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Humic Acid Interactions in the Growth Process

JIŘÍ ŘEŘÁBEK

Department of Plant Physiology and Soil Biology, Faculty of Natural Sciences, Charles University, Praha

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Souhrn

Charakteristickým rysem působení huminových kyselin na růst rostlin je stimulace prodlužování kořenů, vysvětlovaná někdy auxinoidní povahou těchto látek.

Autor prověřil tento předpoklad testem na odřezcích koleoptile pšenice a hypokotylu lnu. Růst odřezků obou typů se v roztocích humátu draselného o koncentraci 10^{-1} až 10^{-7} g/l ve srovnání s kontrolním růstem ve vodě nezměnil.

Dále byla studována interakce huminových kyselin s auxiny. Průběh růstové křivky odřezku do koncentrací auxinu kolem 20 mg/l (len) nebo 50 mg/l (pšenice) přítomnost humátu v testovaném roztoku v koncentraci 10^{-1} g/l neměnila. Při vyšších koncentracích auxinu byl růst odřezků v roztocích auxin-humát podstatně vyšší než v roztoku samotného auxinu. Efekt se snižoval se stoupající koncentrací auxinu (kyselina β-indolyloctová a α-naftyloctová).

Auxinoidní charakter ve vodě rozpustných solí huminových kyselin ani kompetitivní antagonismus ve smyslu koncepce antiauxinu nebyl prokázán.

Summary

A characteristic feature of the effects of humic acids on plant growth is the stimulation of growth in length of the roots. This is sometimes explained as being due to the auxinoid nature of the acids.

The author has examined this assumption by testing segments of wheat coleoptiles and of flax hypocotyls. The growth of segments of both types remained unchanged in solutions of potassium humate in concentrations of 10^{-1} to 10^{-7} g./l. as compared with the controls in water.

A study was also made of the interaction of humic acids with auxins. The growth curves of segments in concentrations of auxin up to about 20 mg./l. (flax) or 50 mg./l. (wheat) were not altered by the presence of a 10^{-1} g./l. concentration of humate in the test solution. With higher concentrations of auxin the growth of the segments in auxin-humate solutions was considerably greater than in solutions containing auxin alone. The effect decreased with rising concentrations of auxins (β-indoleacetic acid and α-naphthylacetic acid).

The auxinoid character of salts of humic acids in water solution was not established, nor was there any confirmation of competitive antagonism in the sense of the antiauxin conception.

Introduction

The favourable effect of humus substances on plant growth has been known for a long time. Root growth is most strongly influenced, the overground parts to a lesser extent. In spite of the fact that considerable efforts have been made to throw some light on the nature of the effects produced by these substances, we have as yet no satisfactory explanation of the mechanism of their action. A number of theories have been advanced, but they have not yet been adequately tested by experiment and are, indeed, often in conflict with facts obtained during the examination of other problems of growth.

However, it would seem that closer attention should be paid to those opinions which regard humus substances as being growth substances. This assumption is already to be found in BOTTOMLEY's work (1917, 1920). This author considered that the active components of humus substances are auximones, i.e. compounds presumably related to the later-discovered auxins. Essentially the same point of view was later held by other authors (BASSALIK and NEUGEBAUER 1933, KUTHY and PECZNIK 1941, CHAMINADE and BOUCHER 1955). Compounds similar to auxins were found in peat, water extracts of which also have a favourable effect on plants. Recently, FLAIG and BREYHAN (1956) have identified indole derivatives in the hydrolysate of humic acids from chernozem. However, we still have no convincing proof of the auxinoid character of humus substances, because they have not been examined by means of the tests commonly used for determination of the activity of growth substances. Only PASZEWSKI, TROJANOWSKI and ŁOBARZEWSKI (1957) have tested the chromatographically separated components of the alcoholic fraction of humus with positive results on wheat coleoptiles; this fraction as a whole was, however, inactive.

