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## The Root-inhibiting Substance of *Allium Cepa**)

by

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INTRODUCTION.

Whereas scientific research on inhibiting substances has mainly occupied itself with the effect of these substances on the germination process, I was able to demonstrate the presence of a root-inhibiting agent during my studies on root formation in *Fuchsia hybrida* and *Pelargonium zonale* (Stolk, 1952). In connection with this previous investigation I tried to find out whether a similar substance is present as well in bulbous plants and, by doing so, as a suitable species for my experiments the Liliaceous *Allium Cepa* was selected. My principal object was to corroborate and, if possible, extend the results obtained with *Fuchsia* and *Pelargonium*. A confirmation appeared highly desirable because the experiments with *Fuchsia* and *Pelargonium* could not possibly be very accurate on account of the direct method of measuring and that is why I used in my experiments with *Allium* a root-auxanometer which will be described in the following section.

Substances retarding germination were demonstrated in the slime of *Viscum album* by WIESNER (1894), in the fruit pulp of ripe tomatoes by OPPENHEIMER (1922), in tomato juice by REINHARD (1933), in other pulpy fruits such as apples, pears, quinces and tomatoes by KÖCKEMANN (1934), in the exocarp of buckwheat by LEHMANN (1937), in the fruits of *Helianthus annuus* and *Avena sativa* by RUGE (1939) and in *Beta* by FRÖSCHEL (1939, 1940). That not one and the same inhibitor is involved, is evident from the fact that the above-mentioned authors found differences between the inhibiting substances they studied in their behaviour towards high temperatures. The inhibiting substances found by OPPENHEIMER and LEHMANN, for instance, are thermolabile, those discovered by REINHARD, KÖCKEMANN and FRÖSCHEL thermostabile. Also in their chemical behaviour certain differences can be demonstrated. Whereas the substance studied by KÖCKEMANN is soluble in ether, this is not the case with the inhibiting substance found by OPPENHEIMER in tomatoes.

*) Received March, 1951.

A short survey of my personal results with *Fuchsia* and *Pelargonium* may serve to give the reader some idea of the inherent properties of root-inhibiting substances:

1. *Pelargonium* cuttings excrete a substance inhibiting the growth of *Pelargonium* roots and *Fuchsia* cuttings produce a substance inhibiting the growth of *Fuchsia* roots: auto-inhibition.
2. The inhibiting substance from *Pelargonium* also inhibits *Fuchsia* roots and acts aspecifically: inhibition sensu stricto.
3. The inhibiting substance from *Fuchsia* is weaker in its action than the one from *Pelargonium*.
4. Both the *Fuchsia* and *Pelargonium* inhibitors are thermolabile, the inhibiting capacities get lost after a treatment at 100° C.
5. If the inhibition has not been lasting for too long a period, it can be stopped by a transfer of the cuttings to fresh water. The inhibition is therefore a reversible process.
6. The inhibiting substance from *Fuchsia* cuttings has no perceptible influence on the formation of lateral shoots.

Although, as was reported before, hardly any publications dealing with substances retarding root growth are available, many observations on growth phenomena in roots can easily be explained in the light of the activity of some growth-inhibiting substance. WEAVER and HIMMEL (1930), for instance, report that the growth of *Phragmites* and *Spartina* roots is strongest in a soil with a high water content. As a measure of the root growth the dry weight of the roots was used. A similar result was obtained by GORDIENKO (1930) when he studied root formation in *Secale cereale, Triticum repens, Pisum sativum* and *Lupinus luteus*. The number of roots formed by these plants was highest in soils with a high water content.

KEEBLE, NELSON and SNOW (1930) believe to have found a traumatic hormone in *Zea Mays* and *Pisum sativum* which, in contradistinction to HABERLANDT'S well-known traumatic hormone (1921), has a negative action by inhibiting root growth. If, namely, they injured roots by decapitation of 1 or 0.5 mm., the roots whose traumatic substance had been rinsed away showed a higher growth rate (6 to 32 per cent higher) than those which had not been subjected to such a treatment. In my opinion it is not necessary to conclude, on the strength of their experiments, that a traumatic hormone is involved, as the occurrence of a growth-inhibiting substance, which is excreted also by intact roots, may also be responsible for the acceleration of growth. This would at the same time explain the controversy between their results and those of HABERLANDT.

The fact that roots are capable of producing certain substances was shown by SOLBERG (1935), among others, in lupins, beans, maize and *Sinapis*. He does not give any particulars as to the nature and the activities of these substances, which have an acid reaction. It seems quite conceivable to me, although I have so far found no evidence to prove this assumption, that SOLBERG was dealing with inhibiting substances.

## MATERIAL AND METHOD.

The material consisted of bulbs of *Allium Cepa*. Whereas for the measurements of the root lengths small onions were used (referred to as : "test onions" and weighing 8 to 13 g.), the "inhibitor onions" which, as the name indicates, were used to produce the required inhibiting substance, were heavier and weighed from about 90 to 185 g. These inhibitor onions were placed on relatively small containers filled with water and kept in the dark so as to allow them to form roots. This water was used as "inhibiting water", but not before the root system had reached a considerable size and, accordingly, might be expected to have produced a sufficient quantity of the inhibiting substance. The test onions were also allowed to strike root in the dark and were transferred to the measuring apparatus as soon as they had formed a straight root measuring 1.5 to 2 cm.

