Botany. — *Researches on plant growth regulators.* XIII. *Leaf growth factors.* I. By W. KRUYT and H. VELDSTRA. (Communicated by Prof. V. J. KONINGSBERGER.)

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Introduction.

The growth which may be observed in different parts of the plant, is a very complicated process, in the regulation of which several phytohormones play a part.

The growth of the leaf is also influenced by these substances, whereby one must distinguish between the growth of the veins which is influenced by auxin (AVERY (1), WENT and THIMANN (2), WENT (3)) and the growth of the mesophyl, which is independent of auxin. WENT showed that in etiolated pea-seedlings the leaf growth is dependent on the growth factors accumulated in the cotyledons. BONNER, HAAGEN–SMIT and WENT (4) in 1939 elaborated a test to be able to compare by means of pieces of leaf blades of *Raphanus* and *Nicotiana* the activity of leaf growth factors.

In 1939 BONNER and HAAGEN–SMIT (5) published a comprehensive article on the activity of several synthetic substances as leaf growth factors. As might be expected the test object also plays an important part. Of the examined amino acids arginine for example proved to be the most active one for the growth of leaf-discs of *Nicotiana sylvestris,* whilst this substance was inactive in *Raphanus.* Therefore caution is a first necessity especially when different authors are comparing their results. Even if experiments are carried out on a similar species there is always a possibility of difference in variety or race.

Of the purine derivatives *adenine* was especially active even in solutions as dilute as 20 μg per litre. BONNER and HAAGEN–SMIT therefore used several other methods to study this exceptional activity. Whole leaves of ten days old etiolated pea-seedlings were cut off and put into different solutions under sterile conditions. After five weeks the growth in "pea-diffusate" proved to give the best results; 2 mg/l adenine added to a mixture of 1 % cane-sugar and inorganic salts caused an inhibition, whereas the addition of 0.2 mg/l adenine caused an important increase in growth with regard to the control. Here the effect of adenine was comparable with that in the leaf growth test.

In the cultivation of pea-roots a stimulation of growth was also noticed though this was not apparent till after six transferences. The effect was less than the increase of growth caused by vitamin B_1 and nicotinic acid.

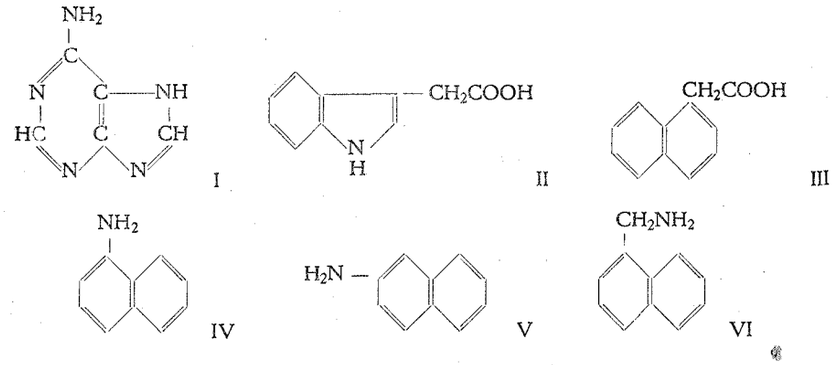
Finally BONNER and HAAGEN–SMIT examined the influence of adenine on the growth of Cosmos plants, cultivated in a hothouse in washed sand,

regularly watered with nutrient-solution. The cultivation took place in daylight, both short and long photoperiods being applied. The first two or three weeks no differences were observed but after that period the plants treated with 0.1 mg/l adenine became steadily larger than the controls, whereby the leaves in particular showed an increase in size. A concentration of 0.5 mg/l already caused inhibition. Under long-day condition the influence of adenine was especially noticeable on the longitudinal growth, whilst under short-day condition the measurements of the leaves were chiefly affected. Thus BONNER and HAAGEN–SMIT stated that the addition of adenine to plants may cause an increase of leaf-surface under certain conditions. A picture showed a very clear difference between leaves of control plants and treated specimens (adenine 0.1 mg/l), five weeks after germination.

As a result of the increase of leaf-surface after addition of adenine there will be an increase in the production of e.g. auxin and vitamin B_1 in these leaves. Therefore the addition of adenine will indirectly influence the growth of stem and root and a better developed plant may result. D. M. BONNER and J. BONNER (6) report that by adding 0.1 mg/l adenine to the nutrient solution in *Cosmos sulphureus* an important increase in the dry weight of the seedling (up to as much as three times) may be the result; the roots would show only a slight increase.