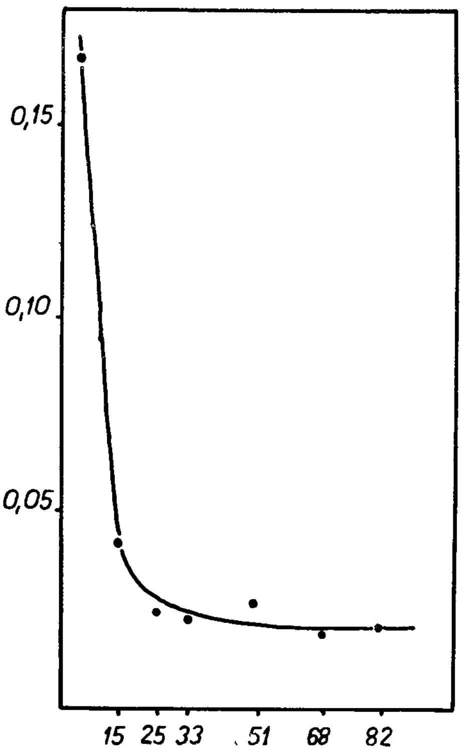
Further, the question of the stimulation of root growth by auxins is still doubtful. In view of this it would be more justified to compare humus substances to antiauxins, one property of which is their favourable effect on root growth.

Experimental investigation of these two assumptions is the aim of the present work.

Material and Methods

The activity of humic acids and their interaction with auxins was determined using segments of etiolated wheat coleoptiles and flax hypocotyls.

The test on wheat coleoptiles was carried out in the normal way. Following 24 hours' swelling in water, wheat grains (*Triticum vulgaris* L. var. Pyšelka) were grown in the dark in damp lime-tree sawdust. Segments of 10 mm. in length were cut off from coleoptiles of similar length (20 to 40 mm. according to the experiment). The first incision was made 2 mm. below the tip of the coleoptile. Equality of length of the segments was ensured by cutting nine at a time with a knife composed of two razor blades fixed at a distance of 10 mm. from each other (BENTLEY and HOUSLEY 1954). Twenty-five to thirty segments were tested for 12 to 36 hours in 25 ml. solution in Petri dishes. The leaves of the segments were not removed and they floated freely in the solution. The number of segments in a dish was established according to the course of Fig. 1 in such a way that the variability of their growth was eliminated. Following the test, the segments were slightly curved, which made direct microscopic measurement of their length very difficult and inaccurate. The outlines of the segments were, therefore, projected on to photographic paper with a 3·5 enlargement. After development the lengths were measured by means of a flexible rule with an accuracy of 0·5 mm.



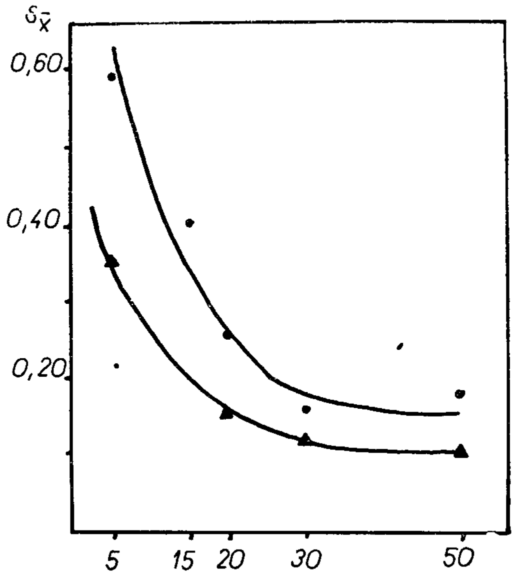


Fig. 2. Dependence of standard error of average length of flax hypocotyl segments on number of individuals in set. 10 mm. segments, 24 hours at 24° C. ●—● segments in distilled water (av. length 12—13 sections); ▲—▲ segments in α-naphtylacetic acid solution 50 mg./l. (av. length 14—15 sections). Ordinate: standard error of average of sections. Abscissa: number of individuals in set.