Before starting the experiment I removed all superfluous and bent roots by means of tweezers, so that as a rule only one root per onion was used. In some cases more roots were in the same stage of development and these could without any difficulty simultaneously be used for measurements.

The measurements were carried out by means of a root-auxanometer which I had especially designed and constructed for the purpose. The

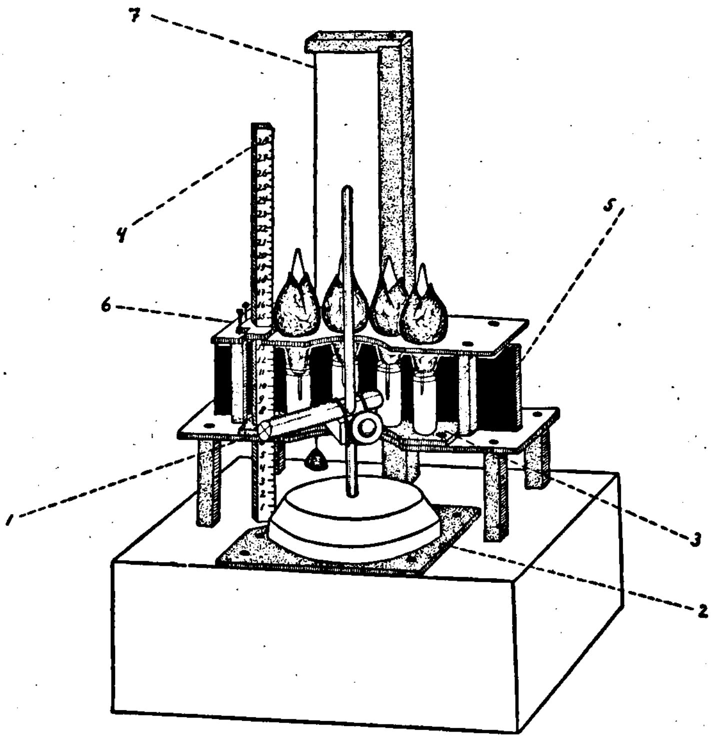



Fig. 1. Root-auxanometer.

EXPLANATION : 1 = pocket telescope, 2 = pedestal, 3 = support for containers, 4 = measuring ruler, 5 =screen, 6 = adjusting arrangement, 7 = plummet. By means of a cover (a wooden frame covered with black paper) the whole apparatus can be placed in the dark. This cover is omitted in the figure for obvious reasons.

apparatus is represented in fig. 1. The pocket telescope (1) is provided at both ends with a reticle made of human hair and can be fixed at various heights by means of a perforated metal block and adjusting screw. The perfectly circular support fits accurately in the circular opening of the pedestal (2) and allow for movement of the telescope in a horizontal plane. Opposite the pocket telescope are two platforms, of which the lower one can hold four small containers (each with a content of about 11 ml.) and the upper one has four openings for the test onions. The containers can be held in place by a support (3), which also has four holes for the purpose. The test onions are kept in place by means of pins and cotton wool. In order to obtain exact readings, a screen (5) which is covered with black paper on the one side and with white on the reverse side is attached to the lower platform behind the experimental containers. It is fixed by means of two wooden swivels and can be removed if desired and eventually turned the other way round. Through the upper and lower platform extends the measuring ruler (4), which can be placed exactly perpendicular by means of the adjusting arrangement (6) consisting of a perforated piece of wood and some nails. A plummet (7) serves for controlling the perpendicularity of the ruler, the telescope support and the containers. It goes without saying that the position of the containers can only be controlled if the screen has been removed. Finally, it should be mentioned that the root-auxanometer was placed under a cover made of a wooden frame covered with black paper, so that the experiments were carried out in complete darkness.

The measurements are performed as follows : The place of the tip of the root is estimated at a certain time by adjusting the pocket telescope in such a way that the centres of both reticles are exactly in line with the root apex. The telescope is subsequently turned about its support and focussed on the graduated scale of the ruler, so that the position of the root apex can be read. From the initial and the ultimate positions of the root apex the growth and by means of a short computation also the speed of growth during a certain period can be estimated. By means of the root-auxanometer it is possible to estimate the position of the root apex very accurately.

In order to demonstrate the presence of an inhibiting substance, several criteria are available. For the study of the inhibiting substance in *Fuchsia* and in *Pelargonium* the following phenomena served as criteria :

1. The presence or absence of roots.

    I started from the assumption, which was justified by subsequent experiments, that in smaller containers the inhibiting substance attains a higher concentration than in larger ones and that, accordingly, root growth is more strongly inhibited in the former.

