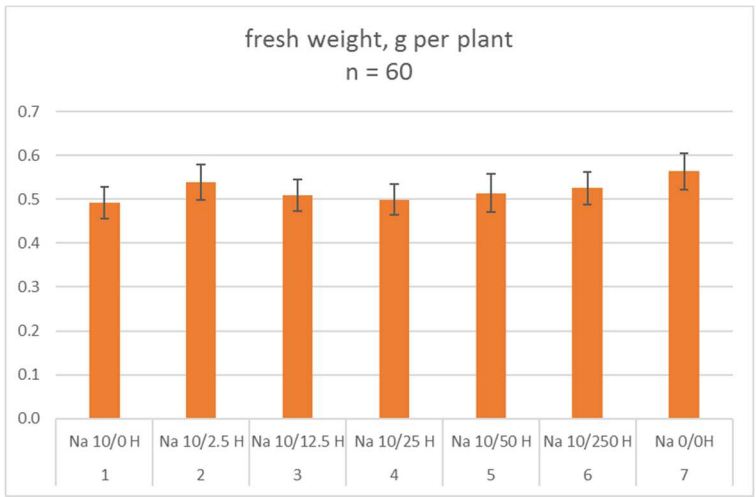

Figure 9 Fresh weight of plants after harvest.

The SPAD measurements of colour did show differences between the treatments, the control without sodium was much greener than the other treatments and the treatments with humate were, in general greener than the treatment without added humate. This was more apparent with a humate concentration of 25 mg/l or more. This is illustrated in *Figure 10*.