Fig. 1. Dependence of magnitude of standard error of average length of coleoptile segments on number of inividuals in set. Ordinate: standard error of average, in mm. gain in 24 hours. Average growth increase of segments in water = 1·50—·1/20 mm./24 hours. Abscissa: number of individuals in given set.

Test with flax hypocotyls (*Linum usitatissimumt* var. Viera), Flax roots are suitable as material for testing growth substances (ÅBERG 1950). It was found in our experiments that segments of etiolated hypocotyls also react to the presence of auxins in the test solution. The method of cultivation has been described by ÅBERG (1950). The period was prolonged to 36 to 40 hours. At the end of this period the hypocotyls measure 15—30 mm. Hypocotyls of equal lengths of 20—25 mm. are selected and their tips are removed about 2 mm. below the bend. A segment measuring 10 mm. is then cut off from the remaining part as in the case of the coleoptiles. The further procedure was similar to that for the test using coleptiles. Fig. 2 shows

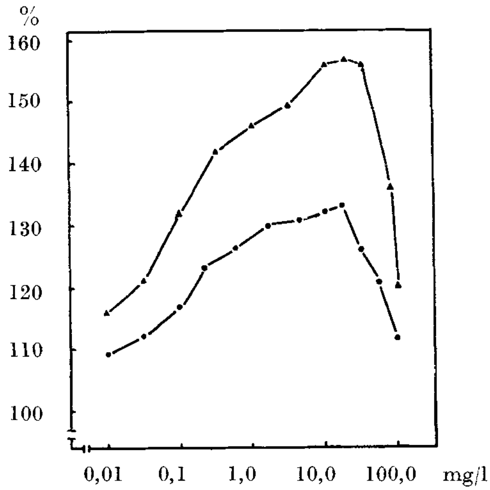
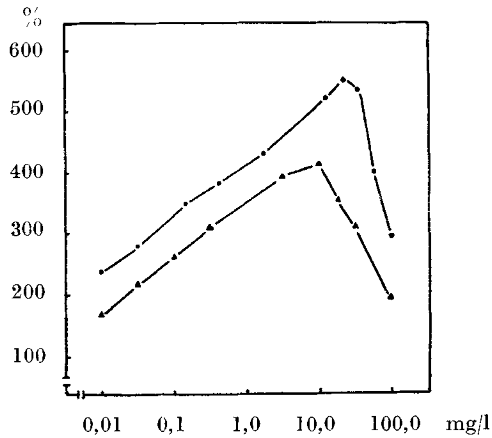


Fig. 3. Growth (24 hours, 24° C) of wheat coleoptile (▲—▲) and flax hypocotyl (●—●) segments in β-indoleacetic acid. Ordinate: length as percentage of control length. Abscissa: auxin/l.

Fig. 4. Growth (24 hours, 24° C) of wheat coleoptile (▲—▲ and flax hypocotyl (●—●) in β-indoleacetic acid. Ordinate: gain in length as percentage of control gain. Abscissa: mg. auxin/l.



that 25 to 30 segments in 25 ml. of solution are necessary for one experimental variant. The period of the test was 24 hours. Measurement of the segments was carried out in a different way. After development of the enlargement of the outlines on photographic paper, lengths were measured by means of sections marked along one side of the photographic outlines with a small cog-wheel. One section corresponds to 1·11 mm. actual length.

Growth of the plants and of the segments was carried out in both cases in the dark at a temperature of 24° C. The segments were cut in orange light (photo-bulb AGFA-ORANGE).

The growth curve obtained for the hypocotyl segments follows the same course as that for coleoptile segments. If the effect of β-indoleacetic acid is expressed as a percentage of the lengths of control segments in distilled water, the segments of flax hypocotyls show a sensitivity in their reaction to auxins of up to one-half less than the coleoptile segments. (Fig. 3.) The increase in length of the segments expressed as a percentage of the increase of the control in water are, however, always higher for flax hypocotyls (Fig. 4). This is mainly due to the slight growth of the control hypocotyl segments. which show a maximum increase of 8 to 10% of their original length after 24 hours. The first procedure is more frequently used and therefore the further results have been dealt with in that way.