Uric acid, the structure of which is closely related to adenine, showed in cultivation experiments with Cosmos an effect similar to adenine, although it had little effect in experiments on leaves (BONNER and HAAGEN–SMIT). Identical results were obtained with *Brassica alba.* The combination of adenine and uric acid caused a result corresponding with the effect of twice the amount of each component separately.

In connection with our investigations on the practical applications of plant growth regulators a further study of the above-mentioned effects of adenine was considered desirable. On account of the high growth substance activity of α-naphthalene acetic acid (III), to be considered as a structural imitation of hetero-auxin (II), we thought it attractive to include some easily accessible substances the structure of which reminds to a certain extent of that of adenine (I).



For this reason α-naphthylamine (IV) was examined and in comparison to it also its β-isomer (V). Because of the typical function of the CH_2-group in the side-chain of the acids, derived from indole or naphthalene and active as growth-substances (see VELDSTRA (7)), α-(aminomethyl-) naphthalene (VI) was added as a counterpart of these acids.

The differences in properties between the ring-systems of indole and naphthalene being certainly less than those between the purine- and naphthalene nuclei, the comparison between adenine and α-naphthylamine may seem to be a risky one. This choice, however, was also deemed justifiable on account of the fact that of the naphthylamines and closely connected compounds typical reactions in other aspects are already known (compare (8)).

Material and methods.

In general we decided to follow the methods used by BONNER and HAAGEN–SMIT (5), i.e. the cultivation in sand, to which nutrient solution is added regularly. The above-named authors, however, have given no further details as to the Cosmos used; from the published photographs it can be concluded only that it was a fine-leaved type. In the article by D. M. BONNER and J. BONNER (6) *Cosmos sulphureus* is mentioned as an object that would react to adenine as a leaf growth factor. Therefore we started with the use of seeds of *Cosmos sulphureus* (harvest 1942) obtained from the Hortus at Leyden. During cultivation, however, it proved not to be a fine-leaved type. Comparison of our material with plants from the State-herbarium at Leyden and with reproductions in Cavanilles Icones (9) in which this plant is described for the first time, shows that *Cosmos sulphureus* Cav. is indeed a broad-leaved type so that we must conclude that BONNER and HAAGEN–SMIT did not use the genuine *Cosmos sulphureus* in their experiments.

On account of the war we had no opportunity of receiving further information from the authors.

After our first experiment with *Cosmos sulphureus* Cav. the work was continued with the seed of the fine-leaved type *Cosmos bipinnatus* Cav. called "Sensation Innocene" (seed Nr 896 of C. G. van Tubergen's Bulb and Seed Trade Ltd, Nursery Zwanenburg — Haarlem), a white flowering Cosmos.

BONNER and HAAGEN–SMIT (5) do not give a detailed specification as to the nutrient-solution used. They speak of a Shive's solution but as there exist several Shive's solutions (10) this description is insufficient. The nutrient-solution which we used (according to Shive) had the following composition: $Ca(NO_3)_2 \cdot 4\,H_2O$, 1.2 g/l; $MgSO_4$. ca $3\,H_2O$, 2.6 g/l; KH_2PO_4, 2.5 g/l; $Fe_2(PO_4)_2$, trace. The cultivation took place in coarse sand which had been well washed with tapwater three times. The Cosmos seeds were first made to germinate in sowing-pans (inside measurements $26 \times 26 \times 6.5$ cm) or in boxes at regular distances (three cm square). It

proved to be of importance not to sow too deep because otherwise the seedlings had too much difficulty in penetrating the sand-layer, which might of course cause differences in development. The various groups of one experiment were watered immediately after the sowing with nutrient-solution with or without the addition of substances in different concentrations, which were to be tested on their leaf growth activity. To begin with cultivation was done under double glass (sometimes with soil-heating), but soon after the germination regular aeration took place and finally the plants were placed on a table in the centre of the hothouse. After some seven days the plants have sufficiently developed to be transplanted. We transferred them into pots (top diameter 9 cm, bottom diam. 5 cm, height 8 cm) with washed coarse sand. Of every group 20 to 40 best specimens were selected. These pots, each containing only one plant, were dug into sand on the cultivation table to prevent evaporation as much as possible. At first we kept every group apart but later on we randomized all the pots of one experiment-series so that only the order and the presence of a coloured stick showed to which group the plant belonged in order that in watering the right nutrient-solutions should be given. Only in this way it is possible to distribute favourable or unfavourable position-influences evenly over all the plants of one series.