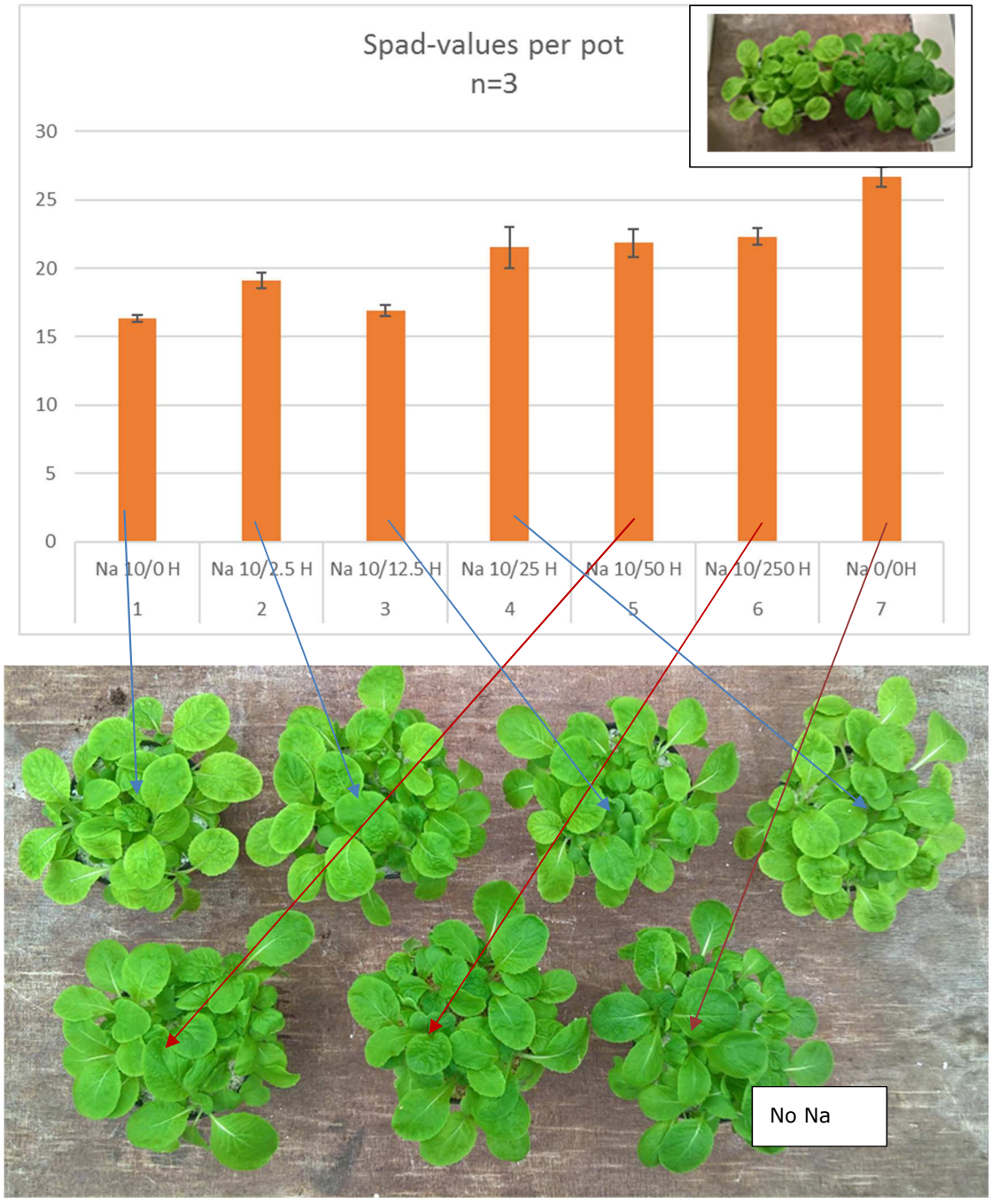

Figure 10 All the plants have 10 mmol/l Na in nutrient solution except bottom right.

3.2.1 Remarks on experiment 2

The results of experiment 2 were interesting enough to warrant a repeat of this experiment. In this repeat experiment less plants per pot will be used to decrease competition between the plants. Additionally an extra control with no sodium and 250 mg/l humate in the nutrient solution will be added. The number of humate concentrations used in the experiment will be decreased. Dried plant material and the perlite will be analysed.

3.3 Experiment 3

This experiment started in week 43 and to ensure sufficient plant material only 10 seeds per pot were sown and the experiment lasted 6 weeks. The humate concentrations were 0 (control 1), 2.5, 25 and 250 mg/l in the nutrient solution and a control 2 without sodium but with a high humate concentration (*Table 6*). Again similar results were found as in experiment 2 but in this experiment the final harvest showed differences in fresh weight.

Table 6 Treatments in experiment 3.

Treatment number	treatment	sodium	Humate
		mmol/l	mg/l
1	control no humate	10	0
2	low humate	10	2.5
3	middle humate	10	25
4	high humate	10	250
5	control no salt no humate	0	0
6	control no salt high humate	0	250

The graph (*Figure 11*) shows that the controls without sodium (last two bars in the graph) give the highest fresh weight per plant (1.37 to 1.31 g). Treatment 1 with sodium and without humate and the treatment with sodium and a low humate level give the lowest fresh weights per plant (0.97 to 1.05 g). The treatments with sodium and a higher level of humate give intermediate fresh weights per plant (1.21 g) which are significantly higher than the treatments with sodium but without or with low humate levels. The dry weight is significantly lower in the treatment with sodium and 2.5 mg/l humate and low in the treatment with sodium without or with intermediate humate level. The highest humate level with sodium gives a significantly higher dry weight than the lower humate level with sodium.