Preparation and application of humus acids. Two kinds of basic material were employed:

1. Trade preparation Humussäure, manufactured by Riedel-Haën, Hannover.
2. Humic acid fraction of oxyhumolite from the mine Osvobození at Řetenicích, south-west Bohemia.

The humic acid fraction was prepared as follows: 500 g. of oxyhumolite was extracted for 45 min. at room temperature with 2500 ml. of 0·1 N KOH with occasional shaking. The in-soluble matter was removed by filtration through glass wool and 200 ml. of concentrated HCL was added to the filtrate. After centrifuging, the coagulate was washed with distilled water until ions of Cl′ had been removed and was then dried at reduced pressure at 30 to 40° C.

The fraction and the Humussäure preparation both contain considerable amounts of substances which are soluble in ethanol. Both preparations were converted to potassium salts by solution in 0·1 N KOH, dialysis with distilled water and evaporation at reduced pressure at 30 to 40° C. These salts are suitable for application in both tests as they are very readily soluble in water. They will be referred to as K-humate Ox (from Osvobození) and K-humate R (from Humussäure).

Auxins used: β-indoleacetic acid (Merck) and α-naphthylacetic acid (Lachema, Brno). The test solutions were prepared by dissolving the substances in redistilled water. The pH values of the solutions did not differ from one another and ranged between 5·0 to 5·2 before and after the test. The water control had the same value.

Results

Segments from coleoptiles of different lengths were tested in solutions of both K-humates. The concentrations used were those which according to published results have a positive effect on growth in root length. Several repetitions of the experiments were made and in addition the growth of the segments was measured at intervals of 12, 24 and 36 hours. The results given in the tables were worked out by analysis of variance. The values of the F-test for the factors involved in the experiment are appended.

Tables 1, 2 and 3 give the growth of coleoptiles. For greater clarity a value C is also given, i.e. the length of segments of the different variants as a percentage of length of control segments. Neither of the humates affected the growth of the segments, for in no case were the differences, amounting to 5 to 6% of the control length, significant. The growth of flax hypocotyl segments also remained unchanged by the application of humates (Table 4).

Table 1. Growth of wheat coleoptile segments in K-humate solutions. 10 mm. segments from 20—25 mm. coleoptiles. Period of test 12—36 hours. Values in mm. = actual length x 3·5 . C = % control

K-humate	12 hours		24 hours		36 hours	
	mm.	C	mm.	C	mm.	C
Control	41·33	100·0	57·88	100·0	62·57	100·0
10^{-5} g./l. Ox	40·03	96·8	59·88	103·4	—	—
R	41·00	99·2	58·86	101·8	63·92	102·1
10^{-3} g./l. Ox	40·03	96·8	56·79	98·1	—	—
R	39·40	95·3	56·85	98·2	63·00	100·6
10^{-1} g./l. Ox	42·57	103·0	61·30	105·9	—	—
R	39·84	96·3	59·52	102·8	62·94	100·5

Analysis of variance: Ox for concentration $F = 1·44$ $P > 0·05$
for hours $F = 205·28$ $P < 0·01$
R for concentration $F = 2·20$ $P > 0·05$
hours $F = 416·17$ $P < 0·01$

Table 2. Growth of wheat coleoptile segments in K-humate solution Ox 10^{-1} g./l. 10 mm. segments from 30—35 mm. coleoptiles. Period of test 12—36 hours. Values expressed as in Table 1

	12 hours		15 hours		24 hours		36 hours	
	mm.	C	mm.	C	mm.	C	mm.	C
Control	39·00	100·0	50·82	100·0	60·14	100·0	58·48	100·0
10^{-1} g./l.	39·50	101·2	48·25	94·1	57·46	95·5	58·70	102·3

Analysis of variance: for concentration F = 4·20 P > 0·05
for hours F = 176·19 P < 0·01