The pots were watered from time to time with equal quantities of nutrient-solution and now and then with distilled water only to prevent accumulation of salts. After five or six weeks the plants of each group were measured and finally the fresh and the dry weight of aerial and subterranean parts were determined separately. Usually a selection of the material took place before the ending of the experiment in order that aberrant badly grown or abnormal specimens could be separated.

Beside in the hothouses of the "De Proeftuin" at Boskoop the experiments were also made in our own hothouse at Lunteren.

Experimental results.

1. *Experiment with Cosmos sulphureus Cav. at Boskoop.*

This experiment was started on March 10th 1943 and ended on April 22nd 1943. In addition to a control group which was watered exclusively with Shive's nutrient-solution there were five other groups to the nutrient-solutions of which adenine 0.1 mg/l and 0.5 mg/l, α-naphthylamine 0.1, 0.5 and 1.0 mg/l was added respectively as a leaf growth factor. This time the pots were not randomized.

At the end of the experiment the control plants did not yet show a flower-bud whereas in the other groups there were 3, 4, 1, 2 and 4 specimens respectively with a visible flower-bud. The results are summarized in table I.

On the whole the addition of α-naphthylamine in a concentration of 0.5 mg/l gave the best results. The longitudinal growth is not greatly influenced, more so, however, is the weight of the shoots and the roots. With the addition of adenine the best figures were noted at the highest concentration, viz. 0.5 mg/l which evidently here caused no inhibition yet.

However, we strongly suspect that differences in position have in-

TABLE I.

Group	Number of plants	Solution applied (March 10th 1943— April 22nd 1943)	Average length (mm)	Fresh weight (g) of:			Dry weight (g) of:	
				shoots	roots	10 leaves of the 10 best specimes	shoots	roots
1	20	Shive (= S)	84	48.3	26.8	4.1	4.97	3.10
2	20	S + adenine 0.1 mg/l	88	51.8	31.2	4.6	5.53	3.24
3	20	S + adenine 0.5 mg/l	90	59.4	36.3	5.2	6.32	3.92
4	20	S + α–N.A. *) 0.1 mg/l	81	52.4	32.4	4.8	5.78	3.83
5	20	S + α–N.A. 0.5 mg/l	80	61.1	40.2	5.4	6.75	4.83
6	20	S + α–N.A. 1.0 mg/l	83	58.0	37.4	5.6	6.42	3.84

*) N.A. = naphthylamine

fluenced the result in favour of the effect of the substances investigated. The six groups had been placed in order of the numbers behind each other whereby group 1 stood at the end of the table, rather close to a glass side-wall facing south, so that during sunshine it was practically all the time in the shade. In this way the other groups received more light as their distance to the wall increased.

2. *Experiment with Cosmos bipinnatus Cav. "Sensation Innocence" at Boskoop.*

The experiment with the fine-leaved Cosmos bipinnatus lasted from May 10th 1943 till June 21st 1943. The treatment of six different groups was quite similar to that of the former experiment. Watering was practically exclusively carried out on Monday, Wednesday and Saturday; each group alternately with the nutrient-solution in question and distilled water. A harmful accumulation of salts, as very likely occurred in a measure in our first experiment (some cases of curled leaves) is well-nigh excluded in this way. Each pot received 50 ml every time. The temperature of the air during the experiment was minimal 11.4° C and maximal 16.1 while the soil temperature was minimal 13.9 and maximal 22.1. The position of the groups was the same as in the preceding test.

At the breaking up of the experiment six weeks after sowing the flower-buds were not yet visible. Of each group the 25 best plants were selected and of these 25 the 10 very best specimens were kept apart. Beside this, one leaf of every third leafpair counting from the bottom (ignoring the seedleaves) was torn off. These leaves have been weighed and photographed separately.

We find the results obtained collected in table II.