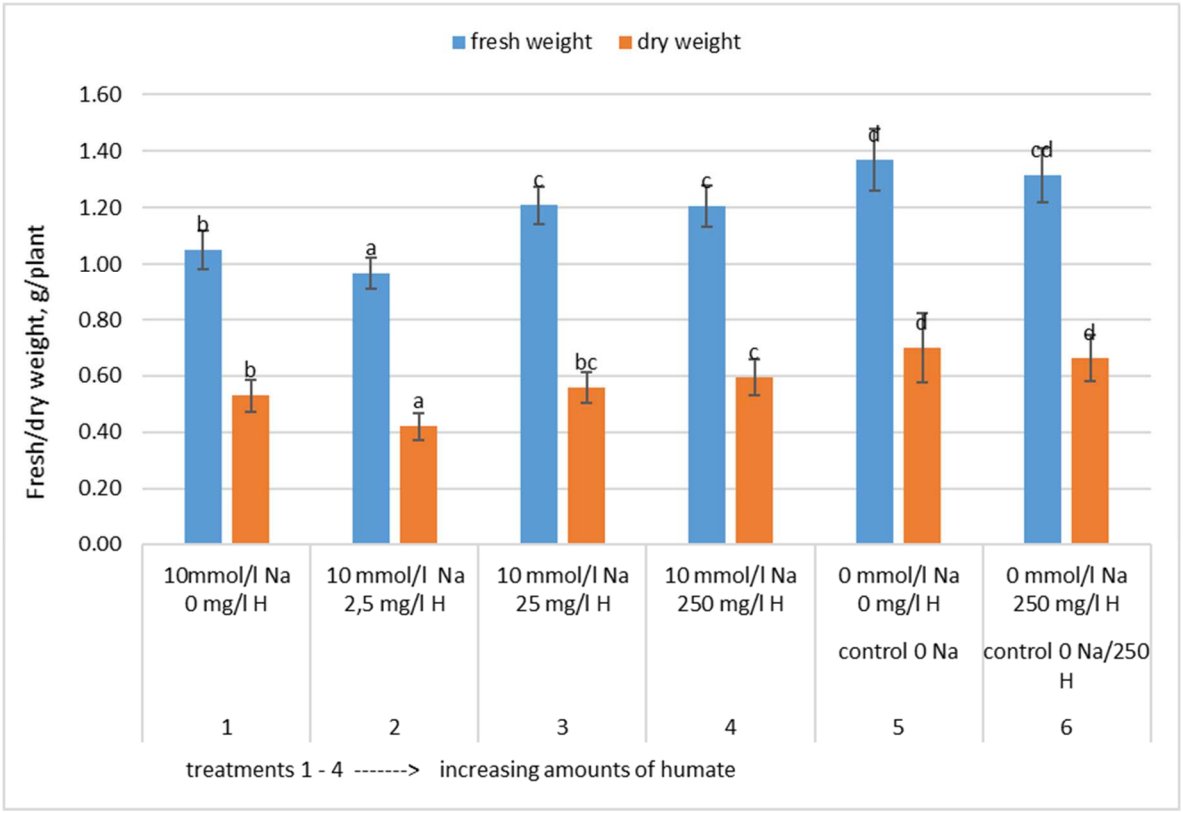

Figure 11 Fresh and dry weight of the plants after harvest. Different letters denote significant differences.

In *Figure 12* the pots after harvest are shown. Although the two treatments with 250 mg/l (4th and 6th row in *Figure 13* seem to differ in colour they showed a similar colour within the pot.

Figure 12 Pots after final harvest, from left to right high sodium with 0 (1 treatment 1), 2.5 (2), 25 (3) and 250 (4) mg/l humate. The last two rows are the controls 0 sodium and 0 humate (5) and 0 sodium and 250 mg/l humate (6).

Figure 13 The contents of the pots both with 250 mg/l, left with 10 mmol/l sodium (4) and right without sodium (6).

The plants in this experiment also showed lighter leaves when sodium was present in the nutrient solution (*Figure 14*). A low level of humate did not improve the colour (*Figure 15*). When humate was added (more than 25 mg/l) the colour of the leaves improved (*Figure 16*). With 10 mmol/l sodium and 250 mg/l humate the colour of the plant was similar to the treatment with no sodium and 250 mg/l humate (*Figure 17*).

Figure 14 Left without sodium (5), right with sodium/0 humate (1).

Figure 15 Left with sodium/0 humate (5), right sodium /2,5 mg/l humate (2).