2. The speed of growth of the roots.
3. The speed of growth of the roots after a change of the liquid medium.

In the experiments with *Allium*, criterion 3 was almost exclusively used, i.e., from the different speed of growth in fresh water and in water containing the inhibiting substance conclusions were drawn regarding the activity of the inhibitor.

An enumeration of the media, with the pertaining symbols used to indicate them in this paper, for the sake of convenience, is given below:

1. F-water= fresh tap water.
2. F100-water= fresh water that had been heated at 100° C.
3. A-water= *Allium* water or inhibiting water, i.e., water that had been in contact with the root system of an "inhibitor onion" for some time. Occasionally the onion had formed its roots in this medium, but this had not been the case in all experiments.
4. A-conc.-water= concentrated *Allium* water, i.e., inhibiting water that had been concentrated by evaporation at a moderate temperature (something like 15 to 20° C.). Sometimes the concentration was known and is indicated in this case.
5. A 100-water= inhibiting water that had been kept at 100° C. for some time. The water evaporated during this treatment was replenished so that the concentration remained unchanged.
6. R-water= root extract water, i.e., fresh water that had been in contact with ground *Allium* roots for some time. Before use the solid matter was filtered off. The root extract water is characterised, apart from the typical onion smell, by an opalescent shine and froths abundantly when shaken.
7. R-100-water= root extract water that had been kept at 100° C. for some time. The water evaporated during this treatment was replenished, so that the concentration remained unchanged.

Finally, a few words to explain the diagrams would not be out of place. The speed of growth-time-diagrams are built up of successive periods of elongation, whose mean speed of growth is indicated. This speed of growth was expressed in units of 10 $\mu$ per h. Upon the whole, each period lasted about 24 hrs. The differences in level between successive periods should not be misinterpreted as being indicative of sudden rises or falls of the speed of growth. These periods are, namely, discontinuous on account of the fact that mean speed of growth of each period was estimated.

A change in the medium is indicated in the diagrams by an arrow with a letter. The symbol $\overset{\uparrow}{\text{F}}$, for instance, means that the root was transferred to fresh water, A $\overset{\uparrow}{100}$ that it was placed in boiled *Allium* water. The symbol without an arrow at the beginning of each diagram indicates the medium in which the root was growing when the experiment started .There is no change in medium in this case, changes only being indicated by an arrow.

The experiments were carried out during the months February, March and April, in a room facing South-West.

## Discussion of experimental results.

The greater part of the diagrams represented are indicative of a retarding action of the *Allium* water on the elongation of *Allium* roots and therefore this water rightfully deserves the name of "inhibiting water". The growth retardation can be a considerable one, such as in the case shown in diagram 1, in which the speed of growth, 48 hrs. after the commencement of the experiment, shows a decrease of 171—55= 116 $\mu$ per h. after application of inhibiting water; and in the case represented

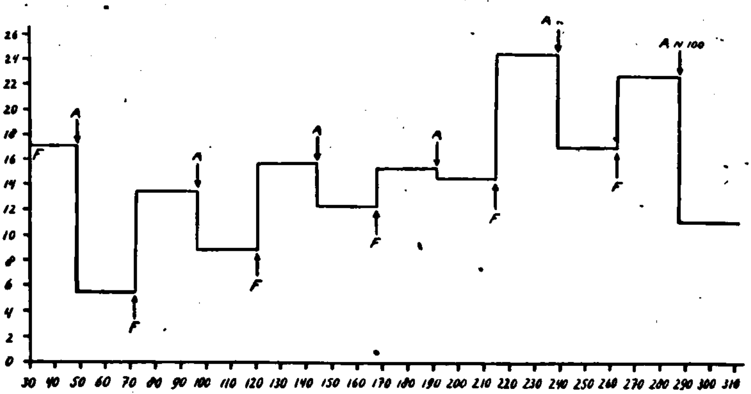



Diagram 1. Inhibition of *Allium* root by *Allium water.*
EXPLANATION OF SYMBOLS: F = fresh water, A = *Allium* water, An = a change of the *Allium-water,* An 100 = An-water, heated at 100° C.
Abscissa: time in hrs., ordinate: mean speed of growth in units of 10 $\mu$ per h. Inactivation of the inhibiting substance in dependence on time (step-by-step phenomenon).

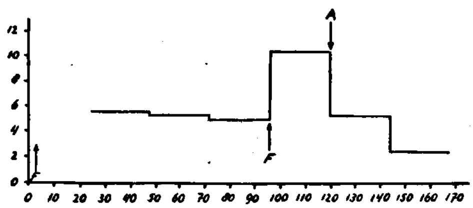



Diagram 2. Inhibition of *Allium* root by a substance excreted by the root itself (auto-inhibition). Application of fresh water increases the mean speed of growth, whereas a subsequent application of *Allium* water decreases the mean speed of growth again.
EXPLANATION OF SYMBOLS: F = fresh water, A = *Allium* water. Abscissa: time in hrs., ordinate: mean speed of growth in units of 10 $\mu$ per h.