Table 3. Growth of wheat coleoptile segments in K-humate solution Ox 10^{-1} g./l. 10mm. segments from 40—45 mm. coleoptiles. Period of test 12—36 hours. Values expressed as in Table 1

	12 hours		24 hours		36 hours	
	mm.	C	mm.	C	mm.	C
Control	47·45	100·0	61·60	100·0	58·65	100·0
10^{-1} g./l.	46·52	98·0	61·70	100·1	61·36	104·6

Analysis of variance: for concentration F = 1 P > 0·05
for hours F = 8·95 P < 0·05

Table 4. Growth of flax hypocotyl segments in K-humate solutions of varying concentration. 10 mm. segments from 20—25 mm. hypocotyls. Period of test 24 hours. Values in sections = = actual length x 1·11. C = % of control

	K-humate Ox			K-humate R		
	Sections	Average Sections	C	Sections	Average Sections	C
Control	11·88 11·88	11·88	100·0	12·26 11·87 11·87	12·00	100·0
10^{-7} g./l.	12·00 11·81	11·90	100·1	12·50 11·68 11·86	12·01	100·0
10^{-5} g./l.	11·88 11·96	11·92	100·3	12·53 11·70 12·07	12·01	100·0
10^{-3} g./l.	11·69 11·71	11·70	98·4	12·21 11·69 12·11	12·00	100·0
10^{-1} g./l.	12·00 11·73	11·86	98·8	12·50 11·85 12·17	12·17	101·4
	Exp. 27A, 27B			4 28A 28B		

Analysis of variance: for concentration F = 1·2 P > 0·05

In the following experiments interaction between humate and the two auxins was followed. The humate concentration was constant in all experiments, while the auxin concentration changed. There resulted concentration curves for β-indoleacetic acid or α-naphtylacetic acid alone or mixed with humate at a concentration of 10^{-1} g./l. The values entered on the graph are the averages of at least three experiments and for each variant, as in the previous experiments, 25 to 30 segments were allowed for. Each average, therefore, represents the growth of approximately 70 to 90 segments.

Fig. 5 and 6 give results for coleoptiles. While at lower auxin concentrations in the mixture the humates showed no influence, at concentrations

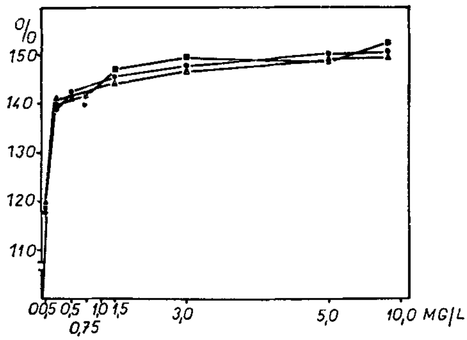


Fig. 5. Growth of wheat coleoptile segments in β-indoleacetic acid (●—●) and in this solution with K-humate Ox 10^{-1} g./l. (▲—▲) and R 10^{-1} g./l. (■—■). 10 mm. segments from 20 to 25 mm. coleoptiles. Period of test 24 hours at 24° C. Ordinate: % of length of control. Abscissa: auxin concentration in mg./l.

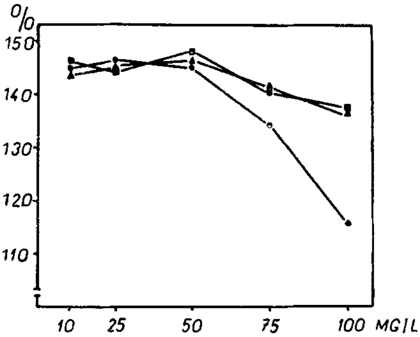


Fig. 6. Growth of wheat coleoptile segments in β-indoleacetic acid (●—●) and in this solution with K-humate Ox 10^{-1} g./l. (▲—▲) and R 10^{-1} g./l. (■—■). See Fig. 5.

above 50 mg./l. auxin activity was altered. The effect of the humate was evident in more intense growth of segments in the solution of the two substances. Differences were significant at concentrations over 75 mg./l. of the test auxin, i.e. β-indoleacetic acid. No difference was found between the effects of the two humates.