The influence of adenine as a leaf growth factor may be practically neglected here and a dry-weight increase of 3 times as was stated by BONNER and BONNER (6) on *Cosmos sulphureus* was not obtained at all in this case. A possible influence of the use of Shive's solution or variety of Cosmos different from those in our experiments, should be taken into consideration, though it is doubtful whether this would have any such great influence.

An increase of rooting after addition of adenine is not found either. α-Naphthylamine reacts favourably on the development of the aerial

TABLE II.

Group	Number of plants	Solution applied (May 10th 1943— June 21st 1943)	Average length (cm)	Fresh weight (g) of:						Dry weight (g) of:	
				10 leaves of the 10 best specimens	25 leaves	shoots of the 10 best specimens	25 shoots	roots of the 10 best specimens	25 roots	25 shoots	25 roots
1	25	Shive (= S)	27	2.6	5.6	39.5	78.7	8.9	18.7	6.2	1.6
2	25	S + adenine 0.1 mg/l	27	3.1	6.8	42.6	90.2	9.5	21.0	6.9	1.7
3	25	S + adenine 0.5 mg/l	26	3.0	6.4	42.7	91.2	10.2	21.6	6.9	1.7
4	25	S + α–N.A. *) 0.1 mg/l	26	3.3	7.4	42.4	93.6	9.9	22.1	7.4	1.9
5	25	S + α–N.A. 0.5 mg/l	27	3.3	7.7	46.8	101.3	11.1	24.6	8.0	1.9
6	25	S + α–N.A. 1.0 mg/l	28	3.9	9.3	54.1	122.4	12.6	29.6	9.7	2.4

*) N.A. = naphthylamine.

parts as well as on the rooting. There is no influence on the longitudinal growth; the treated plants are only more sturdy. In contrast with the results obtained in the experiment with *Cosmos sulphureus* (exp. 1) 0.5 mg/l proves to have not yet an optimal effect, so that the optimum may be at 1 mg/l or more.

The unfavourable influence of the position in the hothouse with respect to the development of the control group is here less obvious than in our first experiment. This may be explained if it is taken into consideration that during this second period the sun was already much higher so that the difference in exposure for the various groups was much less pronounced. We must however, take it into account that in consequence of the position the results may again be flattering for α-naphthylamine.

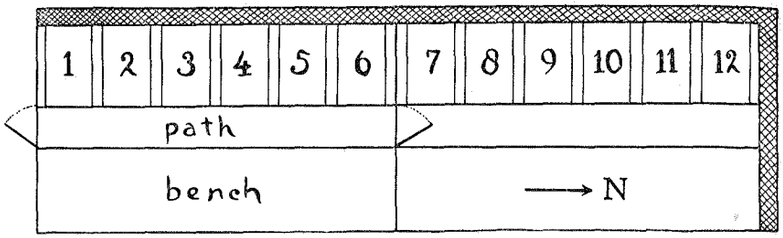
3. *Experiment with Cosmos bipinnatus Cav. "Sensation Innocence" at Lunteren.*

In this experiment which lasted from May 21st 1943 till July 1st 1943, the number of groups was twelve. Beside the control group and the groups treated with adenine in concentrations of 0.1 and 0.5 mg/l, α- and β-naphthylamine and α-(aminomethyl-) naphthalene were tested as leaf growth factors in concentrations of 0.1, 0.5 and 1.0 mg/l. The pots received 50 ml of liquid on Monday, Wednesday and Friday; alternately nutrient-solution and distilled water.

As we shall see from the results the placing of the plants in this experiment was not an ideal one either, so that differences in position should certainly be taken into consideration when judging the results. The place of cultivation was a hothouse situated in a N—S direction which was shut off by a stone wall on the W and the N sides and divided in two by a glass partition. Beside this, part of the roof of the rear half was frosted. The bed of sand lay alongside the western wall so that plants placed in it only received direct sunlight for part of the day. The placing of the different groups in the sand bed was done as is shown in the sketch (see p. 1148). Undoubtedly the glass partition together with the two walls have influenced the development of various groups unfavourably.