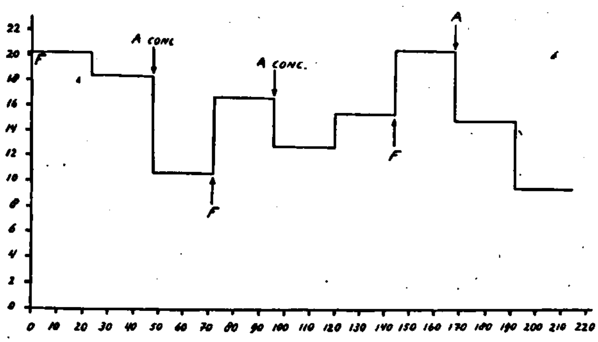



Diagram 3. Inhibition of *Allium* root by concentrated *Allium* water. Inactivation of the concentrated inhibiting agent in dependence on time (step-by-step phenomenon). Continued falling off of mean speed of growth (191¼ hrs. after the commencement of the experiment). EXPLANATION OF SYMBOLS: F = fresh water, A = *Allium* water, A conc. = concentrated *Allium* water. *Abscissa*: time in hrs., ordinate: mean speed of growth in units of 10 $\mu$ per h.

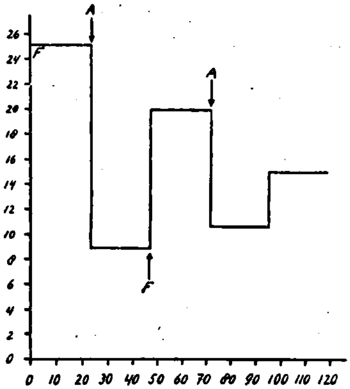
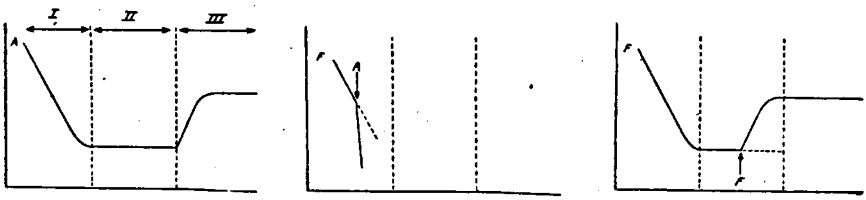



Diagram 4. Increase in mean speed of growth in the second part of the second period of inhibition (95 hrs. after the commencement of the experiment). EXPLANATION OF SYMBOLS: F = fresh water, A = *Allium* water. Abscissa: time in hrs., ordinate: mean speed of growth in units of 10 $\mu$ per h.




A B C

Fig. 2. Diagrams showing the inhibiting action. EXPLANATION OF SYMBOLS: F = fresh water, A = *Allium* water, I = inhibiting action, II = equilibrium, III = recovery. Abscissa = time; ordinate = speed of growth.

A. Inhibition of *Allium* root by root-inhibiting substance of *Allium*.

I. A falling section of curve as a result of the activity of the inhibiting agent. The speed of growth gradually falls off till the minimum is reached.

II. A horizontal section of curve which must be a result of a certain equilibrium. The inhibiting substance is no longer capable of reducing the speed of growth any further, and the root cannot yet increase its growth on account of the after-effect.

III. A rising section of curve indicating recovery. The root recovers from the effect of inhibition and shows an increased speed of growth again.

B. Additional inhibition of *Allium* root by root-inhibiting substance of *Allium*. The inhibiting substance of *Allium* increases the inhibition which had occurred in water already to a considerable extent.

C. Inhibition of *Allium* root in fresh water. The growth retardation must be caused by an inhibiting substance produced by the root itself. In course of time the equilibrium phase is reached (horizontal section). The application of fresh water changes the horizontal section into the rising section indicating a higher speed of growth.

in diagram 4, in which the decrease is 252—89 = 163 $\mu$ per h. when, 23½ hrs. after the experiment started, inhibiting water was supplied.

Diagram 1, however, also teaches us that the growth retardation can only be very small, as, 192 hrs. after the commencement of the experiment, the decrease in speed of growth amounts to 153—145 = 8 $\mu$ per h. These differences in decrease in speed of growth have to be ascribed to different concentrations of the inhibiting substance and, moreover, to the inactivation of this substance in dependence on the temperature. This will be discussed in more detail later on.

The speed of growth may show a still considerable decrease during a second period of application of inhibiting water. It is, however, also quite possible that the speed of growth during this second period is of the same rate as during the first or it can even be higher, which means that the speed of growth has increased in spite of the fact that the root is still in the inhibiting water. The root has seemingly adapted itself and become accustomed to the change in environment. Of these cases some examples are given below.

A continued decrease is clearly demonstrated by diagram 3, in which the speed of growth, after 167¾ hrs., shows a decrease of 204—149= 55 μ per h. after application of inhibiting water, followed by a second decrease, after 191¼ hrs., of 149—96= 53 μ per h. From the fact that the second decrease is practically as great as the first it can be concluded that the speed of growth has regularly decreased as a result of the application of inhibiting water.