Humate was tested on hypocotyl segments with β-indoleacetic acid and α-naphtylacetic acid. Both auxins exhibit a typical effect on the growth of the segments. α-naphtylacetic acid is somewhat less active at lower concentrations; at concentrations over 30 to 40 mg./l. growth in this solution is also lower than in β-indoleacetic acid solutions of equal concentration (Fig. 7 and 9). The effect of the humate does not differ from that in the experiments with coleoptiles. Low auxin concentrations, in this case about 20 to 25 mg./l., are not affected by either of the humates, while the effect in supra-optimal concentrations is again decreased. (Fig. 8. and 10). Differences in growth are, however, somewhat greater than in the experiment with coleoptile segments.

Reduction in auxin activity at super-optimal concentrations is strongly dependent on humate concentration. A concentration of 10^{-1} g./l. is fully effective. A solution diluted by 100 is ineffective.

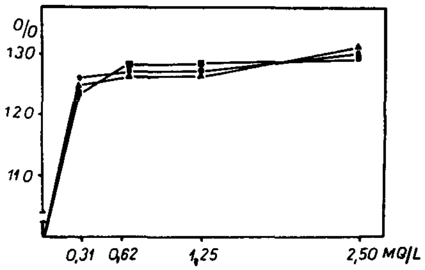
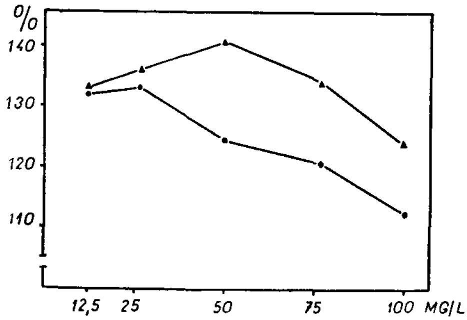


Fig. 7. Growth of flax hypocotyl segments in β-indoleacetic acid (●—●) and in this solution with K-humate Ox 10^{-1} g./l. (▲—▲) and R 10^{-1} g.W. (■—■). See Fig. 8.

Fig. 8. Growth of flax hypocotyl segments in β-indoleacetic acid (●—●) and in this solution with K-humate Ox 10^{-1} g./l. (▲—▲). Segments from 20—25 mm. hypocotyls. Period of test 24 hours. Ordinate: % of control length. Abscissa: auxin concentration in mg./l.



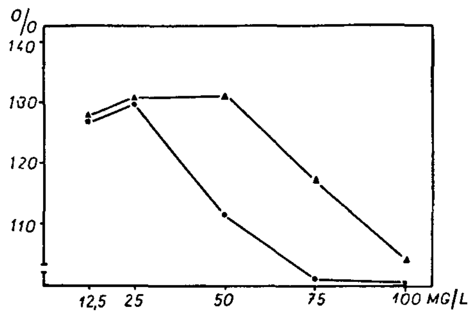


Fig. 9. Growth of flax hypocotyl segments in α-naphtylacetic acid (●—●) and in this solution with K-humate Ox 10^{-1} g./l. (▲—▲). See Fig. 8.

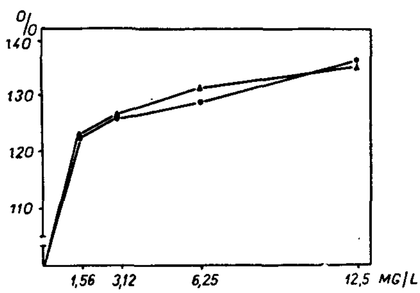


Fig. 10. Growth of flax hypocotyl segments in α-naphtylacetic acid (●—●) and in this solution with K-humate Ox 10^{-1} g./l. (▲—▲). See Fig. 8.