It should be noted that some treatments (especially those with α-naphthylamine 0.5 mg/l) had caused, as early as the seedling stage, a development of a stronger (and more branched) root system. This was observed when the plants were transferred from the sowing trays into the pots. A closer investigation as to the action of α-naphthylamine on the development of the root system of seedlings of Cosmos and other plants might

perhaps produce interesting information. With some experiments which we performed in this direction with Cosmos bipinnatus "Sensation Innocence" and tomato "Potentaat" these results did not always prove to be reproducable. In this matter evidently a number of yet practically unknown factors plays a part and therefore it is not always possible to reach



Arrangement of the groups of experiment 3 on the bench along the side-wall of the greenhouse at Lunteren.

the same starting-point. Meanwhile the reaction of this substance is also studied in the cultivation *in vitro* of pea- and tomato-roots and of pea-embryos.

At the end of the test on July 1st 1943, nearly six weeks after the sowing, the 30 best plants of the 35 specimens of every group were selected. Of these the 10 very best ones were kept apart. Only on one specimen (of group 10) a flower-bud was visible. From each plant one leaf of every third real leaf pair counting from the bottom (ignoring the seed leaves) was torn off. These leaves were again weighed separately while fresh. From each group six of the best plants were photographed and photos were also taken of the picked leaves from the 10 very best plants and of the roots. The results of this experiment are collected in table III.

TABLE III.

The first number in each column refers to the 10 best plants; the second number refers to the whole group of 30 plants.

Group	Solution applied (May 21st 1943—July 1st 1943)	Average length in cm	Fresh weight (g) of:			Dry weight (g) of:	
			leaves	shoots	roots	shoots	roots
1	Shive (= S)	30/28	4.3 /12.0	55.0 /142.5	19.6/51.9	4.8 /12.3	1.6 /4.8
2	S + adenine 0.1 mg/l	31/27	5.15/12.75	66.7 /155.9	27.7/60.2	5.9 /13.7	2.1 /5.3
3	S + „ 0.5 „	31/29	5.1 /12.4	67.35/156.45	26.0/58.9	6.0 /13.6	2.4 /6.05
4	S + α–N.A. *) 0.1 mg/l	30/28	4.2 /10.7	59.2 /149.85	22.3/51.8	5.0 /12.35	1.8 /4.0
5	S + „ 0.5 „	33/31	4.4 /11.8	64.4 /164.6	20.6/50.1	5.35/13.45	1.6 /4.3
6	S + „ 1.0 „	33/30	4.9 /11.2	67.7 /148.2	21.5/43.2	5.5 /11.6	1.85/4.0
7	S + β–N.A. 0.1 mg/l	31/28	3.2 / 7.55	46.3 /109.9	12.9/26.5	3.6 / 8.25	0.95/2.35
8	S + „ 0.5 „	31/29	4.8 /11.4	65.3 /154.4	19.8/48.9	5.4 /12.6	1.8 /4.35
9	S + „ 1.0 „	33/30	4.3 /11.85	66.5 /167.0	21.6/52.3	5.45/13.55	1.7 /4.5
10	S + α–A.M.N. **) 0.1 mg/l	31/27	4.6 /11.3	61.7 /150.5	20.1/46.6	5.05/12.0	1.8 /4.15
11	S + „ 0.5 „	30/28	3.7 / 9.9	50.8 /134.9	14.8/39.0	4.3 /11.15	1.3 /3.75
12	S + „ 1.0 „	26/25	3.5 / 8.6	43.6 /105.6	12.2/28.6	3.5 / 8.35	1.1 /2.45

*) N.A. = naphthylamine.
**) A.M.N. = (aminomethyl-)naphthalene.

The influence of adenine and naphthylamine is here less than it proved to be in the first experiment. At an earlier stage (about a week before the ending of this experiment) the mutual differences between the groups

appeared greater. For a correct judgment of the differences between the activity of α- and β-naphthylamine and α-(aminomethyl-)naphthalene the acquired results are of little use. It is remarkable, however, that 0.5 mg/l α-naphthylamine produces nearly the same effect as 1.0 mg/l β-naphthylamine.

In what degree position differences here have had an influence is very difficult to ascertain. The low figures concerning the fresh- and dry-weights of the aerial parts of group 7 might be ascribed to the influence of the partition in the hothouse. Since, however, the average length of the plants of group 7 corresponds with that of the control group we must certainly be careful with this conclusion. It is quite possible that plants of group 7 have stretched more in length as a result of bad exposure to light than would have been the case under favourable conditions. We believe that the reason for the low figures in group 12 must rather be found in the unfavourable influence of its position in the corner of the hothouse than in too high a concentration of α-(aminomethyl-)naphthalene.