A constancy of speed of growth is seen in diagram 5. After 47½ hrs. a second period of inhibition begins during which the speed of growth is essentially the same as it was during the first (158 and 163 μ per h., respectively).

Finally, the increase in speed of growth in the second period of inhibition is demonstrated by diagram 4 in which after 71½ hrs. the decrease in speed of growth of 200—106= 94 μ per h. is followed after 95 hrs. by a considerable rise of 150—106= 44 μ per h.

On the strength of these data I suggest the following diagrammatical representation of the action of the inhibiting substance (see fig. 2.) In this curve three phases can be distinguished:

1. A falling section of curve as a result of the activity of the inhibiting agent. The speed of growth gradually falls off till the minimum is reached.
2. A horizontal section of curve which must be a result of a certain equilibrium. The inhibiting substance is no longer capable of reducing the speed of growth any further, and the root cannot yet increase its growth on account of the after-effect.
3. A rising section of curve indicating recovery. The root recovers from the effect of the inhibition and shows an increased speed of growth again.

The mutual relations of the three sections of the curve may, among other things, be dependent on the concentration of the inhibiting substance.

The process of retardation is a reversible one. If, namely, the inhibited root is placed in fresh water, an increase in speed of growth is observed. At first the original value of the speed of growth is not reached as is evident from diagram 1, in which 71¾ hrs. after the start of the experiment the speed of growth increases by 135—55= 80 μ per h., whereas the previous decrease, 48 hrs. after the beginning of the experiment, caused by inhibition amounted to 171—55= 116 μ per h.

Diagram 5 shows a case in which after the application of fresh water a higher speed of growth than the one before inhibition is attained. The increase after the change to fresh water (after 120 hrs.) amounts to 200—98= 102 μ per h., against a decrease (after 96 hrs.) during the previous period of inhibition of 179—98= 81 μ per h. This increase even appears to continue during the next period.

The solution of the inhibiting agent is inactivated in course of time, as the inhibiting action decreases in dependence on time. Diagram 1 (from 48 hrs. onwards) gives some idea of this inactivation. The actual values are tabulated in table 1.

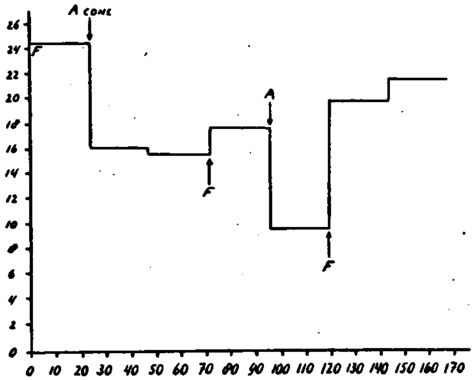



Diagram 5. Inhibition of *Allium* root by concentrated *Allium* water. The mean speed of growth during the second part of the period of inhibition remains practically the same (47½ hrs. after the commencement of the experiment).

EXPLANATION OF SYMBOLS : F = fresh water, A = *Allium* water, A conc. = concentrated *Allium* water. Abscissa : time in hrs., ordinate : mean speed of growth in units of 10 μ per h.

*Table 1.* Inactivation of solution of root-inhibiting substance. Explanation in the text.

| Inhibition period | Decrease of speed of growth |
|---|---|
| I | 171— 55 = 116 μ per hour |
| II | 135— 89 = 46 μ per hour |
| III | 157—123 = 34 μ per hour |
| IV | 153—145 = 8 μ per hour |

As a result of the inactivation of the inhibitor the speed of growth-time-diagram shows a characteristic stepwise succession of levels of inhibiting activity which might be described as "step-by-step phenomenon".

Also diagram 7 illustrates the inactivation of a certain solution of the inhibiting agent. It is an inactivation curve showing the relation between the percentage of inhibition and time. This percentage of inhibition indicates the growth during a period of inhibition, expressed as a percentage of the growth during the preceding fresh water period. The inactivation of the inhibitor is evident from the increase in percentage of inhibition, the latter being directly proportional to the growth of the root. From the concavity of the diagram towards the time-axis it can be concluded that the inactivation is strongest in the beginning and afterwards slightly decreases.

As regards the effect of the temperature on the inhibiting substance the following remarks can be made. It appeared to be possible to concentrate the inhibiting substance at a moderate temperature (15°—20° C.).

Examples of the inhibiting action can be found in diagrams 3 and 5. Diagram 3 shows, in addition, from 47¾ hrs. up to 119¼ hrs. after the beginning of the experiment, the step-by-step phenomenon, so that the concentrated inhibiting agent is also inactivated in dependence on time. The first decrease in speed of growth amounts to 183—106= 77 $\mu$ per h., the second to 166—128= 38 $\mu$ per h. The inactivation diagram (relation between the percentage of inhibition and time) of a concentrated solution of the inhibiting agent is given in diagram 8.