Discussion

As has already been mentioned in the introduction, many authors have assumed that there is an analogy between the effect of humus substances, particularly humic acids, and that of auxins. But the criterion for regarding any compound as an auxin is a positive result with some of the tests used for determining the activity of growth substances. Therefore, it was necessary to treat the humic acids in this way, too. The present experiments have established that this fraction completely lacks the properties of auxins. Growth of segments of wheat coleoptiles and flax hypocotyls was unaltered following the application of a wide range of concentrations of K-humates of various origin. This is in agreement with the results of Paszewski, Trojanowski and ŁOBARZEWSKA (1957), who also used coleoptiles to test the alcoholic fractions of peat and compost humus. The fractions as a whole were also inactive. It is interesting, however, that the chromatographically separated components acted on the curving of the longitudinal parts of cut coleoptiles in a similar way to β-indoleacetic acid.

In opposition to the view that humic acids act on plants like auxins is also the fact of the qualitative difference in the effect of these substances on root growth. While it has been repeatedly demonstrated that humic acids have a favourable effect on root growth, the primary characteristic of auxins is that they inhibit this growth. Stimulation by very low concentrations has not yet, according to ÅBERG (1957), been reliably demonstrated as a general phenomenon. Auxins do not affect the growth of algae (LHOTSKÝ 1952), while humus substances act as strong stimulants (LHOTSKÝ 1954, PRÁT 1955). These differences, particularly as regards roots, indicate an analogy with another group of growth substances—the antiauxins, which also strongly accelerate root growth. They are, however, also characterized by the fact that in various tests they reduce the effect of externally applied auxins (ÅBERG 1950, BURSTRÖM 1950, VAN OVERBEEK et al. 1951). This interaction is particularly evident with sub-optimal concentrations of auxin. It has been found that humic acids do not alter the effect of these concentrations. The growth activity of super-optimal concentrations is, however, considerably reduced by humate. The growth of segments in a solution containing auxin and humate is definitely greater than in a solution of auxin alone. Typical antiauxins, such as 1-naphtylmethylsulphidepropionic acid (ÅBERG and KHALIL 1953) or 4-chlorphenoxyisobutyric acid (FOSTER et al. 1955), do not alter the activity of such concentrations of auxin, insofar as they are not applied in toxic amounts.

The humate-auxin relation, insofar as it has been followed in tests with overground plant parts, does not show a typical antiauxinoid character. The real nature of the relation between the two substances at super-optimal concentrations of auxin, when humate antagonizes its growth activity, remains, however, a problem. The results obtained so far will have to be amplified by investigation of interaction in a suitable root test. It is also necessary to find out whether this action is reversible following further applications of auxin.

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Address: Jiří Řeřábek, prom. biol., Katedra fysiologie rostlin a půdní biologie přírodovědecké fakulty K. U. Praha 2 - Nové Město, Viničná 5

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Влияние гумусовой кислоты на рост растений

Иржи Ржержабек

Кафедра физиологии растений и биологии почвы Естественного факультета Карлова Университета, Прага

Резюме

Характерной чертой действия гумусовых кислот на рост растений является стимуляция роста корней в длину, которая иногда объясняется тем, что эти вещества имеют характер ауксиноидов.

Автор проверил это предположение опытами на отрезанных частях колеоптиля пшеницы и гипокотиля льна. Рост частей того и другого типа в растворах гумата калия с концентрацией 10^{-1}—10^{-7} г/литр не отличался от роста контрольных частей в воде.

Далее изучалось взаимодействие гумусовых кислот и ауксинов. Ход кривой роста отрезанных частей в растворах ауксина с концентрацией до 20 мг/л (лен) или 50 мг/л (пшеница) не зависел от присутствия в растворе гумата в концентрации 10^{-1} г/л. В случае более высоких концентраций ауксина рост отрезанных частей в растворах ауксин-гумат был значительно выше, чем в растворах одного ауксина. Однако, с дальнейшим ростом концентрации ауксина (индолилуксусная и нафтилуксусная кислоты) эти различия проявлялись все слабее.

Таким образом, не удалось доказать ни ауксиноидный характер водорастворимых солей гумусовых кислот, ни компетитивный антагонизм в соответствии с представлениями об антиауксине.