Figure 16 Left with sodium/0 humate (5), right with sodium/ 250 mg/l
Humate (4).

Figure 17 Left with sodium/250 mg/l humate (4), right without sodium/ 250 mg/l humate (6).

3.3.1 Analyses of perlite and of dried plant samples

After the plants were harvested the perlite in the pots was analysed using a 1: 1,5 method (*Table 7*). The EC ranged from 1 to 1.2 and the pH from 5.9 to 7.1. The sodium was between 5.1 and 6.7 mmol/l in the treatments where the 10 mmol/l nutrient solution was used (treatments 1, 2, 3 and 4) and 0.9 to 1.1 mmol/l in the control treatments (5 and 6). The potassium was 40% higher in the controls and this corresponds to the 40% higher potassium levels in the nutrient solution of the control. The reason for this was that a higher potassium was given to ensure that the EC was similar between the treatments. The same observation was also found for calcium, but here the differences were approx. 80%. Magnesium and manganese were higher in the controls (treatments 5 and 6). The only other difference was a higher iron level in the pots with 250 mg/ l humate and 10 mmol/l sodium (treatment 6) but this was not visible in the control with 250 mg/l humate and no sodium.

Table 7 Analysis (1:1,5) of the perlite at the end of the experiment.

	TREATMENT	1	2	3	4	5	6
	Na (mmol/l)	10	10	10	10	0	0
	Humate (mg/l)	0	2.5	25	250	0	250
pHRapport		7.1	6.7	7.1	7.0	5.9	6.0
EC	mS/cm	1.2	1.1	1.2	1.0	1.1	1.0
NH4	mmol/l	< 0.1	< 0.1	< 0.1	< 0.1	0.2	< 0.1
K	mmol/l	2.2	2.2	2.3	2.1	3.8	3.7
Na	mmol/l	**6.7**	**6.1**	**6.3**	**5.1**	**0.9**	**1.1**
Ca	mmol/l	0.3	0.3	0.3	0.3	1.7	1.4
Mg	mmol/l	0.5	0.6	0.6	0.5	0.8	0.7
Si	mmol/l	0.2	0.1	0.2	< 0.1	0.1	0.1
NO3	mmol/l	7.9	7.4	7.3	6.0	7.5	6.6
Cl	mmol/l	0.2	0.2	0.2	0.2	0.3	0.3
SO4	mmol/l	0.5	0.7	0.7	0.5	0.6	0.6
HCO3	mmol/l	0.6	0.6	0.6	0.6	0.2	0.3
P	mmol/l	0.55	0.70	0.75	0.60	0.55	0.45
Fe	µmol/l	3.4	2.6	3.7	7.8	3.9	3.8
Mn	µmol/l	1.7	2.5	2.5	1.8	4.9	3.1
Zn	µmol/l	3.1	3.2	3.1	2.5	2.3	1.5
B	µmol/l	22	25	27	22	25	22
Cu	µmol/l	0.7	0.6	0.8	0.4	0.5	0.2
Mo	µmol/l	0.23	0.25	0.30	0.24	0.16	0.15

The dried plant material was also analysed at the end of the experiment (*Table 8*). The sodium levels found in the dried plant analyses corresponded to the treatment. The plants with sodium in the nutrient solution had a higher sodium level than the control treatments 5 and 6. The potassium was 54% higher in the controls and the calcium was approx. 70% higher in the controls and the reason for this was the higher potassium and calcium levels in the nutrient solution. As stated before this was done to ensure that all the treatments had a similar EC-level. Although the magnesium and manganese were higher in the perlite in the control treatments the levels in the plant material were slightly lower in the control treatments for magnesium and similar for manganese.

Table 8 Analysis of dry plant material of the plants at the end of the experiment.