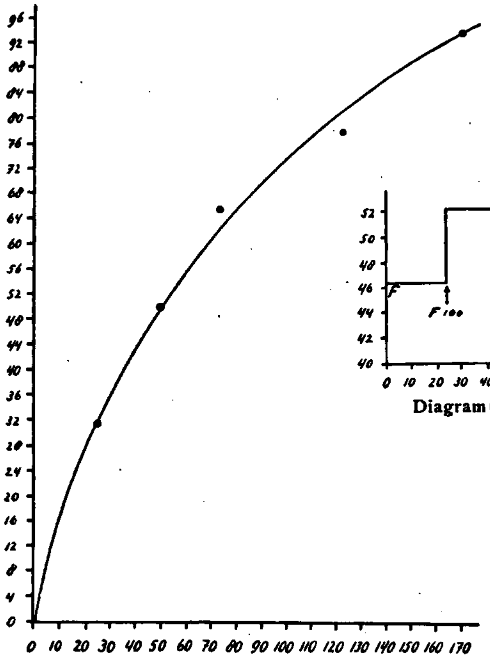



Diagram.7

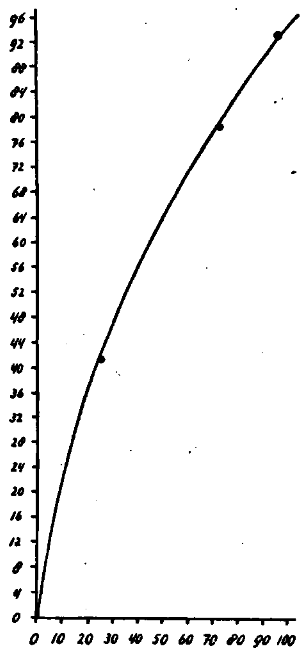


Diagram 8

Diagram 6. Control experiment. Fresh water, heated at 100° C. for some time, does not exert any inhibiting action on the *Allium* root.

EXPLANATION OF SYMBOLS : F = fresh water, F 100 = fresh water, heated at 100° C. for some time. Abscissa : time in hrs., ordinate : mean speed of growth in units of 10 $\mu$ per h.

Diagram 7. Inactivation of root-inhibiting substance of *Allium* in dependence on time. Abscissa : time in hrs., ordinate : percentage of inhibition. The percentage of inhibition indicates the growth during a period of inhibiting, expressed as a percentage of the growth during the preceding fresh water period. In this inactivation diagram the effect of the inhibiting action is inversely proportional to the percentage of inhibition.

Diagram 8. Inactivation of concentrated root-inhibiting substance of *Allium* in dependence on time. Abscissa : time in hrs., ordinate : percentage of inhibition. Cf. diagram 7.

The root-inhibiting substance of *Allium* is also resistant to heating at 100° C., so that it, unlike those of *Fuchsia* and *Pelargonium,* is thermostabile. Whereas the unheated inhibiting water in diagram 1 causes a decrease in speed of growth of 243—170= 73 $\mu$ per h., the decrease caused by the same substance after it had been heated at 100° C. amounts

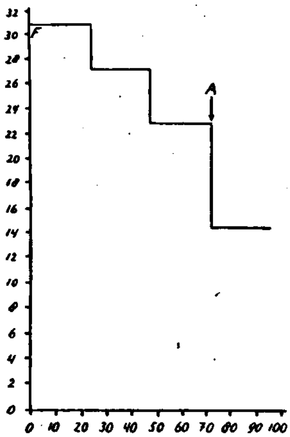



**Diagram 9**

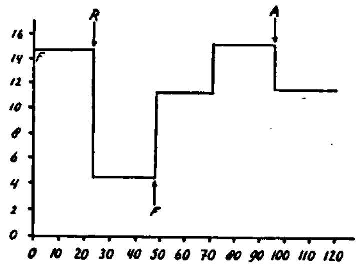



**Diagram 10**

Diagram 9. Inhibition of *Allium* roots by an inhibiting substance produced by the root itself (auto-inhibition). Explanation of symbols: F = fresh water, A = *Allium* water. Abscissa: time in hrs., ordinate: mean speed of growth in units of 10 $\mu$ per h.

Diagram 10. Inhibition of *Allium* root by root extract water. After application of fresh water the original mean speed of growth is attained again. The decrease in mean speed of growth by root extract water is greater than the decrease caused by *Allium* water.

Explanation of symbols: F = fresh water, A = *Allium* water, R = root extract water. Abscissa: time in hrs., ordinate: mean speed growth in units of 10 $\mu$ per h.

to 226—111= 115 $\mu$ per h. The control experiment is represented in diagram 6, which clearly shows that fresh water heated at 100° C. by no means exerts a retarding influence. In the heated tap water the root even shows a slightly increased speed of growth.

The thermostability of the root-inhibiting substance of *Allium* is also clearly demonstrated by diagram 12, in which, after 23½ hrs., the boiled inhibiting water causes a decrease in speed of growth of 813—289= 524 $\mu$ per h. In the next inhibiting period the decrease in speed of growth of 813 $\mu$ per h. was attained in boiled tap water.