	TREATMENT	1	2	3	4	5	6
	Na (mmol/l)	10	10	10	10	0	0
	Humate (mg/l)	0	2.5	25	250	0	250
DS	%	95	95	95	95	95	96
K	mmol/kg ds	1089	1142	1174	1118	2102	1897
Na	mmol/kg ds	**2696**	**2351**	**2394**	**2173**	**299**	**245**
Ca	mmol/kg ds	253	246	248	230	784	769
Mg	mmol/kg ds	355	428	434	347	291	255
N-tot	mmol/kg ds	4720	4694	4703	4733	4897	5042
P-tot	mmol/kg ds	300	292	289	246	257	265
Fe	mmol/kg ds	1.8	1.6	1.6	1.5	1.6	2.1
Mn	mmol/kg ds	1.8	2.5	2.3	1.8	2.1	2.3
Zn	mmol/kg ds	0.81	1.10	0.90	0.68	1.00	0.87
B	mmol/kg ds	5.7	6.4	6.2	5.3	5.7	5.4
Mo	µmol/kg ds	97.0	86.7	79.3	77.7	102.0	101.0
Cu	µmol/kg ds	140	136	220	84	180	88

4 Further discussion

General

In experiments 2 and 3 we chose to use nutrient solutions with similar EC levels to test the effectiveness of humate in the prevention the inhibitory effects of sodium. The EC levels were similar for all treatments and the uptake of sodium by the plant corresponded to the sodium level in the nutrient solutions. The SPAD index measurements in the plants treated with a nutrient solution containing 10 mmol/l sodium increased with the amount of humate added to the nutrient solution. The dry weight production of the plants increased about 10% when 25 mg/L or more of humate was added to the solution. This was not enough to fully counter the production loss by the 10 mmol/L of sodium. The addition of humate reduced the concentration of sodium in the plant material.

Similar effects of biostimulants

The effect of organic biostimulants has recently been investigated in research by Di Stasio et al. (2018). The addition of seaweed extract reduced the sodium uptake while the uptake of other elements were similar or higher. These results were independent of the EC of nutrient solution. The seaweed extract contained inconsequential levels of nutrients. Di Stasio et al. suggested the decrease in sodium concentration in the plants after root application of seaweed bio stimulant may be associated with an increase in the activity of phytohormones which cause a more efficient translocation of minerals independent of the nutrient concentration. They however, could not specify the alleged phytohormones. Nevertheless the parallels with the effects found in this research are striking.

The mechanisms of action

When dry matter yield increases and the concentration of sodium in the dry plant material decreases, several possibilities exist:

a) The absolute amount of sodium taken in is still higher than without humate.
b) The absolute amount of sodium taken in is equal to the amount taken in without humate.
c) The absolute amount of sodium taken in is lower than without humate.

Especially in the last case, the plant does not take in as much sodium, one would expect the sodium to accumulate in the root environment. It is therefore highly interesting to note that the concentration in the root environment actually decreases (Table 8)! To interpret this correctly we have to first compare the absolute amounts of nutrient in the plants. To do so we multiply plant concentration with plant weight. For experiment 3 this is about 2696*0.053 = 143 mmol for treatment 10/0 and 2173*0.060 = 130 mmol for treatment 10/250. This means the absolute intake of sodium really was lower after adding humate. It is of relevance to note the total analysis sample was taken from all of the above ground dry matter! The 13 mmol sodium not taken in by the plant would therefore have to be found in either the non-harvested parts (roots) or in the solution in the root environment. As the root dry mass is expected to amount to only 20% of the above ground dry mass, the root mass is not likely to contain the extra sodium even though the possibility cannot be totally excluded. To estimate the amounts of sodium in the root environment we have to multiply the concentration (Table 8) with the amount of water in the root environment. This quantity of water in the root environment is however not measured. It is estimated to be about 350 ml per container. The quantities in the root solution are therefore estimated to be 6.7*0.350 = 2.3 mmol for treatment 10/0 and 5.1*0.350 = 1.8 mmol for treatment 10/250. Although the calculations to arrive at this result require at least one assumption and the data have unknown standard errors, it is still clear there is a substantial amount of sodium, about 10 mmol per container, which is no longer measured. Possible explanations are :

a) In the root mass (as argued above; possible but not likely)
b) Moisture differences between the treatments. Not possible as the amount recovered are too small, even for the highest possible moisture content (600 ml).
c) The sodium precipitates in the root environment. However sodium does not precipitate under these conditions.
d) The sodium is bound to particles which themselves are filtered off before analysis.

The last possibility could mean sodium binds to humate as humates are large enough to be filtered out by the 40 micrometre paper filters the labs use. To catch 10 mmol sodium (230 mg) we would expect about twice the weight in humate (assuming one negative charge per 50 mg) i.e. 460 mg. This would require 2 litre with 250 mg humate/L.

At this stage the above thoughts do not give precise answers but help us to design a next experiment making sure we can:

- Measure the root mass as well
- Register the supply of solution over the cultivation period
- Measure the water content in the container.
- Ask the lab to partly omit the filtering step.

5 Conclusions

First experiment
The first experiment was used to establish the level of sodium that would inhibit the growth of lettuce, Chinese cabbage and barley. 10 mmol/l sodium was found to inhibit growth and this value was chosen for the subsequent experiments where humate was added to the nutrient solution.

Second experiment
The addition of humate at the concentrations 2.5, 12.5, 25, 50 and 250 mg/l to a nutrient solution containing 10 mmol/l sodium gave an improvement in colour (darker green colour). The chlorophyll (SPAD index) measurements also supported this observation. In the this experiment there was no significant improvement in fresh weight at the end of the experiment with the addition of humate. This was due to the competition between the plants and the large differences in fresh weight of the plants per pot (plant density effects).

Third experiment
Again a level of 10 mmol sodium was used but less plants per pot were grown. The colour of the plants was again improved by adding humate to the nutrient solution and again this effect increased with increasing humate dose. The plants treated with 250 mg/l humate in the 10 mmol/l sodium nutrient solution were darker than the plants treated without humate in the 10 mmol/l sodium. When humate was added at a level of 25 mg/l or more to a solution with 10 mmol/l sodium the colour of the leaves improved and fresh weight improved with about 10%. The highest humate level with sodium gives a significantly higher dry weight than the lowest humate level with sodium. The intermediate humate level of 25 mg/l lies between these two values. The plants treated with humate had lower sodium levels than the plants that were not treated with humate.

General
It seems that the plants treated with the 10 mmol/l solution all accumulate sodium in the plant. The two controls without sodium have much lower sodium concentrations in the leaves. The plants were harvested and dried in experiment 3 and the dried plant material was analysed. The sodium levels found in the dried plant analyses corresponded to the treatment. The dried plant material of the plants treated with sodium and humate had a lower sodium concentration in the plant than the control with sodium but without humate. It seems humates keeps the sodium from entering the plant

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Annex 1 Perlite start experiment

Analsysis of perlite (1:1,5) at start of experiment.

		Perlite
pH		7.6
EC	mS/cm	< 0.1
NH4	mmol/l	< 0.1
K	mmol/l	< 0.1
Na	mmol/l	0.4
Ca	mmol/l	< 0.1
Mg	mmol/l	< 0.1
Si	mmol/l	0.7
NO3	mmol/l	0.1
Cl	mmol/l	0.2
SO4	mmol/l	< 0.1
HCO3	mmol/l	0.3
P	mmol/l	< 0.05
Fe	µmol/l	6.8
Mn	µmol/l	0.4
Zn	µmol/l	< 0.1
B	µmol/l	< 4
Cu	µmol/l	< 0.1
Mo	µmol/l	< 0.1

Corresponding address for this report:
P.O. Box 20
2665 ZG Bleiswijk
The Netherlands
Violierenweg 1
2665 MV Bleiswijk
T +31 (0)317 48 56 06
F +31 0)10 522 51 93
www.wur.eu/greenhousehorticulture

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