Also the extract of ground *Allium* roots appeared to contain a fairly large amount of the inhibiting substance, as administration of the root extract causes a considerable decrease in speed of growth. In diagram 10 the retardation amounts to 149—47= 102 $\mu$ per h. The inhibition is counteracted by the application of fresh water, so that ultimately the speed of growth reaches its original value.

Diagram 11 shows that the retarding action of the root extract is also resistant to the temperature of boiling water, the decrease in speed of growth being practically the same as the one caused by a boiled and diluted root extract. The first, measured after 24 hrs., was 589—404= 185 $\mu$ per h. (diagram 11 A) and the second 569—402= 167 $\mu$ per h. (diagram 11 B).

Diagram 13, finally, shows that the root-inhibiting substance of *Allium* can be concentrated by evaporation at 100° C. After 23½ hrs., the application of inhibiting water concentrated about 2.7 times causes a decrease in speed of growth of 353—21= 332 $\mu$ per h. (diagram 13 A),

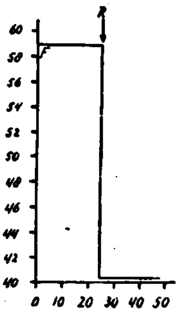



Diagram 11A

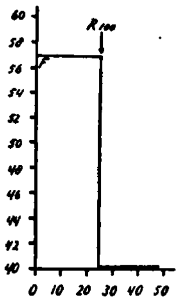



Diagram 11B

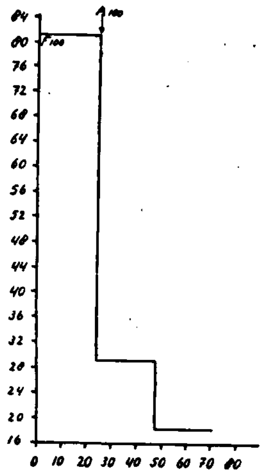



Diagram 12

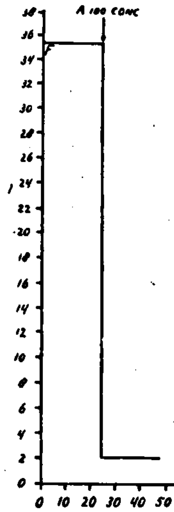



Diagram 13A

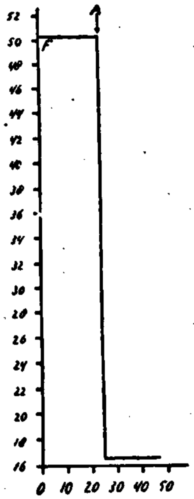



Diagram 13B

Diagram 11. Thermostability of inhibiting substance in aequous extract of *Allium* roots.

A. Inhibition of *Allium* root by root extract water.

B. Inhibition of *Allium* root by root extract water which had been heated at 100° C. for some time. The decrease in speed of growth caused by the root extract water is of the same order of magnitude as the one caused by the heated aequous extract.

Explanation of symbols: F = fresh water, R = root extract water, R 100 = the same heated at 100° C. for some time. Abscissa: time in hrs., ordinate: mean speed of growth in units of 10 $\mu$ per h.

Diagram 12. Thermostability of root-inhibiting substance of *Allium*. Inhibition of *Allium* root by *Allium* water heated at 100° C. for some time.

Explanation of symbols: F 100 = fresh water heated at 100° C. for some time. A 100 = *Allium* water heated at 100° C. for some time. Abscissa: time in hrs., ordinate: mean speed of growth in units of 10 $\mu$ per h.

Diagram 13. Concentration of root-inhibiting substance of *Allium* by evaporation at 100° C.

A. Inhibition of *Allium* root by *Allium* water, concentrated by evaporation.

B. Inhibition of *Allium* root by *Allium* water.

The fall in speed of growth caused by *Allium* water appears to be about the same as the one caused by the concentrated *Allium* water.

Explanation of symbols: F = fresh water, A = *Allium* water, A 100 conc. = *Allium* water concentrated by evaporation at 100° C. Abscissa: time in hrs., ordinate: mean speed of growth in units of 10 $\mu$ per h.

whereas the decrease as a result of the original, not concentrated, solution amounts to 502—166= 336 $\mu$ per h. (diagram 13 B).

These experiments having been taken simultaneously, the external conditions were exactly the same.

In the reported experiments the retardation was always obtained by means of an inhibiting substance produced by a different specimen, as we had made a distinction between "inhibitor onions" and "test onions". It is quite feasible, however, that the speed of growth of the roots also decrease on account of an inhibiting substance produced by these roots themselves. In some cases this was indeed observed. Diagram 9, for instance, shows three successive "fresh water periods" with a considerably decreasing speed of growth. The first decrease amounts to 308—272= 36 μ per h., the second to 272—229= 43 μ per h., whereas the subsequent administration of an inhibiting substance causes a decrease of 229—145= 84 μ per h.

In diagram 2 the speed of growth remains about constant for 72 hrs.: first period 55 μ per h., second period 54 μ per h., third period 50 μ per h. After application of fresh water the speed of growth increases by 104—50= 54 μ per h., so that undoubtedly an inhibiting substance must have played a part.

These two cases can be satisfactorily explained by means of the diagram in fig. 2 A. In diagram 9 the falling section of the curve is involved, which decreases even more rapidly after the application of a concentrated solution of the inhibitor (see fig. 2 B). The approximately stationary speed of growth in diagram 2, on the other hand, leads to the conclusion that the action of the inhibiting substance has proceeded further already and is in the so-called equilibrium phase. The administration of fresh water changes the horizontal section of the curve into the rising part (see fig. 2C).

COMPARISON OF EXPERIMENTAL RESULTS WITH THOSE IN THE EXISTING LITERATURE.

As was the case with my study on *Fuchsia* and *Pelargonium*, I have to rely on the data about inhibiting substances retarding germination for a comparison of my experimental results with the existing literature, only the former having been studied to some extent. Again I am able to find parallelism between FRÖSCHEL's results (1939, 1940), obtained with *Trifolium* and *Beta*, and my results with *Allium*, because the *Allium* root does not only show the phenomenon of self-inhibition discovered by this author, but is also capable of recovering in fresh water after a period of inhibition, so that also in this instance the inhibition can be considered to be a reversible one.

Moreover, the inhibiting substance of *Allium* is thermostabile and its behaviour towards high temperatures is consequently the same as that of the inhibiting substance of *Beta*, this in contradistinction to the inhibiting substances of *Fuchsia* and *Pelargonium* which were shown to be thermolabile. The various substances inhibiting germination and those retarding root growth are tabulated in table 2, in which also their behaviour towards the temperature is reported.

It should be mentioned that I was able to find more evidence for my explanation of the accelerated growth upon application of fresh water from my experiments with *Allium* (see fig. 2). According to this explanation the accelerated growth is caused by elimination of the inhibition. It remains to be seen, however, whether every acceleration of growth has to be considered to be an elimination of inhibition, which theory was put forward by VON VEH (1936), and at any rate this cannot be decided from my experiments with *Allium*.

*Table 2.* Survey of various inhibiting substances.

| Author | Source of inhibiting substance | Nature of inhibiting substance | Behaviour towards temperature |
|---|---|---|---|
| OPPENHEIMER (1922) | Fruitpulp of tomato | Inhibiting germination | Thermolabile |
| REINHARD (1933) | Fruitjuice of tomato | Inhibiting germination | Thermostabile |
| KÖCKEMANN (1934) | Fruitpulp of apple, quince, pear, tomato | Inhibiting germination | Thermostabile |
| LEHMANN (1937) | Exocarp of buckwheat | Inhibiting germination | Thermolabile |
| FRÖSCHEL 1940) | *Beta* | Inhibiting germination | Thermostabile |
| STOLK (1952) | *Fuchsia* and *Pelargonium* | Inhibiting root growth | Thermolabile |
| STOLK (1953) | *Allium* | Inhibiting root growth | Thermostabile |

In the introduction it has already been mentioned that the results obtained by WEAVER and HIMMEL (1930), namely that *Phragmites* and *Spartina* roots show an optimum growth in a soil saturated with water and those obtained by GORDIENKO (1930), namely that a similar soil condition is necessary for the development of a maximum amount of roots of *Secale cereale, Triticum repens, Pisum sativum* and *Lupinus luteus,* are in good agreement with the conception of the occurrence of root-inhibiting substances. However, the observation made by GORDIENKO, that the elongation of the roots of the above-mentioned species is reduced by a higher moisture content, can as yet not be explained by means of the theory of root-inhibiting substances.

SUMMARY OF RESULTS.

1. *Allium* roots excrete a substance retarding the growth of *Allium* roots and, accordingly, shows the phenomenon described by FRÖSCHEL as "self-inhibition".
2. The inhibition can be stopped by a transfer of the inhibited root to fresh water and is, therefore, a reversible one.
3. The root-inhibiting substance of *Allium* is thermostabile. Unlike the root-inhibiting substances of *Fuchsia* and *Pelargonium* the growth-retarding action is not lost by a heating at 100° C.
4. The root-inhibiting substance of *Allium* can be concentrated by evaporation both at a moderate temperature (15—20° C.) and at a higher temperature (100° C.).
5. The root-inhibiting substance of *Allium* does not maintain its inhibiting action indefinitely. In dependence on time inactivation occurs.

6. Also the aequous extract of *Allium* roots retards root growth and must therefore contain an inhibiting substance.
7. The inhibiting action of the aequous extract of *Allium* roots is not lost after heating it at 100° C.: thermostability of the extract of *Allium* roots.
8. The inhibiting action in *Allium* could be represented in a diagram. Most probably three phases have to be distinguished in this process, viz.:
   a. a phase of inhibition in which the inhibiting substance gradually decreases the speed of growth of the roots.
   b. a phase of equilibrium in which the inhibiting substance is not capable of reducing the speed of growth any further, so that the speed of growth remains practically constant.
   c. a phase of recovery in which the roots show an increased speed of growth again.

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